LA FORCE DE RÉACTION AU SOL VERTICALE MAXIMALE COMME TÉMOIN D'EFFETS FONCTIONNELS ET STRUCTURAUX CHEZ DES MODÈLES CANINS D'ARTHROSE : POTENTIEL ENVERS LE DÉVELOPPEMENT THÉRAPEUTIQUE

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Thèse présentée à la Faculté de médecine vétérinaire

en vue de l'obtention du grade de

philosophiae doctor (Ph.D.)

en sciences vétérinaires

option pharmacologie

Décembre, 2014

Résumé

Les modèles animaux d'arthrose permettent d'évaluer le potentiel d'agents thérapeutiques en phase préclinique de développement. Le présent ouvrage tient compte du chien comme modèle d'arthrose naturelle (chez l'animal de compagnie) ou expérimentale (par sectionnement chirurgical du ligament croisé crânial). Au sein des expérimentations, la force de réaction au sol verticale maximale, mesurée lors de l'analyse cinétique de la locomotion, est proposée comme témoin d'effets fonctionnels et structuraux sur ces modèles d'arthrose.

Sur un modèle canin d'arthrose naturelle, le seuil de changement minimal détectable a été déterminé. Les changements au dysfonctionnement locomoteur peuvent désormais être cernés en s'affranchissant de la marge d'erreur inhérente à la mesure de la force verticale maximale. Il en découle l'identification de répondants lors d'essais cliniques entrepris chez le chien arthrosique. Une analyse rétrospective a, par la suite, déterminé un taux de répondants de 62.8% et d'une taille d'effet de 0.7 pour des approches thérapeutiques actuellement proposées aux chiens arthrosiques. Cette analyse détermina également que la démonstration d'une réponse thérapeutique était favorisée en présence d'un fort dysfonctionnement locomoteur.

Sur un modèle canin d'arthrose par sectionnement chirurgical du ligament croisé crânial, la force verticale maximale a démontré une relation inverse avec certains

types de lésions arthrosiques évaluées à l'aide d'imagerie par résonance magnétique. Également, la sensibilité de la force verticale maximale a été mise en évidence envers la détection d'effets structuraux, au niveau de l'os sous-chondral, par un agent anti-résorptif (le tiludronate) sur ce même modèle.

Les expérimentations en contexte d'arthrose naturelle canine permettent de valider davantage les résultats d'essais cliniques contrôlés utilisant la force verticale maximale comme critère d'efficacité fonctionnelle. Des évidences cliniques probantes nécessaires à la pratique d'une médecine basée sur des faits sont ainsi escomptées. En contexte d'arthrose expérimentale, la pertinence d'enregistrer le dysfonctionnement locomoteur est soulignée, puisque ce dernier est en lien avec l'état des structures. En effectuant l'analyse de la démarche, de pair avec l'évaluation des structures, il est escompté de pouvoir établir la répercussion de bénéfices structurels sur l'inconfort articulaire.

Cet ouvrage suggère qu'une plateforme d'investigations précliniques, qui combine le modèle canin d'arthrose par sectionnement chirurgical du ligament croisé crânial à un essai clinique chez le chien arthrosique, soit un moyen de cerner des bénéfices structuraux ayant des impacts fonctionnels. Le potentiel inférentiel de ces modèles canins d'arthrose vers l'Homme serait ainsi favorisé en utilisant la force verticale maximale.

Mots-clés : Analyse cinétique démarche, essais cliniques, médicament, recherche préclinique, douleur, boiterie

Abstract

Animal models of osteoarthritis are useful to evaluate the potential of osteoarthritis therapeutics at the preclinical stage of development. In this thesis, the dog is used as a model of naturally-occurring (*i.e.* companion animal) and experimentally induced (*i.e.* by surgical transection of the cranial cruciate ligament) osteoarthritis. The peak of the vertically-oriented ground reaction force, which is measured during kinetic gait analysis, is proposed to be an indicator of structural and functional benefits in these models of osteoarthritis.

In a canine model of naturally-occurring osteoarthritis, the threshold of the minimal detectable change in peak vertical force was determined. An improvement in the locomotor disability can now be identified according to the measurement error (noise) of the peak vertical force. This allows the identification of responders when the peak vertical force is used as an outcome measure of functional benefits. A retrospective analysis later determined that current therapeutic approaches provided a responder rate of 62.8% with an effect size of 0.7 in dogs with naturally-occurring osteoarthritis. This analysis also determined that the therapeutic response is favored in cases of severe locomotor disability.

In a canine model of osteoarthritis induced by surgical transection of the cranial cruciate ligament, the peak vertical force demonstrated an inverse relationship with different types of structural changes, as evaluated upon magnetic resonance imaging. The sensitivity of the peak vertical force to detect structural benefits on the subchondral bone was also shown in this model using an antiresorptive agent (*i.e.* tiludronate).

The experiments conducted in dogs with naturally-occurring osteoarthritis further validate findings from clinical trials in which the peak vertical force is used as an outcome measure of functional benefits. The practice of an evidence-based medicine is then expected. The experiments conducted in dogs with surgically-induced osteoarthritis support the recording of the locomotor disability, being in line with the level of the structural changes. By performing gait analysis in addition to structural evaluations, it is expected to establish the impact of structural benefits on joint discomfort

This thesis suggests that a platform for preclinical investigations, which combines the canine model of osteoarthritis induced by surgical transection of the cranial cruciate ligament and a clinical trial in dogs with naturally-occurring osteoarthritis, offers the opportunity to discern structural benefits having functional impacts. A better prediction of outcomes for human clinical trials is expected by using the peak vertical force.

Keywords : Kinetic gait analysis, clinical trials, drugs, preclinical research, pain lameness

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% BW:	*Percentage of body weight* – Pourcentage de poids corporel
%:	*Percentage* – Pourcentage
ρ:	*Pearson correlation coefficient* – Coefficient de corrélation de Pearson
µg:	*Micrograms* – microgrammes
Δ:	Delta
∫F:	*Function* – Fonction
°:	Degree – Degré
Θ:	*Thêta angle* – Angle théta
σ:	*Standard deviation* – Écart-type
µm:	*Micrometers* – Micromètres
95% CI:	*95% confidence intervals* – Intervalles de confiance à 95%
ACL:	*Anterior cruciate ligament* – Ligament croisé antérieur
ADAM:	*A disintegrin and metalloprotease*
ADAMTS:	*A disintegrin and metalloproteases with thrombospondin motifs*
AMPA:	Alpha-amino-3-hydroxy-5-méthylisoazol-4-propionate
ANCOVA:	*Analysis of covariance* – Analyse de covariance
ANOVA:	*Analysis of variance* – Analyse de variance
AINS :	Anti-inflammatoire non stéroïdien
AP1:	*Activator protein one*
ARN:	Acide ribonucléique

ASIC: *Acid-sensing ion channels*

ATP: Adénosine triphosphate

AUC: *Area under the curve* – Aire sous la courbe

$a_{vertical}$: *Vertical acceleration* – Accélération verticale

b: *Regression y-intercept* – Intercepte avec l'axe des Y

BCD: *Brachystemma calycinum* D *don*

BDNF: *Brain-derived neurotrophic factor* – Facteur neurotrophique issu du cerveau

BMLs: *Bone marrow lesions* – Lésions de la moelle osseuse

BMU: *Basic multicellular unit* – Unité multicellulaire de base

BPs: *Bisphosphonates* – Biphosphonates

BW: *Body weight* – Poids corporel

CBPI: *Canine brief pain inventory*

CCL: *Cranial cruciate ligament* – Ligament croisé crânial

CCLT: *Surgical cranial cruciate ligament transection* – Sectionnement chirurgical du ligament croisé crânial

CGRP: *Calcitonin gene related peptide*

cm: *Centimeters* – Centimètres

CoD: *Coefficient of dispersion* – Coefficient de dispersion

COF: *Center of force* – Centre de la force

COM: *Center of mass* – Centre de la masse

COX: *Cyclooxygenase* – Cyclooxygénase

CRCHUM: Centre de recherche du Centre Hospitalier de l'Université de Montréal

CSF-1: *Macrophage colony-stimulating factor one*

CSOM: *Case specific outcome measure* – Mesure d'évaluation spécifique au patient

CTR: *Control group* – Groupe contrôle

D: *Day* – Jour

d: *Perpendicular distance from the center of rotation to the force vector* – Distance perpendiculaire au centre de rotation vers le vecteur force

DAAI: *Daily averaged active intensity* – Intensité d'activité quotidienne moyenne

DATI: *Daily averaged total intensity* – Intensité totale quotidienne moyenne

DDAP: *Daily duration of active period* – Durée quotidienne de période d'activité

DDR2: *Discoidin domain receptor two*

DHA: *Docosahexaenoic acid* – Acide docosahexaénoïque

DMOAD: *Disease-modifying OA drug* – Agent structuro-modulateur dans l'arthrose

DRASIC: *Dorsal root acid sensing ion channel*

Dt: *Time derivative* – Dérivée de temps

EDA: *Electrodermal activity* – Conductance électrique du derme

EP: Récepteurs membranaires aux prostaglandines E_2

EPA: *Eicosapentaenoic acid* – Acide eicosapentaénoïque

ES: *Effect size* – Taille de l'effet

F: Force

FRS: Forces de réaction au sol

g0: *Standard gravitational acceleration on earth* – Accélération gravitationnelle standard sur terre

GABA: Acide γ-aminobutyrique

GDU: *Gelatin digesting unit*

GREPAQ: Groupe de Recherche en Pharmacologie Animale du Québec

GRF: *Ground reaction forces*

GRV: *Ground reaction vector* – Vecteur de réaction au sol

HCPI: *Helsinki chronic pain index*

Hz: Hertz

I: *Impulse* – Impulsion

ICC: *Intra-class coefficient of correlation* – Coefficient de corrélation intra-classe

ICE: *Interleukin – 1 beta converting enzyme*

IGF-1: *Insulin-like growth factor one*

IL-1: Interleukine-1 bêta

iNOS: Isoforme inductible de l'enzyme oxyde nitrique synthase

Kcal: Kilocalories

kg: *Kilograms* – Kilogrammes

k_m : *Surface area to weight ratio*

LACIME: Laboratoire de communications et d'intégration de la microinformatique

LOAD: *Liverpool osteoarthritis in dogs*

LOX: Lipoxygenase – Lipoxygénase

m/s: *Meter per second* – Mètre par seconde

M: Masse

m: *Meters* – Mètres

m: *Regression slope* – Pente de régression

MAP: *Mitogen-activated protein*

MCP-1: *Monocyte chimoattractant protein one*

MDC: *Minimal detectable change* – Changement minimal détectable

MDC_{95}: *Minimal detectable change at the 95% confidence level* – Changement minimal détectable à l'intervalle de confiance à 95%

MIA: *Monosodium iodo-acetate*

MITF: *Microphthalmia-associated transcription factor*

mm: *Millimeters* – Millimètres

MMPs: *Matrix metalloproteinase* – Métalloprotéinases matricielles

MRI: *Magnetic resonance imaging* – Imagerie par résonance magnétique

N: Newton

NF-kappa B: *Nuclear factor-kappa B*

ng: *Nanograms* – Nanogrammes

NHPs: *Natural health products* – Produits de santé naturels

Nm: *Newton meters* – Mètres Newton

NMDA: N-méthyl-D-aspartate

NOS: *Nitric oxide synthase* – Enzyme oxyde nitrique synthase

NOx: Nitrites et nitrates

NRS: *Numerical rating scale* – Échelle de cotation numérique

NSAIDs: *Non-steroidal anti-inflammatory drugs* – Agents anti-inflammatoires non stéroïdiens

NY: New York

NK-1 : Neurokinine un

OA: *Osteoarthritis* – Arthrose

OARSI: *OsteoArthritis Research Society International*

Omega-3: *Omega-3 fatty acids* – Acides gras oméga-3

OMERACT: *Outcome Measures in Rheumatology*

ON: Ontario

p: *Linear momentum* – Quantité de mouvement linéaire

p: Momentum – Quantité de mouvement

P: *Power* – Puissance

P: *Probability* – Probabilité

P2X: *Ionotropic ligand-gated purinergic receptors*

PAR-2: *Protease activated receptor two*

pg: *Picograms* – Picogrammes

PGE2: Prostaglandine E2

PNF: *Peak normal force* – Force normale maximale

PUFA: *Polyunsaturated fatty acids* – Acides gras polyinsaturés

PVF: *Peak vertical force* – Force verticale maximale

QC: Québec

RANKL: *Receptor activator of nuclear factor kappa-B ligand*

RCTs: *Randomized controlled trials* – Essais cliniques randomisés

r_s: *Spearman coefficient* – Coefficient de Spearman

RUNX2: *Runt-related transcription factor two*

s: *Seconds* – Secondes

SC: *Subcutaneous* – Sous-cutané

SD: *Standard deviation* – Écart-type

SEM: *Standard error of measurement* – Erreur standard de mesure

SPGR: *Three-dimensional spoiled gradient recalled sequence with fat suppression*

t: *Time* - Temps

T1w-GRE: *T1-weighted three-dimensional fast gradient recalled echo*

T2w-FS: *T2-weighted fast spin echo sequence with fat saturation*

TGF-beta: *Transforming growth factor beta*

TIMPs: *Tissue inhibitor of metalloproteinases*

TLN: Tiludronate

TNF-alpha: *Tumor necrosis factor-alpha* – Facteur nécrosant tumoral alpha

TNF-R1: *Tumor necrosis factor receptor one* – Récepteur au facteur tumo-nécrosant alpha

TRAP: *Tartrate-resistant acid phosphatase*

TREK-1: *TWIK1-related K^+ channel*

TRP: *Transient receptor potential*

TRPM8: *Transient receptor potential melastatin eight*

TRPV1: *Transient receptor potential vanilloid one*

uPA: *Urokinase-type plasminogen activator*

USA: *United States of America* – États-Unis d'Amérique

v: *Velocity* – Vitesse

VAS: *Visual analog scale* – Échelle visuelle analogique

VEGF: *Vascular endothelial growth factor* – Facteur de croissance de l'endothélium vasculaire

VOL: Volume

VTD: *Veterinary therapeutic diet*

W: Watts

W: *Week* – Semaine

WNT: *Wingless integration site*

À ma fille et mes fils,

Puissiez-vous trouver à même cet ouvrage une parcelle d'inspiration au dépassement.
Sachez que ce qui importe n'est pas tant l'arrivée mais bien l'ascension.

Pour Anne,

Je te suis d'une infinie reconnaissance pour ton support infaillible.
Nulle autre que toi n'aura été un si parfait complément durant ce parcours.

Remerciements

L'auteur voudrait remercier :

Docteur **Éric Troncy**

Cet homme d'exception, qui par son esprit de meneur, sa capacité remarquable d'intégration scientifique et son dévouement incomparable, a suscité (et maintenu) chez moi un désir de dépassement et d'accomplissement.

Docteur **Bertrand Lussier**

Pour son encadrement manifeste lors de ce programme de doctorat et pour avoir rendu mémorables nos séjours à l'étranger.

Docteur **Jean-Pierre Pelletier**

Pour son appel téléphonique en 2003 qui a donné l'envol au développement d'une expertise et d'une profonde satisfaction personnelle.

Docteure **Stéphanie Keroack**

Pour avoir teinté ce programme de doctorat d'expériences professionnelles des plus enrichissantes.

Mes amis les Docteurs **Martin Guillot** et **Jérôme del Castillo**

Deux comparses dotés d'un niveau de savoir que je ne peux qu'espérer un jour atteindre.

Mon ami le Docteur **Christian Bédard**

Pour nos distractions entretenues à mon bureau dans l'*underground.*

La subvention de formation des Instituts de recherche en santé du Canada en évaluation et traitement des troubles de la mobilité et posture (Programme MENTOR).

Le Fonds de recherche du Québec – Santé.

ArthroLab.

La force de réaction au sol verticale maximale comme témoin d'effets fonctionnels et structuraux chez des modèles canins d'arthrose: Potentiel envers le développement thérapeutique

Introduction

Le squelette du mammifère forme avec les muscles et les articulations le système myo-arthro-squelettique. Les interactions biomécaniques entre les différents composants de ce système permettent l'exécution du mouvement et l'action locomotrice. L'arthrose est une pathologie du système myo-arthro-squelettique qui afflige de manière fulgurante aussi bien l'humain (Lawrence *et al.*, 2008; Cross *et al.*, 2014) que le chien (*Canis domesticus*) (Johnston, 1997). La présence de dommages arthrosiques altère les propriétés structurelles et le rôle fonctionnel des tissus de l'articulation synoviale, ce qui mène à une perte d'aisance dans l'exécution des mouvements articulaires. Au niveau d'une articulation sollicitée lors de la locomotion, la présence d'arthrose pourra induire une irrégularité de la démarche qualifiée de dysfonctionnement locomoteur (DeCamp, 1997; Constantinou *et al.*, 2014). Ce dysfonctionnement est principalement attribué à un signe clinique prépondérant lors d'arthrose, soit le ressenti douloureux au niveau de l'articulation.

Le développement d'agents anti-arthrosiques (aux effets structuro-modulateurs et/ou analgésiques) requiert des modèles animaux afin d'évaluer le potentiel thérapeutique d'un candidat d'intérêt et ce, en contexte d'arthrose, qu'elle soit d'étiologie naturelle ou expérimentale. Le modèle à préconiser doit être en mesure

de produire fidèlement ce qui sera observé ultérieurement lors d'essais cliniques, particulièrement au regard des effets attendus envers les dommages structuraux et le dysfonctionnement locomoteur.

Le présent ouvrage tient compte du chien comme espèce modèle d'intérêt pour l'ensemble des investigations réalisées en contexte d'arthrose d'étiologie naturelle (chez l'animal de compagnie) ou expérimentale (par sectionnement chirurgical du ligament croisé crânial). Les travaux de recherche, présentés au chapitre Expérimentations, ont comme point commun l'usage de la force de réaction au sol verticale maximale mesurée lors de l'analyse cinétique de la locomotion. La force verticale maximale est proposée comme témoin d'effets thérapeutiques face au dysfonctionnement locomoteur et aux dommages structuraux encourus chez deux modèles canins d'arthrose.

Le Chapitre 1 sera dédié à une recension des écrits avec, en premier lieu, une description du mouvement locomoteur. L'Article I traitera des forces de réaction au sol chez des modèles canins et félins d'arthrose; suivront aussi des notions concernant la physiopathologie de l'arthrose, l'expérience sensorielle et l'instrumentation métrologique. L'Article II traitera de l'influence du gain de poids envers la force verticale maximale chez le chien arthrosique. Finalement, un survol des différents modèles d'arthrose sera effectué. Le modèle canin d'arthrose naturelle sera présenté et supporté par trois essais cliniques contrôlés qui ont utilisé la force verticale maximale comme critère d'efficacité (Articles III, IV, V). Le

modèle félin d'arthrose naturelle sera également présenté en mettant en lumière l'acquisition et la gestion des données de force verticale maximale chez ce modèle (Article VI).

Le Chapitre 2 sera dédié aux expérimentations sur le modèle canin d'arthrose naturelle et sur celui par sectionnement chirurgical du ligament croisé crânial. En premier lieu, les travaux de recherche seront voués à déterminer la marge d'erreur inhérente à la mesure de la force verticale maximale de même que le seuil de changement minimal détectable sur le modèle canin d'arthrose naturelle (Article VII). L'établissement de ce seuil permettra de distinguer les changements au dysfonctionnement locomoteur (amélioration ou détérioration) en s'affranchissant de la marge d'erreur inhérente à la mesure de la force verticale maximale. Ce seuil servira, ultérieurement, à cerner l'impact de l'activité quotidienne par l'analyse télémétrique du mouvement locomoteur réalisée chez le chien arthrosique. Également, ce seuil permettra de définir ce qu'est un répondant thérapeutique dans un contexte d'essais cliniques effectués chez le chien atteint d'arthrose naturelle. Une analyse rétrospective sera par la suite effectuée afin de déterminer la présence d'un lien entre la fréquence de répondants et différents facteurs, tels que le niveau de dysfonctionnement locomoteur initial et le type de traitement administré.

En second lieu, le lien entre le dysfonctionnement locomoteur, représenté par la force verticale maximale, et la sévérité des différents types de dommages

structuraux sera investigué sur un modèle canin d'arthrose par sectionnement chirurgical du ligament croisé crânial (Article VII). Ces travaux de recherche s'avèrent possibles et particulièrement stimulants suite à la récente caractérisation des dommages structuraux à l'aide d'imagerie par résonance magnétique, soit les atteintes au cartilage articulaire (volumétrie et lésions, Annexe II), les lésions à l'os sous-chondral (Annexe III) de même que la taille des ostéophytes et le degré d'effusion articulaire (Annexe IV).

La sensibilité de la force verticale maximale envers la détection d'effets structuro-modulateurs d'une plante thérapeutique au niveau du cartilage articulaire a été récemment démontrée sur le modèle canin d'arthrose par sectionnement chirurgical du ligament croisé crânial (Annexe V). Dès lors, il est proposé, en troisième lieu, de mettre en évidence la sensibilité de la force verticale maximale envers la détection d'effets structuro-modulateurs au niveau de l'os sous-chondral, tel que transmis par un agent anti-résorptif, le tiludronate, chez ce même modèle (Article VIII).

Le Chapitre 3 discutera du modèle canin d'arthrose naturelle et de celui par sectionnement chirurgical du ligament croisé crânial. Il y aura présentation d'arguments critiques envers un effet placebo présumé selon la force verticale maximale (Article IX). Un retour sera effectué sur l'ensemble des travaux de recherche puisqu'ils se proposent d'être inscrits dans une quête de conformité avec la règle des trois « R » qui consiste à Remplacer, Réduire et Raffiner l'emploi d'animaux en recherche (Russell, 1995). Cette thèse s'intègre dans un désir de

présenter la force verticale maximale comme témoin d'effets fonctionnels et structuraux chez des modèles canins d'arthrose. Il est proposé que le développement d'agents anti-arthrosiques, tant pour l'humain que pour l'espèce canine puisse être favorisé.

Des annexes sont présentées à la fin de cet ouvrage. Le lecteur est invité à prendre connaissance de l'Annexe I qui décrit les outils de mesure de la force verticale maximale. Le lecteur est également invité à prendre connaissance d'articles complémentaires, présentés aux Annexes II-VIII, qui abordent divers sujets en lien avec ceux discutés au sein de cette thèse.

CHAPITRE 1. RECENSION DES ÉCRITS

1 Le mouvement locomoteur

Le mouvement se définit comme étant le déplacement d'un corps par rapport à un point fixe situé dans l'espace. La locomotion, pour sa part, est la faculté qu'a un organisme vivant de se mouvoir dans son entier afin d'assurer son déplacement. Du mouvement locomoteur découle un changement de la géométrie du corps et une modification de l'emplacement de son centre de masse, dont la trajectoire définit l'orientation du déplacement dans l'espace.

1.1 La locomotion terrestre

La locomotion d'un organisme est possible dans un milieu terrestre, aquatique et aérien. La locomotion terrestre requiert un contact avec le sol. Elle s'effectue chez l'arthropode à l'aide de membres locomoteurs (communément appelés pattes) du système appendiculaire, qui sont constitués de segments s'articulant entre eux. La locomotion terrestre peut également s'effectuer chez l'apode par un phénomène de reptation.

1.1.1 Étude cinétique de la locomotion terrestre

L'analyse du mouvement locomoteur fascine l'Homme, tel qu'en témoigne l'œuvre la salle des Taureaux (grotte de Lascaux, France). Cette peinture à base de pigments sur des parois rocheuses révèle l'attention particulière de l'Homme envers la locomotion du quadrupède et ce, dès la préhistoire, soit vers 18 000 - 15 000 avant J.C. Cette représentation picturale d'une faune en mouvement démontre une qualité exceptionnelle. Ainsi, l'illustration du mouvement locomoteur au sein de cette

œuvre pariétale est d'une exactitude considérable, dépassant même la qualité des représentations datant de l'âge moderne, soit de la préhistoire jusqu'à l'époque Muybridge (1887, voir ci-dessous) (Horvath *et al.*, 2012).

L'apparition d'instruments spécialisés a permis un gain considérable envers l'approfondissement des connaissances en ce qui entoure l'analyse du mouvement locomoteur. Il est possible de dénoter les travaux d'Eadweard Muybridge (1830-1904) comme étant déterminant envers l'étude du mouvement. Cet homme, décrit comme étant le père du cinéma, utilisa la décomposition photographique comme témoin rigoureux et objectif du mouvement locomoteur. Il confirma dès lors la présence d'une phase de non appui chez le cheval au galop, théorie précédemment émise par Étienne-Jules Marey (1830-1904, voir ci-dessous) (Sadoul, 1966).

Les années 1870-1890 furent fastes en découvertes et s'inscrivirent comme un moment clé envers la compréhension du mouvement locomoteur. Il est intéressant de préciser que l'exactitude dans les diverses représentations artistiques du mouvement locomoteur (peinture, sculpture) fut grandement améliorée suite aux travaux d'Eadweard Muybridge. À ce moment, ce dernier illustra les phases consécutives du mouvement locomoteur de différents quadrupèdes (Muybridge, 1979, Intégrale réimprimée). Par conséquent, l'époque Muybridge (1887) explique l'amélioration significative de la qualité de la description du mouvement locomoteur (Horvath *et al.*, 2012).

Les éléments se rapportant à l'évaluation cinématique, c'est-à-dire la trajectoire d'un point et sa vitesse à un instant donné, sont sous la gouverne de forces qui régissent le mouvement. Tel est le constat d'Étienne-Jules Marey, un médecin et physiologiste français. Ce dernier conçoit un dynamomètre inscripteur pouvant enregistrer la force générée par un sujet stationnaire et non immobile (Marey, 1883). Cet outil est le prototype de ce qui sera plus tard appelé plateforme de force par Herbert Elftman dont ce dernier décrivit le fonctionnement en 1938 (Elftman, 1938). Dès lors, il fut possible de quantifier les forces générées par le déplacement du centre de masse d'un corps en mouvement et ce, dans les trois plans orthogonaux. L'analyse cinétique du mouvement locomoteur voyait ainsi le jour en décrivant les forces qui interviennent dans le mouvement locomoteur.

1.1.2 Le mouvement locomoteur et les forces de réaction au sol

Sir Isaac Newton (1642-1727) présenta ses trois lois du mouvement en 1687 dans un ouvrage intitulé *Principia Mathematica Philosophiae Naturalis* (Newton, 1687). En accord avec la troisième loi du mouvement de Newton, pour chaque action d'une force, il y a une réaction. La réaction est de magnitude égale mais de direction opposée à l'action causale.

En contact avec le sol, le membre locomoteur exerce des forces orientées selon trois plans (orthonormés X, Y et Z) dont la direction et l'intensité varient à chaque instant en fonction de la progression de la locomotion (Kirtley, 2006; Whittle, 2007). L'analyse des forces de réaction au sol cible, en fonction du temps, l'évolution des forces générées lors de la locomotion terrestre (Kirtley, 2006; Whittle, 2007).

1.2 Article I: The kinetic measurements of gait for osteoarthritis research in dogs and cats

Cet article, publié dans *The Canadian Veterinary Journal*, présente la mesure des forces de réaction au sol chez le chien et le chat. Sous forme de synthèse, cet article décrit la nature des forces de réaction au sol de même que différents calculs dérivés. Le but de cet article était de présenter les principes fondamentaux qui régissent la mesure des forces de réaction au sol en contexte d'arthrose naturelle et expérimentale chez le chien et le chat.

Cet article est présenté avec la perspective que la mesure des forces de réaction au sol reflète le dysfonctionnement du membre locomoteur. La capacité fonctionnelle du membre s'avère être particulièrement limitée en présence d'arthrose d'étiologie naturelle ou expérimentale.

M. Maxim Moreau a proposé ce travail ainsi que son contenu, puis rédigé cet article présentement publié (*The Canadian Veterinary Journal,* 55:1057-1065, 2014) et a effectué l'ensemble des travaux d'infographie. L'article a par la suite été dûment révisé et bonifié par l'expertise de chacun des coauteurs.

The Canadian Veterinary Journal
55:1057-1065, 2014

The Kinetic Measurements of Gait for Osteoarthritis Research in Dogs and Cats

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1.3 Abstract

Over the last two decades the measurement of ground reaction forces (GRF) has been extensively used in dogs and cats to gain insights on normal locomotion, discrepancies under pathologic conditions and biomechanical changes following surgical procedures. Ground reaction forces have become a well established outcome measure of pain-related functional impairment in animals affected by experimental and naturally occurring osteoarthritis. This paper comprehensively reviews the nature of the GRF and presents arguments regarding its measurement in osteoarthritis research.

1.4 Résumé

Au cours des deux dernières décennies, la mesure des forces de réaction au sol (FRS) a été largement utilisée chez les chiens et les chats afin de mieux comprendre la locomotion normale, les anomalies en conditions pathologiques et les changements biomécaniques suivant une procédure chirurgicale. Les FRS sont devenues un critère d'évaluation bien connu de la limitation fonctionnelle liée à la douleur chez l'animal atteint d'arthrose expérimentale et naturelle. Le présent manuscrit dresse un aperçu de la nature des FRS et présente les arguments qui supportent son usage dans un contexte de recherche sur l'arthrose.

1.5 Introduction

Animals move from one point to another by means of sequential and coordinate motion of jointed appendages called limbs. Every time a limb interacts with the ground, the animal's body is subjected to ground reaction forces (GRF) in response to muscular and inertial forces the limb exerts (1).

Ground reaction forces combined with the downward effect of gravity are external forces acting on a moving body (2). The study of GRF involves the field of kinetics, which addresses forces associated with movement (3). Over the last two decades the measurement of GRF has increased in dogs and cats, particularly to gain insight on normal locomotion and discrepancies under pathologic conditions (4). In companion animals, GRF are commonly measured using a force platform, accounting prominently for a large fraction of the overall publications in this field (4). Furthermore, when coupled to kinematic analyses, the measurement of the GRF also provides input for a complete description of the entire mechanical processes of locomotion (5).

Osteoarthritis is a highly prevalent musculoskeletal condition, which involves structural changes and disability at the level of the affected joint (6). The GRF have become well-established outcome measures of functional impairment in dogs and cats affected by osteoarthritis. This comprehensive review aims to describe the nature of the GRF and derivatives and to present the fundamentals regarding their

measurement in dogs and cats affected by experimental and naturally occurring osteoarthritis. The authors propose the use of GRF as an outcome measure, which reflects pain-related functional impairment in the context of osteoarthritis.

1.6 Nature of GRF

1.6.1 Weight and force

There is a clear distinction between mass and weight. The mass of an object, denoted as m and expressed in kg refers to the amount of matter contained in it. The weight of an object is the force directed downward in the vertical axis ($F_{vertical}$) in response to the gravitational acceleration of the body's center of mass (COM) (Equation 1) (3).

(1) Weight of an object = $m \times g_0$ = Downward $F_{vertical}$
where g_0 is the standard gravitational acceleration on earth.

Weighing an animal accurately isn't an easy task as the reading changes according to the animal's movement. When the mass of the body is constant, fluctuation in the measurement of the body weight involves additional acceleration (downward or upward) of the whole body. For a moving object, the net $F_{vertical}$ corresponds to the weight of the body and additional force due to inertia (Equation 2) (1).

(2) Net downward $F_{\text{vertical}} = (m \times g_0) + (m \times a_{vertical})$

where $a_{vertical}$ is the acceleration (m/s^2) of the COM in the vertical axis. The direction of the additional acceleration (downward or upward) governs the net $F_{\text{vertical,}}$ which could therefore be higher or lower than the weight of the moving animal.

1.6.2 Action and reaction forces

Each time an object hits the ground or another object, 2 forces are in opposition: the action and the reaction forces. The relationship between an action and its reaction is given by Newton's third law of motion (3). Hence, for each action, there is a reaction equal in magnitude and opposite in direction. For a body in contact with the ground, the downward F_{vertical} generates the same reaction back on the body. This reaction force is referred to as GRF. When vertical displacement of the COM occurs, the effect of inertial force has to be considered (Equation 3).

(3) Net downward $F_{\text{vertical}} = (m \times g_0) + (m \times a_{vertical}) = Upward\ \text{GRF}_{\text{vertical}}$

1.7 Ground reaction force patterns

The gait of a quadruped is defined as a manner of moving which can differ according to sequence and rhythm of footfalls and to the number of support limb(s) in each stage of 1 cycle of footfalls (7). Whatever the type of gait involved, a series of rhythmic, alternating movements of the body results in the forward progression of the COM along a horizontal trajectory (8). The linear movement of the COM is

disrupted by the alternation of footfalls. In humans as well as in dogs and cats, the COM executes a sinusoidal displacement in the 3 orthogonal plans from which the direction and the magnitude of the net acceleration govern the presence of GRF in causal relationships (2). For an object having complex shape and different densities such as an animal's body, direct measurement or calculation is required to precisely determine limb inertial parameters and thereby the COM. In dogs, although the COM is in close relationship with the length of the neck (7), the common position of the COM is illustrated in **Figure 1** as previously described (9).

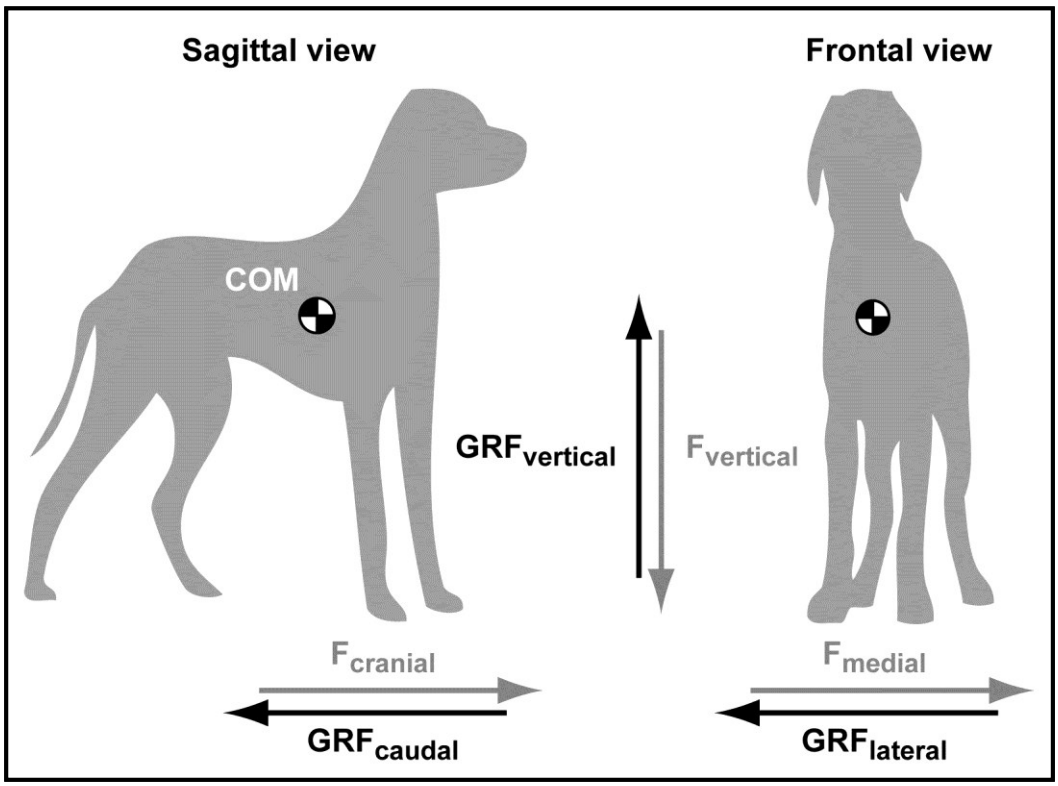

Figure 1. Sagittal and frontal views of a dog in a standing position

The three orthogonal force (F) vectors are paired with their oppositely directed ground reaction forces (GRF). The center of force (COM) is located according to previously published scheme (9) and is only indicative of its exact position. The F_{medial} *refers to the right limbs.*

Movements of the COM give rise to $F_{craniocaudal}$, $F_{mediolateral}$ and $F_{vertical}$ (**Figure 1**). As a result, $GRF_{craniocaudal}$, $GRF_{mediolateral}$ and $GRF_{vertical}$ are produced during the stance phase. Typical GRF *versus* time curves are illustrated in **Figure 2**. The GRF vectors are expressed herein as positive when directed upward, cranially and medially.

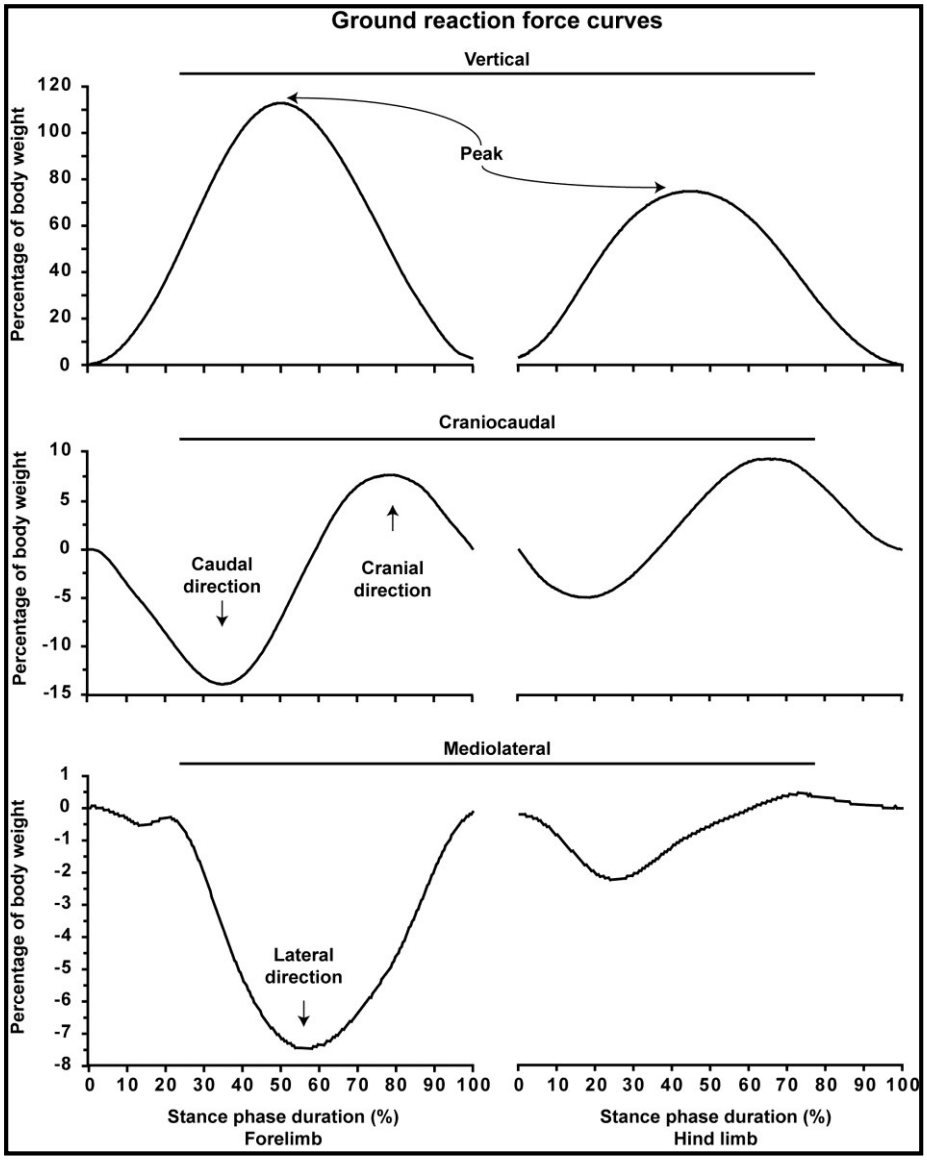

Figure 2. Typical curves of the ground reaction forces

Measurements are from a 27-kg golden retriever dog crossing the force plate at a trotting gait velocity (2.0 m/s). Ground reaction forces are considered positive when directed upward, cranially and medially. The stance phase is expressed for the forelimb and hind limb to ease their distinction. Peaks denote the points of maximal values of the fore and hind limb GRFvertical.

1.7.1 Vertical ground reaction force

At the initiation of the stance phase, the $GRF_{vertical}$ begins to be measured as the mass of the dog in motion is gradually supported by the limb (**Figure 2**). Soon after, a maximal point is attained which is the highest product of the mass of the body and the net vertical acceleration of the COM. This point is referred as the peak of the $GRF_{vertical}$ (*i.e.* peak vertical force). The $GRF_{vertical}$ then decreases until toe-off, which defines the end of the stance phase. In some situations the pelvic limb begins the stance phase when the thoracic limb is still in contact with the ground. This overlap explains why the $GRF_{vertical}$ doesn't fall to zero when the thoracic limb has left the ground.

For a dog standing still on his 4 limbs, the body weight is expected to be supported at 30% by each thoracic limb and at 20% by each pelvic limb (10). The COM being closer to the thorax contributes to this imbalance in pelvic-to-thorax weight distribution when standing (10). As illustrated in **Figure 2Erreur ! Source du renvoi introuvable.**, the $GRF_{vertical}$ of the thoracic limb reaches a maximal point (*i.e.* 113% of body weight), which is in accordance with the literature (11) (see Equation 3). Hence, the body absorbs a high level of forces in the vertical axis reaching more than 3 times the one observed at the stance (*i.e.* 30% *versus* 113% of body weight).

1.7.2 Craniocaudal and mediolateral ground reaction forces

The pattern of the $GRF_{craniocaudal}$ involves successive caudal and cranial components **Figure 2**. The GRF_{caudal} is in fact generated as a reaction to the force applied in the direction of movement (*i.e.* cranially or forward). Hence, as a result of the $F_{cranial}$, a GRF is generated and directed caudally to the dog (*i.e.* backward) which decelerates the dog's motion. During the second half of the stance phase, the $GRF_{cranial}$ propels the dog forward.

A third force vector is also depicted during the gait of dog. **Figure 2** illustrates the $GRF_{mediolateral}$, which involves medial and lateral components. Although the pattern of $GRF_{mediolateral}$ grossly mimics those reported in the literature (12), there is no clear waveform established for $GRF_{mediolateral}$ in dogs and cats due to inconsistent results (10,12).

1.8 Ground reaction vector

The three GRF components are directed in opposite directions by 90° (**Figure 1**). The resultant GRF, which is the net effect of the three orthogonal GRF components, is called the ground reaction vector (GRV). In the sagittal plane, a right-angled triangle is formed by the $GRF_{craniocaudal}$ (adjacent side) and the $GRF_{vertical}$ (opposite side) of the angle θ (**Figure 3**) (1). The hypotenuse, which defines the magnitude and orientation of the GRV in the sagittal plan, can be resolved according to Pythagoras theorem (Equations 4 and 5) (1).

(4) Magnitude of the GRV (sagittal plan) = Square root ($GRF_{vertical}^2$ + $GRF_{craniocaudal}^2$)

(5) Direction of the GRV (*i.e.* angle θ at the vertex) = $Tangent^{-1}$ $GRF_{vertical}/GRF_{craniocaudal}$

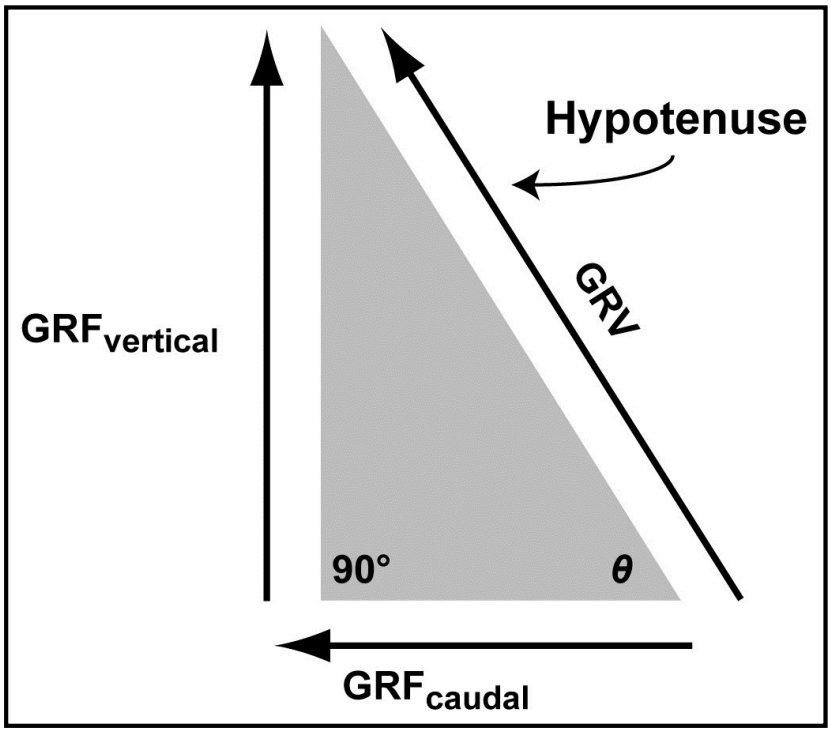

Figure 3. Ground reaction vector

The concept of sagittal ground reaction vector (GRV) is illustrated using a right angled triangle according to Pythagoras theorem. In the sagittal plane, vertical ground reaction force ($GRF_{vertical}$) and $GRF_{craniocaudal}$ (caudal component) are orthogonal, giving the magnitude of the GRV as the square root of $GRF_{vertical}^2$ + $GRF_{craniocaudal}^2$. The angle θ refers to the direction of the GRV obtained by the $tangential^{-1}$ of $GRF_{vertical}/GRF_{craniocaudal}$.

The sagittal GRV derived from the measures of a typical dog are illustrated at selected time points in **Figure 4**. As shown, the direction and the magnitude of the forelimb sagittal GRV evolve over time. At 60% of the stance phase, the sagittal GRV is perpendicular to the $GRF_{craniocaudal}$ while being directed cranially thereafter. This force vector diagram can be used to determine where the path of the GRV travels according to anatomical structures or joints at specific points of the stance phase (*i.e.* caudal to the brachialis muscle, through the carpal joint). In cats, the GRF vector was used to investigate walking strategy and muscle activity under different conditions (13). The GRV can also serve to calculate moment of force, which is the tendency of a force to rotate an object. A moment of force is denoted as M and expressed in newton-metres (Nm) (Equation 6) (3).

(6) $M = F \times d = GRV \times d$

where d is the perpendicular distance from the center of rotation to the force vector.

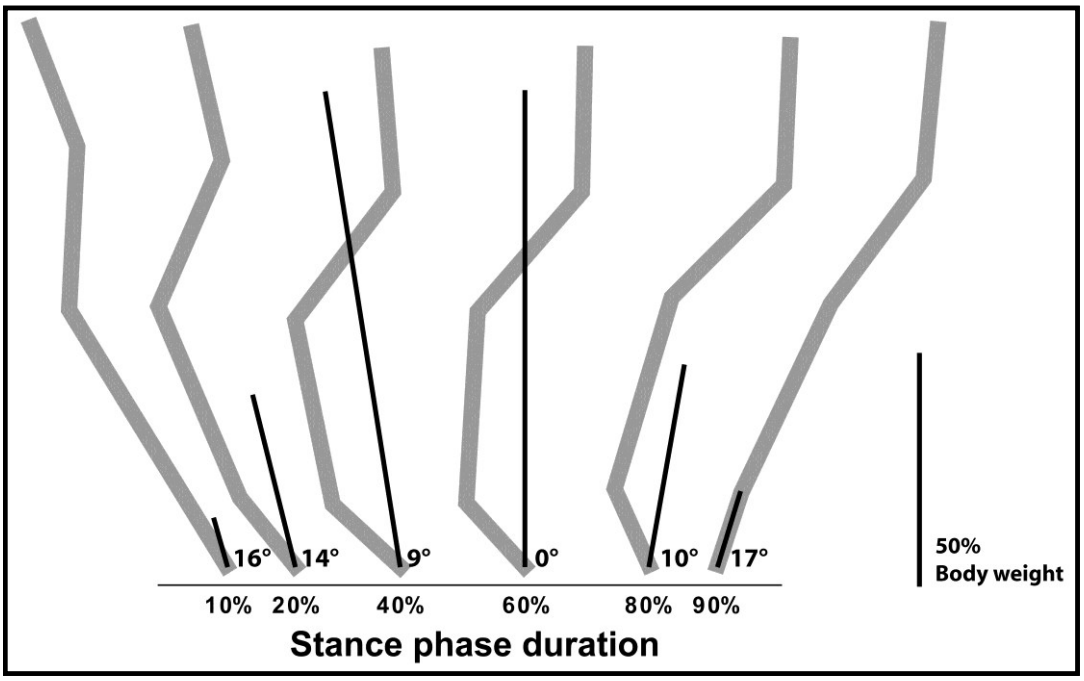

Figure 4. Progression of the ground reaction vector

Illustration of the progression of the ground reaction vector (GRV) for a 27-kg dog crossing the force plate at a trotting gait velocity (2.0 m/s). The measures are for the forelimb. The degrees indicate the direction of the GRV at specific points of the stance phase. The length of the lines indicates the magnitude of the GRV. The direction and magnitude of the GRV are calculated according to the ground reaction force value presented in ***Figure 2****. In the background, forelimb bone position is detailed in gray as previously illustrated and is only indicative of its exact position. The length of the bar indicates 50% of body weight.*

1.9 Center of force

The center of force (COF) is a coordinate pair (*x,y*) located within the surface of any parts of the body which are in contact with the ground. The COF corresponds to the average location where all the $F_{vertical}$ act. When an animal is moving, the distribution of the $F_{vertical}$ within the paw is modified and the COF changes accordingly. The COF can also be depicted as a trajectory according to its displacement during the stance phase. In humans, the path of the COF moves in a curvilinear fashion from heel to toes (14). In the dog, little is known about the COF trajectory. However, the $F_{vertical}$ appears to show a distribution pattern among the pads in the dogs (15). The COF can serve to determine limb positioning, and serve to precisely define where the GRV takes its origin (1,16).

1.10 Impulse, linear momentum and power

The area under any force *versus* time curve represents the impulse denoted as *I* and expressed in N × s or in kg × m/s. Impulse is the time integral of a force (Equation 7) (3).

(7) $I = \int F dt$

where *t* is the time (s).

The linear momentum of a moving object is the product of the mass and velocity and is denoted as *p* and expressed in N × s or in kg × m/s (Equation 8) (3).

(8) $p = m \times v$

where *v* is the velocity (m/s).

According to the impulse-momentum relationship, the impulse for a given time interval represents the change in linear momentum (equation 9) (17).

(9) $I = \Delta p = m \times \Delta v$

It is common to report the impulse of the measured $GRF_{vertical}$ and $GRF_{craniocaudal}$ which reflects the transfer of momentum from a moving limb to the ground. As illustrated in **Figure 2**, the forelimb undergoes craniocaudal transfer of linear momentum which is higher in the caudal direction. Conversely, the hind limb has a net linear momentum in the cranial direction. The overall net momentum (*i.e.* fore

and hind limbs) has to be null in the craniocaudal axis to maintain a constant gait velocity, otherwise the dog undergoes acceleration or deceleration (16).

During gait, muscles generate or absorb power due to their ability to perform concentric or eccentric activities (3). Power, denoted as P and expressed in watt (W) or in Nm/s is the product of the force and its velocity (Equation 10) (1).

(10) $P = F \times v$

Using Equation 9, the net power output of a limb can be resolved by multiplying the sum of the GRF (*i.e.* $GRF_{craniocaudal}$, $GRF_{mediolateral}$ and $GRF_{vertical}$) by the velocity of the animal (18).

1.11 Ground reaction force measurement devices

Force transducers are typically designed to measure the strain in a material under load. An electrical output proportional to the applied force is then generated and amplified. The most common type of device used to measure the GRF exerted by the body during locomotion is the force platform, which consists of a steel plate with force transducers at each corner.

The force platform allows the measurement of GRF using either strain gauges or piezoelectric crystals as a sensing element (3). As GRF have components in the 3 orthogonal planes, the force platform should have sensing elements arranged in a manner to capture all oriented strains for a complete measurement of the GRF

generated during the locomotion of the animal. Force platforms can be used alone (10) or combined to allow the simultaneous measurement of the GRF among the 4 limbs (19). Suitable and reproducible measurement of the $GRF_{vertical}$ is also possible when integrated into a treadmill (20).

There is another type of device, which allows the measurement of the $GRF_{vertical}$ as well as the surface of the body in contact with the ground (see Additional material 1). This involves the concept of pressure, defined as a force expressed per unit area (3). Using sensitive elements, which base their properties on the presence of conductive material and pressure-sensitive ink, it is possible to obtain the force/pressure-distribution pattern of a given paw during the stance phase. When integrated into a portable walkway (several feet long, few millimeter thick), the plantar force/pressure measurement system allows the acquisition of $GRF_{vertical}$ from simultaneous and consecutive footfalls over several strides, which is particularly relevant for small to medium sized quadrupeds (21,22). This device restricts their measurement to the $GRF_{vertical.}$ A secondary role for the $GRF_{craniocaudal}$ and $GRF_{mediolateral}$ is therefore assumed when using this device.

1.12 Ground reaction force acquisition setting

A typical force platform requires a long walkway (6-10 meters) with the force platform (Annexe I) positioned near the center to favour free motion and to avoid intuitive braking at the end of the runaway. The force platform can be either

embedded in the floor or mounted flush with the surface of an elevated (5-8 cm) walkway. The walkway must be carefully made to avoid any vibration or echo, which can alter the signal (back noise) and disturb the animal. Effort must be addressed to avoid visual recognition or texture difference between the surfaces of the floor and the force platform.

At the trot, a typical acceptable trial involves a single thoracic limb on the force plate followed by its pelvic counterpart (**Figure 5**). The limb must be in full contact with the surface of the force platform. Visual inspection or *a posteriori* video monitoring ensures quality of the trial and limb distinction. After unsuccessful attempts to obtain the desired limb on the force plate, the handler may have to modify the starting position. In numerous published investigations, 5 valid trials are acquired to record the GRF before being averaged or used as per trial measurements.

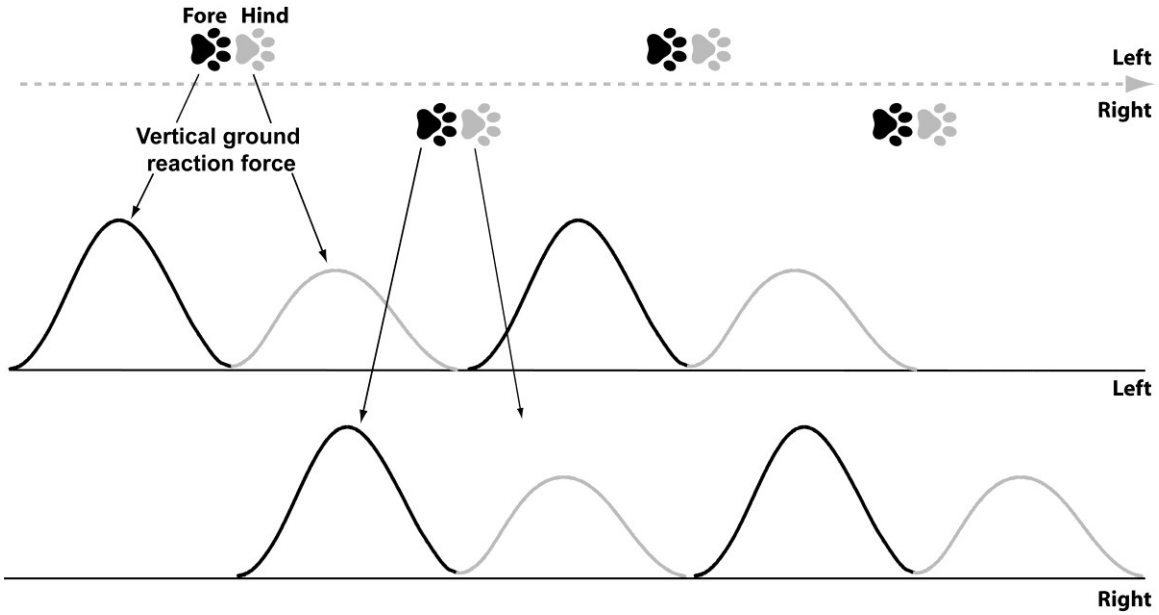

Figure 5. Patterns of footfalls

Illustration of patterns of footfalls from a 23-kg dog crossing a force/pressure measurement system at a trotting gait velocity (2.0 m/s). The corresponding typical curves of the vertical ground reaction forces are also illustrated. For this dog, a cycle of footfalls has a 0.5 second duration and 0.5 meter long. Both fore and hind limb footfalls usually overlap, which is not illustrated here for clarity purposes.

The use of common force platform is generally restricted to large quadrupeds (>20 kg). In smaller dogs and in cats, the presence of more than a paw in contact with the force platform compromises the measurement by a summation process. Specially designed devices are required to record GRF in such animals (13,23,24).

The general reluctance of cats to be handled with a leash also contributes to the paucity of force platform data in this species. Researchers use a plantar force/pressure measurement system in freely moving or thoroughly conditioned cats (21) (Annexe I).

The repeatability of the GRF measurement is of paramount importance. The peak of the $GRF_{vertical}$ has shown low dispersion in dogs (25) and thoroughly conditioned cats using a plantar force/pressure measurement system (26). Critical factors that need to be kept constant when measuring GRF are the velocity and acceleration of the whole body (27), which can be monitored using a set of 3 to 5 photoelectric cells. In the plantar force/pressure measurement system, the velocity of the animal is computed using time and distance indices.

To further improve the quality of the GRF measurement, the animals must be morphologically identical; however, the *in-vivo* reality brings high mass and anatomical size heterogeneities. According to the theory of dynamic similarity, cursorial quadrupeds move in a dynamically similar fashion. Therefore, GRF measures can be compared between different species as diverse as dog, camel and rhinoceros (28). However, care should be taken to ensure that animals move at the same relative 'body size-normalised' velocity (11), which is achieved by multiplying linear dimensions, time intervals and GRF measures by different constants (29,30). Accordingly, it is common to normalise the peak of the GRF to the body weight of the animal and later express this measure in percentage of body

weight. This normalisation technique improves the homogeneity of the peak of the $GRF_{vertical}$ measured between animals while remaining subjected to intrinsic variation in animals (dog mainly) showing atypical body conformations (11).

1.13 Ground reaction force measurement in experimental model of osteoarthritis

1.13.1 Joint inflammatory pain

Osteoarthritis is a musculoskeletal disease, which involves pain, functional limitation and structural changes to the cartilage and other joint structures. This disease is highly prevalent in companion animals (31). The hallmark clinical manifestation of osteoarthritis is joint pain, which occurs through inflammation (32) and a potentially associated peripheral and/or central nociceptive sensitization (33). Crippling joint pain has the potential to alter normal function, particularly locomotion. The phenomenon is referred to as pain-induced functional impairment, which parties one of the clinical signs of osteoarthritis (34).

Joint pain causes alteration in the normal use of the limb as demonstrated following injection of noxious substances into the synovial space of the stifle (35). The inflammation-related pain in this model is reflected to the musculoskeletal system by reluctance to support the body weight and inertial force, compromising standing and leading to a change in the gait pattern.

The transient decrease in $GRF_{vertical}$ induced in this model is used as an indicator of pain-related functional impairment to evaluate the potential of compounds having analgesic claims in dogs and cats (36). An accelerated restoration of function toward initial levels is then expected from the analgesia provided.

1.13.2 Structural changes of osteoarthritis

Structural osteoarthritis changes can be induced by surgical procedures such as meniscal lesions, varus and valgus osteotomy, myectomy, patellectomy, cartilage scarification and transarticular impacts (37). In dogs and cats, a well-known surgical model of osteoarthritis is the cranial cruciate ligament transection (CCLT) of the stifle joint. The instability caused by sectioning of this passive primary stabilizer induces deleterious mechanical alterations, particularly excessive rotational and translational movements (38). Abnormal loading of specific regions of the joint, along with the release of catabolic and inflammatory mediators, disrupts the normal subchondral bone remodeling process, damages the cartilage and inflames synovial tissues in a manner similar to human post-traumatic osteoarthritis (23,38, 39).

Early after CCLT, there is an acute inflammatory process and a severe decrease in $GRF_{vertical}$ followed by gradual improvement over time (40,41). Up to 4 years after surgery, the GRF remain altered in dogs (42), while recovering to near pre-surgical level in about 1 year in cats (24). In addition to impairment of the surgically-altered stifle, abnormalities also occur in the controlateral limb as denoted by an increase in

$GRF_{vertical}$. The redistribution of body weight and inertial forces to the controlateral limb were reported to be under the control of sensory feedback from the unstable joint to protect against a worsening of structural changes (43). This transfer however invalidates the use of the controlateral limb as a normal control in this model (44).

The $GRF_{vertical}$, which was shown to be a reliable measure in dogs undergoing CCLT (42) was used as an indicator of pain-related functional impairment in this model (45). Hence, beside the conventional tests for benefits associated with structural joint changes of osteoarthritis, investigators can gain insights on another feature of the disease process using GRF measurement; the level of disability associated with the use of the limb. This was recently confirmed by our group (46) demonstrating a structure – function (pain) relationship in the canine CCLT model, similar to the one observed in human beings. Structure was assessed noninvasively by magnetic resonance imaging while the GRF measurement allowed a serial evaluation of pain-related functional impairment. This could explain the impressive translational predictability recorded from this model with products subsequently tested in human (46). Other experimental models of osteoarthritis have been investigated using GRF to validate alteration in kinetics, including a postponed disruption of the CCL by monopolar radiofrequency (47).

1.14 Ground reaction force measurement in naturally-occurring osteoarthritis

Force platforms provide objective, repeatable and clinically meaningful information about the functional abilities in dogs and cats affected by naturally-occurring osteoarthritis (21, 48). The $GRF_{vertical}$ has been shown to be abnormally lower in these species when radiographic signs of osteoarthritis are present (21,25,48). However, the relationship between severity of the structural changes of osteoarthritis, as assessed on radiographs and the presence of abnormal GRF measurement is poor (49) and therefore not well understood. Moreover, the level of disability seems to be different according to the joint in which the structural changes of osteoarthritis are present. Hence, dogs affected by osteoarthritis at the stifle have a more severe decrease in the $GRF_{vertical}$ compared to those having osteoarthritis of the hip joint (25).

The pain generated by osteoarthritis has the potential to alter the normal function of the limb, a phenomenon referred to as functional allodynia. The peak of the $GRF_{vertical}$ showed sensitivity and responsiveness for therapeutic approaches purported to alleviate the pain-related functional impairment in dogs and cats affected by osteoarthritis. Therefore, drug research and development often rely on randomized, placebo-controlled clinical trials with sequential GRF measurements to support pain alleviating properties of therapeutic compounds under investigation. In most cases, improvement is translated as an increment over the initial condition, as previously noted following non-steroidal anti-inflammatory drugs (50-53)

complementary and alternative medicine (50,54,55) and veterinary therapeutic diets (56). Clinical improvement is further indicated when the change in GRF exceeds the one observed with a negative control (placebo) or when similar to a positive control such as a homologated treatment (55). The improvement in GRF measurement is not systematic, and the range of efficacy could reflect the nature of the tested product adapted (or not) to the inflammatory or neuropathic component of pain (46).

Other factors influence the pain-related functional impairment in the presence of osteoarthritis. These factors need to be taken into account. Recently, it was shown that changes in the body mass interfere with the measurement of the $GRF_{vertical}$ in dogs. Hence, when an increase in body mass occurred, dogs did not experience a proportional increase in the peak of the $GRF_{vertical}$ (57) suggesting a change in the gait pattern in the presence of osteoarthritis. In line with this finding, the level of impairment improved in obese osteoarthritic dogs when a decrease in body mass occurred (58). The impact of exercise in osteoarthritic dogs and cats (26) was also evaluated using GRF measurement. Hence, while daily leash walks improve the abnormal level of $GRF_{vertical}$ (59), care should be directed to avoid intensive exercise, which exacerbates limb impairment (60).

1.15 Conclusion

The musculoskeletal systems of quadrupeds such as dogs and cats are able to modify the gait pattern in response to painful stimulation of the joint. In both experimental and clinical contexts of osteoarthritis, measurement of GRF allows quantification of the functional impairment. The measurement of GRF is therefore an outcome measure in osteoarthritis research, which reflects pain-related functional impairment.

Some issues need to be resolved. The first and by far the most critical one is identification of the structural changes that intervene in the pain-related functional impairment. The role of cartilage thinning and focal lesion, bone and meniscus alteration and osteophyte growth as assessed using sensitive methods needs to be put in relationship with the GRF. Some work has been initiated on this issue (46). Whether or not the impairment is exclusively due to pain or involves biomechanical alterations in the normal dynamics and lubrication of the joint is unknown and remains of particular interest for osteoarthritis research.

The measurement of GRF leads to several raw parameters and derivatives. Precautions must be taken to determine a valid (or clinically significant) primary outcome measure. The peak of the $GRF_{vertical}$ is often selected to discern treatment efficacy in osteoarthritis. Measurement error (noise) and minimal detectable change for this endpoint have been established in osteoarthritic dogs (46), but not osteoarthritic cats. Such information is critical to discern that a change is

meaningful and not a difference that might be reasonably expected from the measurement error. Without this information, investigators are unable to distinguish a placebo (or a nocebo) effect from normal variation. How the changes in GRF measurement in dogs and cats affected by osteoarthritis evolves in response to sustained daily life activity is also relevant and has to be determined to improve the quality of data and conclusions that arise from randomized controlled trials.

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2 La physiopathologie de l'arthrose

L'arthrose est un désordre du système myo-arthro-squelettique hautement prévalent tant chez l'humain (Lawrence *et al.*, 2008; Cross *et al.*, 2014) que chez le chien (Johnston, 1997). L'âge, le genre féminin, l'obésité et les traumatismes articulaires sont des facteurs de risque qui prédisposent à l'apparition de dommages articulaires (Blagojevic *et al.*, 2010; Zhang *et al.*, 2010b; Li *et al.*, 2013). La présence de dommages arthrosiques altère les propriétés structurelles et le rôle fonctionnel des tissus, ce qui mène à une perte d'aisance dans l'exécution des mouvements, laissant place à des limitations fonctionnelles ainsi qu'à de l'inconfort, le tout portant inévitablement préjudice à la qualité de vie de l'être atteint.

La destruction progressive du cartilage articulaire et la présence d'un remodelage osseux défaillant sont des éléments caractéristiques d'arthrose. Ils témoignent d'altérations aux propriétés structurelles ayant des répercussions néfastes sur l'accomplissement du rôle fonctionnel des tissus (Martel-Pelletier *et al.*, 2010). L'activation de processus inflammatoires est également prépondérante dans ce désordre (Kapoor *et al.*, 2011), ce qui contribue non seulement au ressenti douloureux mais également à l'ensemble des processus dégénératifs à ce jour irréversibles. Lors d'arthrose, la présence d'une membrane synoviale inflammée (synovite) et d'excroissances osseuses au pourtour de la surface articulaire (ostéophytes) est également notoire (**Figure 6**).

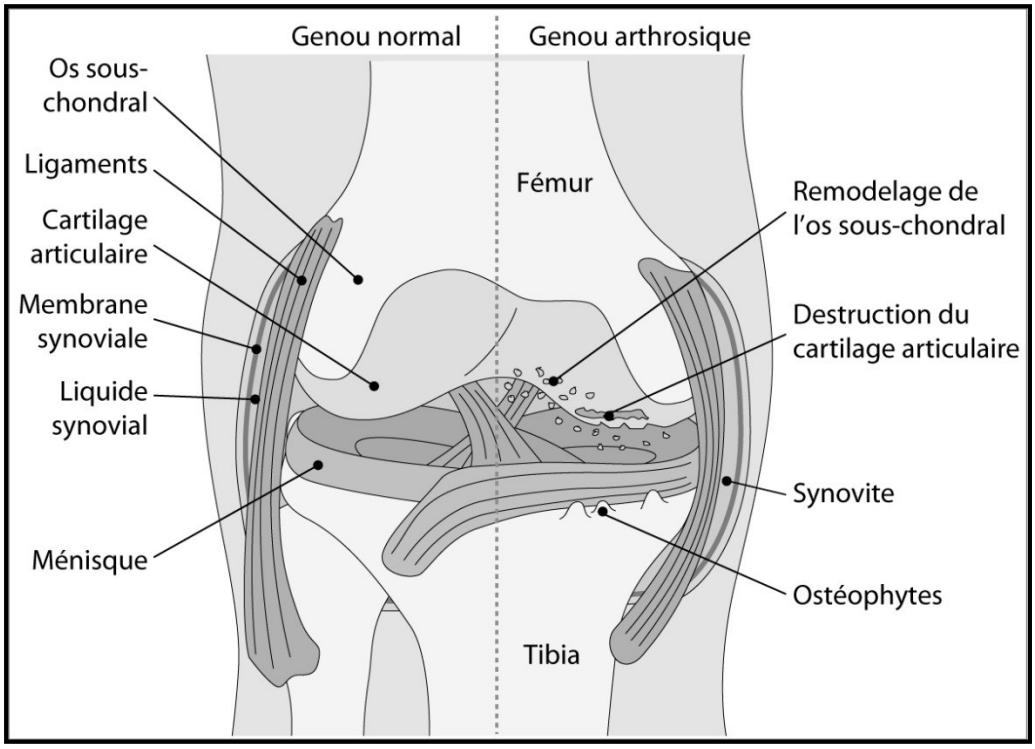

Figure 6. Schématisation des éléments caractéristiques d'arthrose

Adapté de Hunter, 2006 (Hunter *et al.*, 2006).

L'étiopathogénèse de l'arthrose n'est pas clairement définie. Deux concepts ont été postulés, basés sur la mise en jeu d'un stress mécanique soit :

- Une sollicitation mécanique excessive (absolue) tenant compte de structures articulaires normalement constituées (Dijkgraaf *et al.*, 1995; Brandt *et al.*, 2008; Brandt *et al.*, 2009).
- Une sollicitation mécanique excessive (relative) tenant compte de structures articulaires altérées (Dijkgraaf *et al.*, 1995; Brandt *et al.*, 2008; Brandt *et al.*, 2009).

En regard à l'étiopathogenèse de l'arthrose chez le chien, la dysplasie de la hanche et la rupture de ligament croisé crânial (naturelle ou expérimentale) sont reconnues comme source évidente de sollicitation mécanique excessive (Alexander, 1992; Tashman *et al.*, 2004; Pozzi *et al.*, 2013). Cette recension de littérature est rédigée afin de mettre en relief l'implication de la sollicitation mécanique excessive envers la physiopathologie de l'arthrose, en ciblant le phénomène de la dégradation du cartilage. Au préalable, le cartilage articulaire en condition d'homéostasie est présenté.

2.1 Le cartilage articulaire en condition d'homéostasie

2.1.1 Organisation structurelle

Le cartilage articulaire (hyalin) est un tissu de soutien poreux, bi-phasique (phase solide et phase aqueuse) dépourvu de vaisseau sanguin, de vaisseau lymphatique et de neurone afférent primaire (**3.3.2 ci-dessous - Chapitre 1**). Ce tissu recouvre les structures osseuses d'une articulation de type synoviale. Il est constitué d'un enchevêtrement ordonné de divers composants et ce, de la surface articulaire jusqu'en profondeur. Le cartilage articulaire est divisé en trois zones, soit superficielle, intermédiaire et radiale (**Figure 7**).

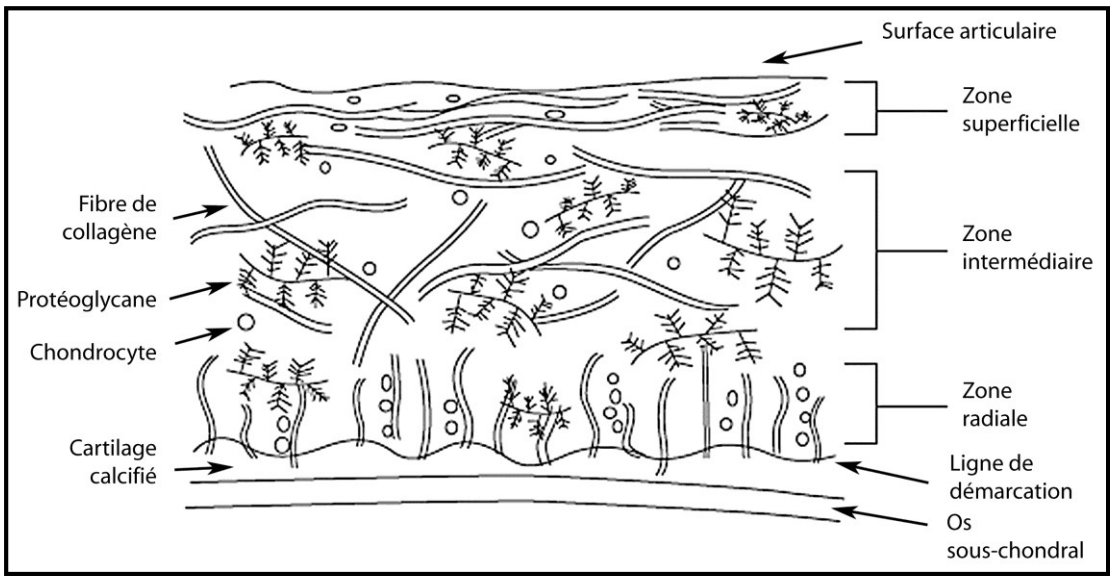

Figure 7. Schématisation de la structure et des principaux composants du cartilage articulaire

Adapté de Setton, 1999 (Setton *et al.*, 1999).

La zone superficielle, la plus mince, est principalement responsable de la résistance aux diverses contraintes mécaniques qu'impose la mise en charge de l'articulation. À ce niveau, le cartilage articulaire est en contact avec le liquide synovial. Ce liquide est un filtrat plasmatique dont la viscosité est assurée par l'acide hyaluronique. L'acide hyaluronique est un polymère de disaccharides (soit l'acide D-glucuronique et le D-N acétylglucosamine) que l'on nomme glycosaminoglycanes (**2.1.1.2 ci-dessous - Chapitre 1**). Les synoviocytes de type B synthétisent et sécrètent de l'acide hyaluronique ainsi que des facteurs cataboliques (**2.2.2.1 ci-dessous - Chapitre 1**). Les synoviocytes de type A phagocytent les produits de dégradation du cartilage. Outre les synoviocytes, la membrane synoviale (**2.4.1 ci-dessous - Chapitre 1**) inclut des plexus de vaisseaux

sanguins et des nocicepteurs. Le rôle du liquide synovial est la lubrification, la résistance aux contraintes mécaniques, la nutrition et l'hydratation des surfaces cartilagineuses. L'acide hyaluronique est également retrouvé au niveau du cartilage articulaire sous forme d'une macromolécule (agrégat, **2.1.1.2 ci-dessous - Chapitre 1**).

La zone radiale prend siège au-dessus du cartilage calcifié (également considéré comme une quatrième zone) et est séparée de ce dernier par la ligne de démarcation. Cette ligne délimite la région minéralisée (cartilage calcifié) du cartilage articulaire (non-minéralisé). La présence de neurones afférents primaires (**3.3.2 ci-dessous - Chapitre 1)** et de vaisseaux sanguins se retrouve plus en profondeur, soit au niveau de l'os sous-chondral.

2.1.1 Composants extracellulaires

La matrice extracellulaire du cartilage articulaire comprend des fibres (majoritairement des fibres de collagène de type II) ainsi qu'un gel (substance fondamentale **2.1.1.2 ci-dessous - Chapitre 1**) hautement hydraté (Martel-Pelletier *et al.*, 2008).

2.1.1.1 Fibres de collagène

Le collagène est une protéine structurelle retrouvée sous forme de fibres dont la structure implique trois chaînes polypeptidiques torsadées. Les fibres de collagène représentent de 50 à 60 % de la masse dessiquée du cartilage. Le collagène de type

II est spécifique au cartilage articulaire et s'avère être le type le plus abondant (90–98 %).

Plusieurs autres types de fibres de collagène sont impliqués, à des degrés divers, dans le maintien de la structure de la matrice extracellulaire. Ainsi on y retrouve les fibres de collagène de type XI (3 % des fibres de collagène) et le type IX (3 % des fibres de collagène). En conditions normales, le collagène de type X sera absent. Lors d'arthrose, ce type de collagène est cependant retrouvé aux régions attenantes aux chondrocytes. Même si certains types de fibres de collagène sont quantitativement secondaires, l'utilité structurelle de ces intervenants fibreux n'est pas négligeable. Ainsi, grâce à des interactions chimiques et des liens mécaniques la substance fondamentale (**2.1.1.2 ci-dessous - Chapitre 1**) sera circonscrite par le treillis fibreux formé de l'enchevêtrement ordonné de divers types de fibres de collagène (Cohen *et al.*, 1998). Au niveau de la zone superficielle, les fibres de collagène de type II sont orientées parallèles à la surface et deviennent graduellement perpendiculaires en profondeur (zone radiale). Les fibres de collagènes sont sensibles à l'effet de certaines enzymes de dégradation, telles que les métalloprotéinases matricielles (MMPs) de type MMP-1, MMP-8 et MMP-13 (**2.2.2.1 ci-dessous - Chapitre 1**).

2.1.1.2 Substance fondamentale

La substance fondamentale se compose de molécules formées de chaînes saccharidiques (glycosaminoglycanes), de chaînes peptidiques (protéines), d'eau (75 % de la masse du cartilage) et de sels (Cohen *et al.*, 1998). Les charges

négatives présentes sur certains glycosaminoglycanes fixent chimiquement une partie de l'eau, procurant ainsi l'hydratation et l'aspect gélatineux de la substance fondamentale, ainsi qu'une résistance au contraintes de compression (Grimsby *et al.*, 2009). Si les fibres de collagène forment un véritable réseau assurant le support structurel au cartilage (en réponse notamment aux contraintes mécaniques de tension), alors les macromolécules polysaccharidiques assurent une réponse à celles de compression.

Le complexe constitué de protéines et de glycosaminoglycanes se nomme protéoglycane. Les protéoglycanes représentent de 5 à 10 % de la masse du cartilage hydraté (Martel-Pelletier *et al.*, 2008). L'aggrécane est un immense protéoglycane retrouvé en abondance au niveau du cartilage, particulièrement au niveau de la zone radiale. Ce composant est d'une importance critique au maintien du rôle fonctionnel du cartilage articulaire *via* la résistance aux contraintes mécaniques.

L'aggrécane est constitué de plusieurs enchaînements de kératanes sulfates, d'héparines-sulfates et de chondroïtines sulfates, des glycosaminoglycanes, greffés à un cœur protéique. Ces cœurs protéiques s'attachent sur l'acide hyaluronique *via* une protéine de liaison. L'aggrécane est doté de la capacité de former une macromolécule (agrégat) en s'agrégeant de manière non-covalente à l'acide hyaluronique qui s'entrelace dans le réseau de fibres collagène (Kiani *et al.*, 2002). Les agrégats sont emmaillotés dans ce réseau.

2.1.2 Le chondrocyte

Le chondrocyte est l'entité cellulaire que l'on retrouve au niveau du cartilage (Martel-Pelletier *et al.*, 2008). Les chondrocytes s'avèrent être plus nombreux au niveau de la zone superficielle qu'en profondeur (zone radiale) (**Figure 7**).

La fonction primaire du chondrocyte est la préservation d'une matrice extracellulaire dont la teneur en ses divers composants est optimale afin de permettre d'assurer le maintien de l'intégrité structurelle et ainsi, soutenir la sollicitation mécanique encourue lors de la mise en charge de l'articulation (rôle fonctionnel).

Il est important de spécifier que le cartilage est un tissu qualifié de braditrophique suite au renouvellement relativement lent des composants de la matrice extracellulaire (Mueller *et al.*, 2011). Ainsi, la demi-vie de l'aggrécane est estimée à 120 jours (ce qui implique un renouvellement aux 240-280 jours chez le chien adulte) alors que celle des fibres de collagène de type II est de 117 ans (Verzijl *et al.*, 2000). En condition d'homéostasie, la matrice extracellulaire accuse un renouvellement perpétuel de ses composants.

Au niveau du cartilage articulaire sain, le chondrocyte est dans un état qualifié de quiescent. Cet état se caractérise par un équilibre entre l'activité anabolique (synthèse) de composants de la matrice extracellulaire et le catabolisme (dégradation) de ceux-ci. Le catabolisme s'effectue majoritairement par des

enzymes de dégradation (**2.2.2.1 ci-dessous - Chapitre 1**) qui contribuent au renouvellement normal des composants. A l'inverse, la synthèse des composants par le chondrocyte est favorisée par la libération de facteurs anaboliques (Martel-Pelletier *et al.*, 2008). Afin de stimuler l'anabolisme, les facteurs de croissance, tels que l'IGF-1 (de l'anglais *Insulin-like growth factor one*) (Schmidt *et al.*, 2006) et le TGF-bêta (*Transforming growth factor beta*) (Martel-Pelletier *et al.*, 2008) sont libérés à cette fin. Ce dernier est cependant doté d'une action catabolique en favorisant la présence d'enzymes de dégradation du cartilage (Moldovan *et al.*, 1997). Le TGF-bêta est également associé à la présence d'ostéophytes (**Figure 6**) (Blaney Davidson *et al.*, 2007).

La mise en charge de l'articulation est nécessaire au maintien d'un état d'équilibre entre le catabolisme et l'anabolisme du cartilage (Houard *et al.*, 2013). Ainsi, plusieurs expérimentations *in-vivo* suggèrent la présence de dommages articulaires, dont la perte en protéoglycanes, suivant l'immobilisation d'un membre (Leong *et al.*, 2011). Cette dégradation du cartilage implique l'action excessive de facteurs cataboliques, particulièrement certaines enzymes de dégradation (Leong *et al.*, 2010).

La mise en charge de l'articulation assure également un échange de fluides entre le cartilage et le liquide synovial ce qui a pour effet, en mettant en jeu des forces d'imbibition, de nourrir et d'assainir le cartilage (O'Hara *et al.*, 1990). Les glycosaminoglycanes contribuent fortement à ce phénomène en étant des molécules

chargées négativement. Ainsi, des ions tels le sodium et le calcium, en solution au sein de la phase aqueuse du cartilage fixent les glycosaminoglycanes, créant de ce fait un échange de fluides par osmose (Grimsby *et al.*, 2009).

Le cartilage étant avasculaire, les chondrocytes résident donc dans un milieu hypoxique. L'oxygène et les nutriments proviennent de l'apport vasculaire au sein de la membrane synoviale, de la capsule articulaire et de l'os sous-chondral.

2.1.3 Rôles et propriétés du cartilage articulaire

Le rôle principal du cartilage est de permettre un glissement entre les surfaces articulaires sous de minimes contraintes frictionnelles, tout en limitant la transmission de l'impact de la sollicitation mécanique aux structures osseuses sous-jacentes. Le cartilage articulaire, dont l'épaisseur varie entre 2-4 millimètres chez le chien sain de 20 kg, s'avère être soumis à des pressions maximales intermittentes variant entre 2.4-18 méga pascals (soit entre 35-260% de poids corporel par centimètre carré chez l'humain de 70 kg) (Hodge *et al.*, 1986; Hodge *et al.*, 1989). La fréquence annuelle de sollicitation articulaire est de l'ordre du million (Silva *et al.*, 2002).

Le faible coefficient de friction entre les surfaces articulaires est tributaire de phénomènes tels que la lubrification en mince film de liquide synovial (Dowson *et al.*, 1969; Hui *et al.*, 2012), la lubrification de surface à l'aide de lubricine (glycoprotéine sécrétée par les synoviocytes, les chondrocytes et par les cellules des

ménisques) (Schmidt *et al.*, 2007) et la lubrification par pressurisation des liquides cartilagineux (pression hydrostatique, turgidité tissulaire) (Krishnan *et al.*, 2004). Le cartilage articulaire est doté de la capacité de se remodeler de manière à supporter les contraintes extrêmes associées à la mise en charge de l'articulation. À titre d'exemple, chez l'haltérophile, le cartilage articulaire du genou démontre une adaptation structurelle en étant plus épais aux régions de contact (Grzelak *et al.*, 2014).

L'architecture et la nature des différents composants de la matrice extracellulaire confèrent au cartilage articulaire la propriété de résister aux contraintes mécaniques qu'impose la mise en charge de l'articulation. Plus particulièrement, les fibres de collagène de type II procurent au cartilage articulaire la capacité de résister aux contraintes mécaniques de tension (Elliott *et al.*, 1999). Les glycosaminoglycanes hautement hydratés permettent de résister aux contraintes de compression et agissent afin d'absorber la sollicitation mécanique (Maldonado *et al.*, 2013). Le complexe glycosaminoglycanes/fibres de collagène de type II contribue pour sa part à doter le cartilage articulaire d'une résistance aux contraintes de cisaillement (Zhu *et al.*, 1993).

2.2 La dégradation du cartilage articulaire lors d'arthrose

L'arthrose peut être définie comme étant une réponse pathologique de l'articulation synoviale face à une agression à prépondérance mécanique. En accord avec cette

définition, cette arthropathie chronique dégénérative représente une tentative d'ajustement à la sollicitation mécanique excessive en mettant de l'avant un processus de réparation de dommages, particulièrement suite à la perte en protéoglycanes (Brandt *et al.*, 2008). Un point de non-retour (vers l'irréversibilité des dommages cartilagineux) est atteint lors de dommages aux fibres de collagène de type II (Karsdal *et al.*, 2008). L'amincissement, voire la disparition focale du cartilage articulaire qui en résulte est un déterminant important d'une articulation arthrosique.

Le cartilage articulaire est un tissu temporaire lors du phénomène d'ossification endochondrale qui se déroule au stade de développement embryonnaire. À ce stade, les chondrocytes démontrent des phénotypes d'hypertrophie et ultérieurement, un état d'apoptose (processus par lequel une cellule déclenche son autodestruction en réponse à un signal) qui précédera la minéralisation du cartilage (van der Kraan *et al.*, 2012). Le cartilage articulaire (mature) est un tissu permanent. Il démontre une structure stable en étant constitué de chondrocytes quiescents.

Le chondrocyte assure le maintien de la matrice extracellulaire en condition normale de renouvellement (équilibre entre synthèse et dégradation). Lors d'arthrose, les chondrocytes quittent l'état quiescent et deviennent activés, démontrant ainsi des changements phénotypiques d'hypertrophie en réponse à leur environnement. Au sein de cet environnement, il est possible de dénoter l'implication (non mutuellement exclusive et inter-reliée) de la sollicitation

mécanique excessive, des composants de la matrice extracellulaire et des intervenants pro-inflammatoires comme source d'activation du chondrocyte (Goldring *et al.*, 2011).

2.2.1 Implication de la sollicitation mécanique excessive

Le chondrocyte activé démontre des changements phénotypiques tels que la prolifération cellulaire, la formation de grappes de même que des changements dans la quantité, la composition, la distribution et la synthèse de composants de la matrice extracellulaire (van der Kraan *et al.*, 2012; Houard *et al.*, 2013). Particulièrement observé au niveau des zones superficielles et intermédiaires, ce phénotype partage des similitudes avec celui du chondrocyte au stade de développement embryonnaire (ossification endochondrale). Cette activation cellulaire est vouée à pallier à la forte demande en composants de la matrice extracellulaire en réponse à une sollicitation mécanique excessive. Ceci s'effectue cependant en vain; le cartilage arthrosique démontre une teneur faible en protéoglycanes dont certains s'avèrent être non agrégés à l'acide hyaluronique (Pearle *et al.*, 2005; Martel-Pelletier *et al.*, 2008). En découle l'augmentation de la perméabilité du cartilage articulaire ce qui altère non seulement la structure, mais également le rôle fonctionnel de ce tissu. Un degré de résistance aux contraintes de compression est alors observé, ce qui rend le cartilage articulaire davantage vulnérable à la sollicitation mécanique excessive (Maldonado *et al.*, 2013).

La sollicitation mécanique excessive stimule également le chondrocyte à libérer des facteurs cataboliques du cartilage (**2.2.2.1 ci-dessous - Chapitre 1**) et ce, *via*

l'activation de facteur de transcription tel que le RUNX2 (*Runt-related transcription factor two*) (Wang *et al.*, 2004; Troeberg *et al.*, 2012). Un facteur de transcription est une protéine nécessaire à l'initiation ou à la régulation de la transcription génétique, permettant d'obtenir de l'ARN (acide ribonucléique) qui sera ensuite traduit afin d'obtenir une protéine.

Les changements cartilagineux surviennent majoritairement au niveau de la couche superficielle et sont localisés au lieu d'application des contraintes mécaniques associées à la mise en charge de l'articulation (Andriacchi *et al.*, 2004). Chez un modèle canin d'arthrose par sectionnement chirurgical du ligament croisé crânial, l'évolution temporelle de la perte en cartilage a été décrite à l'aide d'imagerie par résonance magnétique (Annexe II). Le chapitre Expérimentations de cet ouvrage présente la relation entre la perte de cartilage (analyse volumétrique) et le degré de dysfonctionnement locomoteur, reflété par la mesure de la force verticale maximale, chez ce modèle (**10.4.1.2 ci-dessous - Chapitre 2**).

2.2.2 Implication des composants de la matrice extracellulaire

Les chondrocytes sont dotés de récepteurs pour les composants de la matrice extracellulaire. Les récepteurs transmembranaires d'adhésion cellulaire, comme les intégrines, possèdent une extrémité qui interagit avec l'extérieur de la cellule et l'autre, avec des composants intracellulaires. Ce type de récepteur génère donc une réponse suite à l'interaction entre la cellule et son milieu (Goldring *et al.*, 2011).

Ainsi, l'activation de ces récepteurs par des fragments de fibres de collagène (issus de la sollicitation mécanique excessive) mène à la libération excessive de facteurs cataboliques du cartilage, ce qui porte à sa destruction (Almonte-Becerril *et al.*, 2014).

Les interactions entre les composants de la matrice extracellulaire et les chondrocytes touchent non seulement des fragments de la matrice mais également des fibres intactes et ce, *via* des récepteurs cellulaires comme le DDR2 (*Discoidin domain receptor two*). L'expression de ce récepteur s'avère être augmentée lors d'arthrose (Goldring *et al.*, 2011). Sous l'influence d'une sollicitation mécanique excessive, le DDR2 transmet la libération de facteurs cataboliques du cartilage (Xu *et al.*, 2011).

Des irrégularités au niveau des composants de la matrice extracellulaire sont également observées lors d'arthrose, ce qui favorise l'activation de chondrocytes. Ainsi, la diminution de la synthèse des fibres de collagène de type II est dénotée au profit de la synthèse de fibres de type X. Ce dernier est malheureusement dépourvu de la capacité à fixer de manière optimale les protéoglycanes. Par conséquent, le cartilage articulaire exhibe une perte d'élasticité et ainsi, une prédisposition à la fibrillation et aux fissures (Silver *et al.*, 2002). De plus, les protéoglycanes nouvellement synthétisées n'ont pas la capacité de s'agréger afin de former une macrostructure en s'agrégeant à l'acide hyaluronique (Szabo *et al.*, 1995). Ce

phénomène prédispose le cartilage articulaire à subir davantage de dommages lors de la mise en charge de l'articulation.

2.2.2.1 Facteurs cataboliques

Les facteurs cataboliques du cartilage sont majoritairement des enzymes de dégradation, dont l'action protéolytique touche les fibres de collagène et les protéoglycanes. Les MMPs sont des enzymes de dégradation caractérisées par la présence d'un ion zinc au niveau de leur site catalytique. Les MMPs sont secrétées sous formes inactives et requièrent une action protéolytique afin d'être opérationnelles. Cette activation peut être effectuée *via* d'autres MMPs ou *via* des régulateurs, comme l'uPA (*Urokinase-type plasminogen activator*) (Kim *et al.*, 2012). La présence de ce dernier s'avère être en lien avec la sévérité des dommages arthrosiques (Pap *et al.*, 2000), ce qui suggère un rôle dans la physiopathologie de l'arthrose de même qu'une avenue thérapeutique potentielle (Hsieh *et al.*, 2007).

L'activité protéolytique des MMPs est également sous la gouverne d'inhibiteurs endogènes comme les TIMPs (*Tissue inhibitor of metalloproteinases*) dont la libération est cruciale afin de maintenir la matrice extracellulaire exempte d'un remodelage excessif (Sahebjam *et al.*, 2007). Sous l'influence de la sollicitation mécanique excessive, l'activation des MMPs peut être accentuée, contribuant ainsi à la destruction du cartilage articulaire observée lors d'arthrose (Chen *et al.*, 2013). Récemment, l'activation des MMPs a été démontrée *via* l'implication d'un

récepteur au niveau des chondrocytes soit le PAR-2 (*Protease activated receptor two*) qui s'avère être surexprimé lors d'arthrose (Boileau *et al.*, 2007).

Chez un modèle canin d'arthrose par sectionnement chirurgical du ligament croisé crânial, l'inhibition du PAR-2 a été soulignée comme étant une avenue thérapeutique potentielle (Annexe V). Ainsi, en parallèle à la préservation des structures cartilagineuses, le degré de dysfonctionnement locomoteur, reflété par la mesure de la force verticale maximale, fut moindre sous un effet thérapeutique chez ce modèle (Annexe V). L'effet thérapeutique fut également observé, chez un modèle canin d'arthrose naturelle, suite à l'amélioration de la force verticale maximale utilisée comme critère primaire d'efficacité (**6.1.2 ci-dessous - Chapitre 1**).

Les MMPs démontrent une spécificité envers certains substrats. Ainsi, l'aggrécane peut être assujetti à une dégradation enzymatique par l'action de divers types de MMPs, soit les MMP-1, MMP-2, MMP-7, MMP-8, MMP-9 et MMP-13 (Troeberg *et al.*, 2012). Les fibres de collagène de type II sont sensibles principalement à l'action protéolytique des MMP-1, MMP-8 et particulièrement à celles des MMP-13 (Amalinei *et al.*, 2010; Kim *et al.*, 2012). La cathepsine K, une enzyme protéolytique retrouvée en quantité accrue lors d'arthrose, est également en mesure de dégrader les fibres de collagène de type II et les aggrécanes (Konttinen *et al.*, 2002).

Les aggrécanes sont parmi les premiers composants du cartilage articulaire à être altérés au stade précoce d'arthrose, portant ainsi atteinte au rôle fonctionnel de ce tissu. Les aggrécanes sont sensibles à l'action protéolytique de deux enzymes membres de la famille des ADAM (*A disintegrin and metalloprotease*) soit l'ADAMTS-4 (*A disintegrin and metalloproteases with thrombospondin motifs four)* et l'ADAMTS-5 (Verma *et al.*, 2011). Cette dernière enzyme de dégradation est reconnue comme étant celle la plus impliquée dans la destruction des aggrécanes lors d'arthrose (Goldring *et al.*, 2011).

La présence supranormale des MMP-1 (Moreau *et al.*, 2006), MMP-3 (Pelletier *et al.*, 1993) et MMP-13 (Moreau *et al.*, 2006), de l'ADAMTS-5 (Moreau *et al.*, 2006) de même que la cathepsine K (Connor *et al.*, 2009) au sein du cartilage a été démontrée sur un modèle canin d'arthrose chirurgicale. Sous un effet thérapeutique, la présence de ces facteurs cataboliques fut moindre et ce, en parallèle à une réduction des lésions cartilagineuses. Ceci suggère un rôle prépondérant de ces facteurs cataboliques lors d'arthrose de même qu'une avenue thérapeutique potentielle (Moreau *et al.*, 2006; Boileau *et al.*, 2009; Connor *et al.*, 2009).

2.2.3 Implication des intervenants pro-inflammatoires

La sollicitation mécanique excessive altère les interactions entre les composants de la matrice extracellulaire et les chondrocytes. Des composants habituellement fixés deviennent alors fragmentés et solubles. Ils parviennent à interagir avec le chondrocyte et à induire des actions inflammatoires délétères pour le cartilage.

Ainsi, *via* des voies de signalisation intracellulaire, comme celle des protéines kinases MAP (*Mitogen-activated protein*) et l'implication de facteurs de transcription comme le NF-kappa B (*Nuclear factor-kappa B*) et l'AP1 (*Activator protein one*) le chondrocyte est en mesure de démontrer des changements phénotypiques. De tels changements impliquent l'expression aberrante de gènes menant à l'obtention de facteurs cataboliques du cartilage (comme MMP-13 et ADAMTS-5), à la libération d'intervenants pro-inflammatoires et à l'apoptose du chondrocyte (Goldring, 2000; Henrotin *et al.*, 2003; Ding *et al.*, 2010b; Maldonado *et al.*, 2013; Pulsatelli *et al.*, 2013). Les chondrocytes libèrent plusieurs intervenants pro-inflammatoires lors d'arthrose. Les principaux intervenants sont l'interleukine-1 bêta (IL-1 bêta), le facteur tumo-nécrosant alpha (TNF-alpha, *Tumor necrosis factor alpha*), les eicosanoïdes et l'oxyde nitrique (Abramson, 2008).

2.2.3.1 L'interleukine-1 bêta

L'interleukine-1 bêta est considérée comme l'une des cytokines (molécule responsable de la communication entre cellules) pivot dans la pathogénèse de l'arthrose. L'interleukine-1 bêta est à l'origine une protéine précurseur qui subit une protéolyse intracellulaire effectuée par l'enzyme de conversion de l'IL-1 bêta (ICE, *interleukin-1 beta converting enzyme*).

Au niveau de l'articulation synoviale, l'IL-1 bêta est libérée par les chondrocytes, les synoviocytes, les ostéoblastes (**2.3.2.1.2 ci-dessous - Chapitre 1**) et les cellules mononucléaires présentes au niveau de la capsule articulaire. Lors d'arthrose, la

présence d'IL-1 bêta est élevée au niveau du liquide synovial, de la membrane synoviale, du cartilage et de l'os sous-chondral (Wojdasiewicz *et al.*, 2014).

Au niveau de la membrane d'une cellule cible, la présence du récepteur à l'IL-1 bêta (IL-1R1) permet de transmettre le message intracellulaire *via* des voies de signalisation comme celles des protéines kinases MAP et l'implication de facteurs de transcription (comme le NF-kappa B et l'AP-1). En découle une cascade d'évènements intracellulaires menant à la diminution des composants de la matrice extracellulaire et à l'augmentation de la libération de MMPs (MMP-1, MMP-3 et MMP-13), d'ADAMTS-4 et ADAMTS-5. À ceci s'ajoute la synthèse de TNF-alpha, de prostaglandine E_2 (**2.2.3.3.1 ci-dessous - Chapitre 1**) et d'oxyde nitrique (**2.2.3.4 ci-dessous - Chapitre 1**) (Wojdasiewicz *et al.*, 2014).

Chez un modèle canin d'arthrose par sectionnement chirurgical du ligament croisé crânial, l'IL-1 bêta est libérée à des niveaux supranormaux au niveau du cartilage et de la membrane synoviale. Par contre, l'inhibition de l'ICE chez ce même modèle permet de préserver l'intégrité des structures cartilagineuses, suggérant ainsi une avenue thérapeutique potentielle (D'Lima *et al.*, 2006). Des niveaux élevés d'IL-1 bêta sont également retrouvés en contexte d'arthrose naturelle par rupture traumatique du ligament croisé crânial chez le chien (Hegemann *et al.*, 2005).

2.2.3.2 Le facteur tumo-nécrosant alpha

Tout comme l'IL-1 bêta, le TNF-alpha est considéré comme une cytokine pivot dans la pathogénèse de l'arthrose. Le TNF-alpha est à l'origine une protéine précurseur qui subit une protéolyse intracellulaire effectuée par une MMP.

Au niveau de l'articulation synoviale, le TNF-alpha est libéré par les mêmes cellules que son homologue l'IL-1 bêta et est également sous la gouverne du facteur de transcription NF-kappa B. Par conséquent, le TNF-alpha se retrouve principalement libéré concomitamment à l'IL-1 bêta.

Au niveau de la membrane d'une cellule cible, la présence de récepteur au TNF-alpha (soit le TNF-R1) permet de transmettre le message intracellulaire *via* des voies de signalisation (telles que celles des MAP kinases) et certains facteurs de transcription (comme le NF-kappa B et l'AP-1). En découle une cascade d'évènements intracellulaires menant à la diminution des composants de la matrice extracellulaire, à l'augmentation de la libération de MMPs (MMP-1, MMP-3 et MMP-13), de prostaglandine E_2 (**2.2.3.3.1 ci-dessous - Chapitre 1**) et d'oxyde nitrique (**2.2.3.4 ci-dessous - Chapitre 1**) (Wojdasiewicz *et al.*, 2014).

2.2.3.3 Les eicosanoïdes

Les eicosanoïdes constituent une vaste famille de molécules dérivées de l'oxydation d'acides gras polyinsaturés comme l'acide arachidonique ou certains acides gras oméga-6 et oméga-3. Les eicosanoïdes regroupent les leucotriènes (dont la

leucotriène B4) et les prostanoïdes (thromboxanes, prostacycline et autres prostaglandines).

2.2.3.3.1 Prostaglandine E2

La prostaglandine E_2 est un acide gras polyinsaturé qui joue un rôle important dans le processus inflammatoire associé à l'arthrose. La prostaglandine E_2 a comme précurseur l'acide arachidonique que l'on retrouve principalement au niveau de la bicouche lipidique des membranes cellulaires. Une série de transformations enzymatiques mène à l'obtention d'une prostaglandine intermédiaire, soit la prostaglandine H_2. L'enzyme responsable de ce processus enzymatique est la cyclooxygénase (COX). Plusieurs isoformes de la COX existent (soit la COX-1, COX-2 et COX-3). Cependant, c'est la forme inductible (COX-2) qui est libérée de manière prépondérante lors d'arthrose (Martel-Pelletier *et al.*, 2003). L'enzyme prostaglandine E synthase transforme, au final, la prostaglandine H_2 en prostaglandine E_2 (Kojima *et al.*, 2005).

Lors d'arthrose, la présence de l'enzyme COX-2 est augmentée, ce qui mène à l'obtention accrue de prostaglandine E_2 (Martel-Pelletier *et al.*, 2003). La surabondance de COX-2 est induite par des médiateurs pro-inflammatoires comme l'IL-1 bêta et le TNF-alpha (Martel-Pelletier *et al.*, 2003). Les fonctions cellulaires de la prostaglandine E_2 sont transmises par le biais de récepteurs membranaires, dont quatre sous-types sont connus soit l'EP1, l'EP2, l'EP3 et l'EP4. Au niveau du cartilage, la prostaglandine E2 génère des effets cataboliques *via* un sous-type de

récepteur soit l'EP4 en augmentant la libération de facteurs cataboliques (comme MMP-13 et ADAMTS-5) et en limitant la synthèse de protéoglycanes (Attur *et al.*, 2008; Zweers *et al.*, 2011). Par contre, *via* le récepteur EP4, des effets anaboliques ont été démontrés au niveau du cartilage articulaire (Mitsui *et al.*, 2011).

L'expression de COX-2 est augmentée au niveau du cartilage en contexte d'arthrose naturelle associée à la dysplasie de la hanche chez le chien (Lascelles *et al.*, 2009). Chez un modèle canin d'arthrose par sectionnement chirurgical du ligament croisé crânial, la teneur en prostaglandine E_2 au niveau du liquide synovial est en lien avec le degré de dysfonctionnement locomoteur, tel que reflété par la mesure de la force verticale maximale chez ce modèle (Trumble *et al.*, 2004). L'inhibition concomitante des prostanoïdes (incluant prostaglandine E_2) et des leucotriènes (leucotriène B_4) chez ce modèle canin procure des effets bénéfiques au niveau du cartilage, suggérant de ce fait une avenue thérapeutique potentielle (Moreau *et al.*, 2006). Ceci est d'autant plus pertinent puisque sur un modèle canin d'arthrose naturelle, l'inhibition des prostanoïdes et des leucotriènes a amélioré le dysfonctionnement locomoteur, tel que reflété par la mesure de la force verticale maximale (Moreau *et al.*, 2007).

2.2.3.4 L'oxyde nitrique

L'oxyde nitrique est une molécule synthétisée par l'enzyme oxyde nitrique synthase (NOS *Nitric oxide synthase*) à partir de l'acide aminé arginine et de l'oxygène. L'isoforme inductible de l'enzyme NOS (iNOS) est associé à la synthèse d'oxyde

nitrique lors d'arthrose (et d'inflammation), soit en réponse à la stimulation par des cytokines comme l'IL-1 bêta et le TNF-alpha. L'oxyde nitrique réduit la synthèse des protéoglycanes (effet anti-anabolique), favorise la libération supranormale de MMPs (effet catabolique) et de prostaglandine E_2, inhibe la prolifération des chondrocytes et induit l'apoptose de ces derniers (Lee *et al.*, 2002; Vuolteenaho *et al.*, 2007; Martel-Pelletier *et al.*, 2012). Au niveau de l'articulation, l'oxyde nitrique est libéré par les chondrocytes et les synoviocytes (Henrotin *et al.*, 2014) ce qui a pour effet de soutenir l'activation du facteur de transcription NF-kappa B. Par conséquent, la réponse inflammatoire est perpétuée en favorisant la transcription de gènes pro-inflammatoires menant à la présence d'IL-1 bêta, de TNF-alpha, de COX-2 et d'iNOS (Berenbaum, 2004; Abramson, 2008).

Chez un modèle canin d'arthrose par sectionnement chirurgical du ligament croisé crânial, la présence d'oxyde nitrique est associée à un niveau élevé de chondrocytes apoptotiques (Pelletier *et al.*, 2001). Par contre, l'inhibition de l'iNOS chez ce modèle permet de préserver l'intégrité des structures cartilagineuses, ce qui suggère une avenue thérapeutique potentielle (Pelletier *et al.*, 1998)

2.2.4 Conclusion

Le chondrocyte assure l'intégrité des composants de la matrice extracellulaire, assurant ainsi la préservation des structures et leurs rôles fonctionnels. Lors de sollicitation mécanique excessive, le cartilage démontre une capacité sous optimale à résister aux contraintes de cisaillement, de tension et de compression et génère des niveaux supranormaux de facteurs cataboliques et inflammatoires. L'effet

néfaste d'une sollicitation mécanique excessive ne se limite pas qu'au cartilage articulaire. Elle porte également atteinte à l'os sous-chondral. Les prochaines sections présentent un survol des divers intervenants impliqués dans le processus de remodelage osseux en condition d'homéostasie et lors d'arthrose.

2.3 L'os sous-chondral

En regard à la physiopathologie de l'arthrose, les investigations de l'os sous-chondral ont longtemps été dans l'ombre d'une altération focale au niveau du cartilage articulaire. Bien qu'intimement lié au cartilage qui le chapeaute, l'origine de l'intérêt envers le tissu osseux comme facteur clé dans la progression de cette condition est issue des travaux de Radin et collègues (Radin *et al.*, 1970b). La séquence d'apparition des dommages structuraux (os sous-chondral *versus* cartilage) demeure incertaine et requiert de plus amples investigations. Néanmoins, il s'avère que les changements de l'os sous-chondral soient définis comme étant des facteurs de risque envers la progression de l'arthrose (Ding *et al.*, 2010a), signifiant de ce fait un rôle majeur dans l'initiation de ce processus.

Afin de cerner l'implication de l'os sous-chondral dans la physiopathologie de l'arthrose, il est impératif de détailler l'organisation de ce tissu de même que le phénomène du remodelage osseux.

2.3.1 Organisation structurelle

L'os sous-chondral inclut la plaque sous-chondrale (os dense) et l'os sous-chondral trabéculaire. Ce tissu est innervé et vascularisé. La présence de canalicules y est

aussi notoire, permettant un contact entre la moelle osseuse et le cartilage articulaire. La distribution de ces canaux est associée aux régions sujettes à de fortes contraintes lors de la mise en charge de l'articulation. À travers cette canalisation, la micro vascularisation est possible, pouvant ainsi procurer l'apport nutritionnel essentiel au cartilage mais également, permettre aux cellules mésenchymateuses (précurseur donnant entre autres les chondrocytes) d'accéder au cartilage. Le trafic de molécules (incluant des enzymes de dégradation) est également possible entre ces deux tissus (Guevremont *et al.*, 2003).

La structure de l'os sous-chondral est optimisée afin de pallier aux contraintes mécaniques associées à la mise en charge de l'articulation. La capacité adaptative de l'os se réfère à la loi de Wolff (Ding, 2010). Cette loi propose l'architecture des trabécules en fonction des contraintes mécaniques. Cette architecture requiert également la présence de contraintes pour être optimisée. L'arrangement des trabécules est anisotropique, décrivant une orientation particulière afin d'optimiser sa résistance (particulièrement à la compression). Ainsi, l'os sous-chondral atténuera 30 % des contraintes de compression associées à la mise en charge de l'articulation (Radin *et al.*, 1970a).

Au sein des composants extracellulaires de l'os sous-chondral, les fibres de collagène de type I sont responsables de la ductilité. Pour sa part, la matrice minéralisée procure une résistance aux contraintes de compression (Ding, 2010). Les régions associées à la mise en charge de l'articulation, dotées d'une forte

minéralisation, témoignent d'une épaisseur accrue et d'une densité élevée afin de pallier de manière optimale aux contraintes mécaniques (Madry *et al.*, 2010).

2.3.2 Le remodelage de l'os sous-chondral

L'os est un tissu dynamique qui subit une adaptation continuelle afin de préserver une géométrie et l'intégrité des structures. Deux processus distincts mais intimement liés régissent le développement et le maintien osseux : le modelage et le remodelage (Raggatt *et al.*, 2010).

Le modelage est responsable de la croissance de l'os et de l'adaptation aux contraintes mécaniques. Il requiert un processus de résorption et de formation de l'os. Bien que coordonnés, les deux processus agissent sur des sites anatomiques distincts. Le remodelage osseux pour sa part est responsable de la résorption et de la réparation de l'os endommagé afin de maintenir son architecture et l'homéostasie minérale. Le processus de remodelage fait appel à des intervenants cellulaires qui coordonnent les activités de résorption et de synthèse de manière séquentielle et ce, au même site anatomique (Raggatt *et al.*, 2010).

2.3.2.1 Entités cellulaires et mécanismes

2.3.2.1.1 Ostéoclastes

Les ostéoclastes sont des cellules myéloïdes différenciées, terminales, multi-nuclées ayant la caractéristique de résorber la matrice minéralisée. Le phénotype propre de l'ostéoclaste est l'expression d'enzymes de dégradation, telle que la TRAP, (*Tartrate-resistant acid phosphatase*) et de récepteurs à la calcitonine (avec un rôle

anti-résorptif, elle est un antagoniste physiologique de la parathormone). Les ostéoclastes ont la capacité d'acidifier l'environnement par l'action de pompe à protons hydrogène (H^+).

Les précurseurs cellulaires des ostéoclastes requièrent l'action de molécules afin d'assurer la survie, mais aussi leur différenciation en ostéoclastes matures à même d'effectuer la résorption osseuse. Le CSF-1 (*Macrophage colony-stimulating factor one)* et le RANKL (*Receptor activator of nuclear factor kappa-B ligand)* sont des molécules clés intervenant dans le processus de l'ostéoclastogenèse.

La présence d'ostéoprotégérine (récepteur soluble qui se lie au RANKL) est un puissant inhibiteur de la résorption osseuse qui agit afin de limiter l'ostéoclastogenèse. Le ratio RANKL/ostéoprotégérine est ciblé comme facteur clé de l'ostéoclastogenèse supranormale associée à une résorption excessive de l'os sous-chondral lors d'arthrose (Tat *et al.*, 2009).

Les précurseurs des ostéoclastes nécessitent l'activation de facteurs de transcription. Le facteur AP-1 et le MITF (*Microphthalmia-associated transcription factor*) sont responsables de l'ostéoclastogenèse et de l'obtention d'ostéoclastes capables d'initier la résorption par synthèse d'enzymes de dégradation, telles que la TRAP et la cathepsine K. Il y aura également expression de récepteurs cellulaires au RANKL (soit le RANK) et au CSF-1 (soit le CSF-1R). L'ostéoclastogenèse est aussi stimulée *via* la sécrétion de CSF-1.

2.3.2.1.2 Ostéoblastes

L'ostéoblaste est une cellule spécialisée issue d'une cellule souche pluripotente qui exprime le récepteur à la parathormone. Celle cellule contribue à l'ostéoclastogenèse, produit la matrice osseuse (ostéoïde; majoritairement collagène de type I, protéoglycanes et glycosaminoglycanes) et la matrice minérale (majoritairement l'hydroxyapatite). Les précurseurs des ostéoblastes nécessiteront l'activation de facteurs de transcription comme le RUNX2, dont l'absence chez la souris inhibera toute forme de minéralisation (Komori *et al.*, 1997).

2.3.2.1.3 Ostéocytes

Durant la synthèse de l'ostéoïde, certains ostéoblastes différenciés deviennent engouffrés à même cette matrice. Après la minéralisation, ces cellules deviennent des ostéocytes, représentant plus de 90 % de la population osseuse. Les corps cellulaires des ostéocytes gisent à même des logettes (ostéoplastes) qui sont remplies de fluide. Les prolongements cellulaires des ostéocytes sont localisés dans des canalicules anastomosés, entourés d'une matrice minéralisée**.** La présence de canalicules permet aux ostéocytes d'établir un réseau, interagissant avec d'autres ostéocytes de même que des ostéoblastes. Les ostéocytes répondent au flux de liquide interstitiel induit par les contraintes mécaniques véhiculées à travers le réseau de canalicules. En réponse à ce phénomène, les ostéocytes produisent des molécules telles que la prostaglandine E_2, l'oxyde nitrique et l'adénosine triphosphate (ATP).

La morphologie de l'ostéocyte lui confère la capacité d'être sensible au fluide en aval distinctement du fluide en amont. La présence d'intégrines permet à l'ostéocyte d'orchestrer le remodelage osseux en fonction des contraintes liées à la mise en charge de l'articulation afin de conférer à l'os un rôle fonctionnel de soutien et de résistance (Klein-Nulend *et al.*, 2005). L'ostéocyte secrète également les récepteurs cellulaires RANK et CSF-1.

2.3.2.1.4 Lymphocytes de type B

Ce sont des cellules immunitaires contribuant à restreindre l'ostéoclastogenèse en synthétisant de l'ostéoprotégérine.

2.3.2.1.5 Macrophage osseux

Ce sont des cellules pouvant être des précurseurs des ostéoclastes. Le macrophage est nécessaire à la différentiation et à l'activité des ostéoblastes. Cette cellule est impliquée dans le processus de remodelage osseux en recouvrant le site (fosse) de résorption.

2.3.2.2 Phases du processus de remodelage de l'os sous-chondral

L'ensemble du processus de remodelage osseux s'échelonne sur plusieurs semaines. Les différents intervenants cellulaires sont regroupés dans une unité multicellulaire de base (BMU, *basic multicellular unit*) qui est en soit une structure temporaire permettant de marier l'action des ostéoblastes à celle des ostéoclastes afin d'assurer un degré de remodelage optimal (Hauge *et al.*, 2001; Raggatt *et al.*, 2010). L'arrangement de la BMU est critique et s'effectue dans une fosse de résorption. Elle inclut un front de résorption, des cellules en réversion et une queue

d'ostéoblastes qui assurent le dépôt d'ostéoïde et sa minéralisation subséquente. Le processus est constitué de cinq phases (**Figure 8**) (Raggatt *et al.*, 2010).

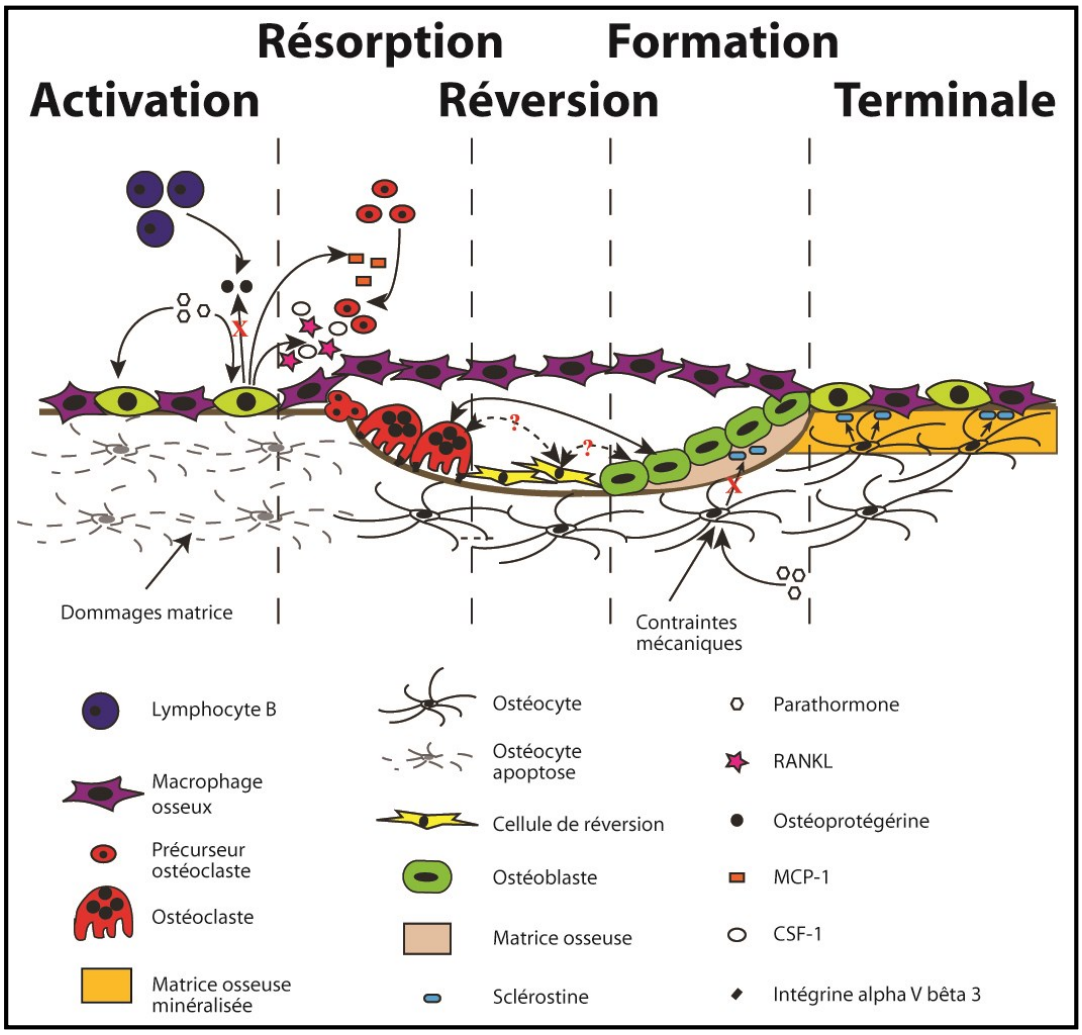

Figure 8. Phases du processus de remodelage de l'os sous-chondral

Adapté de Raggatt, 2010 (Raggatt *et al.*, 2010).

2.3.2.2.1 Phase I : Activation

L'activation débute par la détection d'un signal de remodelage, qui peut être direct (contraintes mécaniques, dommages structuraux) ou bien systémique en impliquant la parathormone (rôle pro-résorptif, est un antagoniste physiologique de la calcitonine) ou la diminution en œstrogènes (ménopause). L'apoptose de l'ostéocyte peut survenir suite à une sollicitation mécanique excessive, à l'absence de contrainte (vol spatial), à l'immobilisation ou à des microfissures de l'os sous-chondral (Verborgt *et al.*, 2002; Aguirre *et al.*, 2006; Bonewald, 2007). La mort cellulaire cessera l'expression basale du TGF-bêta (inhibiteur de l'ostéoclastogenèse) ce qui inhibera la résorption osseuse. Pour ce qui est du rôle de la parathormone, sa liaison aux récepteurs présents sur l'ostéoblaste activera des facteurs de transcription permettant le recrutement, la différenciation et l'activation d'ostéoblastes (Raggatt *et al.*, 2010).

2.3.2.2.2 Phase II : Résorption

L'ostéoblaste activé libère le *MCP-1 (Monocyte chimoattractant protein one)* qui attire les précurseurs d'ostéoclastes au site de résorption et augmente la libération de RANKL, de CSF-1 tout en diminuant l'ostéoprotégérine contribuant ainsi à l'ostéoclastogenèse (Ma *et al.*, 2001). L'apposition de l'ostéoclaste à la matrice osseuse est optimisée lorsque l'ostéoblaste y a libéré un ancrage spécifique suite à l'action d'enzymes protéolytiques. L'intégrine alpha V bêta 3 assure un lien optimal entre la matrice et l'ostéoclaste. Ce lien permet de sceller le site de dégradation afin d'acidifier le milieu suite à la libération de protons H^+. Le milieu

acide (pH entre 4-5) dégrade la matrice minérale tout en favorisant l'action d'enzymes de dégradation comme la cathepsine K (Saftig *et al.*, 1998). Il se forme ainsi un trou nommé lacune de Howship (Teitelbaum, 2000). Il y a par la suite apoptose de l'ostéoclaste (Raggatt *et al.*, 2010).

2.3.2.2.3 Phase III : Réversion

Au sein des lacunes de Howship, le restant de matrice déminéralisée est ingéré par les cellules de réversion par phagocytose. Au sein de la BMU, il y a alors transition de la résorption vers la formation de matrice osseuse (Everts *et al.*, 2002; Raggatt *et al.*, 2010).

2.3.2.2.4 Phase IV : Formation

Des mécanismes sont proposés comme source de signalement mutuel entre l'ostéoblaste et l'ostéoclaste, comme le couplage entre le ligand Ephrin B2 (ligand exprimé à la surface des ostéoclastes) et le récepteur Eph B4 (récepteur exprimé à la surface des ostéoblastes) (Zhao *et al.*, 2006). Le couplage de ces deux effecteurs permet la transmission d'un signal conjoint, arrêtant la résorption de l'ostéoclaste d'une part et initiant la formation par l'ostéoblaste (Raggatt *et al.*, 2010).

La présence de sclérostine (molécule soluble sécrétée par les ostéocytes) contribue à la synthèse de la matrice osseuse par les ostéoblastes. Ainsi, en condition basale, l'ostéocyte exprime la sclérostine (van Bezooijen *et al.*, 2004). Celle-ci inhibe la voie de signalisation WNT (*Wingless integration site*) dont l'effet sera d'inhiber la formation osseuse. L'absence de gène permettant l'obtention de sclérostine mime,

chez la souris, la sclérostéose caractérisée par l'augmentation de la densité osseuse (Li *et al.*, 2005; Papapoulos, 2011).

Lorsque l'ostéocyte est soumis à des contraintes mécaniques ou à la présence de parathormone, il arrête l'expression de sclérostine (Robling *et al.*, 2008), ce qui cesse le rôle inhibiteur de la sclérostine envers la signalisation WNT et donc, permet la synthèse de matrice osseuse. Le phénomène de minéralisation n'est pas élucidé entièrement. Cependant, l'ostéoblaste est en mesure de synthétiser une quantité de composants organiques (collagène type I, protéoglycanes) et d'intervenants, tels la phosphatase alcaline et l'ostéocalcine (marqueur de l'activité des ostéoblastes) afin d'instaurer les conditions nécessaires à la minéralisation (Harmey *et al.*, 2004; Murshed *et al.*, 2005). La minéralisation comprend l'incorporation d'hydroxyapatite (phosphate minéral).

2.3.2.2.5 Phase V : Terminale

Le cycle se termine lorsque la minéralisation est suffisante, dont l'arrêt est probablement signalé par l'ostéocyte. Par le suite, la synthèse de sclérostine reprend, les ostéoblastes deviennent soit en apoptose, soit ils redeviennent quiescents ou soit ils se différencient en ostéocytes (Raggatt *et al.*, 2010).

2.3.3 Le remodelage de l'os sous-chondral lors d'arthrose

L'os sous-chondral est intimement relié au cartilage et fonctionne en symbiose avec ce dernier afin de pallier aux contraintes mécaniques que génère la mise en charge de l'articulation (Ding, 2010). Ce tissu doit démontrer un degré de remodelage

optimal, une viabilité cellulaire de même qu'une architecture optimisée afin de contribuer à préserver son rôle fonctionnel et ainsi, l'intégrité du cartilage. Lors d'arthrose, les changements de l'os sous-chondral sont notoires, impliquant l'attrition osseuse en début de processus, et par la suite d'accrétion au stade avancé (Kwan Tat *et al.*, 2010).

2.3.3.1 Altérations structurelles

Un remodelage osseux défaillant mène l'architecture de l'os sous-chondral à démontrer des altérations caractéristiques propres à l'arthrose. L'épaississement de l'os sous-chondral, l'augmentation de la densité apparente (représentée par la sclérose en imagerie) ainsi qu'une hypo-minéralisation marquée associée à une faible densité matérielle sont observés au stade clinique d'arthrose (Ding, 2010; Burr *et al.*, 2012). La modification de l'architecture des trabécules inclut l'épaississement tridimensionnel et la morphologie en plaque/disque (la morphologie normale étant en bâtonnet).

Des anomalies au niveau du collagène de type I, tributaire de la déficience en minéralisation et, par conséquent, de la perte de propriétés mécaniques, sont également rapportées (Couchourel *et al.*, 2009). Bien que léger, le changement dans le degré d'anisotropie (changement vers l'isotropie) peut tout de même suggérer un réarrangement en fonction des contraintes mécaniques observées particulièrement au niveau du compartiment médial. Le compartiment médial est assujetti à de fortes contraintes mécaniques, comme en témoigne l'analyse cinématique de la démarche

(Bennell *et al.*, 2010). L'abondance d'ostéoïdes non-minéralisés est signe d'accrétion osseuse et prend l'apparence de sclérose en imagerie (densité apparente élevée). D'un regard mécanique, l'os sous-chondral arthrosique est moins raide que la normale, pour une densité apparente donnée et ce, en lien avec un degré de minéralisation sous optimale (Coats *et al.*, 2003; Burr *et al.*, 2012). L'os sous-chondral acquiert tout de même de la raideur par l'abondance d'ostéoïde. Un lien étroit existe entre l'élasticité de ce tissu et la présence de dommages au cartilage articulaire (Day *et al.*, 2001). Ainsi, des contraintes de cisaillement affecteraient le cartilage aux points focaux où l'os sous-chondral démontre de l'élasticité (Radin *et al.*, 1986).

Les lésions de la moelle osseuse sont des observations faites en imagerie par résonance magnétique au niveau de l'os sous-chondral. Les lésions de la moelle osseuse sont impliquées dans le processus arthrosique, plus particulièrement au niveau du ressenti douloureux (Hunter *et al.*, 2011), de la perte de cartilage (Doré *et al.*, 2010) et au risque d'implantation d'une prothèse (Tanamas *et al.*, 2010) tout en étant associées au degré de mise en charge de l'articulation (Bennell *et al.*, 2010). En histologie, les lésions de la moelle osseuse contiennent des régions de fibrose et de nécrose, des anomalies au niveau des trabécules et des micro-fractures caractéristiques d'un remodelage répété (Zanetti *et al.*, 2000; Taljanovic *et al.*, 2008).

En présence de lésions de la moelle osseuse, la nécrose osseuse démontre un lien avec l'hypoxie des tissus. La réponse phénotypique de l'ostéocyte suite aux changements dans son environnement (changements de pression hydrostatique et d'écoulement des fluides et présence d'hypoxie) pourrait mener aux lésions cartilagineuses dont l'apparition est temporellement retardée mais géographiquement associée aux lésions de la moelle osseuse (Aaron *et al.*, 2007).

Chez un modèle canin d'arthrose par sectionnement chirurgical du ligament croisé crânial, l'évolution temporelle des lésions de la moelle osseuse a été décrite à l'aide d'imagerie par résonance magnétique (Annexe III). Le chapitre Expérimentation de cet ouvrage présente la relation entre les lésions de la moelle osseuse et le degré de dysfonctionnement locomoteur, reflété par la mesure de la force verticale maximale, chez ce modèle (**10.4.1.2 ci-dessous - Chapitre 2**)

2.3.3.2 Fonctionnements cellulaires anormaux

Les altérations structurelles de l'os sous-chondral impliquent des entités cellulaires et des voies de signalisation intracellulaire qui interviennent dans le processus de remodelage osseux.

Lors d'arthrose, l'ostéoblaste synthétise un niveau anormalement élevé d'inhibiteurs de la voie de signalisation du développement osseux (voie WNT), soit le Dickkopf-2 et la sclérostine (Kwan Tat *et al.*, 2010; Chan *et al.*, 2011). Le phénotype anormal de l'ostéoblaste (phénotype pro-résorptif) peut contribuer à

l'exacerbation de la résorption osseuse et la faible minéralisation. La libération accentuée par l'ostéoblaste de RANKL n'étant pas contrecarrée par l'ostéoprotégérine, l'ostéoclastogenèse est ainsi anormalement induite (Kwan Tat *et al.*, 2010). Le métabolisme altéré de l'os sous-chondral est aussi une conséquence de l'action délétère de facteurs cataboliques. Ainsi, l'augmentation d'enzymes de dégradation, comme la cathepsine K et la MMP-13 par les ostéoclastes, est néfaste.

La zone radiale du cartilage articulaire normalement avasculaire, peut démontrer des invasions vasculaires lors d'arthrose, et ce, en lien avec le remodelage excessif de l'os sous-chondral (Burr *et al.*, 2012). La libération par les ostéoblastes de niveaux supranormaux du facteur de croissance de l'endothélium vasculaire (VEGF, *Vascular endothelial growth factor*) contribue à la néovascularisation et pourrait stimuler le chondrocyte à exprimer des facteur cataboliques du cartilage (Brown *et al.*, 1983; Burr *et al.*, 2012; Karsdal *et al.*, 2014). La présence concomitante de neurones afférents primaires (**3.3.2 ci-dessous - Chapitre 1**) à même les infiltrations (néo) vasculaires contribue au ressenti douloureux lors d'arthrose (Suri *et al.*, 2007; Hunter *et al.*, 2011).

Des facteurs de croissance sont aussi libérés en abondance par l'ostéoblaste et interviennent dans le processus de remodelage excessif de l'os sous-chondral lors d'arthrose. L'augmentation du TGF-bêta, associée à la défaillance du collagène de type I, contribue à la minéralisation sous optimale de l'os sous-chondral effectuée par l'ostéoblaste (Couchourel *et al.*, 2009).

La stimulation conjointe entre l'ostéoblaste et l'ostéoclaste que permet le couplage du ligand Ephrin B2 avec le récepteur Eph B4 permet de régulariser le processus de remodelage en inhibant des facteurs cataboliques impliqués lors d'arthrose (Kwan Tat *et al.*, 2008; Kwan Tat *et al.*, 2009). La synthèse d'eicosanoïdes (prostaglandine E_2, leucotriène B_4) en excès par l'ostéoblaste est également dénotée, ce qui porte atteinte à l'équilibre du processus de remodelage.

Sur un modèle canin d'arthrose par sectionnement chirurgical du ligament croisé crânial, l'amincissement de l'os sous-chondral (résorption excessive) est observé dans les semaines suivant l'induction (Sniekers *et al.*, 2008), tout en démontrant une perte de raideur (Boyd *et al.*, 2002). Ultérieurement, l'os sous-chondral démontre une augmentation marqué du volume et du niveau d'ostéogénèse (Brandt *et al.*, 1991). La sollicitation mécanique excessive pourrait induire des changements phénotypiques favorisant la résorption excessive de l'os sous-chondral et l'ostéoclastogenèse (Burr *et al.*, 2012; Sanchez *et al.*, 2012). Également chez ce modèle, l'inhibition de prostaglandine E_2 et de leucotriène B_4 par les ostéoblastes limite le remodelage excessif et ainsi, préserve les structures articulaires chez ce modèle (Pelletier *et al.*, 2004).

2.3.4 Conclusion

Le rôle de l'os sous-chondral est de distribuer, de manière optimale, les contraintes mécaniques associées à la mise en charge de l'articulation de manière à prévenir d'éventuels points focaux de stress. Il va de soi que l'intégrité du cartilage

articulaire dépend d'un remodelage optimal de l'os sous-chondral afin de préserver l'intégrité structurelle et son rôle fonctionnel. L'atteinte au cartilage seule n'est pas systématiquement associée à la présence de lésions d'arthrose. L'atteinte au niveau de l'os sous-chondral, comme lors d'un remodelage anormal, est nécessaire (Burr *et al.*, 2012).

2.4 Synovite

L'arthrose est considérée comme étant une arthropathie non inflammatoire par l'absence de neutrophiles au sein du liquide synovial et de manifestation d'inflammation systémique. Par contre, la présence d'inflammation synoviale (synovite) est substantielle lors d'arthrose et est responsable de caractéristiques cliniques comme l'effusion et la douleur articulaire.

2.4.1 Membrane synoviale

La membrane synoviale est une structure spécialisée qui tapisse la couche interne de la capsule articulaire. Cette membrane est d'environ 10 à 20 micromètres d'épaisseur et comprend une à trois couches de cellules spécialisées, à savoir les synoviocytes de type B (67 %) et une proportion plus faible de synoviocytes de type A (Momberger et al., 2005). L'activité principale des synoviocytes de type B est la libération de composants du liquide synovial, et celle des synoviocytes de type A est la phagocytose des débris au niveau du liquide synovial (Iwanaga et al., 2000). La membrane synoviale est semi-perméable. Elle contrôle l'entrée et la sortie de molécules au sein de l'espace synovial et régule la composition du liquide synovial. Lors d'inflammation, la perméabilité de la membrane synovial est altérée,

ce qui modifie la composition du liquide synoviale, particulièrement la teneur en lubricine et en acide hyaluronique, affectant ainsi le rôle fonctionnel de ce liquide (Scanzello *et al.*, 2012).

2.4.2 Changements inflammatoires

Lors d'arthrose, des changements de nature inflammatoire surviennent au sein de la membrane synoviale. De tels changements incluent l'hypertrophie et l'hyperplasie de la couche bordande de même que l'infiltration par des cellules inflammatoires (Sellam *et al.*, 2010; Scanzello *et al.*, 2012).

La sollicitation mécanique excessive induit la fragmentation des composants de la matrice extracellulaire conséquemment aux contraintes mécaniques que subit le cartilage. Les produits de cette dégradation se retrouvent solubles au niveau du liquide synovial et parviennent à activer les synoviocytes. La synovite implique la libération d'intervenants pro-inflammatoires par les synoviocytes. Tout comme le cartilage articulaire, les intervenants pivots sont l'IL-1 bêta et le TNF alpha de même que la prostaglandine E_2 et l'oxyde nitrique. Des facteurs cataboliques (comme les MMPs) sont également libérés (Goldring *et al.*, 2011). La présence au sein du liquide synovial de tels intervenants contribue à la dégradation du cartilage articulaire, ce qui supporte le lien entre la synovite et la progression des lésions (Ayral *et al.*, 2005; Scanzello *et al.*, 2012; Henrotin *et al.*, 2014)

L'effusion articulaire est également source de douleur lors d'arthrose. Ainsi, secondaire à la libération de substances vasodilatatrices comme la prostaglandine $E_{2,}$ l'extravasation de protéines plasmatiques survient au sein de la membrane synoviale. Ceci crée l'exsudation de fluides suite à des changements de pression oncotique. La résultante est une augmentation de la pression intra-articulaire et subséquemment, un ressenti douloureux (McDougall, 2006).

Chez un modèle canin d'arthrose par sectionnement chirurgical du ligament croisé crânial, il y a apparition d'une synovite en lien avec la présence de facteurs cataboliques au niveau du cartilage (Pelletier *et al.*, 1985). Les changements inflammatoires chez ce modèle demeurent présents plus de quatre ans après l'induction d'arthrose (Brandt *et al.*, 1991). Également chez ce modèle, l'évolution temporelle de l'effusion articulaire a été décrite à l'aide d'imagerie par résonance magnétique (Annexe IV). Le chapitre Expérimentations de cet ouvrage présente la relation entre l'effusion et le degré de dysfonctionnement locomoteur, reflété par la mesure de la force verticale maximale, chez ce modèle (**10.4.1.2 ci-dessous - Chapitre 2**).

2.5 Ostéophytose

Un ostéophyte correspond à une excroissance osseuse coiffée de fibrocartilage qui prend naissance en marge de l'articulation synoviale (Wang *et al.*, 2014b). Les ostéophytes peuvent être considérés comme une réaction adaptative de l'articulation afin de faire face à un degré anormal de laxité. L'ostéophyte peut donc jouer un rôle

stabilisateur et ainsi limiter une sollicitation biomécanique excessive, protégeant de ce fait le cartilage articulaire (Menkes *et al.*, 2004). La taille des ostéophytes est en lien avec le degré de désalignement du membre chez l'humain (Nagaosa *et al.*, 2002), ce qui supporte l'implication de la sollicitation mécanique face à la genèse d'excroissances osseuses.

Sur un modèle canin d'arthrose par sectionnement chirurgical du ligament croisé crânial, il y a apparition d'ostéophytes dans les jours suivant l'induction (Gilbertson, 1975). Les ostéophytes peuvent être considérés comme une réaction adaptative de l'articulation afin de faire face à un degré anormal de laxité. En se basant sur des observations chez ce modèle d'arthrose (Marshall *et al.*, 1971; Olsson *et al.*, 1972), l'ostéophytose pourrait jouer un rôle de stabilisateur articulaire et ainsi, assurer la protection du cartilage articulaire (Menkes *et al.*, 2004). Également chez ce modèle, l'évolution temporelle de la présence d'ostéophytes a été décrite à l'aide d'imagerie par résonance magnétique (Annexe IV). Le chapitre Expérimentations de cet ouvrage présente la relation entre la présence d'ostéophytes et le degré de dysfonctionnement locomoteur, reflété par la mesure de la force verticale maximale, chez ce modèle (**10.4.1.2 ci-dessous - Chapitre 2**).

3 L'expérience sensorielle

Les informations sensorielles sont à la base des activités neuronales générées par la stimulation de l'organisme. On dénote depuis Aristote (384–322 avant Jésus-Christ) cinq sens au sein du mammifère soit l'ouïe, le toucher, la vision, l'olfaction et le

goût. Une définition des sens établis selon un regard neurobiologique plus actuel permet d'y d'inclure certaines modalités nécessaires à la fonction de l'organisme, telles que les sensations viscérales, vestibulaires et somatiques (Kandel *et al.*, 2012).

3.1 Le système somatosensoriel

Le système somatosensoriel est à même de capter une multitude de stimuli en provenance de l'organisme et de son environnement. De tels stimuli incluent le toucher, la proprioception (phénomène nécessaire au mouvement), les démangeaisons histaminiques, les perturbations viscérales de même que les stimuli algiques (Kandel *et al.*, 2012). L'afférence des stimuli somatosensoriels est également sous la gouverne de ce système.

Afin d'être ressenties, les stimuli somatosensoriels requièrents la présence de récepteurs qui se déclinent en divers types et se distinguent par la nature des stimuli à même de générer une sensation. Ainsi, il existe des mécanorécepteurs qui relaient l'information relatives aux stimuli mécaniques (étirement), des thermorécepteurs au chaud ou au froid, des propriocepteurs qui relaient l'information relative à la géométrie du corps dans l'espace et des nocicepteurs (Kandel *et al.*, 2012). Ces derniers relaient l'information relative aux stimuli potentiellement douloureux.

3.2 La douleur

La douleur peut être définie comme étant une expérience sensitive et émotionnelle associée à un dommage tissulaire actuel ou potentiel (IASP, 1979; Carrasquillo *et al.*, 2008). Cette expérience est, par définition, fortement subjective et s'avère être influencée par la perception de l'évènement *a priori* nocif et par les expériences antérieures de l'individu (Marchand, 2009). Le ressenti d'une douleur qualifiée de physiologique (ou de normale) aura pour effet de protéger l'organisme envers un stimulus potentiellement délétère. Ce type de douleur sera responsable d'un réflexe de retrait, comme celui engendré lors d'une brûlure. L'absence pathologique de cette action protectrice, dans le cas par exemple d'une insensibilité congénitale à la douleur, aura pour effet de favoriser l'apparition de lésions, autrement évitables (Marchand, 2009).

De contraste avec le rôle essentiel que procure un ressenti douloureux qualifié de physiologique, une douleur qui persiste au-delà du temps nécessaire à la guérison est pratiquement dépourvue de fonction bénéfique. Cette douleur récurrente ou chronique se voit, dans ce cas, qualifiée de pathologique, dont l'ampleur des perturbations engendrées dépasse même l'agent causal (Marchand, 2009). Ce type de douleur implique un processus de sensibilisation périphérique et/ou centrale (**3.4.1 ci-dessous - Chapitre 1**), ce qui souligne la capacité de remodelage (ou plasticité) du système somatosensoriel nociceptif.

La douleur ressentie, qu'elle soit physiologique ou pathologique, sera le résultat d'une interaction complexe entre les différents composants du système somatosensoriel nociceptif. La perception unique de l'individu sera considérée *via* l'implication du système nerveux central. À la base du ressenti douloureux réside le processus neuronal de la nociception.

3.3 La nociception

La nociception est un phénomène de perception de stimuli thermiques, mécaniques ou chimiques par des neurones afférents primaires. Elle représente l'activité neuronale engendrée par une stimulation *a priori* nocive pour l'organisme. La nociception implique, d'abord et avant tout, que le stimulus soit transduit en un signal chimio-électrique neuronal pour être ensuite transmis vers les centres supérieurs du système nerveux central et ce, *via* des voies nociceptives afférentes. Les informations attenantes à l'intensité, la qualité et la localisation du stimulus nocif sont alors modulées à différents étages, puis intégrées et traitées au niveau des centres supérieurs du système nerveux central.

3.3.1 Transduction d'un stimulus nocif

La transduction neuronale est le processus par lequel un stimulus (particule odorante, lumière, pression, *etc*.) génère au sein d'un neurone une réponse (signal) chimio-électrique *via* la modification de la conductance ionique membranaire. La bicouche lipidique qui forme la membrane de l'ensemble des cellules de l'organisme est imperméable aux ions. Le transport d'ions de part et d'autre de la membrane ne se fait qu'au niveau de protéines transmembranaires spécialisées

(canaux ioniques). Le transport d'ions issus du processus de transduction affectera la polarité de la membrane cellulaire allant même jusqu'à l'obtention d'un potentiel d'action (**3.3.4 ci-dessous - Chapitre 1**).

La transduction d'un stimulus *a priori* nocif pour l'organisme requiert la présence d'un élément transducteur (récepteur nociceptif, **3.3.3 ci-dessous - Chapitre 1**) situé au niveau des terminaisons libres des neurones afférents primaires (**Figure 9**). Les terminaisons périphériques des neurones afférentes primaires sont qualifiées de libres par l'absence de récepteurs encapsulés, comme ceux de Ruffini par exemple, responsables de la proprioception au niveau de la capsule articulaire (Schenk *et al.*, 1996).

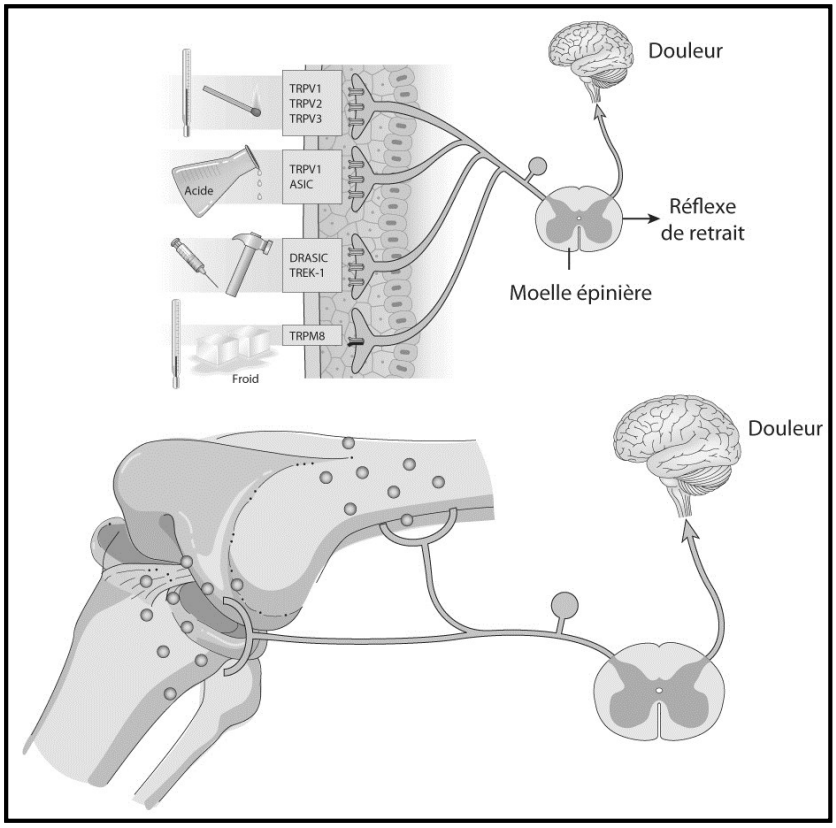

Figure 9. Schématisation de l'afférence nociceptive périphérique vers les centres supérieurs du système nerveux central

*Adapté de Scholz, 2002 (Scholz et al., 2002). La transduction d'un stimulus (physique, thermique ou chimique) vers la génération d'un potentiel d'action se déroule à la terminaison périphérique des neurones afférents primaires par le biais de récepteurs nociceptifs (**3.3.3 ci-dessous - Chapitre 1**). Un axone périphérique conduit le potentiel d'action (**3.3.4 ci-dessous - Chapitre 1**) vers le corps cellulaire jusqu'à l'axone central qui pénètre la moelle épinière au niveau de la racine dorsale. Un neurone afférent secondaire achemine le signal chimio-électrique neuronal vers le thalamus puis vers le cortex somatosensoriel (centres supérieurs du système nerveux central) via un neurone afférent tertiaire.*

3.3.2 Afférences sensorielles primaires

Les voies afférentes primaires transmettent le signal neuronal depuis la périphérie vers la moelle épinière *via* des neurones afférents primaires. Ce type de neurone est qualifié de pseudo-unipolaire par la présence de deux branches (ou axones). Ainsi, une branche projette en périphérie (axone périphérique) et une seconde projette vers la moelle épinière (axone central). Les deux branches sont jointes au niveau du corps cellulaire (Kandel *et al.*, 2012) (**Figure 10**).

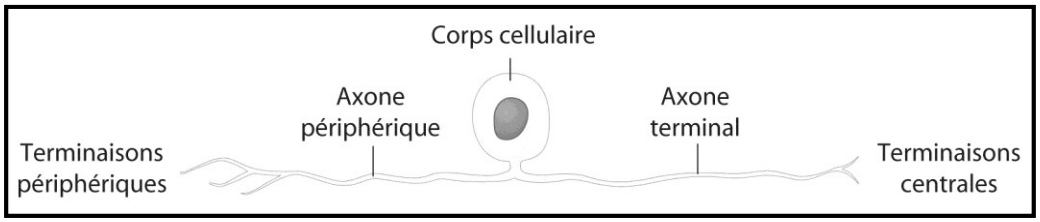

Figure 10. Schématisation d'un neurone afférent primaire

Un regroupement d'axones forme un nerf. Au niveau de la moelle épinière, chaque nerf (spinal) est formé par la réunion de deux racines, soit l'une ventrale (efférence motrice) et l'autre dorsale (afférence somatosensorielle). Au niveau de la racine dorsale de la moelle épinière, le regroupement de corps cellulaires des neurones afférents primaires crée une structure, nommée ganglions des racines dorsales (Kandel *et al.*, 2012). L'afférence somatosensorielle du corps se retrouve au niveau des ganglions des racines dorsales à l'exception des afférences de la tête, dont les corps cellulaires sont situés au niveau des ganglions trigéminaux dits de Gasser chez l'humain.

Sur un modèle canin d'arthrose par sectionnement chirurgical du ligament croisé crânial, l'ablation de l'afférence nociceptive du membre lésé (*via* une gangliectomie des racines dorsales) a démontré l'exacerbation des lésions d'arthrose habituellement rencontrées chez ce modèle. L'aggravation des lésions fut en lien avec un faible dysfonctionnement locomoteur, reflété par un niveau élevé de force verticale maximale (O'Connor *et al.*, 1999).

La classification des neurones afférents primaires est possible en regard à l'aspect de l'axone et à la rapidité de propagation du signal chimio-électrique neuronal.

- **Les neurones de Type A-bêta** possèdent un axone de fort diamètre doté d'une gaine de myéline permettant de propager un signal associé à un stimulus principalement mécanique (proprioception, toucher) à une vitesse entre 35-75 m/s (Almeida *et al.*, 2004; Marchand, 2009).
- **Les neurones de type A-delta** possèdent un axone doté d'une gaine de myéline permettant de propager un signal associé à un stimulus thermique et nociceptif (thermique ou mécanique) à une vitesse entre 5-30 m/s. Le signal porté par ces neurones induit un ressenti douloureux bref et bien localisé. Ce type de neurones contribue à générer le réflexe de retrait (Almeida *et al.*, 2004; Marchand, 2009).

- **Les neurones de type C** possèdent de fins axones dépourvus de myéline et propagent un signal associé à un stimulus (nociceptif) chimique, thermique ou mécanique à une vitesse entre 0.5-2 m/s. Le signal porté par ces neurones induit un ressenti de douleur tardif et diffus (Almeida *et al.*, 2004; Marchand, 2009).

Certains neurones afférents primaires sont activés par plus d'un stimulus et qualifiés de polymodaux. C'est le cas pour la majeure partie des neurones de type C. La présence de différents récepteurs nociceptifs (**3.3.3 ci-dessous - Chapitre 1**) explique une sensibilité hétérogène à divers stimuli. Les neurones afférents primaires peuvent également être qualifiés de silencieux. Les neurones afférents primaires silencieux ont la capacité d'être activés en réponse à des stimuli non nocifs uniquement en présence de substances algésiogènes issues de processus inflammatoires ou lésionnels. Autrement, ces derniers demeurent dans un état de quiescence face à des stimuli non nocifs (Schaible *et al.*, 1985; Rukwied *et al.*, 2008). Certains neurones afférents primaires sont qualifiés de peptidergiques en lien avec leur capacité à libérer des neuropeptides en périphérie, comme la substance P.

Les voies afférentes primaires transmettent le signal chimio-électrique neuronal depuis la périphérie vers les centres supérieurs du système nerveux central *via* différentes voies, soit la voie lemniscale (proprioception, stimuli mécanique) et les voies spinothalamiques et spinoparabrachiales (stimuli nociceptifs). Un neurone

afférent secondaire achemine par la suite le signal vers le thalamus, puis vers le cortex somatosensoriel *via* un neurone afférent tertiaire (Marchand, 2009).

3.3.3 Les récepteurs nociceptifs

Les sensations somatiques sont liées à la perception de plusieurs stimuli *via* des protéines transmembranaires spécialisées. Ces structures forment des canaux ioniques retrouvés à la terminaison périphérique des neurones afférents primaires. Les récepteurs nociceptifs (ou capteurs somatosensoriels) sont, en fait, des canaux ioniques dont l'ouverture est gouvernée par un stimulus.

Plusieurs types de canaux ioniques existent en fonction du mécanisme régissant leurs ouvertures (Kandel *et al.*, 2012).

- Canaux activés par un ligand (récepteur ionotrope)
- Canaux activés en fonction du voltage
- Canaux activés par le degré d'étirement de la membrane
- Canaux activés par un second messager intracellulaire
- Canaux activés par le changement de volume cellulaire

L'afférence nociceptive vers les centres supérieurs du système nerveux central est schématisée (**Figure 9**). Les canaux ioniques TRPV1 (*Transient receptor potential vanilloid one*) sont activés par des substances agonistes telles que la capsaïcine, la leucotriène B4 et des dérivés de gingembre et également par la chaleur (>43°c), *via* un changement de pression osmotique. D'autres canaux ioniques au sein des TRP

sont également thermosensibles dont le TRPV2, le TRPV3, et le TRPM8 (*Transient receptor potential melastatin eight*) (Liedtke, 2006). Les canaux ioniques ASIC (*Acid-sensing ion channel*) sont activés par l'acidification extracellulaire (présence d'ions H^+). Les canaux ioniques TREK-1 (*TWIK1-related* K^+ *channel*) (Maingret *et al.*, 1999) et les DRASIC (*Dorsal root acid sensing ion channel*) (Price *et al.*, 2001) sont sensibles à des stimuli mécaniques.

Des substances chimiques, présentes au niveau de l'espace extracellulaire à la suite d'un dommage tissulaire (par exemple l'histamine) ou lors d'inflammation (prostaglandine), démontrent un potentiel algésiogène. La liaison entre ces substances et leurs récepteurs respectifs parvient à altérer la conductance membranaire et ainsi, à générer un potentiel d'action. Contrairement aux récepteurs ionotropes, les récepteurs métabotropes, comme les récepteurs à l'histamine et aux prostaglandines, ne contiennent pas de canaux ioniques. Ces derniers agissent plutôt en activant des voies de signalisations intracellulaires. La cohabitation entre les récepteurs métabotropes et des canaux ioniques (incluant des récepteurs ionotropes) permet, lors de leur activation, de mettre en branle une cascade d'évènements intracellulaires menant à la genèse d'un potentiel d'action.

3.3.4 Potentiel d'action

La bicouche lipidique de la membrane cellulaire d'un neurone permet d'isoler deux milieux conducteurs, soit le fluide extracellulaire et le cytosol, ce qui contribue à établir une différence de potentiel. Ainsi, le cytosol est doté d'une charge négative de l'ordre de -60 à -70 millivolts qui correspond au potentiel de repos de la

membrane (Kandel *et al.*, 2012). Ce potentiel de repos négatif est le résultat d'une perméabilité sélective envers certains ions (sodium, potassium, chlore) et de l'activité des protéines transmembranaires, particulièrement des pompes sodium-potassium. L'activité de ces dernières consiste à concentrer le potassium à l'intérieur de la cellule et le sodium à l'extérieur, en utilisant l'énergie cellulaire. Les ions sont également véhiculés de part et d'autre de la membrane selon des forces chimiques (gradient de concentration), des forces électriques (différence de potentiel) et également en fonction de la conductance membranaire (*via* des canaux ioniques) (Kandel *et al.*, 2012). Quand le potentiel membranaire s'achemine vers des valeurs positives, la membrane est dite dépolarisée.

En réponse à un stimulus, l'activation des canaux ioniques (*via* un ligand agoniste par exemple) aura pour effet de permettre l'entrée massive de cations (sodium, calcium) vers le cytosol. Ceci provoquera la dépolarisation de l'axone par un déséquilibre de charges ioniques. Un seuil critique détermine si la dépolarisation est suffisante à la genèse d'un potentiel d'action. Autrement, la membrane se repolarise et atteint de nouveau son potentiel de repos. Au-delà d'un seuil critique (par exemple -55 millivolts), le cytosol se voit être fortement dépolarisé, ce qui a pour effet d'activer d'autres canaux ioniques, soit ceux dont l'ouverture dépend du potentiel. C'est la sommation temporelle et spatiale de l'activation de tous ces canaux qui permet d'atteindre la dépolarisation cellulaire.

Les canaux ioniques dépendants du potentiel, lorsqu'ils sont activés, subissent un changement de conformation. Ce changement facilite le passage de cations à travers le canal vers le cytosol, ce qui a pour effet de générer un potentiel membranaire positif (40 millivolts) (Kandel *et al.*, 2012). Pour acheminer le signal chimio-électrique neuronal de la périphérie vers le système nerveux central, le potentiel d'action doit se propager dans l'axone du neurone afférent primaire. La propagation du potentiel d'action se fait de manière unilatérale. Ainsi, en amont, réside une période réfractaire qui empêche la propagation du potentiel d'action, tandis qu'en aval, le potentiel d'action peut se propager en dépolarisant (réaction en chaîne) la partie du cytosol au repos (polarisé). Le potentiel d'action atteint ensuite l'axone terminal situé au niveau de la moelle épinière. Il entre alors en contact avec le neurone afférent secondaire.

3.3.5 Neurone afférent secondaire

Les neurones afférents primaires projettent le signal chimio-électrique neuronal au niveau de la moelle épinière, plus particulièrement au niveau de la substance grise. À ce niveau, il y a synapse (échange entre deux entités cellulaires permettant de véhiculer un message) entre le neurone afférent primaire et le neurone afférent secondaire.

La génération d'un potentiel d'action au niveau des neurones afférents primaires, suivant l'activation par un stimulus nociceptif, induit la libération de neurotransmetteurs par l'axone terminal. Ainsi, au niveau de la fente synaptique,

l'axone terminal libère des neurotransmetteurs peptidiques, tels que la substance P et le CGRP *(Calcitonin gene related peptide*) et des acides aminés excitateurs comme le glutamate.

Le glutamate joue un rôle clé dans la transmission du signal chimio-électrique neuronal. Il est un neurotransmetteur libéré par les neurones afférents primaires de type A-delta et de type C et parfois A-bêta. Suivant sa libération, le glutamate est véhiculé au niveau de la fente synaptique puis, ce ligand, trouve assise au niveau de récepteurs ionotropes présents sur les neurones afférents secondaires. Ces récepteurs incluent ceux de type AMPA (alpha-amino-3-hydroxy-5-méthylisoazol-4-propionate), kaïnate et NMDA (*N*-méthyl-D-aspartate). Le glutamate est également le ligand de certains récepteurs métabotropes (Chiechio *et al.*, 2012).

La substance P et le CGRP, lorsqu'ils sont libérés par des neurones afférents primaires de type A-delta ou de type C, trouvent une assise au niveau de récepteurs métabotropes NK-1 (Neurokinine un) et CGRP, respectivement. Il en découle des potentiels post-synaptiques excitateurs et la génération subséquente d'un second potentiel d'action. Le signal chimio-électrique neuronal est ensuite acheminé vers les centres supérieurs du système nerveux central *via* les voies spinothalamiques et spinoparabrachiales (Okuse, 2007).

Au niveau de la moelle épinière, le point de synapse entre le neurone afférent primaire et le secondaire est également le lieu de différents processus de

modulation du signal neuronal nociceptif. La modulation de la réponse face à un stimuli nocif implique des interneurones aux rôles activateurs et inhibiteurs (Marchand, 2009). Ainsi, des interneurones qui ont pour effet d'activer des neurones efférents, permettront de générer le réflexe de retrait *via* l'action musculaire. Le rôle inhibiteur de certains interneurones s'effectuera grâce à la libération de neurotransmetteurs au niveau de la fente synaptique, tels que les enképhalines (action *via* récepteur opioïde), la noradrénaline (action *via* les récepteurs alpha-2), ou encore l'acide γ-aminobutyrique (GABA, action *via* récepteurs GABA). L'action inhibitrice aura pour effet d'hyperpolariser la membrane du neurone secondaire, ce qui augmentera le seuil d'activation et ainsi, limitera la transmission du signal chimio-électrique neuronal. Cette action inhibitrice est effectuée *via* des voies inhibitrices descendantes sous dominante sérotoninergique, opioïdergique ou noradrénergique (Marchand, 2009).

3.4 Arthrose et plasticité du système somatosensoriel

Au sein de l'articulation synoviale, les muscles, la membrane synoviale, la capsule articulaire, les tendons, les ligaments et l'os sous-chondral sont dotés d'afférences primaires. Seul le cartilage articulaire en est dépourvu. Le ressenti douloureux lors d'arthrose s'effectue *via* des processus complexes qui ne sont pas rigides mais plutôt assujettis à un phénomène de remodelage. Ainsi, lorsque le stimulus devient chronique, des changements inadaptés et persistants au niveau du système somatosensoriel sont responsables d'un ressenti douloureux pathologique,

découlant entre autres, de la plasticité du système somatosensoriel (Carrasquillo *et al.*, 2008).

Une propriété importante des neurones afférents primaires et secondaires est leur capacité à se sensibiliser. La sensibilisation se développe communément à des atteintes tissulaires, ou lors d'inflammation, importantes en intensité ou répétées, et se caractérise par un abaissement du seuil d'activation et une augmentation de la réponse à un stimulus nocif. De la plasticité du système somatosensoriel nociceptif découle la notion d'hypersensibilité.

Lors d'arthrose, les altérations au niveau de l'afférence somatosensorielle sont notoires et mènent à un état d'hypersensibilité. Dans cet état, un simple mouvement d'amplitude normale (non nocive) sera source d'un ressenti douloureux. Ce phénomène se nomme allodynie et se produit lorsque le seuil d'activation permettant la genèse d'un potentiel d'action est diminué (dépolarisation de la membrane facilitée). Lorsqu'un mouvement articulaire intense est source d'un ressenti douloureux exagéré, il y a hyperalgésie qui correspond à la genèse d'intenses potentiels d'action à un seuil normal d'activation (Basbaum *et al.*, 2009).

Un état d'hypersensibilité a été récemment démontré chez le chien en contexte d'arthrose naturelle associée à une rupture traumatique du ligament croisé crânial (Brydges *et al.*, 2012). L'état d'hypersensibilité englobe deux facteurs clés dans le ressenti douloureux, soit la sensibilisation périphérique et la sensibilisation centrale.

3.4.1 Sensibilisation périphérique

Le phénomène de sensibilisation périphérique résulte communément de changements dans le milieu qui entoure le neurone afférent primaire. Ce dernier se retrouve ainsi plongé dans une soupe inflammatoire qui est riche en substances algogènes issues de la réaction inflammatoire associée aux dommages arthrosiques (Basbaum *et al.*, 2009).

La sensibilisation périphérique représente une forme de plasticité des neurones afférents primaires induite par des stimuli qui sont, dans ce cas, des substances algogènes (prostaglandine E_2, bradykinine, ATP, protons H+). Les substances algogènes présentes au sein de la soupe inflammatoire activent les canaux ioniques en étant des modulateurs allostériques directs (protons H+). D'autres substances se lient à leur récepteurs respectifs, par exemple la bradykinine *via* un récepteur couplé à une protéine G, et modulent l'ouverture des canaux par le biais de voies de signalisations intracellulaires (Basbaum *et al.*, 2009). Dans les deux cas, il y aura un abaissement du seuil d'activation des canaux ioniques (allodynie). Autrement dit, la membrane se dépolarise et atteint un niveau de dépolarisation qui se rapproche du seuil d'activation du potentiel d'action.

Lors de sensibilisation périphérique, le neurone afférent primaire peut subir un remodelage et ainsi, voir des changements phénotypiques apparaître, comme la présence de récepteurs nociceptifs de type TRPV1 issus de l'activation de facteurs de transcription (Carlton *et al.*, 2001). Également, des neurones de type A-Beta,

dont l'afférence est normalement dédiée à la transmission d'informations proprioceptives au sein de l'articulation, subissent des changements phénotypiques caractéristiques de neurones de type C, comme la présence du neuropeptide substance P dans des vésicules terminaux (Woolf *et al.*, 1999). En présence de cette soupe inflammatoire, l'activation de neurones afférents primaires qualifiés de silencieux est également un changement phénotypique lors d'arthrose (Schaible *et al.*, 1985; Rukwied *et al.*, 2008). Ces changements phénotypiques contribuent au phénomène d'hyperalgésie.

Dans son ensemble, la sensibilisation périphérique contribue, en favorisant le relargage de neurotransmetteurs activateurs au sein de la fente synaptique, à instaurer le phénomène de sensibilisation centrale.

3.4.2 Sensibilisation centrale

Lorsque l'état d'hypersensibilité persiste, suite à la chronicité de la douleur, le ressenti douloureux prend une importance exagérée. La sensibilisation centrale se caractérise par un état d'hyperexcitabilité du système nerveux central. Plusieurs mécanismes expliquent cet état tels que l'implication de la transmission glutamatergique (récepteur NMDA), la perte de contrôle inhibiteur et l'interaction avec les cellules gliales (Basbaum *et al.*, 2009).

Le récepteur NMDA, qui est lié à l'entrée de calcium dans le cytosol, contribue à la sensibilisation centrale. Lors de l'afférence nociceptive (physiologique), ce dernier

est inactif suite à la présence d'un ion magnésium à l'entrée du canal ionique. Ce blocage peut cependant être éliminé lors de la dépolarisation de la cellule par l'action post-synaptique de la substance P. De cette action découle l'entrée massive d'ions calcium dans le cytosol, ce qui dépolarise davantage la membrane du neurone. L'activation concomitante de récepteurs à la substance P et de récepteurs métabotropes contribue à l'excitabilité du neurone secondaire. Il en découle une série d'évènements intracellulaires qui amplifie et facilite la transmission du signal neuronal nociceptif jusqu'aux centres supérieurs du système nerveux central (Basbaum *et al.*, 2009).

La perte du contrôle inhibiteur contribue à la sensibilisation centrale. Lors de l'afférence nociceptive (physiologique), la transmission du ressenti douloureux est modulée par la libération de neurotransmetteurs inhibiteurs, tel le GABA par des interneurones. Cependant, l'hyperalgésie survient lorsqu'il y a cessation de ce phénomène inhibiteur, tel qu'observé lors d'arthrose (Rakel *et al.*, 2014). La perte du contrôle inhibiteur contribue également à l'allodynie en favorisant l'interprétation nociceptive pour un signal neuronal qui, en fait, provient d'une afférence primaire non nocive transmise par des neurones de type A-bêta (proprioception). En présence de sensibilisation centrale, l'activation de certains facteurs de transcription mènera à l'activation de gènes qui codent pour l'obtention, par exemple, de COX-2. En découlera la production accentuée de la substance algogène prostaglandine E_2. Au sein de la fente synaptique, la libération de

prostaglandine E_2 favorisera l'excitation post-synaptique et diminuera l'action inhibitrice d'interneurones (Scholz *et al.*, 2002).

Les cellules gliales (microglies et astrocytes) présentes au niveau de la moelle épinière contribuent à la sensibilisation centrale. Ces dernières seront activées par la libération de substances algogènes, comme l'ATP. La libération d'autres substances algogènes, comme le facteur neurotrophique issu du cerveau (*Brain-derived neurotrophic factor*, BDNF) *via* des canaux ioniques purinergiques, aura pour effet d'augmenter l'excitabilité du neurone afférent secondaire. Cet effet favorisera la genèse d'un potentiel d'action en dépolarisant la membrane du neurone afférent secondaire (Basbaum *et al.*, 2009).

3.4.3 Conclusion

Lors d'arthrose, le système somatosensoriel subit un remodelage dû à la présence d'un ressenti douloureux qui persiste. Les afférences nociceptives sont favorisées et amplifiées, ce qui exacerbe le ressenti douloureux au niveau de l'articulation, induisant l'allodynie et l'hyperalgésie.

3.5 Ressenti douloureux et l'os sous-chondral

Le remodelage excessif au niveau de l'os sous-chondral participe à la genèse d'un ressenti douloureux (Mantyh *et al.*, 2002; Luger *et al.*, 2005) (**Figure 11**). La présence de protons H^+ (milieu acide, pH entre 4-5), que nécessite l'activité résorptive des ostéoclastes, aura pour effet d'activer des récepteurs nociceptifs de

type TRPV1 et ASIC3. Également, l'apoptose cellulaire en présence d'une sollicitation mécanique excessive, contribuera à favoriser l'acidité du milieu et l'activation de récepteurs nociceptifs (Luger *et al.*, 2005). La synthèse de substances algogènes, particulièrement la prostaglandine E_2 par les ostéoblastes (Lajeunesse, 2004), aura pour effet de dépolariser la membrane et de favoriser la genèse d'un potentiel d'action (hyperalgésie). Les dommages structuraux au niveau de l'os sous-chondral, issus d'un remodelage excessif, sont également algogènes. Ainsi, l'augmentation de la pression hydrostatique au niveau des canalicules, en lien avec l'atteinte structurelle, aura pour effet d'activer des récepteurs nociceptifs sensibles à la déformation mécanique. Également, sous l'effet d'une sollicitation mécanique excessive, les ostéocytes et les ostéoblastes libèreront de l'ATP ce qui aura pour effet d'activer des canaux ioniques purinergiques, comme le $P2X_3$ (Bonewald, 2006).

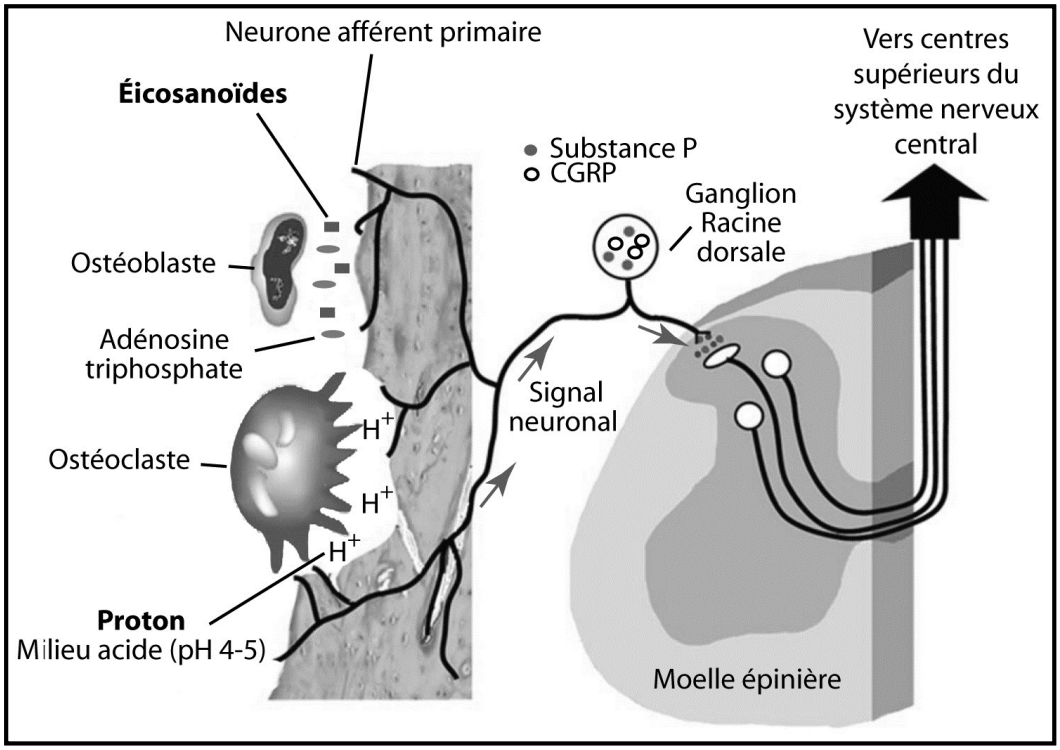

Figure 11. Schématisation de l'afférence nociceptive de l'os sous-chondral

Adaptation de Luger, 2005 (Luger *et al.*, 2005). *Calcitonin gene related peptide (CGRP).*

D'autres changements au niveau de l'os sous-chondral sont également sources d'un ressenti douloureux lors d'arthrose. La présence d'infiltrations vasculaires au niveau du cartilage calcifié, concomitante à l'infiltration par des neurones afférents primaires, contribue à favoriser ce ressenti (Suri *et al.*, 2007; Houard *et al.*, 2013). L'infiltration vasculaire et sensitive est également observée au niveau des ostéophytes (Suri *et al.*, 2007; Houard *et al.*, 2013).

4 Instruments métrologiques de l'atteinte fonctionelle en contexte d'arthrose naturelle chez le chien

L'usage des forces de réaction au sol, particulièrement la force verticale maximale, s'inscrit comme un moyen d'évaluer l'effet thérapeutique découlant de diverses modalités contre l'arthrose canine (**6.1 ci-dessous - Chapitre 1**). L'analyse cinétique du mouvement n'est pas le seul instrument métrologique qui cible le dysfonctionnement locomoteur. L'analyse cinématique ou encore télémétrique du mouvement locomoteur renseigne également sur ce point.

4.1 L'analyse télémétrique du mouvement locomoteur

L'analyse télémétrique du mouvement locomoteur est réalisée à l'aide d'accéléromètre triaxial. La majorité des instruments utilise des accéléromètres de type piézoélectrique basé selon le principe de masse-ressort. La propriété de certains cristaux et céramiques de se charger électriquement lorsqu'ils sont soumis à une déformation est mise à profit. Le dégré et l'intensité du mouvement locomoteur sont alors transduits en signaux électriques pour ensuite être intégrés de manière numérique.

La durée et la fréquence d'enregistrement peuvent être paramétrées afin de procurer un suivi continu du mouvement locomoteur sur plusieurs jours. Il en découle différentes mesures dont la durée quotidienne d'activités (**10.3.2.4 ci-dessous -**

Chapitre 2). L'enregistrement du mouvement locomoteur est effectué dans les trois axes orthogonaux, ce qui diffère d'un pédomètre usuel.

Chez un modèle canin d'arthrose naturelle, l'analyse télémétrique du mouvement locomoteur a permis de détecter une réponse thérapeutique (Brown *et al.*, 2010; Rialland *et al.*, 2013). Le chapitre Expérimentations de cet ouvrage présente la relation entre l'analyse télémétrique du mouvement locomoteur et le degré de dysfonctionnement locomoteur reflété par la force verticale maximale (**10.4.2.2 ci-dessous - Chapitre 2**).

4.2 Appréciations à l'aide d'un tiers

La communication entre l'humain et le chien est limitée par l'incapacité de ce dernier à exprimer pleinement son ressenti face à sa condition arthrosique. L'évaluation de l'incapacité fonctionnelle et de la qualité de vie du chien arthrosique fait donc appel à des appréciations effectuées à l'aide d'un tiers. Ce tiers peut être, soit un professionnel de la santé vétérinaire, soit le propriétaire de l'animal. Dans le cas du propriétaire, ce dernier dispose de divers instruments psychométriques, sous la forme d'échelles composites multiparamétriques, tels que le LOAD (*Liverpool osteoarthritis in dogs*) (Walton *et al.*, 2013), le CBPI (*Canine brief pain inventory*) (Brown *et al.*, 2008), le HCPI (*Helsinki chronic pain index*) (Hielm-Bjorkman *et al.*, 2009) et le CSOM (*Case specific outcome measure*) (Rialland *et al.*, 2012). Pour ce qui est du professionnel de la santé vétérinaire, ce

dernier possède également des instruments métrologiques comme l'échelle de cotation numérique tirée de Vasseur, 1995 (Vasseur *et al.*, 1995).

À l'aide de ces instruments métrologiques, l'exercice consiste à procurer des réponses à des questions fermées. La question fermée oblige le tiers à répondre à partir d'options prédéfinies. Les options peuvent être semblables ou pas, c'est-à-dire qui diffèrent en terme de magnitude d'effet. Afin d'orienter la réponse du tiers, d'autres échelles sont également disponibles.

- Échelle visuelle analogue. Quantification du paramètre sur une ligne de 10 centimètres. La distance entre l'extrémité gauche (0 = aucune douleur) et le point donne la valeur.
- Échelle de cotation numérique. Quantification du paramètre à l'aide d'un nombre entier.
- Échelle descriptive simple. Quantification du paramètre à l'aide de descriptions courtes.

Pour le propriétaire, il est également possible de répondre à des questions ouvertes, comme il en est le cas avec l'instrument psychométrique CSOM. A l'aide de ce type d'instruments, le propriétaire définit une activité problématique associée à l'état arthrosique de l'animal. Par la suite, une échelle de cotation numérique est utilisée afin de quantifier la magnitude du problème (**6.1.1.3.4 ci-dessous - Chapitre 1**).

4.3 Concepts métrologiques appliqués à la force verticale maximale

Au cours des deux dernières décennies, la mesure des forces de réaction au sol, particulièrement la force verticale maximale, a été largement utilisée afin de décrire le dysfonctionnement locomoteur associé à la présence d'arthrose chez le chien. Certains critères doivent être respectés lors de la mesure de la force verticale maximale afin de s'affranchir d'une source potentielle d'erreurs et ainsi, assurer un degré de fiabilité acceptable et une validation des interprétations qui en découlent.

Le processus de validation est considérée comme étant crucial au succès des résultats qui découlent d'une mesure (Kelly *et al.*, 2005). Ce processus requiert de l'inférence au sujet de la véracité de la mesure dans son ensemble. Il s'obtient de manière progressive, c'est-à-dire au fil des évidences de validité. La validité n'est pas une caractéristique de l'outil de mesure. La validité est plutôt une propriété de l'interprétation des résultats de cette mesure (Kelly *et al.*, 2005; Cook *et al.*, 2006). Ainsi, l'expression fictive suivante est à éviter : *la mesure de la force verticale maximale est validée chez le chien arthrosique*. L'expression suivante est à favoriser : *l'interprétation d'une force verticale maximale anormalement faible, comme étant une caractéristique d'un état arthrosique, est valide*.

Pour une mesure quantitative directe, comme la force verticale maximale (ou toute autre quantité physique), l'interprétation de la mesure propre est intuitive et confirmée (Laurencelle, 1998). Cependant, l'erreur de mesure sera un point critique à considérer pour cette mesure quantitative. L'erreur de mesure est la différence entre la mesure vraie et celle obtenue par l'instrument. L'erreur de mesure peut être systématique (erreur qui varie de façon prévisible en contexte de mesures répétées) (VIM, 2012), ou aléatoire (erreur qui varie de façon imprévisible en contexte de mesures répétées) (VIM, 2012).

La validité de l'interprétation qui découle d'une mesure est donc en relation étroite avec l'erreur de mesure. Par conséquent, une mesure observée résultera de la somme de la valeur vraie et de l'erreur de mesure (Higgins *et al.*, 2006). Le concept de validité repose sur la solidité des arguments supportant la réduction de l'erreur de mesure (systématique et aléatoire) afin d'extrapoler les résultats à l'ensemble de la population (Cook *et al.*, 2006). Ainsi, a priori d'une interprétation valide, l'outil de mesure devra démontrer son exactitude (étroitesse de l'accord entre une valeur mesurée et une valeur réelle) (VIM, 2012). L'exactitude d'une mesure est définie par le degré de justesse (étroitesse de l'accord entre un nombre infini de mesures répétées et une valeur de référence) (VIM, 2012) et de précision (étroitesse de l'accord entre les mesures du même objet) (VIM, 2012). Un appareil procure donc des mesures exactes lorsque celles-ci sont à la fois justes et précises. A l'aide de procédures d'étalonnage, il est possible de déterminer l'exactitude d'un appareil,

par exemple une plateforme de mesure de forces de réaction au sol (Collins *et al.*, 2009), et d'appliquer des méthodes de correction lorsque c'est nécessaire.

Le tableau suivant (**Tableau I**) présente le concept général de validation en fonction de l'erreur de mesure, qu'elle soit systématique ou aléatoire (Higgins *et al.*, 2006). Les différents critères décrits à ce tableau peuvent être pris en considération afin d'obtenir une inférence valide au sujet, par exemple, de l'efficacité d'une approche thérapeutique (Hoogeboom *et al.*, 2012) ou d'un modèle d'arthrose (Kim *et al.*, 2013).

Au chapitre Discussion générale, la nature évaluative de la force verticale maximale sera décrite chez le modèle canin d'arthrose naturelle à l'aide du concept général de validation (**Tableau I**). Plus particulièrement, il y aura argumentation favorable envers la validation de la force verticale maximale comme critère d'efficacité lors d'essais cliniques contrôlés chez le chien arthrosique.

Tableau I. Concept de validation intégré à l'erreur de mesure

Erreur systématique (Hoogeboom *et al.*, 2012)	**Validité du contenu**	Recension littéraire Réflexions personnelles Analyses critiques d'experts
	Validité de critères	Relation concomitante Relation convergente/divergente Relation prédictive Analyse factorielle
	Validité interne	Évènement concurrent Effet maturation de la condition Effet habituation du sujet Régression envers la moyenne Sélection des sujets Attrition de sujets Ambiguïté sur la relation causale Égalité compensatoire des traitements Rivalité des sujets Démoralisation chez les sujets contrôles négatifs
	Validité externe	Interaction sélection du sujet *versus* traitement Interaction installation *versus* traitement Interaction circonstances *versus* traitement Interaction avec de multiples traitements
	Validité des conclusions statistiques	Puissance statistique Respect des pré-requis statistiques Seuil alpha pour comparaisons multiples Fiabilité des outils de mesure Qualité du traitement et assiduité Installation/environnement hétérogène Hétérogénéité des sujets
Erreur aléatoire (Hoogeboom *et al.*, 2012)	**Consistance interne***	Coefficient alpha de Cronbach
	Stabilité	Coefficient de corrélation intra-classe (test-retest)
	Fidélité*	Pourcentage d'accord Graphique Bland-Altman

*Description non exhaustive

4.4 Biais de mesure

Le biais de mesure est une source d'erreur apparentée à un manque de justesse. Ce biais offre une estimation de l'erreur de mesure systématique (VIM, 2012) et s'avère être indépendant de l'erreur de mesure aléatoire (Oort *et al.*, 2009). Autrement dit, l'erreur due au biais de mesure transgresse la portion invariable de la mesure. Cette portion devient alors atteinte de variance qui ne s'explique pas par le degré habituel d'erreur de mesure aléatoire. La relation entre l'attribut, la mesure et une variable est schématisée (**Figure 12**).

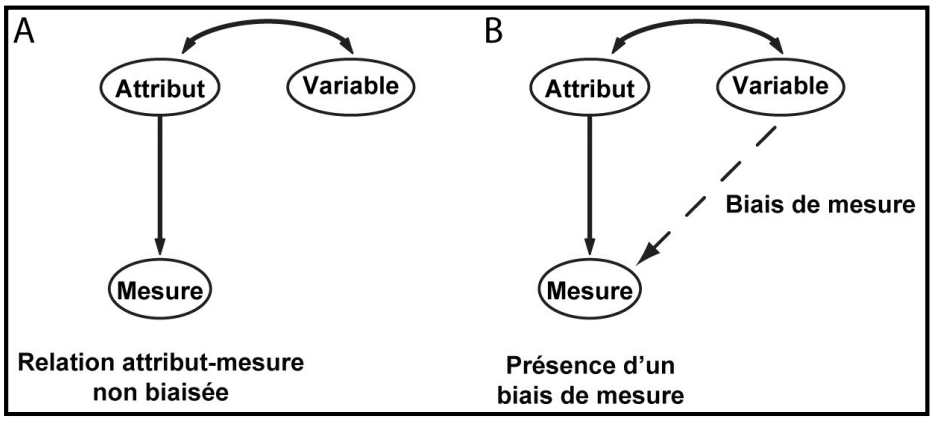

Figure 12. Schématisation de la relation entre attribut, variable et mesure

Adapté de Oort, 2009 (Oort et al., 2009). A) La mesure est déterminée par l'attribut, comme l'indique la flèche unidirectionnelle. La variable est en relation avec l'attribut (flèche bidirectionnelle) et démontre un effet sur la mesure via l'attribut. La relation entre l'attribut et la mesure n'est donc pas affectée directement par la variable. B) La relation entre la mesure et la variable n'est pas

complètement expliquée par la relation entre la variable et l'attribut ni par celle entre l'attribut et la mesure. La présence d'un biais de mesure est ainsi observée.

4.4.1 Effet de l'exercice intense sur la mesure de la force verticale maximale du chien arthrosique

Une récente étude réalisée chez le chien arthrosique (Annexe VI) a permis de déterminer l'impact d'un exercice intense sur la mesure de la force verticale maximale. L'effet de l'exercice intense représente une source de biais en présence d'arthrose au membre locomoteur. De ce biais découlent des mesures de force verticale maximale qui sont significativement moindres que celles obtenues avant l'exercice. Ceci suggère l'exacerbation du dysfonctionnement locomoteur suivant un exercice intense. Ce biais de mesure est présent uniquement chez les chiens arthrosiques.

4.4.2 Article II: Influence of changes in body weight on peak vertical force in osteoarthritic dogs: A possible bias in study outcome

Cet article, publié dans *Veterinary Surgery,* présente la relation entre le gain de poids corporel et la mesure de la force verticale maximale chez le chien arthrosique. Le but était de démontrer statistiquement qu'un gain de poids corporel, secondaire à la prise d'une diète thérapeutique, affecte la force verticale maximale chez des chiens arthrosiques.

Le gain de poids corporel s'avère être un biais envers la mesure de la force verticale maximale chez le chien arthrosique. De ce biais découlent des valeurs de force verticale maximale qui sont significativement moindres que celles obtenues avant ce gain de poids. Ceci suggère l'exacerbation du dysfonctionnement locomoteur en dépit de l'expression de la force verticale maximale relative au poids de l'animal (pourcentage de poids corporel).

M. Maxim Moreau a participé au design expérimental, à l'acquisition des données, à l'analyse de ces dernières et a rédigé cet article présentement publié (*Veterinary Surgery*, 39:43–47, 2010). M. Moreau a également effectué l'ensemble des travaux d'infographie. L'article a par la suite été dûment révisé et bonifié par l'expertise de chacun des coauteurs.

Veterinary Surgery
39:43–47, 2010

Influence of Changes in Body Weight on Peak Vertical Force in Osteoarthritic Dogs: A Possible Bias in Study Outcome

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4.4.2.1 Abstract

Objective- Force platform gait analysis is a recognized clinical evaluation tool that captures and documents the in vivo pathomechanics of osteoarthritis (OA). In a clinical trial designed to evaluate the impact of two specific diets, an increase in body weight (BW) was observed in lame client-owned dogs. Covariance analysis was done to evaluate the interference of BW changes toward the evolution of peak vertical force (PVF) values. These secondary findings are reported in the present study.

Study design- Prospective study

Animals- Twenty six lame client-owned dogs

Methods- Dogs showing radiographic evidence of OA and low PVF values were fed with two specific diets for subsequent periods of 30 and 60 days. PVF and BW were recorded at baseline, day 30 (D30), and D90. Mean±Standard Deviation values are presented.

Results- PVF values (%BW) did not differ significantly overtime (D0: 63.9±17.2; D30: 65.5±17.4; and D90: 66.5±20.1). In contrast, BW (kg) was significantly higher at D90 (41.3±7.9) when compared to D30 (39.9±8.4) and D0 (40.0±8.7). Upon covariance analyses, BW changes interfere significantly with PVF values already normalised in %BW (p=0.013). Values of PVF adjusted using BW as a covariate were then 63.4±17.1 (D0), 65.0±17.3 (D30) and 67.6±20.5 (D90), whereas D90 was significantly higher than D0.

Conclusion & clinical relevance- These findings highlighted the interference of changes in BW toward the ambulation of OA dogs when using PVF values normalised in %BW. Exacerbation of lameness when a gain in BW occurred was also sustained, raising a possible bias in clinical study outcomes.

4.4.2.2 Introduction

Osteoarthritis (OA) stands among the most common joint diseases encountered in veterinary clinical practice and remains highly prevalent in dogs.[1,2] Efforts have been directed to alleviate the crippling pain emerging secondary to this whole joint organ affliction, mainly through the control of recurrent inflammation.[3]

To capture and document therapeutic efficacy, kinetic evaluation using force platform gait analysis is an established gold standard in dogs, being an objective biomechanical method to evaluate therapeutic efficacy upon forces generated during motion. The later are three-dimensionally oriented and refer as the ground reaction forces; from those the recording of vertically-oriented force has generated interesting findings.[4-9] Due to a refined method addressed to enhance quality of gait's data, to keep variation as low as possible and to limit study bias, clinical trials with low sample size (<40 subjects) may be sufficient to demonstrate clinically meaningful results.[4,10-14]

Recently our team completed a clinical trial designed as a proof of concept efficacy study for specific diets purported to improve clinical signs of dogs with OA. This trial failed since more than half of the dogs received less diet than the amount

defined by the investigators. This lack of compliance occurred when owners observed an increase in the body weight (BW) condition of their dogs. This was later confirmed by a significant increase in BW at study completion. In addition, the recording of peak vertical force (PVF) values discerned as the primary study outcome did not show significant changes. It was recently shown that weight losses substantially improve major clinical signs of canine OA,[15-17] referred as lameness, thus corroborating similar human clinical findings.[18,19] As weight control could improve the gait of afflicted dogs, it seems reasonable and clinically valuable to anticipate an opposite effect, meaning an exacerbation of lameness related to a change in BW, precluding to an absence of significant changes in PVF values. Using covariance analysis, our objective was to evaluate the interference of BW changes toward PVF values expressed relatively to the dog's BW. We raised the hypothesis that a BW gain interferes significantly and negatively with the gait pattern of a lame dog, as shown by a decrease in PVF.

4.4.2.3 Methods

The initial clinical trial was approved by the Institutional Animal Care and Use Committee in accordance with the guidelines of the Canadian Council on Animal Care. An informed consent was completed by each owner for each dog prior to study beginning. Both diets amount (kcal/kg) were defined according to manufacturer's recommendations.

The clinical trial began with a 30-day period during which all dogs received the first diet. After this period, dogs received a second diet for a subsequent 60 days

period. In the first two weeks of each period, dogs were fed gradually to ensure acclimation with the diet used. A unilateral cross-over design was chosen for the clinical trial, explaining the use of two specific diets. However, rationale, details on diets content and overall findings are not within the scope of the present report.

4.4.2.3.1 Cohort description

Client-owned dogs weighing more than 20 kg and older than 12 months were recruited for the study. Owners had to report a chronic and stable lameness. All dogs had radiographic evidence of OA in one or more joints (hip, stifle, elbow, shoulder or tarsus) and of which OA was the cause of lameness, as detected by force platform gait analysis and by a complete orthopedic examination performed by a veterinary surgeon certified by the American College of Veterinary Surgeons. Dogs with cranial cruciate ligament deficiency were included when surgical stabilization was done more than one year prior to the first evaluation. Dog did not have gross instability of the stifle upon orthopedic examination. Dogs were free of any other orthopedic disease or abnormality. Prior to the first evaluation, wash out periods were respected for pharmaceutical treatments, nutraceuticals, fatty acid supplement and OA therapeutic diets or treats, as done in previous clinical trials.[5-9]

4.4.2.3.2 Force platform gait analysis

Gait analysis was performed at D0, D30 and D90 as described.[6-8] Trot was maintained, at a velocity between 1.9 and 2.2 m/sec, using 3 sets of photoelectric cells. The five first valid trials for each limb were recorded and considered for the analysis. Peak vertical force normalized relatively to BW (%BW) was reported to

characterize the pathomechanic effect of OA upon dog's ambulation. At D0, dogs had an abnormally lower PVF recorded on at least one limb according to the following cut-off values: 99.1 %BW for forelimb lameness and 62.2 %BW for hind limb lameness. These cut-off values correspond mathematically to the PVF values of normal dogs acquired at the trot minus 1.5 time standard deviation.[20] In the event of bilateral lameness, the lowest PVF determined which limb was selected for evaluation.

4.4.2.3.3 Statistical analysis

The evolution of BW and PVF were evaluated using repeated measures general linear model analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison test. To evaluate the interference of BW changes on the evolution of PVF values (expressed in %BW), repeated measures general linear model analysis of covariance (ANCOVA) was performed using BW as the covariate. ANCOVA was followed by Tukey-Kramer multiple comparison test. Spearman correlation was used to test the association between the changes in BW observed following the first diet (BW at D0 minus BW at D30) toward similar changes in PVF values (%BW). Changes following the administration of the second diet were also tested. Significant level was set at 5%. Data are reported as mean±standard deviation.

4.4.2.4 Results

Twenty-six lame client-owned dogs weighing 40.0±8.7 kg and 72.4±28.7 months old were evaluated. Nineteen dogs were evaluated for hind limb lameness and the remaining 7 dogs had forelimb lameness.

Peak vertical force values were not significantly different between D0 (63.9±17.2 %BW), D30 (65.5±17.4 %BW) and D90 (66.5±20.1 %BW). However, a significant increase in BW was recorded (p<0.001). On average, dogs had higher BW at D90 (41.3±7.9 kg) when compared to D30 (39.9±8.4 kg) and D0 (40.0±8.7 kg). Between D30 and D90, 17 dogs had an increase in BW (2.7±1.4 kg) while decreasing BW was observed in the remaining 9 dogs (-1.1±0.7 kg).

Interference of changes in BW toward PVF values was further scrutinised first between D0 and D30 and then between D30 and D90. A significant correlation (p<0.001, r_s=-0.61) was only observed between D30 and D90 and presented in **Figure 13**. In this graphic, dots included in the upper left panel correspond to a decrease in PVF values when an increase in BW occurred, while the opposite is shown on bottom right panel. More specifically, from the 26 dogs, 9 had an increase in BW (3.2±1.5 kg) associated to a decrease in PVF values (-3.8±2.8 %BW). Inversely, 8 dogs had a decrease in BW (-1.2±0.5 kg) and showed an increase in PVF values (5.4 ± 4.0 %BW).

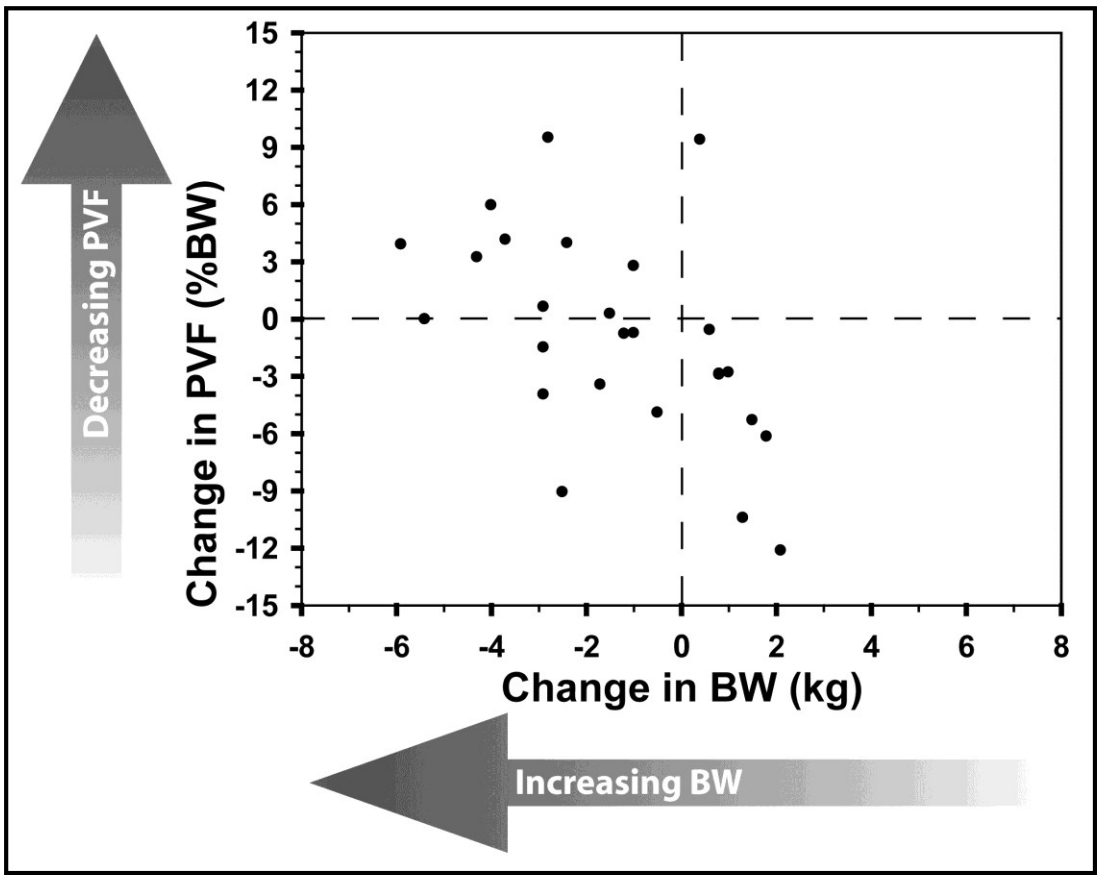

Figure 13. Scatter plot of the changes (D30 minus D90) in body weight (BW) against changes in peak vertical force (PVF) values

Zero values delineate panels using short dashed line.

Based on covariance analyses (n=26), BW changes significantly interfered with PVF values already normalised in %BW (p=0.013). When PVF was adjusted according to BW changes, values were then 63.4±17.1 %BW (D0), 65.0±17.3 %BW (D30), and 67.6±20.5 %BW (D90) and were significantly different overtime (p=0.018) meaning that adjusted PVF values were higher at D90 when compared to D0.

4.4.2.5 Discussion

We hypothesised that changes in BW have the potential to alter the gait of dogs afflicted by OA. The present study supports this hypothesis as changes in BW clearly influence the limb function of OA dogs, even when using PVF values already expressed relatively to the dog's BW. Therefore, a change in BW is identified as a confounder overtime when PVF is recorded to determine treatment efficacy.

Lameness may reflect compensatory changes in response to underlying pathologies, such as OA-associated to acquired cruciate ligament diseases or developmental joints abnormalities.[21,22] As an investigative tool exempt of subjective errors, force platform gait analysis has been used extensively to describe the functional aspect of ambulation in dogs, reaching the state of gold standard. Hence, this tool allows a thorough documentation of kinetic benefits provided by pharmaceutical, nutraceutical or physical therapies towards orthopedic condition with regards to forces generating movement. The PVF reflects the maximal point of the vertically-oriented force exerted by the dog on the ground during the stance phase of the stride. Vertical force can therefore be confounded as the weight support of a given limb during locomotion. In dogs with heterogeneous BW, and by extend different axial loading, PVF recorded in similar conditions will be theoretically constant when normalized relatively to dog's BW. This supports the need to express force platform values relatively to BW (%BW), to allow accuracy between and within subjects comparisons.

As shown in **Figure 13**, the increase in BW by 3.2 kg at D90 significantly exacerbated the lameness observed at D30. At the opposite, clinically meaningful improvements regarding PVF values were observed from relatively low (1.2kg) decreasing BW.

Hence, a relationship exists between body mass and functional aspect in dogs with OA, as represented by the level of lameness. To the authors' knowledge, this is the first report on the detrimental effects of weight gain on limb function in dogs afflicted by OA. We could propose some hypotheses to explain the negative impact of BW gain on PVF values, and oppositely the improvement in weight support when BW decreased. As OA and weight excess are often linked, a mutual amplification can be pinpointed. Indeed, pain surrounding OA may predispose to sedentarity and inactivity, leading to a decrease in muscle mass (disuse atrophy) while favouring weight gain and, by extent lead to a weight gain. A decrease in muscle mass associated with excess weight may contribute to excessive joint load. As a consequence, supraphysiologic joint stresses may then alter intrinsic physical properties and promote tissue depletion throughout pathophysiological pathways occuring in OA. However, it was demonstrated that healthy cartilage will buffer the increase in joint load while diseased cartilage will not fill its buffer role even in face of normal loading.[23] To a certain extent, abnormal motion causing a shift load to non-weight bearing regions may promote the onset and progression of the disease.[23] To avoid excessive joint load and further connective tissues damages, biomechanical strategies might be put forward to slow down disease progression.

Such phenomenon was previously reported, suggesting ability of the central nervous system to protect joint from a rapid tissue breakdown.[24] Therefore, an increase in BW leads to a decrease in relative PVF that may be explained by a protective adaptation to weight bearing. Also, considering that joint loading is a painful action when OA is present, load redistribution could therefore be beneficial to alleviate pain enhanced by excessive joint load. In contrast, an improvement in pain perception following OA analgesic therapy will increase load support, the later reaching normal values in favourable conditions. [2,25] Finally, the impact of excess weight is typically recognised as being driven by biomechanical aspects.[26] Nevertheless, adipose tissues are able to mediate inflammation and to play an undeniable role in the pathophysiology of OA, particularly with the contribution of an adipokine.[27-30]

Optimal BW maintained throughout lifetime diet restriction was shown to provide benefits by reducing abnormal mechanical stress that favours the expression of OA degenerative changes.[2,25] This clinically meaningful information highlights the importance of strict daily feeding habits, from the initial stage of development to senescence. This fact is particularly relevant considering that dietary guidelines are not scrupulously respected by all dog's owners, meaning *ad libitum* feeding in some cases. Food intake is an important environmental factor for OA development, and this supports current management approaches with nutrigenomics. In addition, a study by Impellizeri and colleagues have shown that in overweight dogs with hindlimb lameness secondary to hip OA, a reduction in weight (by 11 to 18%)

accounted for substantial improvement in clinical signs of OA.[16] This finding was also supported by another study that reports on the beneficial effects of weight management and intense physiotherapy for dogs affected with single joint OA.[17] The influence of weight gain on limb function is advocated by the data of the current study demonstrating a negative relationship between changes in BW and PVF. A similar observation was derived by the study of Messier and colleagues whom highlighted a four-fold benefit exerted toward knee-joint load for a given BW loss in obese OA human patients.[18] From a clinical perspective, such information is valuable to practitioners as they can counsel dog owners based on objective data, enhancing the quality of life of their dogs by privileging optimal BW and muscle mass, thus breaking down the vicious cycle of excessive BW and sedentarity. Maintaining a lean BW is crucial in dogs already afflicted by OA; it reinforces the importance of rigorous weight management as a core component in the multimodal management of this orthopedic pathology.

Based upon covariate analysis, present findings also suggest that normalisation of kinetic end points relatively to the dog's BW remains susceptible to an intrinsic variation in OA dogs BW. However, as it was recently reported, normalisation of force relatively to BW was recommended among other data transformations to compare human subjects having different body weight.[31] By highlighting the interference of changes in BW toward the condition of OA dogs, this study raised that a possible bias in orthopedic study outcomes may occur when gait analysis is chosen to mirror therapeutic efficacy. Aside from the statistical perspective, it

remains important to exactly denote any minor change in the loading of a painful limb. Hence according to previous OA clinical trial[5-8], the improvement in PVF usually denoted is relatively low, being of the magnitude of 3-4% when normalized to the dog's BW. However such increase may represent more than 1kg of additional weight applied on the alleviated limb. The use of covariate analysis appears interesting in a manner to enhance the power to discern statistically significant and clinically relevant changes in the gait of dogs afflicted by OA.

The present study has some limitations. 1- The study was initially designed to provide strong scientific basis supporting the use of a specific diet rather than to demonstrate the interference of BW changes on PVF evolution. Therefore the reported findings are based on secondary observations. 2- Despite data reached significant level, some dogs increased their BW without showing a decrease in PVF. The interference of BW changes on PVF values may be induced by other source of variability, supporting the need for further study. 3- Osteoarthritis is a degenerative disease and may preclude to an exacerbation of the condition over a 90 day period without being related to an increasing BW. 4- The use of client-owned dogs is heterogenic in respect to the degree of disease affliction, joint involvement, activity level and duration predisposing to variation in data recording. 5- Findings where based only on PVF values discerned at the primary study outcome. However PVF data recording was previously demonstrated with low variability and great ability to discern hind limb lameness.[20]

Therefore, the current findings demonstrated originally the major influence of BW changes (gain or loss) on joint loading. Moreover, they highlight that future clinical trials should closely monitor dogs' BW overtime, allowing emphases on subjects susceptible to alter their BW, as is the case with tested therapeutic diets. In particular cases, the use of BW as a covariate may provide assurance of accurate evaluation and exact study conclusions.

4.4.2.6 Conclusion

The present study demonstrated that a gain in BW was detrimental in dogs afflicted by OA. These findings sustained the well recognised assumption of experts in the orthopedic field about the deleterious effects of BW gain for joint integrity, joint support and its potential to exacerbate lameness associated to OA.

4.4.2.7 Reference (Article II)

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5 Modèles animaux d'arthrose expérimentale

Depuis toujours, l'animal est au côté de l'Homme à titre de compagnon, d'outil de travail et de réservoir nutritionnel. Dès l'aube du premier millénaire, Claudius Galen (130-210), un des pères de la pharmacie moderne, expérimenta la physiologie comparée et produisit des ébauches scientifiques. L'expérimentation animale fut en essor dès le XX^{e} siècle. Ainsi, plus de cinq millions d'animaux ont été voués annuellement à l'expérimentation à partir de 1945 aux États-Unis d'Amérique, atteignant un sommet inégalé avec plus de 30 millions en 1965 (Neyt *et al.*, 1998).

L'usage de modèle d'arthrose permet d'investiguer l'évolution des changements structuraux et l'implication de diverses substances pro-/anti-inflammatoires et ce, dès le stade précoce. Généralement, l'usage de modèle d'arthrose se situe en phase préclinique de développement thérapeutique afin de supporter l'hypothèse d'un effet bénéfique envers une voie pathologique clé. Les modèles d'arthrose peuvent être catégorisés selon la taille de l'espèce animale. Il est également possible de catégoriser les modèles d'arthrose selon la procédure (ou l'absence de procédure) utilisée afin d'induire les lésions dites arthrosiques.

Les sections suivantes dressent un survol des différents types de modèle d'arthrose expérimentale sans égard à l'espèce.

5.1 Modèles par injection intra-articulaire d'agents délétères

L'injection d'agents délétères au niveau de l'articulation, comme des enzymes protéolytiques (papaïne, trypsine, collagènase, hyaluronidase), aura pour effet d'altérer les composants articulaires, principalement le cartilage. La particularité de l'injection de collagènase intra-articulaire est l'atteinte au ligament qui en découle. Ceci induit de l'instabilité articulaire et, par conséquent, une sollicitation mécanique excessive (van der Kraan *et al.*, 1989). L'injection de saline hypertonique est source de dommages structuraux, d'inflammation synoviale et d'ostéophytose (Vasilev *et al.*, 1992). L'injection de mono-iodoacétate, un poison cellulaire qui agit comme inhibiteur de la glycolyse, est responsable de dommages sévères à l'articulation en induisant la mort cellulaire (Strassle *et al.*, 2010). C'est la procédure la plus communément utilisée pour tester les propriétés analgésiques face à un modèle standardisé de douleur arthrosique chez le rat. Toutefois, le modèle induit une altération temporaire et ne répond pas systématiquement à toute méthode d'évaluation (Gervais *et al.*, 2015).

5.2 Modèles par altération fonctionnelle

L'altération de la dynamique fonctionnelle d'un membre locomoteur instaure des changements caractéristiques d'arthrose et ce, généralement, *via* une sollicitation mécanique excessive. Une liste non exhaustive des modèles d'arthrose est présentée au tableau suivant (**Tableau II**). Les modèles de cette catégorie requièrent

généralement l'expertise chirurgicale, de même qu'une analgésie périopératoire conséquente.

Tableau II. Modèles d'arthrose par altération fonctionnelle d'un membre

Procédures
Sectionnement chirurgical du ligament croisé crânial/caudal (Davis *et al.*, 1973; Pond *et al.*, 1973)
Rupture du ligament croisé crânial par des ondes électromagnétiques (Lopez *et al.*, 2003)
Sectionnement chirurgical du ligament croisé crânial et gangliectomie (O'Connor *et al.*, 1999)
Méniscectomie (Moskowitz *et al.*, 1973)
Déstabilisation ménisque médial (Glasson *et al.*, 2007)
Ostéotomie (Panula *et al.*, 1997)
Lésions au cartilage (scarification) (Meachim, 1963; Marijnissen *et al.*, 2002)
Lésions osseuses (trauma) (Lahm *et al.*, 2005)
Sollicitation de l'articulation fréquente et répétée* (Radin *et al.*, 1984)
Exercice exagéré* (Videman *et al.*, 1984)
Immobilisation d'un membre* (Evans *et al.*, 1960)
Amputation (Wei *et al.*, 2001)
Myectomie (Arsever *et al.*, 1986)
Sectionnement chirurgical du ligament collatéral médial (Hulth *et al.*, 1970)
Patellectomie (Garr *et al.*, 1973)
* Ne requiert pas de chirurgie

5.2.1 Modèle canin d'arthrose par sectionnement chirurgical du ligament croisé crânial

Chez le chien, le modèle d'arthrose le plus commun est celui induit par sectionnement chirurgical du ligament croisé crânial (Pelletier *et al.*, 2010). Le sectionnement peut être effectué à l'aveugle (selon des repères anatomiques) ou chirurgicalement lors d'une approche médiale ou latérale (Visco *et al.*, 1996). Bien que ces approches mènent au même degré de lésions d'arthrose, l'hémostase doit

être favorisée (électrocautère) afin de limiter l'inflammation synoviale (Visco *et al.*, 1996). Les altérations structurelles et les changements biochimiques engendrés par ce modèle miment les dommages encourus lors d'arthrose chez l'humain (Visco *et al.*, 1996). Les dommages sont également similaires à ceux encourus lors d'une rupture traumatique du ligament croisé crânial chez le chien. L'impact fonctionnel de ce modèle se fait ressentir à long terme (4 ans) par un dysfonctionnement locomoteur reflété par la force verticale maximale (Budsberg, 2001). Il est également possible d'induire la rupture du ligament croisé crânial par l'énergie thermique produite par des ondes électromagnétiques (Lopez *et al.*, 2003).

Le chapitre Expérimentations de cet ouvrage présente le potentiel de la force verticale maximale comme témoin d'effets fonctionnels et structuraux chez le modèle canin d'arthrose par sectionnement chirurgical du ligament croisé crânial. Le caractère inférentiel de ce modèle est souligné dans le but de promouvoir le développement préclinique de modalités thérapeutiques.

5.3 Modèle par altération physiologique

L'estrogène joue un rôle important dans l'intégrité de l'articulation, assurant ainsi l'homéostasie tissulaire particulièrement au niveau de l'os sous-chondral. L'ablation chirurgicale des ovaires permet d'induire une déficience en œstrogène. La déficience en œstrogène sera par la suite responsable de lésions arthrosiques (Hoegh-Andersen *et al.*, 2004).

5.4 Modèle par altération génétique

La souris est représentée de manière prépondérante chez ce type de modèles. Des dommages arthrosiques seront observés chez une lignée de souris mâles génétiquement modifiée (STR/ort) suivant un dérèglement métabolique (Chambers *et al.*, 1992; Mason *et al.*, 2001). Cette lignée est prédisposée aux dommages structuraux au niveau du compartiment patello-fémoral et ce, *via* la sollicitation mécanique excessive qui résulte de la dégénérescence du tendon patellaire.

A l'aide d'altération génétique, l'implication d'un intervenant spécifique est possible en induisant son expression. Ainsi, des souris transgéniques qui synthétisent en abondance l'enzyme de dégradation cathepsine K, démontreront des altérations osseuses, cartilagineuses et de l'inflammation synoviale (Morko *et al.*, 2005). Dans ce même ordre d'idées, la suppression génétique de l'expression de l'enzyme de dégradation ADAMTS-5 préviendra l'apparition de dommages au niveau du cartilage (Glasson *et al.*, 2005).

6 Modèle canin d'arthrose naturelle

L'arthrose prévaut de manière importante chez le chien, atteignant 80% dans la population gériatrique (Johnston, 1997). Des arthropathies, telles que la dysplasie de la hanche et la rupture traumatique du ligament croisé crânial, sont des sources de sollicitations mécaniques excessives et responsables d'altérations structurelles et fonctionelles (Alexander, 1992; Tashman *et al.*, 2004; Pozzi *et al.*, 2013). Il est important de spécifier que seulement 20% des atteintes au ligament croisé crânial sont associées à des traumatismes. La majorité des atteintes est donc associée à des facteurs développementaux, comme la conformation de l'articulation, du membre et des muscles (Griffon, 2010).

6.1 La force verticale maximale comme témoin d'effets thérapeutiques en contexte d'arthrose naturelle chez le chien

L'usage des forces de réaction au sol, particulièrement la force verticale maximale, a permis de discerner le potentiel bénéfique de plusieurs modalités thérapeutiques, comme en témoignent de récentes révisions systématiques de la littérature (Aragon *et al.*, 2007; Sanderson *et al.*, 2009; Vandeweerd *et al.*, 2012). Les sections suivantes (**6.1.1, 6.1.2 et 6.1.3 ci-dessous - Chapitre 1**) présentent des essais cliniques contrôlés qui utilisent l'analyse cinétique de la démarche en contexte d'arthrose naturelle chez le chien. Ces articles démontrent que la force verticale maximale est un témoin d'effets fonctionnels en contexte d'arthrose naturelle. Ils

n'ont pas été placés dans la section Expérimentations de ce travail car ils ne cherchaient pas à démontrer la validité de la méthode de mesure, mais simplement à en réaliser une utilisation pratique dans différentes conditions d'évaluation thérapeutique. Une attention particulière sera attribuée sur le **Tableau VI** inclus dans l'Article IV **(6.1.2 ci-dessous - Chapitre 1)** car il synthétisait à cette époque toutes les publications dans la littérature vétérinaire démontrant un effet thérapeutique sur la base d'une bonification de la force verticale maximale de chiens arthrosiques, que ce soit pour des traitements à base d'anti-inflammatoires non stéroïdiens (AINS), de médecine alternative et complémentaire, ou encore de diètes thérapeutiques vétérinaires.

Le chapitre Expérimentations de cet ouvrage présente le potentiel de la force verticale maximale comme témoin d'effets fonctionnels chez le modèle canin d'arthrose naturelle. Le caractère inférentiel de ce modèle est souligné dans le but de promouvoir le développement thérapeutique.

6.1.1 Article III: Effects of feeding a high omega-3 fatty acids diet in dogs with naturally occurring osteoarthritis

Cet article, publié dans *Journal of Animal Physiology and Animal Nutrition*, présente un essai clinique contrôlé qui utilise la force verticale maximale comme critère primaire d'efficacité thérapeutique. Le but de cet article était de supporter les bienfaits que procure l'administration d'une diète riche en acides gras oméga-3 envers le dysfonctionnement locomoteur du chien atteint d'arthrose naturelle.

Cet essai clinique de phase IV étoffe la littérature existante au sujet du potentiel thérapeutique d'une diète riche en acides gras oméga-3. Il procure au clinicien des évidences cliniques probantes qui sont utiles à la pratique d'une médecine vétérinaire basée sur des faits.

M. Maxim Moreau a participé au désign expérimental, à l'acquisition des données, à l'analyse de ces dernières et a rédigé cet article présentement publié (*Journal of Animal Physiology and Animal Nutrition* 97:830–837, 2013). M. Moreau a également effectué l'ensemble des travaux d'infographie. L'article a par la suite été dûment révisé et bonifié par l'expertise de chacun des coauteurs.

Journal of Animal Physiology and Animal Nutrition
97:830–837, 2013

Effects of Feeding a High Omega-3 Fatty Acids Diet in Dogs With Naturally Occurring Osteoarthritis

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6.1.1.1 Summary

The aim of this randomized, placebo-controlled and double-blinded trial was to compare the effect of a veterinary therapeutic diet (VTD) rich in omega-3 fatty acids (omega-3) from fish origin to a regular diet used as control (CTR) over a period of 13 weeks in dogs afflicted by naturally-occurring osteoarthritis (OA).

Thirty privately-owned dogs were selected. Dogs had lameness confirmed by an orthopaedic examination, had stifle/hip OA and had locomotor disability based on the peak of the vertically-oriented ground reaction force (PVF) measured using a force platform. At Baseline, all owners were asked to determine 2-5 activities of daily living that were the most impaired. Activities were scores (0-4) in accordance with severity using case-specific outcome measures (CSOM). The PVF was also measured. Dogs (15/group) were then randomly assigned to receive either the CTR or the VTD. The CSOM was completed twice weekly. The recording of PVF was repeated at Week 7 and 13.

The VTD fed dogs showed a significantly higher PVF at Week 7 ($p<0.001$) and at Week 13 ($p<0.001$) when compared to Baseline. From Baseline to Week 13, VTD fed dogs had a mean (±SD) change in PVF recording of 3.5 ±6.8 % of body weight (%BW) compared to 0.5 ±6.1 %BW ($p=0.211$) in CTR fed dogs. This change in primary outcome was consistent with an effect size of 0.5. Conversely, dogs fed the CTR did not show significant change in PVF measurements. At the end of the study, the CSOM was significantly decreased ($p=0.047$) only in VTD fed dogs.

In lame OA dogs, a VTD, which contains high level of omega-3 from fish origin, improved the locomotor disability and the performance in activities of daily living. Such nutritional approach appears interesting for the management of OA.

6.1.1.2 Introduction

Canine osteoarthritis (OA) is one of the most common orthopaedic disorders in dogs (Johnston 1997). Systematic reviews of the therapeutic management of canine OA have been recently published, addressing the innovative use of Veterinary therapeutic diet (VTD) in afflicted dogs (Aragon et al. 2007; Sanderson et al. 2009). Such nutritional approaches are appealing to dog owners, as they provide substances claimed to possess beneficial properties beyond supplying the daily nutrient needs. Among these supplemented substances are long chain polyunsaturated omega-3 fatty acids (omega-3), which are highly concentrated in flaxseed, fish and their derivatives (Folador et al. 2006; Bauer 2007; Biondo et al. 2008). Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are among the omega-3 recognized to provide benefits against an array of disorders and conditions in canines (Miller 1992; Brown et al. 2000; Bauer 2007; Biondo et al. 2008; Hansen et al. 2008; Laurent et al. 2008; LeBlanc et al. 2008; Kirby et al. 2009).

For veterinarians having to manage dogs afflicted by OA, optimal decision-making may be compromised by the absence of randomized, controlled and double-blinded clinical trials for every specific VTD available. As it was the case for several

cyclooxygenase-2 specific inhibitors (Aragon et al. 2007; Sanderson et al. 2009), evidence-based approaches to veterinary medicine based on level III or IV clinical trials are required to determine the ameliorative effects of VTD. Afflicted patients will then benefit from standard of care.

The aim of this randomized, controlled and double-blinded clinical trial was to determine whether a VTD, which contains high level of omega-3 (1.08% of eicosapentaenoic and docosahexaenoic acid) from fish origin (rainbow trout; *Onchorynchus mykiss*), improves the functional disability of lame privately-owned dogs afflicted by OA. We hypothesized that the continuous intake of a VTD rich in omega-3 should improve the limb function to a greater extent than would a control diet devoid of the active nutrient (omega-3). A quantitative measure of functional outcome as provided by the recording of the peak of the vertical ground reaction force (PVF) and a case-specific outcome measures (CSOM) (Gingerich and Strobel 2003; Lascelles et al. 2008), were used to further validate the use of this VTD as an effective therapeutic approach.

6.1.1.3 Materials and methods

6.1.1.3.1 Experimental design

A randomized, controlled and double-blinded clinical trial was undertaken to evaluate the efficacy of a VTD rich in omega-3. This trial was approved by the Institutional Animal Care and Use Committee in accordance with the guidelines of the Canadian Council on Animal Care. All owners gave informed consent for their

participation in the trial. They were also requested to avoid any intense activities (jumping, running, throwing ball or Frisbee, etc) potentially deleterious for their OA dogs during the study duration.

Sample size was estimated according to previous work done in similar conditions (Moreau et al. 2007). Thirty privately-owned dogs diagnosed with OA were randomly allocated to 2 groups of 15 dogs each. A restricted randomisation process (random allocation rule) was used to ensure equal group sizes at the end of the trial. Treatment sequence was determined using computer generated random number. Therapeutic group, hereafter-coded VTD (JM Joint mobility; Nestlé Purina PetCare Co) was a commercially available therapeutic diet formulated for dogs with OA.

The control group, hereafter-coded CTR (experimental formulation; Nestlé Purina PetCare Co) was an experimentally diet formulated to be similar in nutritional content to the VTD, at the exception of the omega-3 content. The CTR was considered as a regular diet. The feeding guideline for both diets was based upon the following equation: Maintenance energy requirement =100 x (body weight (BW) [Kg])$^{0.75}$. During the study, the daily amount of diet was divided into two meals. Incomplete feed uptake was documented. An adaptation phase of 3 days to gradually acclimate dogs to the diets was considered, beginning at Day 1. During this adaptation phase, the amount of diet was 20%, 40% and 80% in the first 3 days, and then 100% until study completion. Owners were asked to respect good nutritional habit, avoiding any other food than the tested-diets. The VTD diet

contained 1.08% of eicosapentaenoic and docosahexaenoic acid as active nutrients (**Tableau III**). Dogs were weighted at Baseline, Week 7, and Week 13.

Tableau III. Summary of the diet's content based on dry matter basis

Ingredients	**VTD***	**CTR†**
Protein (%)	33.9	34.4
Carbohydrate (%)	41.5	41.6
Fat (%)	15.1	15.1
Total long chain polyunsaturated omega-6 fatty acids (omega-6) (%)	1.9	2.4
Total long chain polyunsaturated omega-3 fatty acids (omega-3) (%)	1.5	0.2
Omega-6 /omega-3 ratio	1.3	13.6
Eicosapentaenoic and docosahexaenoic acid (%)	1.1	0.1
Glucosamine (%)	0.1	0.1

*VTD Veterinary therapeutic diet †CTR Control diet

6.1.1.3.2 Animal selection

Adult (>12 months) dogs weighing >20 kg having radiographic evidence of OA (see computed radiographs section) at the hip or stifle joints were eligible to the clinical trial. Clinical signs of osteoarthritis, including pain or restricted motion upon orthopaedic examination of the hip or stifle were confirmed by a certified veterinary surgeon (Lussier). To be eligible, dogs had to have a decrease in the normal mobility and function of a limb (*i.e.* lameness) that was visually observed at the level of the hind limb. The elbows as well as others joints had to be normal upon orthopaedic examinations.

Exclusion criteria were as follows: dogs undergoing surgery for a cranial cruciate ligament rupture (CCLR) within 1 year prior to study initiation, dogs receiving a natural health product (including omega-3) supplement or a VTD for OA management 6 weeks prior to study initiation and dogs receiving NSAID 4 weeks prior to study initiation. Pregnant bitches, dogs having received polysulphated glycosaminoglycans or corticosteroids at any time before study initiation and those suffering from neurologic or other musculoskeletal lesions were excluded. Dogs that underwent orthopaedic surgery within the past year and dogs with CCLR having gross instability (positive drawer motion) were not eligible.

6.1.1.3.3 Peak vertical force measurement

Measurements were performed at Baseline, Week 7, and Week 13 at the trot (speed 1.9-2.2 m/s, acceleration ±0.5m/s^2) using a force platform, as previously described (Moreau et al. 2010). The PVF was reported and defined as the primary outcome of interest. Normalized PVF as percentage of body weight (%BW) from the first five valid trials were used for statistical purposes. To be eligible, dogs must have PVF value less that 66 %BW, which is consistent to minus 1 SD of the value measured in normal dogs (Madore et al. 2007). When bilateral lameness was observed, the hind limb having the lowest PVF, in accordance with the orthopaedic exam findings, determined which one was selected for evaluation; otherwise the dog was not eligible.

6.1.1.3.4 Case-specific outcome measures

At Baseline, all owners were asked to determine between 2-5 activities of daily living that were the most impaired (Gingerich and Strobel 2003; Lascelles et al. 2008) by the disease. Owner determined each activity according to his own perception of what characterises the disability of his dog. Activities were then scored on a five-point scale of severity (0 to 4) as follows: No problem (0), minor disability (1), moderate disability (2), severe disability (3) and complete incapacity (4). A score of 0 cannot be attributed at Baseline. Each activity was assessed twice weekly using a specific form that was kept at home by the owner. For each dog, the median of the scores was determined for all evaluations (total of 27 evaluations).

6.1.1.3.5 Computed radiographs

For all dogs, computed radiographs (hips, stifles, and elbows) were systematically obtained under sedation as described (Moreau et al. 2010). All radiographs were reviewed by a certified veterinary surgeon (Lussier). Presence of radiographic evidences of OA (osteophytes, enthesiophyte and sclerosis) exclusively observed at the level of the stifle or hip was a prerequisite for inclusion. Dogs with elbow abnormalities graded as borderline (*i.e.* between normal and grade 1 elbow dysplasia according to the International elbow working group standardized screening procedure) were eligible. Eligible dogs were free of forelimb muscular atrophy, pain or lameness during the orthopaedic examination.

6.1.1.3.6 Statistical analyses

Per trial PVF (log-transformed) data were analyzed with a repeated-measures general linear mixed model that evaluated the fixed effects of Time (temporal evolution), Group (diets) and the Time×Group interaction, with trials (PVF) and dogs nested in treatment group as random effects. A second linear mixed model was conducted on PVF data (log-transformed) for the difference between Week 13 and Baseline. The mean of the five trials was used and tested for Group as a fixed effect. Body weight was analyzed similarly to the first linear model used for PVF, excluding Week 7 data. When necessary, the best covariance structure for fixed and random effects was chosen (Littell et al. 2000) and included in the model. A repeated-measures generalized linear model was used to analyze CSOM data (median of the score given to each selected activities) under Poisson distribution function to evaluate the fixed effects of Time (temporal evolution), Group (diets) and the Time×Group interaction. Scale factor was estimated by Pearson's chi-square. All *post hoc* analyses were done with appropriate Bonferroni adjustments. For CSOM, *post hoc* analyses were done to compare both groups at the 1^{st}, 13^{th} and 27^{th} assessments. Also, in each groups, assessments (from the 2^{nd} to the 27^{th}) were exclusively compared to Baseline (1^{st}). Significant level was set at $p<0.05$. Data are presented as mean ±SD. The last-observation-carried-forward approach was applied in the event of missing data. Statistical analyses and graphics were done using SPSS Statistics software, version 17.0 (SPSS Inc, Chicago, IL USA) and SigmaPlot, version 10.0 (Systat software Inc, Chicago, IL, USA) respectively.

6.1.1.4 Results

6.1.1.4.1 Animal description

Characteristics of the study cohort are presented (**Tableau IV**). Lameness was confirmed by recording abnormally low PVF using a force platform. Owners reported reluctance to perform daily life activity as recorded during CSOM. Daily feed uptake was reduced when a gain in BW >1 kg occurred. Feed uptake was reduced in two CTR fed dogs (by 20 and 12.5%, respectively) and in 3 VTD fed dogs (by 33, 12.5 and 12.5%, respectively). Significant difference was not observed within and among groups for the level of BW (Group effect; p=0.166, Time effect; p=0.079, Time×Group interaction; p=0.805).

Tableau IV. Baseline characteristics of the dogs

Groups	Age (year)	Male dog/total dog	BW (kg)
CTR[†]	6.6 (3.3)	12/15	41.0 (8.2)
VTD*	6.4 (2.7)	8/15	36.7 (12.3)

*VTD Veterinary therapeutic diet [†]CTR Control diet

Data are presented as mean (SD). 15 dogs per group.

6.1.1.4.2 Missing data

A patient lost to follow-up occurred in the CTR group because the owner lost his dog during the study. A patient lost to follow-up occurred in the VTD group when an owner decided to withdraw his dog from the trial for personal reasons unrelated

to the study. Also in this group, a CSOM form was lost by the owner, meaning that only the initial data were accessible.

6.1.1.4.3 Primary study outcome: Peak vertical force

In dogs, the PVF represents the force generated by the painful limb during the stance phase of the stride. The dispersion of data for the recording of the PVF for each group at each time session is illustrated (**Figure 14**). The PVF was increased over time (Time effect; $p<0.001$) without a significant Group effect ($p=0.147$). Groups evolved differently over time (Time×Group interaction; $p=0.006$). According to *post hoc* analyses, PVF at Week 7 (58.4 ±8.0 %BW, $p<0.001$) and at Week 13 (59.5 ±8.5 %BW, $p<0.001$) was higher than Baseline (56.0 ±7.3 %BW) in VTD fed dogs. From Week 7 to Week 13, PVF did not increase significantly ($p=0.457$). In control dogs, PVF at Week 7 (54.0 ±9.6 %BW) and at Week 13 (53.8 ±10.5 %BW) were not significantly increased compared to Baseline (53.3 ±8.3 %BW, $p=0.999$). There was no significant difference between CTR and VTD groups at Baseline, Week 7 and at Week 13. **Figure 15** presents the individual change in PVF recorded at Week 13. From Baseline to Week 13, VTD fed dogs had a mean change of 3.5 ±6.8 %BW. This change was not significantly different compared to the one of CTR fed dogs (0.5 ±6.1 %BW, $p=0.211$).

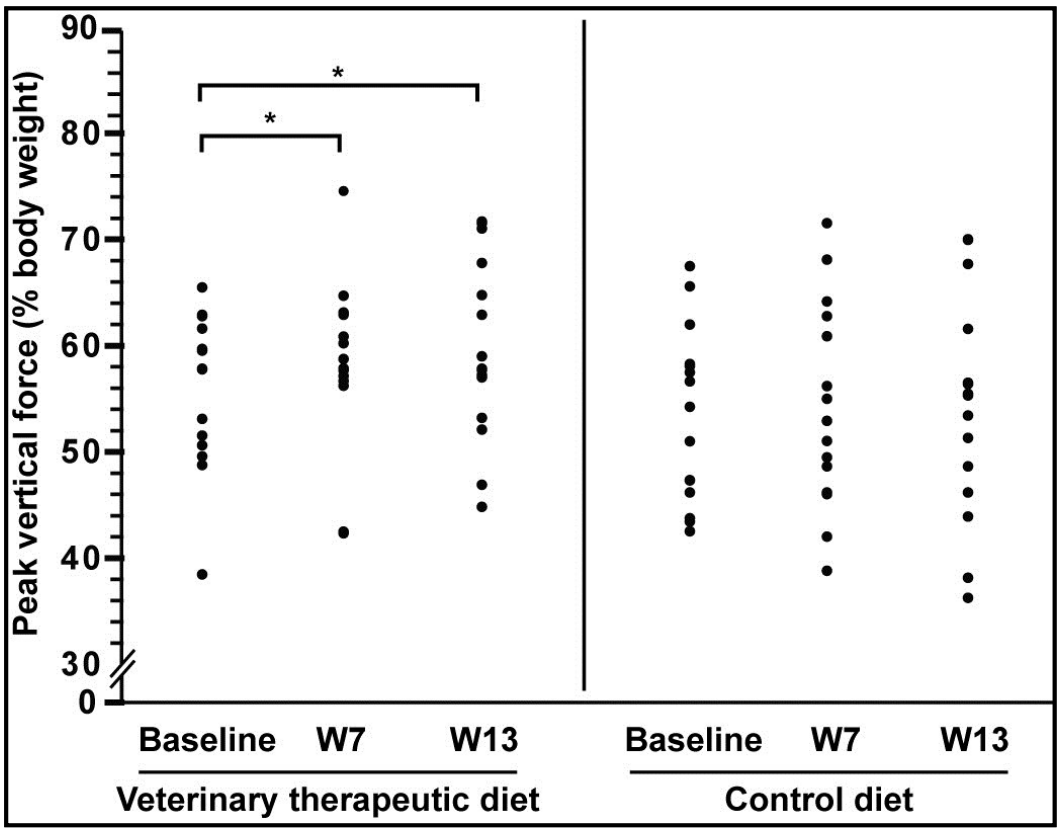

Figure 14. Individual dot plot of peak vertical force recorded in privately-owned dogs with naturally occurring osteoarthritis after 7 and 13 weeks of feeding either Control diet or a Veterinary therapeutic diet rich in omega-3

*Dots are PVF recorded in each dog (15 dogs per group, per time). *Significantly different compared to Baseline $p<0.05$*

6.1.1.4.4 Case-specific outcome measures

The CSOM recorded the owners' assessment of the severity of daily life impairment in their dogs based upon preselected activities reported to be problematic and painful. For CSOM, a lower value means better activity performance.

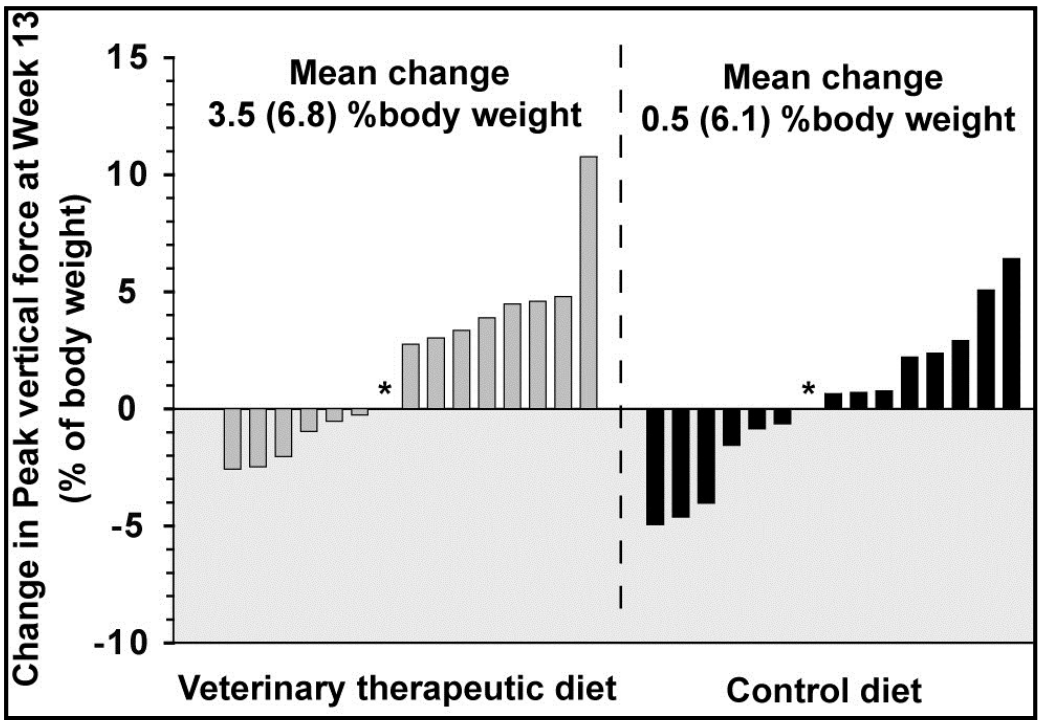

Figure 15. Individual changes in peak vertical force recorded in privately-owned dogs with naturally occurring osteoarthritis after 13 weeks of feeding either Control diet or a Veterinary therapeutic diet rich in omega-3

*Mean (SD) changes were the difference between Week 13 versus Baseline. *Incomplete data were managed using last data carried forward method. Grey zone represent negative change (i.e., deterioration).*

The evolution of the CSOM is illustrated (**Figure 16**). There was a decrease in CSOM over time (Time effect; $p<0.001$), without a significant Group effect ($p=0.245$). Groups evolved differently over time (Time×Group; $p<0.001$). Hence, *post hoc* analyses did not reveal a significant decrease in CSOM for CTR fed dogs when compared to Baseline. Conversely, significant decreases in CSOM were observed for VTD fed dogs at the 25th ($p=0.047$), 26th ($p=0.047$) and 27th ($p=0.047$)

assessments (**Figure 16**). There was no significant difference between CTR and VTD groups for the selected assessments.

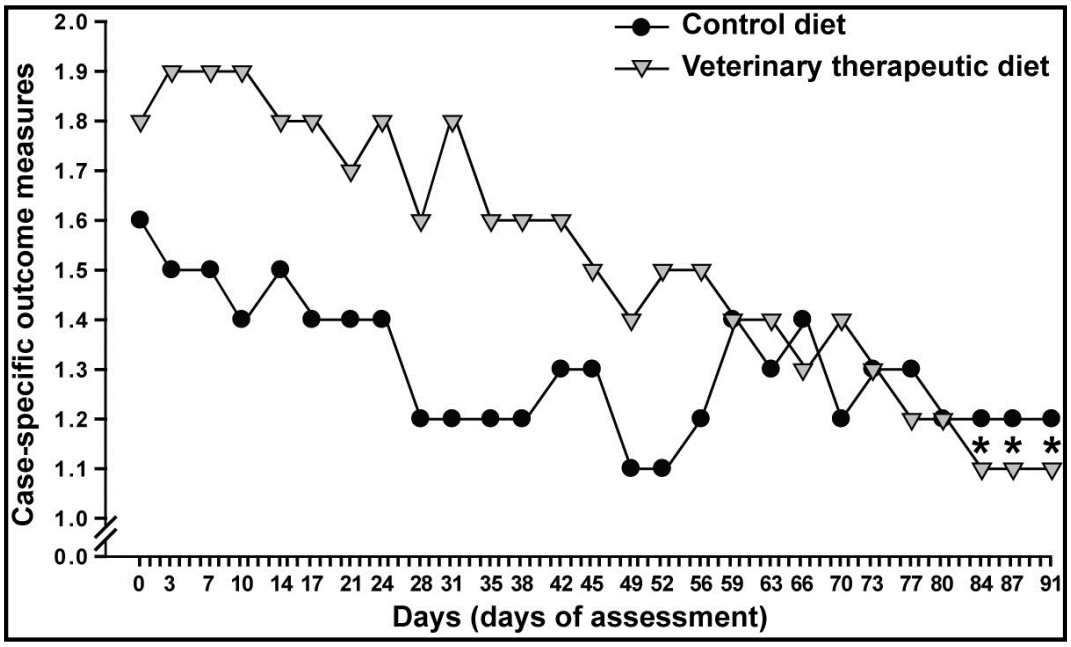

Figure 16. Temporal evolution of the CSOM recorded in privately-owned dogs with naturally occurring osteoarthritis after 7 and 13 weeks (W) of feeding either Control diet or a Veterinary therapeutic diet rich in omega-3

*Assessments were performed twice weekly. Each data point represents the mean (15 dogs per group, per assessment). *Significantly different compared to Baseline $p<0.05$.*

6.1.1.5 Discussion

This clinical trial evaluated the functional outcomes of lame privately-owned dogs afflicted by OA following a 13 weeks of feeding with a VTD containing high levels of omega-3. According to the primary study outcome (PVF) OA dogs were significantly improved ($p<0.001$) 7 weeks after the beginning of a dietary modulation. The improvement in the functional disability was maintained through the 13 weeks duration, achieving a mean improvement of 3.5 ±6.8 %BW when compared to initial limb support ($p<0.001$). When expressed relatively to Baseline (pre-treatment) values, the improvement corresponded to 6.9 ±12.2 %. The ameliorative effect of VTD was in accordance with previous trials done by our group (Moreau et al. 2003; Moreau et al. 2004; Moreau et al. 2007) and others (Budsberg et al. 1999) using NSAID and a powder of elk velvet antler. Moreover, the level of improvement represented an increment of 1.4 kg applied on the afflicted and painful limb for a dog of 36.7kg. The effect size of the VTD improvement was 0.5 that was consistent with a moderate therapeutic effect (Cohen 1992).

The primary study outcome was identified as PVF, which reflects the maximal limb support recorded during the stance phase of the stride. Recording of the PVF has been shown to express low coefficient of variation in OA dogs (Madore et al. 2007). Among all data recorded by a force platform, *a priori* determination of a primary outcome ensures good practice of clinical trial reporting (Budsberg 1997). Hence to avoid a high rate of type I error because of multiple tests and to avoid

searching for significant data, it was established to focus only on PVF instead of analyzing all force platform data.

Based on the subjective assessment, an improvement was also observed in dogs fed a diet rich in omega-3 following over a 13-week period ($p=0.047$). Hence, owners of VTD fed dogs recognized that their dog had better performance in activities of daily living determined to be impaired by the painful disease. The determination of abnormal behaviour potentially linked to pain is believed to represent a key point in the management of OA (Hellyer et al. 2007). In this way, the VTD was able to improve the quality of life by reducing the daily disability.

The limb impairment was improved in dogs fed the VTD according to the primary study outcome ($p<0.001$). However, our hypothesis was not supported: the magnitude of the change in the VTD group (3.5 ±6.8 %BW) did not statistically exceed the one of the CTR group (0.5 ±6.1 %BW). In addition, as illustrated in **Figure 15**, 8/15 (53 %) CTR fed dogs had an increase in PVF at Week 13. Therefore, the present study cannot convincingly claim that VTD improved the limb function to a greater extent than a regular diet used as negative (placebo) control. We believed that the positive change observed in several CTR fed dogs and consequently the large data variation has altered the statistical power necessary to support our hypothesis. According to sample size estimate, it appears that 73 dogs per group are necessary to achieve 80 % power to detect a significant difference between 3.5 ±6.8 %BW *versus* 0.5 ±6.1 %BW.

In the present study, the use of a regular diet given under strict feeding guidelines provided a favourable response in some CTR fed dogs. These findings were consistent with the better limb function observed in a large proportion of dogs (38 %) (Roush et al. 2010) as well as to the reduction of carprofen dosage in dogs fed a regular diet (Fritsch et al. 2010). The natural fluctuation in disease severity (maturation effect) upon force platform measurement is presently not fully documented in placebo-treated dogs. In a manner to optimize the design of clinical trial, it is necessary to better document the evolution of OA dogs as to whether or not their functional outcome remain stable over time.

Exacerbation of lameness related to a gain in BW was previously demonstrated using force platform to record peak vertical force, while benefits occurred when BW was decreased (Moreau et al. 2010). In the present study, the BW of the dogs in their respective group was not modified when both diets were administered according to strict and balanced feeding guidelines. Since OA dogs are mostly sedentary, it is crucial that maintenance energy requirement be adjusted accordingly.

The present study has been conducted based on the following limitations: 1) Data were limited to dogs with hind limb OA and cannot necessarily be extrapolated to those being afflicted at other sites. 2) The study duration is limited to 13 weeks, while OA is a chronic persistent condition. 3) The study cohort was selected based on force platform measurement that may be unrepresentative of the clinical portrait

of OA in dogs. 4) The present study examined the effect of a VTD as a monotherapy even though canine OA treatment is multimodal. 5) Intense activities were reported to be deleterious in dogs afflicted by OA (Beraud et al. 2010). Avoiding intense activity may therefore have precluded to a bias in the study outcome. In addition, whether or not an increment in PVF similar to what observed in VTD fed dogs (3.5 ±6.8 %BW) is clinically relevant or simply a statistical artefact remains to be challenged. Hence, despite the fact that force platforms are widely used in the orthopaedic field, guidelines to ensure adequate data management, interpretation and translation to clinical setting are still lacking.

Based on clinical evidences from objective functional outcome and continuous owner's assessments, the use of a VTD which contains high level of omega-3 from fish origin, could be considered as a component in the multimodal management of canine OA.

6.1.1.6 Acknowledgement

Authors are grateful to Mrs Katherine Bernier and Anne-Andrée Mignault for their outstanding technical assistances. This study was supported by a grant (B. Lussier) from Nestlé Purina PetCare Company, St. Louis, MO, USA, an ongoing New Opportunities Fund grant (E. Troncy) from the Canada Foundation for Innovation (#9483) for the pain / function equipment, and a Discovery grant (E. Troncy) from the Natural Sciences and Engineering Research Council of Canada (#327158-2008) as operating fund.

6.1.1.7 References list (Article III)

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6.1.2 Article IV. *Brachystemma Calycinum* D. Don effectively reduces the locomotor disability in dogs with naturally occurring osteoarthritis: A randomized placebo-controlled trial

Cet article, publié dans *Evidence-Based Complementary and Alternative Medicine*, présente un essai clinique contrôlé qui utilise la force verticale maximale comme critère primaire d'efficacité thérapeutique. Le but de cet article était de démontrer les bienfaits que procure l'administration d'une plante médicinale envers le dysfonctionnement locomoteur du chien atteint d'arthrose naturelle.

Cet essai clinique s'intègre dans un effort de promouvoir la mesure de la force verticale maximale chez le modèle canin d'arthrose naturelle lors du développement d'agents anti-arthrosiques pour l'Homme. Indirectement, cet essai procure des évidences cliniques probantes qui sont utiles à la pratique d'une médecine vétérinaire basée sur des faits.

M. Maxim Moreau a participé au design expérimental, à l'acquisition des données, à l'analyse de ces dernières et a rédigé cet article présentement publié (*Evidence-Based Complementary and Alternative Medicine*, Volume 2012, Article ID 646191). M. Moreau a également effectué l'ensemble des travaux d'infographie. L'article a par la suite été dûment révisé et bonifié par l'expertise de chacun des coauteurs.

Evidence-Based Complementary and Alternative Medicine
Volume 2012, Article ID 646191

Brachystemma calycinum D. Don Effectively Reduces the Locomotor Disability in Dogs with Naturally Occurring Osteoarthritis: A Randomized Placebo-Controlled Trial

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6.1.2.1 Abstract

Objective The aim of this randomized placebo-controlled trial was to evaluate the beneficial effect of a whole plant extract of *Brachystemma calycinum* D don (BCD) in naturally-occurring osteoarthritis (OA) in dogs.

Methods Dogs had stifle/hip OA and poor limb loading based on the peak of the vertically-oriented ground reaction force (PVF) measured using a force platform. At Baseline, PVF and case-specific outcome measure of disability (CSOM) were recorded. Dogs (16 per group) were then assigned to receive BCD (200 mg/kg/day) or a placebo. The PVF was measured at Week (W)3 and W6. Locomotor activity was recorded throughout the study duration using collar-mounted accelerometer, and CSOM was assessed bi-weekly by the owner.

Results BCD-treated dogs had higher PVF at W3 and W6 when compared to Baseline ($p<0.001$) and at W6 when compared to placebo-treated dogs ($p=0.040$). Higher daily duration ($p=0.024$) and intensity ($p=0.012$) of locomotor activity were observed in BCD-treated dogs compared to Baseline. No significant change was observed in either group for CSOM.

Conclusions Treatment with BCD improved the limb impairment and enhanced the locomotor activity in dogs afflicted by naturally-occurring OA. Those preclinical findings provide interesting and new information about the potential of BCD as an OA therapeutic.

6.1.2.2 Introduction

A group of experts recently emphasized the uses of companion animal suffering from naturally occurring diseases to accelerate the development of human therapeutics [1]. Hence to fulfill preclinical data, undertaking a trial in companion animals may represent an interesting way to provide additional evidence on the therapeutic potential of a new drug in development.

Naturally occurring osteoarthritis (OA) is common in companion animals, particularly in dogs. One study estimated that 20 per cent of dogs over one year of age are afflicted by the condition [2]. Traumatic insults to the cranial cruciate ligament (CCL) and hip dysplasia are among the arthropathies considered to be etiopathogenic of OA in this specie [3, 4]. Biological and biomechanical factors merge to induce and perpetuate OA, generating pain, lameness, and limb dysfunction [5, 6].

As for human, the therapeutics modalities to manage canine OA remains largely palliative, from which non-steroidal anti-inflammatory drugs (NSAIDs) take place as the first line of treatment [7]. Only the alleviation of pain-related clinical sign(s) is claimed by actual modalities; structural benefits cannot be expected from current evidences and still represent a clinical challenge [8]. Therefore, there is an unmet need for OA therapeutics that combines disease modifying properties and the capacity to improve the locomotor disability.

Surgical transection of the CCL in dogs impairs the limb function and creates lesions that mimic those encountered in human [9, 10]. In this model, the extract of *Brachystemma calycinum* D don (BCD), an indigenous plant of southwestern China (the Himalayas) has shown promising disease modifying potential against the development of OA lesions and limb impairment [11]. It was reported that the decrease in the levels of protease activated receptor 2 (PAR-2), inducible nitric oxide synthase (iNOS) and matrix metalloprotease 13 (MMP-13) were the key factors tributary of the effect of BCD. However, whether BCD is effective against the locomotor impairment that prevails in dogs naturally afflicted with OA could be informative on its curative potential and need to be scrutinized.

With the idea of providing preclinical data to enhance the development of human therapeutics, the objective of this trial was to determine whether BCD can improve the locomotor disability seen in naturally-occurring OA in dogs.

6.1.2.3 Materials and methods

6.1.2.3.1 Design and subject selection

This study was a randomized, double-blind, parallel-group, placebo-controlled trial lasting 6 weeks. The trial was conducted under the approbation of the Institutional Animal Care and Use Committee (#Rech 1434) in accordance with the guidelines of the Canadian Council on Animal Care. All owners provided written informed consent.

Adult dogs weighed more than 20 kg and had radiographic evidence of OA exclusively at the hip or stifle joints. Radiographs (hips, stifles, and elbows) were obtained under sedation as described [12]. Hind limb lameness in association with the presence of OA was confirmed by a certified veterinary surgeon (Lussier). At the time of screening, all dogs were free of any compound purported to relief the clinical signs of OA according to washout periods ranging between 4 to 12 weeks. Hence, a 4-week wash out period was respected for oral NSAIDs and a 6-week period for natural health products including fatty acid supplement, OA therapeutic diets or treats. Dog having received injectable pentosan polysulfate sodium or corticosteroid one year before the screening visit was not eligible. A 12-week period was requested for injectable polysulfated glycosaminoglycan and hyaluronan, and for oral or topical corticosteroid. During the study, dogs were free of any type of medication except those prescribed for exo- and endoparasite control. Additional exclusion criteria were as follows: dogs with surgical repair of the CCL within 1 year prior to study initiation, dogs suffering from neurologic or other musculoskeletal lesions, dogs that underwent orthopaedic surgery within the past year and dogs with CCL disease having gross instability (positive drawer motion upon orthopaedic exam).

6.1.2.3.2 Complete blood count and biochemistry panel

Each dog underwent routine blood hematology and biochemistry analyses (Bédard) in order to evaluate health status at study entrance and to ensure that physiologic disturbance did not occur following treatment administration (W6).

6.1.2.3.3 Randomization, blinding and therapy regimen

Thirty-two privately-owned dogs were randomized. The restricted randomisation process was defined as a random permuted blocks randomisation, which included a block size of four, with two treatments (A and B) distributed in one-to-one ratio. In blocks of four, there are six possible block allocation sequences: 1)AABB; 2)ABAB; 3)ABBA; 4)BBAA; 5)BABA and 6)BAAB. The treatment allocation sequence was defined using a list of true random integers from 10 to 99 (www.random.org). The block allocation sequence was defined using the first eight single digit of the true random numbers list, omitting numbers outside the range 1 to 6. Among the eight designated blocks of four, a true random integer from 1 to 8 served to define which block was excluded from the balanced attribution of locomotor activity recording. In each of the seven remaining blocks, a true random integer from 1 to 2 served to allocate motor activity recording to treatment A (*i.e.* when a 1 was generated, motor activity recording was allocated to the first treatment A for a given block). The same procedure was repeated to allocate locomotor activity recording to treatment B, leading to the randomized attribution of seven dogs in each treatment group for monitoring locomotor activity. The 32 treatment allocations (with or without locomotor activity recording) were transcript on individual cards in sequentially numbered, sealed, opaque envelopes to ensure concealment. The person responsible of the randomization process (Gauvin) and the treatment preparation was not involved in the enrolment and follow-up. The test agent or the placebo were blinded to Treatment A or B by a third party (Troncy). At trial site, both treatments were labelled exclusively as Treatment A or Treatment B

and were encapsulated identically. The trialists (Lussier, Moreau), the veterinary technicians and all dog owners were blinded to which treatment (A or B) was given to each randomized subject. The key code revealing what referred to treatment A and B (BCD or placebo) was kept confidential by the third party and was revealed only after study completion and preliminary analyses.

The test agent (BCD extract) was obtained as previously described [11]. The test agent or the placebo (corn starch) was given at a dosage of 200 mg/kg/day (minimum 197 mg/kg/day, maximum 240 mg/kg/day) using a combination of capsules (Torpac Inc., NJ, USA) that contains between 1 to 5 g/capsule. Initially, some dogs (n=14) received a 5-day placebo treatment to establish Baseline values, particularly of locomotor activity (see 2.5 below). Treatments were given in the morning, before or after meal. Treatments were encapsulated in the same fashion. The dose of BCD was the one used previously in experimental CCL-sectioned dogs, and was based on the dose administered in humans (120 mg/kg) according to the following formula: human dose × [$k_{m\ human}$ / $k_{m\ animal}$] where k_m is the surface area to weight ratio.

6.1.2.3.4 Force platform measurement

Peak of the vertically-oriented ground reaction force (PVF) was measured at Baseline (Day 0), W3 (Day 21), and W6 (Day 42) at the trot gait (1.9-2.2 m/s) using a force platform, as previously described [12]. The PVF was reported and defined as the primary outcome of interest. Normalized PVF in percentage of body

weight (%BW) from the first five valid trials were used for statistical purposes. To be eligible, dogs must have hind limb PVF value less that 66 %BW which is consistent to minus one SD of the value measured in normal dogs [13]. When bilateral lameness was observed, the hind limb having the lowest PVF, in accordance with orthopaedic exam findings, determined which one was selected for evaluation, otherwise the dog was excluded. The change in PVF was the mean difference between a given week value *versus* Baseline.

6.1.2.3.5 Locomotor activity recording

Accelerometer-based motor activity recording was done using Actical® system (Bio-Lynx Scientific Equipment Inc., Canada) as described [14]. According to the balanced attribution of motor activity recording, collar-mounted accelerometers were worn by seven dogs per group for the entire treatment duration (42 days, 24 hour/day). In addition, collar-mounted accelerometers were worn during a short period (5 days) that preceded real treatment initiation. This period was used to establish Baseline level of locomotor activity recording before treatment administration. During this period, the entire 14 dogs were attributed to receive a placebo treatment managed by the person responsible of the randomization process (Gauvin). The duration and intensity of motion were continuously monitored and expressed as counts every 2 minutes, giving 720 counts per day. Daily duration of active period (DDAP) referred to the time spent (expressed in hour) when the count exceeded 30 in term of intensity. This cut off value was based on intern data and was used to discern active from inactive period [14, 15]. Daily averaged total

intensity (DATI) referred to the mean of all counts per day (unitless). Among the 47 days of continuous recording, three periods of 120 hours were predefined: Baseline (Day -5 to Day 0), first period (Day 17 to Day 21) and the second period (Day 38 to Day 42). Owners of dogs were requested to come at Day -5 and back at Day 0 to the investigation site to acquire other Baseline data.

6.1.2.3.6 Case-specific outcome measure of disability

Assessment of at-home functional disability was done using CSOM as previously described [14]. Owners assessed the ability of their dogs to perform between two to five activities using a 5-points scale for each activity that ranged from no problem (0) to incapacity (4). Each activity was selected by the owner according to his/her own perception of what characterise(s) the disability of the dog. Assessments were done twice weekly using a specific form that was kept at home by the owner. For each dog, medians of the activity scores were determined at each assessment (13 assessments) and were then used for statistical purposes. Among the 13 assessments, three periods were predefined: Baseline (1st assessment done at Day 0), first period (Day 3 to Day 21) and the second period (Day 24 to Day 42).

6.1.2.3.7 Statistical analysis

All statistical tests were two-tailed with significance determined by reference to the 5% threshold. Equality of efficacy was the null hypothesis based on the primary endpoint (PVF). Per trial log-transformed PVF data were analyzed with a repeated-measures general linear mixed model that includes two fixed factors (Time and Group) and their interaction (Time×Group interaction), with trials and dogs nested

in treatment group as random effects. The compound symmetry covariance structure was used for this analysis. *Post hoc* analyses were done with appropriate Bonferroni adjustments. Log-transformed DATI and DDAP were analyzed similarly to PVF (Time [Period] and Group as fixed factors) and their interaction (Time×Group interaction) with days and dogs nested in treatment group as random effects. A repeated-measures generalized linear model was used to analyze median CSOM data under Poisson distribution function using independent working matrix. Fixed factors were Time (Period) and Group and their interaction (Time×Group interaction) with assessments and dogs nested in treatment group as random effects. Scale factor was estimated by Pearson's chi-square. The last recording was carried forward in the event of missing data. Data are presented as mean (standard deviation).

6.1.2.3.8 Sample size calculation

According to previous works done in similar conditions [15], a sample size of 16 dogs/treatment group ensured that a difference of 4.2 %BW in the primary endpoint (PVF) between BCD and control (placebo) dogs could be detected assuming 75% power, a SD of 4.5 and a 5% significance threshold.

6.1.2.4 Results

6.1.2.4.1 Animal description

No clinically relevant changes were observed on physical examination, observation, haematological and biochemical analyses in the entire study cohort. Baseline characteristics of the dogs stratified per group are presented in **Table 4**. Groups

were well balanced according to the outcomes of interest as significant difference was not observed for the levels of PVF (p=0.452), locomotor activity recording when expressed as DDAP (p=0.751) and DATI (p=0.869), and also for CSOM (p=0.194).

Tableau V. Baseline characteristics of the dogs stratified per group

Characteristics	**Groups**	
	Placebo	***Brachystemma calycinum* D don**
Age (year)	5.9 (1.9)	5.6 (2.5)
Sex (male/female)	6/10	8/8
Body weight (kg)	42.5 (7.5)	40.3 (11.4)
Peak vertical force (%BW)	56.3 (6.4)	58.2 (6.7)
Locomotor activity recording (over 5-day)		
Daily duration of active period (h)	6.7 (1.8)	6.5 (1.5)
Daily averaged total intensity (unitless)	191 (66)	194 (79)
Case-specific outcome measure of disability	1.9 (0.6)	1.6 (0.5)
Osteoarthritis-afflicted joint		
Hip (count)	10	11
Stifle (count)	13	12
Hip and Stifle (count)	7	7

6.1.2.4.2 Study withdrawal

The numbers of dogs that were screened, randomly assigned, and analysed in each group are detailed in **Figure 17**. Last data (PVF and CSOM) were carried forward when incomplete data set were encountered.

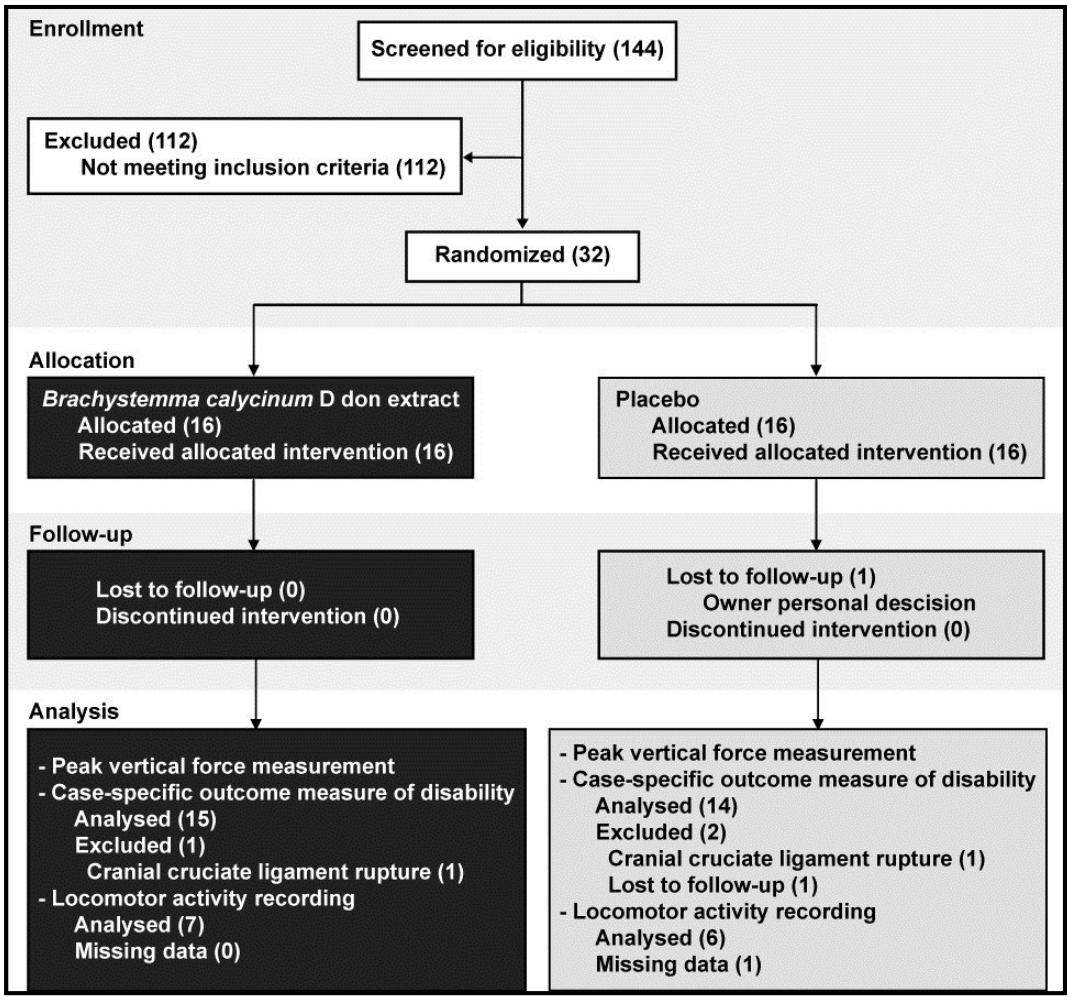

Figure 17. Flow chart of the study enrolment, randomization, follow-up and analysis

6.1.2.4.3 Peak vertical force measurement

The PVF (primary endpoint) generated by the disabled hind limb during the stance phase of the stride was increased in the overall study cohort (Time effect; $p<0.001$), without significant Group effect ($p=0.129$). Increment in PVF was mostly attributed to the changes observed in BCD-treated dogs. Hence, a significant Time×Group interaction ($p<0.001$) was observed which means that groups evolved distinctively from Baseline to the end of the study. More specifically, analyses revealed that the PVF of the BCD-treated dogs was significantly increased at W3 ($p=0.001$) and at W6 ($p<0.001$), when compared to Baseline (**Figure 18**). At the opposite, neither W3 nor W6 value was significantly different than baseline in placebo-treated dogs. Analyses revealed that the change in PVF in BCD-treated dogs showed a tendency to be higher than placebo at W3 ($p=0.099$), reaching significant level at W6 ($p=0.040$). **Figure 19** presents the respective individual changes in PVF recorded at W6 as well as the mean change denoted in each group after 6 weeks of treatment.

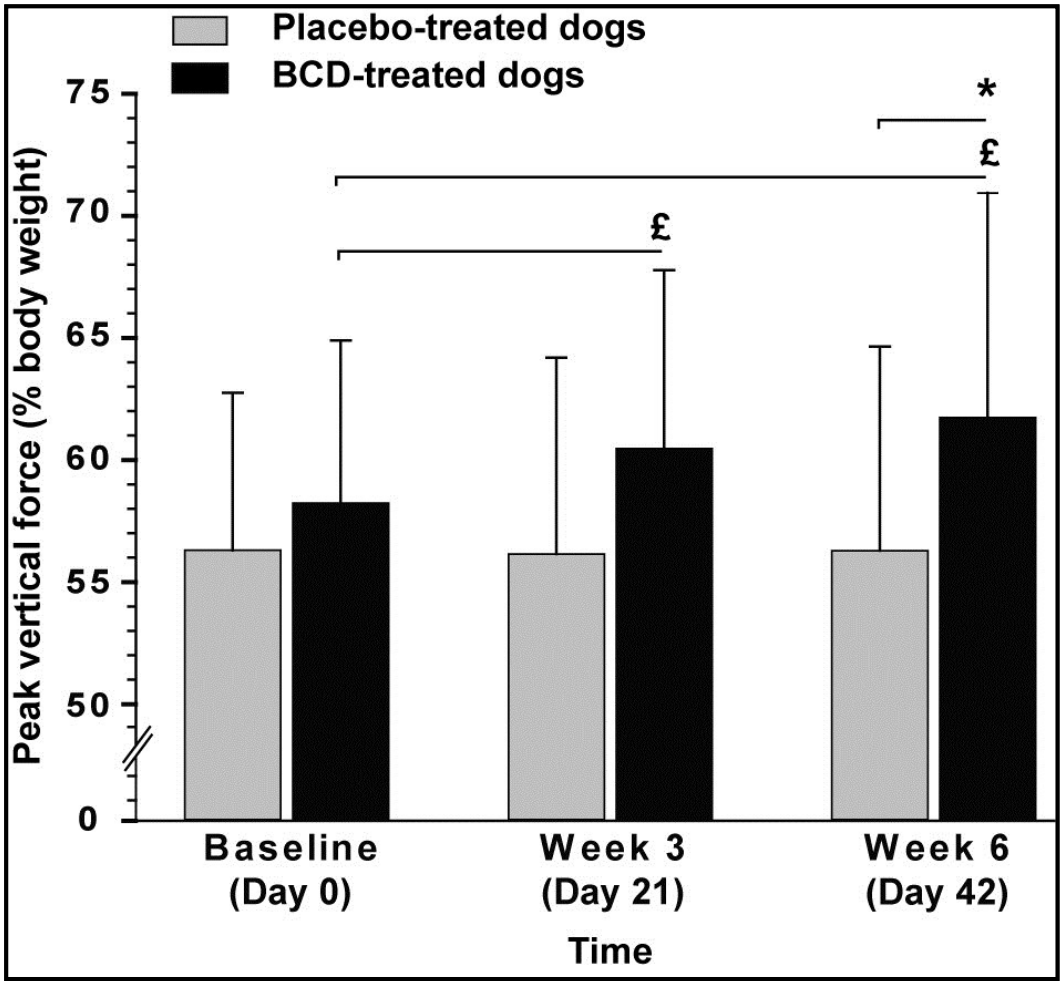

Figure 18. Mean (standard deviation) peak vertical force recorded in dogs having received either *Brachystemma calycinum* D don (BCD) or a placebo

*Values are expressed as percentage of body weight. *Significantly different compared to placebo-treated dogs. £Significantly different compared to Baseline.*

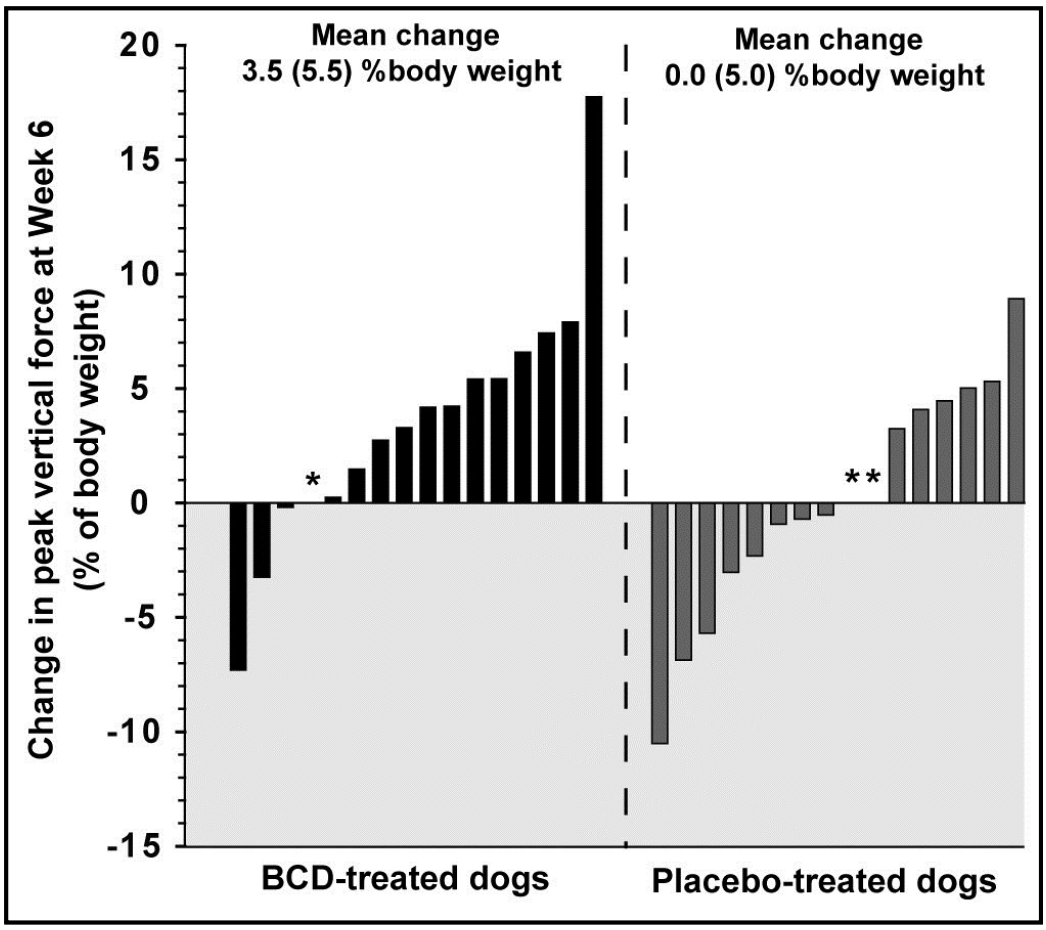

Figure 19. Individual changes in peak vertical force after 6 weeks of treatment with *Brachystemma calycinum* D don (BCD) or a placebo

*Changes were the difference between Week 6 versus Baseline. *Incomplete data were managed using last data carried forward method. Grey zone represent negative change (i.e., worsening).*

6.1.2.4.4 Sensitivity analyses

Sensitivity analyses were done using alternative forms of imputation to confirm the robustness of the results analysed with the last-observation-carried-forward method. Data management conducted with the exclusion of dog having incomplete data set provided an increase in PVF of 3.7 (5.6) %BW at W6 (*post hoc* comparison between groups at W6; p=0.045). When positive data (+3.5% BW) were used to replace missing data, results were consistent with an increase in PVF of 3.7 (5.5) % BW at W6 (*post hoc* comparison between groups at W6; p=0.040). When negative results (-3.5% BW) were used to replace missing data, results supported an increase in PVF of 3.3 (5.7) % BW at W6 (*post hoc* comparison between groups at W6; p=0.043).

6.1.2.4.5 Locomotor activity recording

The accelerometer recorded the motion of the dogs over the entire daily duration. The continuous recording was successful in 7 BCD- and 6 placebo-treated dogs. For DDAP, statistical findings were as follows: Time effect (p=0.032), Group effect (p=0.575) and Time×Group interaction (p<0.001). Analyses revealed a tendency for higher DDAP in BCD-treated dogs during the first period (7.3 (1.7) h, p=0.068), reaching significant level at the second period (7.4 (1.3) h, p=0.024) when compared to Baseline (**Table 4**, **Figure 20**). Placebo-treated dogs had DDAP values at the first [6.8 (1.4) h] and the second period [6.2 (1.7) h] that did not differ from Baseline (**Table 4**, **Figure 20**).

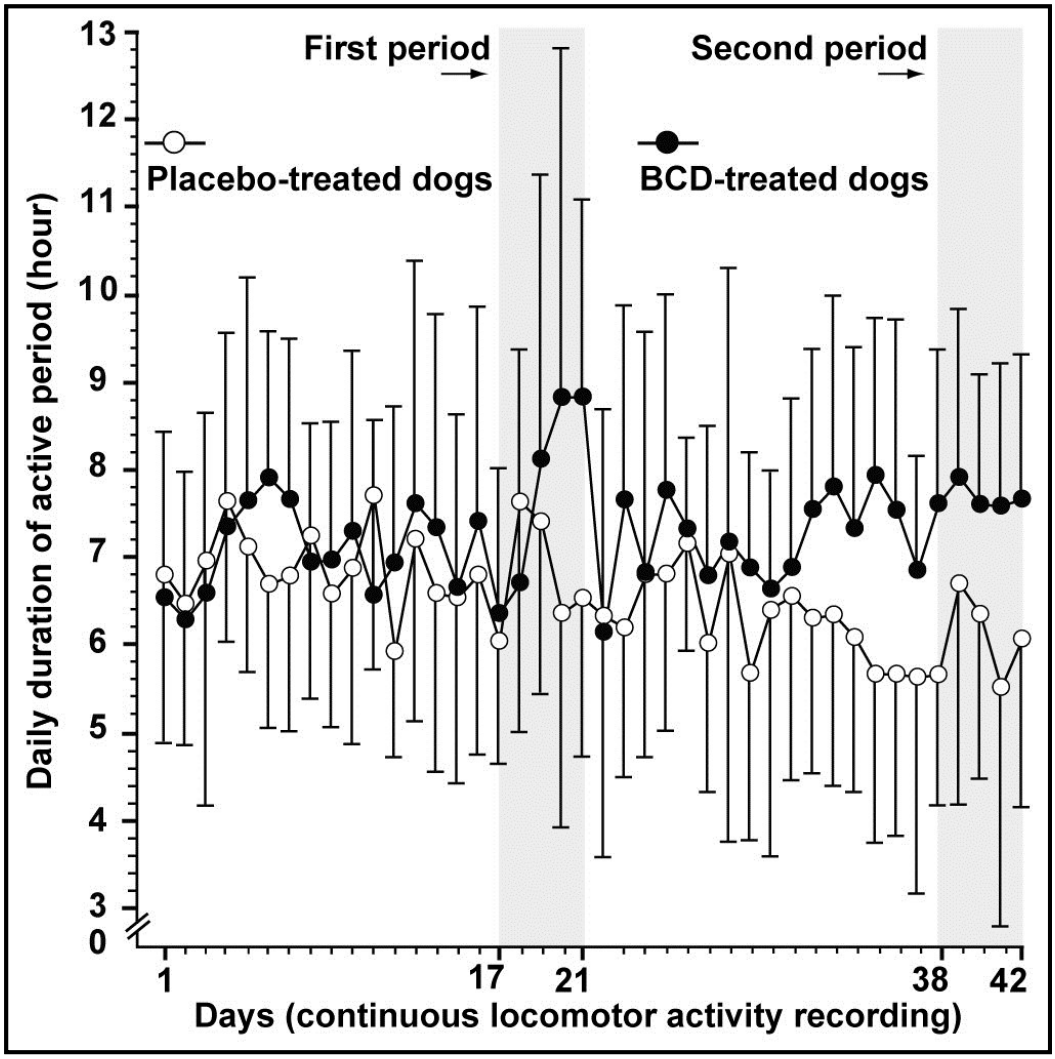

Figure 20. Temporal evolution of the locomotor activity recording over a 6-week period (42 days) in dogs receiving either treatment with *Brachystemma calycinum* D don (BCD) or a placebo

Data are the daily duration of active period and are expressed as mean (standard deviation). Periods were Baseline (Day -5 to Day 0, not shown), first period (Day 17 to Day 21) and the second period (Day 38 to Day 42). At the second period, BCD-treated dogs had significantly higher daily duration of active period (p=0.024) when compared to Baseline.

According to DATI, statistical findings were as follows: Period effect (p=0.103), Group effect (p=0.722) and Period×Group interaction (p=0.006). In BCD-treated dogs, analyses revealed significantly higher DATI during the first period (233 (98), p=0.042) and the second period (229 (85), p=0.012) when compared to Baseline (see **Tableau V**). Placebo-treated dogs had DATI values at the first [199 (66)] and the second period [185 (75)] that did not differ from Baseline (see **Tableau V**). Neither DDAP nor DATI denoted significant difference between groups at Baseline, as well as during the first and second period.

6.1.2.4.6 Case-specific outcome measure

The CSOM assessed the severity of daily life disability in accordance with specific activities reported to be problematic, altered and/or painful. Statistical findings were as follows: Time effect (p=0.003), Group effect (p=0.149) and Time×Group interaction (p=0.732). **Figure 21** presents the evolution of the CSOM recorded twice weekly over a 6-week period. Over time, no significant change in either group was observed. Placebo-treated dogs had mean CSOM of 1.6 (1.0) and 1.5 (0.9) at the first and second period, respectively. Mean values for BCD-treated dogs were 1.3 (0.8) and 1.1 (0.8) at the first and second period, respectively.

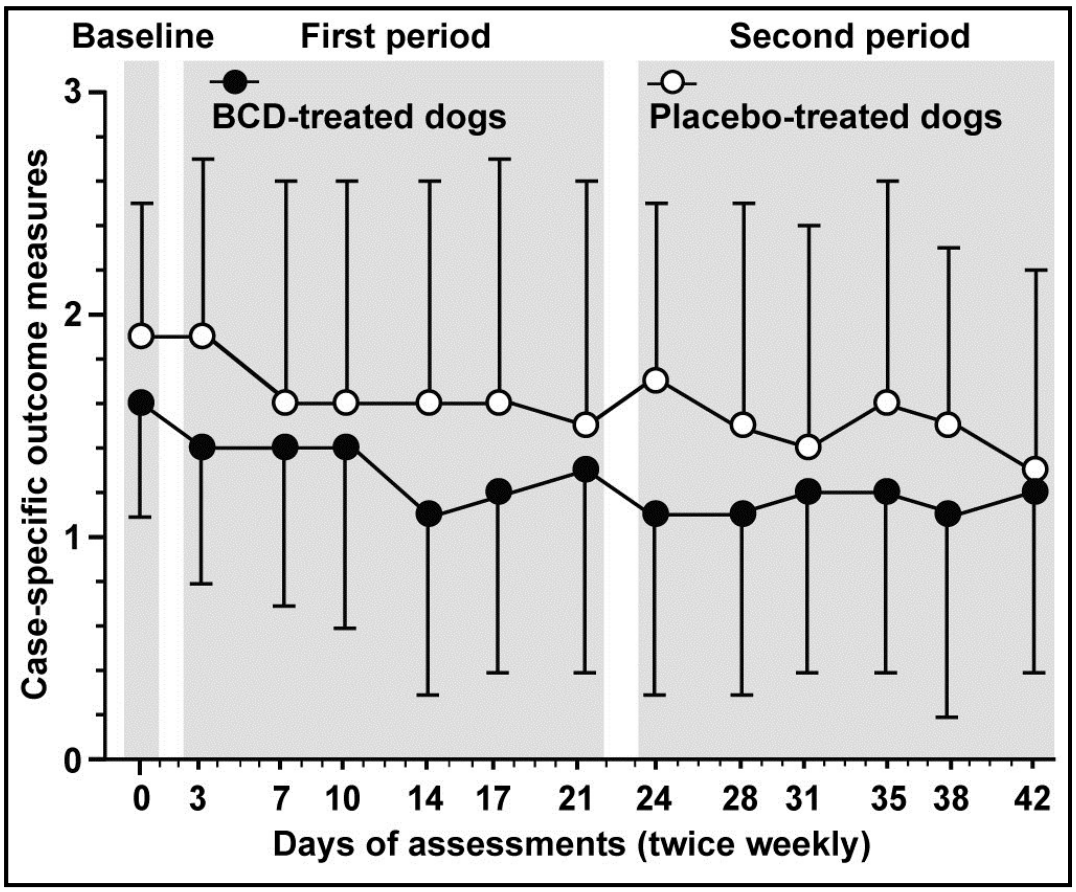

Figure 21. Temporal evolution of the case-specific outcome measures of disability (CSOM) over a 6-week period in dogs receiving either treatment with *Brachystemma calycinum* D don (BCD) or a placebo

Data are expressed as mean (standard deviation). Periods were Baseline (Day 0), first period (Day 3 to Day 21) and the second period (Day 24 to Day 42).

6.1.2.5 Discussion

In the experimental dog CCL model of OA, it was previously demonstrated that BCD treatment helps to reduce cartilage loss and improved functional disability [11]. According to those findings, hypothesis was then raised about the therapeutic potential of BCD in dogs naturally afflicted by the OA disease under curative

conditions. Therefore, a randomized, double-blind, placebo-controlled trial was undertaken with the idea to fulfill pre-clinical evidences and to promote clinical trial in human OA. Based upon the measurement of the PVF defined as the primary endpoint, BCD improved the limb disuse in dogs afflicted by hind limb OA. When given once daily, improvements were seen as early as 3 weeks and reached further gain by a 6-week period. The magnitude of the therapeutic benefits was moderate in accordance with an effect size of 0.7 (95% confidence interval, 0.0-1.4).

Force platform is a recording instrument that measures the forces (such as vertical force) generated by the musculoskeletal system in close relationship with acceleration and mass of the body. Such platform has been considered as objective measure of gait disability in OA patient [16-19]. In dogs with OA, pain-related limb disuse is discriminated by abnormally low PVF. An improvement is translated when an increment over initial condition occur, as denoted following current therapies (**Tableau VI**). In respect with those clinical findings, the change in PVF provided by BCD was within the expected level of improvement provided by non-steroidal anti-inflammatory drugs [20-22], COX-LOX inhibitor [15], complementary and alternative medicine [21, 23] and veterinary therapeutic diets [24-26]. The improvement demonstrated herein was translated into a willingness to load an average of ±1.4 kg on the painful afflicted limb.

Tableau VI. Selected studies that reported statistically significant changes (i.e. improvement) in peak vertical force following different therapeutic approaches in dogs afflicted by osteoarthritis.

Therapeutic approaches	Authors	Changes in peak vertical force (%BW)	Trial duration (sample size)
Non-steroidal anti-inflammatory drugs			
Etodolac	Budsberg et al.[20]	2.3 (0.4)	8 days (34)
Carprofen	Moreau et al.[22]	2.4 [-3.4 to 17.0]	60 days (16)
Meloxicam	Moreau et al.[22]	4.7 [-4.9 to 92.2]	60 days (16)
Licofelone	Moreau et al.[15]	2.9 ± 1.7	28 days (13)
Carprofen	Hielm-Björkman et al.[21]	3.2 [-8.2 to 11.8]	56 days (15)
Complementary and alternative medicine			
Elk velvet antler	Moreau et al.[23]	2.4 ± 0.7	60 days (25)
Herbal preparation	Troncy Eric (unpublished data)	2.6 (2.1)	56 days (13)
Homeopathic preparation	Hielm-Björkman et al.[21]	2.3 [-3.4 to 10.2]	56 days (14)
Veterinary therapeutic diets			
Omega-3 fatty acids	Roush et al.[26]	3.9 ± 1.3	90 days (22)
Green lipped mussel	Bichot et al.[24]	2.5 (4.2)	60 days (23)
Omega-3 fatty acids	Moreau et al.[25]	3.5 (6.8)	90 days (14)

%BW stands for percentage of body weight. Mean (standard deviation). Median [minimum to maximum]. Mean ± standard error of the mean

The outcome measurements of the present study were in agreement with the pain, physical function, and patient global assessment included in the OMERACT-OARSI responder criteria [27]. There are actually no such criteria for OA clinical trials in dogs. Development of such an approach would be most useful to monitor the beneficial effects in a randomized clinical trial such as ours. In the absence of such consensus, an increment in PVF was instinctively considered as a positive response. According to **Figure 19**, 66% of the overall responders were BCD-treated dogs. At the opposite, 73% of dogs having worsened their condition probably due to natural fluctuations in disease severity (maturation effect) were placebo-treated.

The monitoring of ambulatory activities using accelerometers is a reliable technique, providing continuous, unsupervised, objective, monitoring of mobility [28]. In the field of OA, it is well known that afflicted patients suffer limitations in their walking ability as monitored using accelerometer [29-31]. In dogs, this device was deemed adequate for at-home activity monitoring [32] while being a valid tool to document the therapeutic outcome of an OA management [33].

In the present trial, we denoted that BCD-treated dogs had higher locomotor activity (intensity and duration) at the end of the treatment duration. While placebo dogs had similar intensity and duration of active period, BCD-treated dogs reached higher levels, gaining an hour of activity per day. Aerobic and strengthening exercises are beneficial in reducing pain caused by OA [34-36]. Therefore, the effect of BCD could have been translated into more active dog that rehabilitated the

painful and disused limb toward better muscular strength, allowing animal to load more weight on the afflicted limb. Such association was previously demonstrated in OA dogs by our group [37]. Hence, higher levels of daily motion have been mirrored by an improvement in limb loading, which supports the benefits of physical rehabilitation [38].

The way both groups evolved according to the objective measures of function was not replicated by the assessment of daily life activity performance. Rather, the study cohort demonstrated an overall decrease in CSOM, without specific changes in the condition of BCD- and placebo-treated dogs. Longer treatment administration may preclude to a full monitoring of treatment efficacy for such level of PVF improvement, as previously denoted following 3 months of feeding a therapeutic diet in OA dogs [39]. Of note, CSOM was defined as complementary to PVF measurement, providing insights on different clinical aspects of the OA disease [14].

Whether BCD-treated dogs were improved through the preservation of joint structure, the relief of pain or *via* a combination of these main aspects of the OA disease cannot be answered with regards to the present trial. However, from the previous study CCL model of OA [11], it was shown that action on key inflammatory mediators, such as iNOS and PAR-2, was tributary of the therapeutic potential of BCD. Under BCD treatment, lower levels of these mediators were

encountered consistently with a protection against cartilaginous changes and better limb remission in CCL-deficient dogs.

Evidences indicate that PAR-2 participates in the development of experimental OA [40, 41]. Furthermore, PAR-2 activation has been shown to sensitize peripheral nociceptive receptors such as vanilloid, ATP-gated ion channels, and glutamate types [42-45]. Such sensitization contributes to the mechanical hypersensitivity and pain-related dysfunction that is pathognomonic of OA [46, 47]. When integrated, those findings support the putative therapeutic target of PAR-2 to limit the functional disability as well as the structural changes of OA.

A number of important limitations need to be considered; 1) The short duration of the study (6 weeks) for a chronic disease such as OA; 2) The absence of pharmacokinetic data on this plant extract may have precluded to suboptimal dosage; 3) The imputation method for missing data was the last-observation-carried-forward method. This approach preserved the sample size, assuming that the response remains constant at the last observed and that missing data were at random. Underestimation or overestimation of the treatment effect may have occurred. Noteworthy, data managed using different imputation methods provided similar results, supporting the robustness of the primary endpoint results.

6.1.2.6 Conclusion

This study provided clinical evidences of the beneficial effect of BCD extract through its improvement to the locomotor disability associated with naturally-occuring OA in dogs. Using objective measure of spontaneous mechanical allodynia and discomfort, the daily administration of 200 mg/kg/day of BCD extract was efficient, enough to improve the limb disuse and to enhance the locomotor activity. These preclinical findings hopefully may eventually prove to have relevance for the treatment of OA in man.

6.1.2.7 Acknowledgments

Authors would like to acknowledge Katherine Bernier and Anne-André Mignault for their technical support. This study was funded in part by a grant from Vita Green Pharmaceutical (HK) Limited, ArthroLab Inc., an ongoing New Opportunities Fund grant (Eric Troncy) from the Canada Foundation for Innovation (#9483) for the pain / function equipment, and by the Osteoarthritis Chair of the University of Montreal Hospital Centre, Université de Montréal. Vita Green Pharmaceutical (HK) Limited participated in the decision to submit the manuscript for publication but was not involved in the study design, acquisition, analysis and interpretation of data, or the writing of the manuscript. Maxim Moreau received a Doctoral Scholarship from the Canadian Institutes of Health Research – Strategic Training Program (MENTOR).

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6.1.3 Article V. A medicinal herb-based natural health product improves the condition of a canine natural osteoarthritis model: A randomized placebo-controlled trial

Cet article, publié dans *Research in Veterinary Science*, présente un essai clinique contrôlé qui utilise la force verticale maximale comme critère primaire d'efficacité thérapeutique. Le but de cet article était de démontrer les bienfaits que procure l'administration d'un produit de santé naturel, à base de plantes médicinales, envers le dysfonctionnement locomoteur du chien atteint d'arthrose naturelle.

Cet essai clinique s'intègre dans un effort de promouvoir la mesure de la force verticale maximale chez le modèle canin d'arthrose naturelle lors du développement d'agents anti-arthrosiques pour l'Homme. Indirectement, cet essai procure des évidences cliniques probantes qui sont utiles à la pratique d'une médecine vétérinaire basée sur des faits.

M. Maxim Moreau a participé au design expérimental, à l'acquisition des données, à l'analyse de ces dernières et a rédigé cet article présentement publié (*Research in Veterinary Science*, 97:574-81, 2014). M. Moreau a également effectué l'ensemble des travaux d'infographie. L'article a par la suite été dûment révisé et bonifié par l'expertise de chacun des coauteurs.

Research in Veterinary Science
97:574-81, 2014

A Medicinal Herb-based Natural Health Product Improves the Condition of a Canine Natural Osteoarthritis Model: A Randomized Placebo-controlled Trial

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6.1.3.1 Abstract

The present trial evaluated a herb-based natural health product (NHP) in the canine natural osteoarthritis model. At Baseline, the peak of the vertically-oriented ground reaction force (PVF, primary endpoint) and case-specific outcome measure of disability (CSOM) were recorded in privately-owned dogs. Dogs (16/group) were randomized to receive NHP formulations or a negative control (placebo) over eight weeks (W). The PVF was measured at W4 and W8. Daily locomotor activity was recorded using collar-mounted accelerometer. The CSOMs were assessed bi-weekly by the owner. The NHP-treated dogs (n=13) had higher PVF at W4 (p=0.020) and W8 (p<0.001) when compared to Baseline. The changes (W8 *minus* Baseline) were higher than control dogs (n=14, p<0.027) and consistent with an effect size of 0.7 (95% confidence interval: 0.0 to 1.5). The NHP-treated dogs had higher locomotor activity at W8 (p=0.025) compared to Baseline. No significant change was observed for the CSOM. Administration of NHP improved the clinical signs of osteoarthritis in this model.

6.1.3.2 Introduction

Osteoarthritis (OA) is by far the most common human musculoskeletal disease, affecting million of peoples worldwide (Lawrence et al., 2008). The prevalence of OA in dogs is also high, particularly in geriatric animals being estimated to be five times that observed in mature adults (Shearer, 2011). In dogs, OA results mainly from traumatic insults to the cranial cruciate ligament (CCL), and hip or elbow dysplasia (McLaughlin, 2001; Roush, 2001). Cascades of biological and biomechanical events then merge to induce and perpetuate structural changes at the level of the entire joint, which, as in human beings, lead to crippling pain, disability and poor quality of life (Madsen and Svalastoga, 1994; Martinez, 1997; Martinez and Coronado, 1997; Johnston, 2001; Cook, 2010).

Naturally-occurring models of OA have been proposed to accelerate the development of human therapeutics (Pelletier et al., 2010), and a recent review of experimental data underlined the high translationability of outcomes obtained from canine OA models, in particular for the response to treatment (Moreau et al., 2013). Undertaking a trial in privately-owned dogs afflicted by natural OA may therefore represent an interesting way to obtain preclinical data, providing additional evidence on the therapeutic potential of new compounds under development. Of note, the potential of several therapeutic approaches has been tested in different randomized controlled trials (RCTs) in the canine natural OA model using force platform gait analysis as an outcome measure of pain-related functional impairment. These tested compounds include non steroidal anti-inflammatory drugs

(NSAIDS) (Budsberg et al., 1999; Moreau et al., 2003; Moreau et al., 2007), therapeutic diets (Roush et al., 2010; Moreau et al., 2012b; Rialland et al., 2013) as well as natural substances (named too naturaceuticals) used to restore or maintain good health status (Moreau et al., 2004; Hielm-Bjorkman et al., 2009; Moreau et al., 2012a). The later therapeutic class is considered by the authors as natural health products (NHPS) which origin from plants, fruits and vegetables, animals, microorganisms and marine sources.

Currently, no effective therapy seems able to alleviate the clinical signs of OA in human or dogs. As relief of pain and the preservation of joint structure cannot be claimed with certainty for currently approved treatments, there is an unmet need for effective strategies to improve the condition of afflicted patients.

Medicinal herbs have long been used in traditional medicine and there is considerable evidence that such NHP and their derivatives may play beneficial roles in OA (Mobasheri, 2012). *Harpagophytum procumbens*, also known as devil's claw is a South African plant which includes harpagoside as one of its major biologically active phytochemical compounds. A large body of evidence supports the efficacy of harpagoside and related extracts in alleviating symptoms of OA in humans (Gagnier et al., 2004). Resin extracts from the *Boswellia serrata* tree have been demonstrated to be effective in alleviating the clinical sign of OA in human (Kimmatkar et al., 2003) and dogs (Reichling et al., 2004). Active phytochemical compounds isolated from *Ribes nigrum* leaves showed anti-inflammatory properties

in-vivo in chrondrocyte assays (Garbacki et al., 2002) while its seed oil was an effective treatment for active rheumatoid arthritis (Leventhal et al., 1994). *Salix alba* extracts have recently been reported to have in vitro chondroprotective properties in primary canine articular chondrocyte culture (Shakibaei et al., 2012). These extracts seem also to be potent in counteracting low back pain, as recently reviewed (Gagnier et al., 2007). In rodent models of inflammation, an extract from *Tanacetum parthenium* demonstrated antinociceptive and anti-inflammatory effects (Jain and Kulkarni, 1999). Classified as a herb, bromelain is a digestive enzyme found in the stem and the fruit of *Ananas comosus.* This herb has been shown to have anti-inflammatory properties mediated through prostaglandin synthesis (Lotz-Winter, 1990). Finally, curcumin, which is the main biologically active phytochemical compound of *Curcuma longa*, showed inhibitory actions against major inflammatory mediators (Mathy-Hartert et al., 2009; Mobasheri et al., 2012; Aggarwal et al., 2013; Henrotin et al., 2013) while being effective in reducing pain in OA knee patients (Kuptniratsaikul et al., 2009; Madhu et al., 2013). In agreement with those findings, a recent Cochrane systematic review concluded to potential benefits of topical herbal medicines, being as effective as a gel containing NSAID (Cameron and Chrubasik, 2013). However, as also deplored in veterinary medicine (Vandeweerd et al., 2012), further high quality, fully powered studies are required to gain insight in the therapeutic potential of medicinal plants as well for other NHPS.

These studies suggest that NHP formulations containing the aforementioned medicinal herbs as principal ingredients might be useful in the management of OA. Whether or not such formulations are effective against the functional impairment that prevails in a model of natural OA needs to be scrutinized rigorously. With the scope of providing strong evidence-based findings, the aim of this RCT was to assess NHP formulations in the canine natural OA model when compared with dogs receiving a placebo over an eight-week duration.

6.1.3.3 Materials and methods

6.1.3.3.1 Design and subject selection

This study was a randomized, double-blind, parallel-group, placebo-controlled trial. Dogs were evaluated over either 56 or 61 days depending on the balanced attribution of locomotor activity recording (see Section 6.1.3.3.3). The trial was conducted under the approbation of the Institutional Animal Care and Use Committee (#Rech-1437) in accordance with the guidelines of the Canadian Council on Animal Care. All owners provided written informed consent.

Adult dogs weighed more than 20 kg and had radiographic evidence of OA exclusively at the hip or stifle joints. Radiographs (hips, stifles, and elbows) were obtained under sedation as previously described (Moreau et al., 2010). Hind limb lameness in association with the presence of OA was confirmed by veterinary surgeons. At the time of screening, all dogs were free of any compound purported to relieve the clinical signs of OA according to washout periods ranging between

four to 12 weeks. Hence, a four-week washout period was respected for oral NSAIDS and a six-week period for NHPs including fatty acid supplements, OA therapeutic diets or treats. Dogs having received injectable pentosan polysulfate sodium or corticosteroid one year before the screening visit were not eligible. A 12-week washout period was requested for injectable polysulfated glycosaminoglycan and hyaluronan, and for oral or topical corticosteroid. During the study, dogs were exempted from the administration of any type of medication except those prescribed for exo- and endoparasite control. Additional exclusion criteria were as follows: dogs with surgical repair of the cranial cruciate ligament within one year prior to study initiation, dogs suffering from neurologic or other musculoskeletal lesions, dogs that underwent orthopaedic surgery within the past year and dogs with CCL disease having gross instability (positive drawer motion upon orthopaedic examination).

6.1.3.3.2 Complete blood count and biochemistry panel

To ensure that some parameters were within normal limits during the study, each dog underwent routine blood haematological and biochemical analyses in order to evaluate health status at study initiation (Baseline, Day 0) as well as at Week 4 (Day 28) and Week 8 (Day 56). A veterinary clinical pathologist examined all blood counts and biochemistry panels.

Many herbs can increase the risk of bleeding through anti-platelet properties (Samuels, 2005). The buccal mucosal bleeding time is a simple test commonly used in the clinical setting to detect platelet dysfunction in dogs (Callan and Giger, 2001). Each dog underwent a buccal mucosal bleeding time procedure at Baseline and at Week 8. Mucosal punctures were performed on the upper labial mucosa, using a disposable, fully automated incision device (Surgicutt® Bleeding Time device, International Technidyne Corporation, USA). This device provided a controlled incision of 1.0 mm (depth) per 3.5 mm (length). The time of incision was noted, and circular filter paper (Whatman®, USA) was held 1-2 mm away from the incision to blot the blood, taking care not to disrupt the clot, or to allow blood to drip into the dog's mouth. The end point was when the incision stopped bleeding. Normal buccal mucosal bleeding time is defined to be less than three minutes.

6.1.3.3.3 Randomization, blinding and therapy regimen

Thirty-two privately-owned dogs were randomly allocated in two equal groups (placebo or NHP) according to a permuted-block randomization procedure, which included six blocks of four treatment possibilities (A or B) distributed in a one-to-one ratio (i.e. AABB, ABAB, ABBA, BBAA, BABA and BAAB). Among those blocks, eight were randomly selected using random integers to define the treatment allocation sequence. Also, seven blocks were randomly selected using random integers to allocate seven motor activity recordings to treatment A and seven others to treatment B. The 32 treatment allocations (with or without locomotor activity

recording) were transcribed on individual cards in sequentially numbered, sealed, opaque envelopes to ensure concealment. A third party was responsible for the randomization process and for the treatment preparation. At the trial site, both treatments were labelled exclusively as Treatment A or Treatment B and were encapsulated identically. The trialists, the animal health technicians and all dog owners were blinded to which treatment (A or B) was given to each dog. The key code revealing what referred to treatment A and B remained confidential with the third party and was revealed only after study completion and preliminary analyses.

The ingredients of the NHP formulations are described in **Tableau VII**. Dogs allocated to the NHP formulations received the *Alpha* formulation from Day 1 to Day 29, and then received the *Beta* formulation from Day 29 to Day 56. The dosing regimen was as follows: one capsule for dogs <25.0 kg; two capsules for dogs 25.0-39.9 kg; three capsules for dogs 40.0-49.9 kg; four capsules for dogs 50.0-59.9 kg and five capsules for dogs >59.9 kg. Dogs allocated to the negative (placebo) control received capsules filled of excipient to match the amount of the NHP formulations. The negative control (placebo) was given under the same dosing regimen as for the NHP formulations.

Tableau VII. Ingredients includes in each natural health products formulations

Ingredients (mg/capsule)	Formulations		Minimal contents
	Alpha	***Beta***	
Medicinal herbs			
Harpagophytum procumbens	240.0	60.0	Harpagosides 2.7%
Boswellia serrata	240.0	180.0	Boswellic acid 79.2%
Ribes nigrum	60.0	60.0	Rutines 1%
Salix alba	50.0	---	Salicin 1%
Tanacetum parthenium	50.0	---	Parthenolide 0.2%
Ananas comosus	---	40.0	2000-2500 GDU
Curcuma longa	---	35.0	Curcuminoids 95%
Omega-3 PUFA			
Total	40.0	40.0	
Eicosapentaenoic acid	0.4	0.4	
Docosahexaenoic acid	9.0	9.0	
Others			
Glucosamine sulphate	---	300.0	
Methylsulfonylmethane	---	90.0	
Chondroitin sulphate	---	60.0	
L-Glutamine	---	30.0	
Hyaluronic acid	---	15.0	
Excipient	228.0	280.0	
Total weight/capsule	**908.0**	**1190.0**	

Gelatin digesting unit (GDU)
Polyunsaturated fatty acids (PUFA)

6.1.3.3.4 Force platform measurement

Peak of the vertically-oriented ground reaction force (PVF) was measured at Baseline (Day 0), Week 4 (Day 28) and Week 8 (Day 56) at the trot (1.9-2.2 m/s) using a force platform, as previously described (Moreau et al., 2010). The PVF was defined as the primary endpoint of the study. Normalized PVF values in percentage of body weight (%BW) from the first five valid trials were used for statistical purposes. To be eligible, dogs must have at least one hind limb with PVF value lower than 66.0 %BW. This value was consistent to minus one standard deviation (SD) of the PVF value measured in normal dogs (Madore et al., 2007). When unilateral or bilateral lameness was observed, the hind limb having the lowest PVF value determined which one was selected for evaluation. This limb was defined as the most affected limb and was used in the subsequent follow-up of the study. The hind limb selected for evaluation must have been in accordance with orthopaedic examination findings, otherwise the dog was excluded. The change in PVF was the mean difference between Week 8 *minus* Baseline values.

6.1.3.3.5 Locomotor activity recording

Accelerometer-based motor activity recording was accomplished using the Actical® system (Bio-Lynx Scientific Equipment Inc., Canada) as described (Rialland et al., 2012). According to the balanced attribution of motor activity recording, collar-mounted accelerometers were worn by fourteen dogs for the entire treatment duration (61 days, 24 hour/day) which included a Baseline period (Day -4 to Day 0) that preceded the initiation of treatment administration. This period was

used to establish Baseline level of locomotor activity recording before treatment. Over the 61 days, the motion was continuously recorded every two minutes, giving 720 recordings per day. Daily duration of active period (DDAP) referred to the time spent (expressed in hour per day) when the recording exceeded 30 (no unit) in term of intensity. This cut-off value was based on intern data and was used to discern active from inactive period (Moreau et al., 2011; Rialland et al., 2012). Among the 61 days of continuous recording, three periods were predefined: Baseline (Day -4 to Day 0), Week 4 (Day 26 to day 28) and Week 8 (Day 54 to Day 56). Owners of dogs allocated to the locomotor activity recording were requested to come for an additional fourth appointment.

6.1.3.3.6 Case-specific outcome measure of disability (CSOM)

Assessment of at-home functional disability was accomplished using CSOM as previously described (Moreau et al., 2012a; Rialland et al., 2012; Rialland et al., 2013). Owners assessed the ability of their dogs to perform described by two to five activities, and scored on a five-point Likert-type scale for each activity that ranged from no problem (zero) to full incapacity (four). Each activity was selected by the owner according to his/her own perception of what characterised the disability of the dog. Assessments were conducted twice weekly using a specific form that remained in the possession of the owner. For each dog, median of the activities scores was determined for each assessment, giving a total of 17 median CSOM scores over the study. Among all the assessments, three periods were predefined:

Baseline (assessment on Day 0), Week 4 (assessments on Days 24, 28 and 31) and Week 8 (assessments on Days 49, 52 and 56).

6.1.3.3.7 Statistical analysis

All statistical tests were two-tailed with significance determined by reference to a 5% threshold. Normality of the data was tested using Shapiro-Wilk test. Data were log-transformed when requested to assure transformed data Gaussian distribution. Equality of efficacy was the null hypothesis based on the PVF (primary endpoint) as measured for the hind limb having the lowest value. Per trial log-transformed PVF values were analysed with a repeated-measures general linear mixed model that included two fixed factors (time and group) and their interaction (time×group interaction), with trials and dogs nested in treatment group as random effects. The change in log-transformed PVF values (Week 8 minus Baseline) were analysed with a repeated-measures general linear mixed model that included group as fixed factor with trials as random effect. Log-transformed DDAP were analysed similarly to PVF (period and group as fixed factors) and their interaction (period×group interaction) with days and dogs nested in treatment group as random effects. A repeated-measures generalized linear model was used to analyse median CSOM data under Poisson distribution function using independent working matrix. Fixed factors were period and group and their interaction (period×group interaction) with assessments and dogs nested in treatment group as random effects. Scale factor was estimated by Pearson's chi-square. Covariance structures were defined as

recommended (Littell et al., 2000). All *post hoc* analyses were conducted with appropriate Bonferroni adjustments. Data are presented as mean (SD).

6.1.3.3.8 Sample size calculation

According to previous works conducted under similar conditions (Moreau et al., 2007), a sample size of 16 dogs/treatment group ensured that a difference of 4.2 %BW in the primary endpoint (PVF) between groups could be detected assuming 75% power, a SD of 4.5 and a 5% significance threshold.

6.1.3.4 Results

6.1.3.4.1 Animal description

No clinically relevant changes were obtained from haematological and biochemical analyses in the entire study cohort. In addition, abnormal buccal mucosal bleeding times were not observed during the study. The number of dogs screened, randomly assigned, and analysed in each group is detailed in **Figure 22**. The NHP dog with persistent diarrhoea was diagnosed to have gastrointestinal intolerance. Complete CCL rupture (n=2) and humeral bone inflammation were tributary of acute lameness leading to early withdrawal for these dogs.

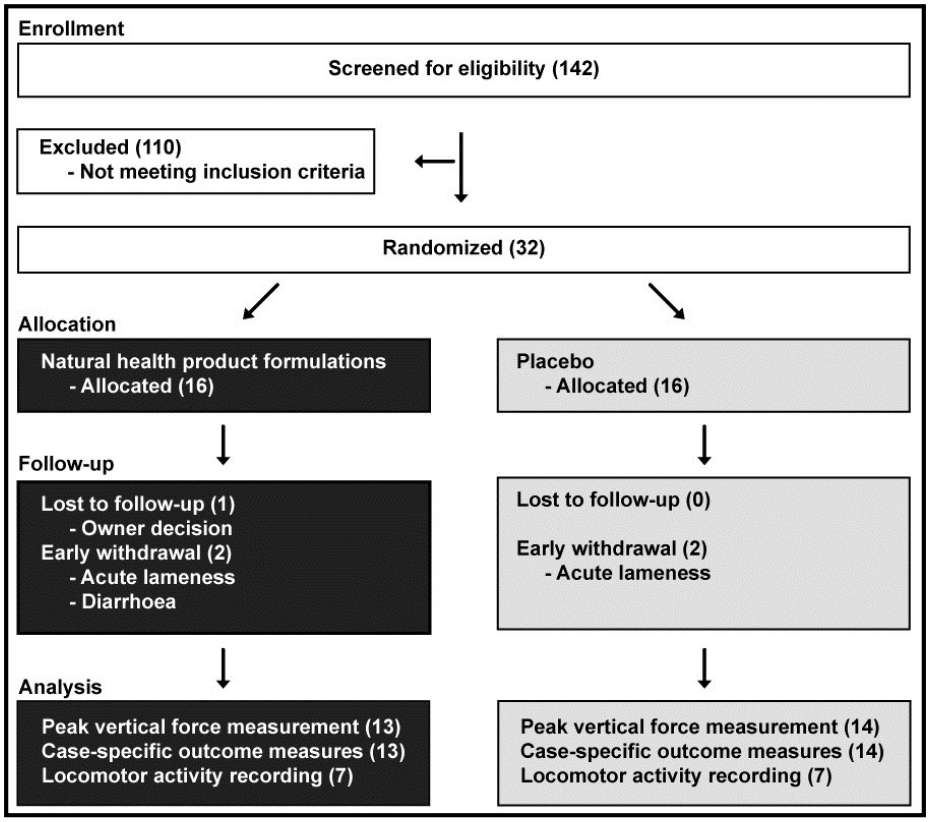

Figure 22. Flow chart of the study enrolment, allocation, follow-up and analysis

Baseline characteristics of the dogs stratified per group are presented in **Tableau VIII**. Groups were well balanced according to the outcomes of interest, as significant difference was not observed for the level of PVF, DDAP and CSOM recorded at Baseline. It should be noted that in each group, the dogs did not experience significant change in BW over time.

Tableau VIII. Baseline characteristics of the dogs stratified per group

Characteristics	Groups (n=16/group)	
	Placebo	Natural health product formulations
Age (months)	71.1 (22.6)	70.8 (33.5)
Sex (male/female)	7/9	10/6
Body weight (kg)	40.7 (8.5)	39.7 (10.8)
Peak vertical force (% body weight)	56.5 (6.2)	56.9 (5.3)
Daily duration of active period (hour/day)	6.7 (1.7)	6.9 (2.4)
Case-specific outcome measure of disability	1.6 (0.6)	1.6 (0.6)
Osteoarthritis-afflicted joint (most affected limb)		
Hip (count)	3	4
Stifle (count)	6	8
Hip and stifle (count)	7	4

6.1.3.4.2 Peak vertical force measurement

The PVF generated by the disabled hind limb was increased in the overall study cohort (time effect; p=0.016), without significant group effect (p=0.299) (**Figure 23**). Increment in PVF was mostly attributed to the changes observed in the NHP-treated dogs. Hence, a significant time×group interaction (p<0.001) was observed which means that groups evolved distinctively from Baseline to the end of the study. More specifically, analyses revealed that the PVF of NHP-treated dogs (n=13) was significantly increased at Week 4 [58.9 (5.4) %BW, p=0.020] and at Week 8 [59.8 (6.3) %BW, p<0.001], when compared to Baseline 57.3 (4.9) %BW. Placebo dogs (n=14) did not have significantly different values at Week 4 [56.4 (5.8) %BW] or Week 8 [56.9 (6.8) %BW] than Baseline [57.2 (4.5) %BW]. Both groups did not differ significantly at Week 8. **Figure 23** presents the respective individual changes in PVF recorded over the study (*i.e.*, Week 8 *minus* Baseline) as

well as the mean change denoted in each group. The mean changes in PVF values were significantly different between groups (p=0.027).

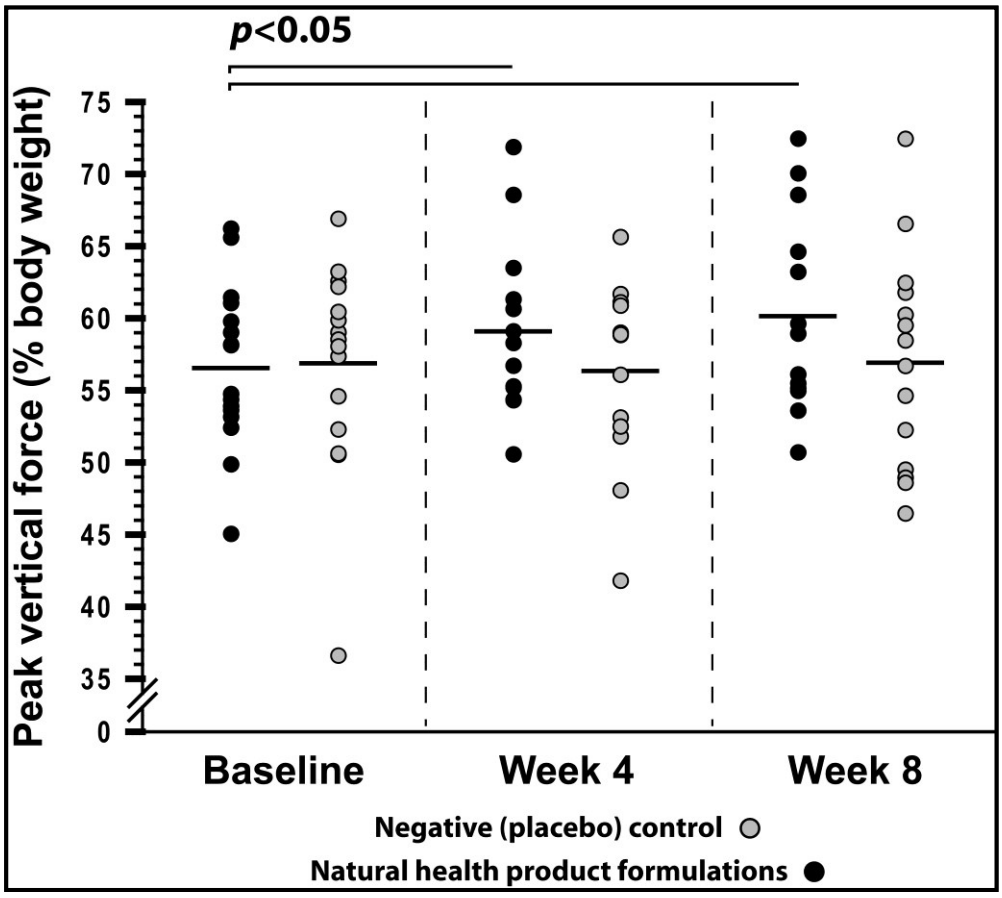

Figure 23. Peak vertical force

Individual peak vertical force values recorded in dogs having received either natural health product formulations or a negative (placebo) control. Peak vertical force values are expressed as percentage of body weight. The short horizontal lines denote mean group values. For the natural health product formulations group, values at Week 4 and Week 8 were significantly different (p<0.05) than Baseline.

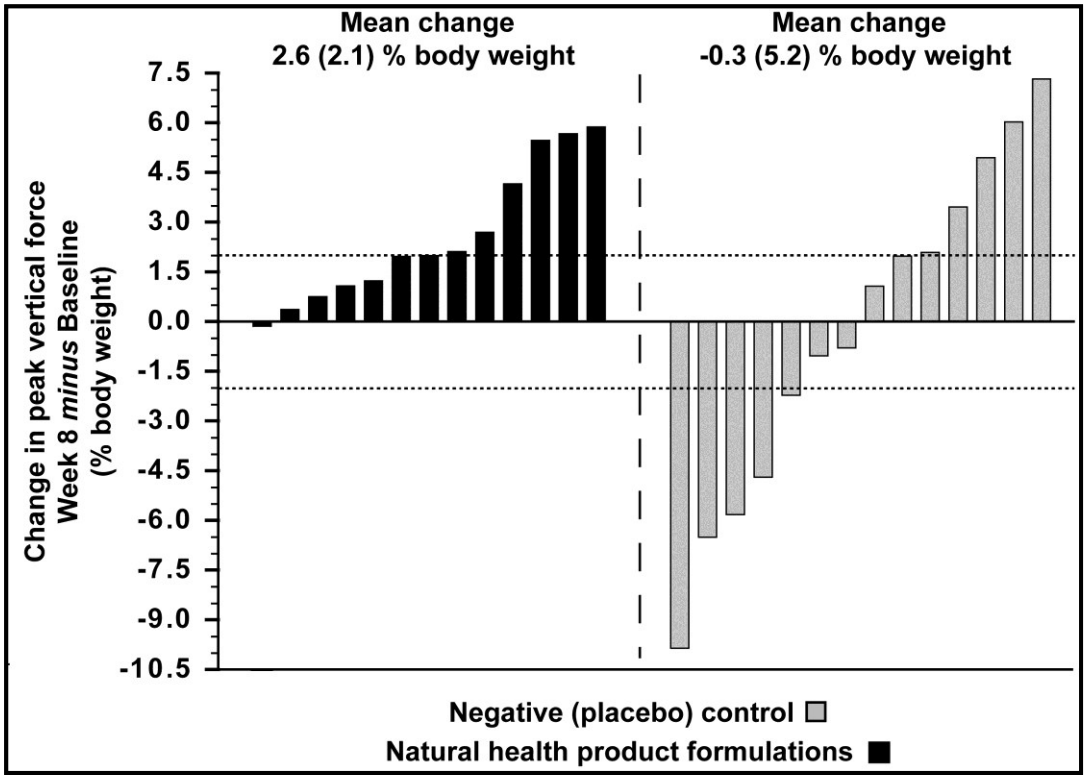

Figure 24. Changes in peak vertical force

6.1.3.4.3 Locomotor activity recording

Individual changes in peak vertical force recorded in dogs having received either natural health product formulations or a negative (placebo) control over eight weeks. Changes are the differences between Week 8 minus Baseline. Negative changes represent a decrease in peak vertical force values at Week 8 (i.e., worsening). Dotted lines delineate responders versus non-responders according to the minimal detectable change at 95% confidence interval (Moreau et al., 2013).

The analysis of DDAP indicated no significant period (p=0.862), or group (p=0.414) effect, but a significant period×group interaction (p<0.001). Analyses revealed that the Week 4 period [7.3 (1.9) h/day] in NHP-treated dogs (n=7) was not significantly different than the Baseline (**Tableau VIII**), reaching significant increase for the Week 8 period [8.2 (3.4) h/day, p=0.025] (**Figure 25**). The DDAP values of placebo dogs (n=7) at the Week 4 [6.7 (2.1) h/day] and Week 8 [6.0 (2.3) h/day] periods were not significantly different than the Baseline (**Tableau VIII**, **Figure 25**). A statistical trend (p=0.064) was observed for a difference in DDAP values between-groups over the study (*i.e.*, Week 8 minus Baseline).

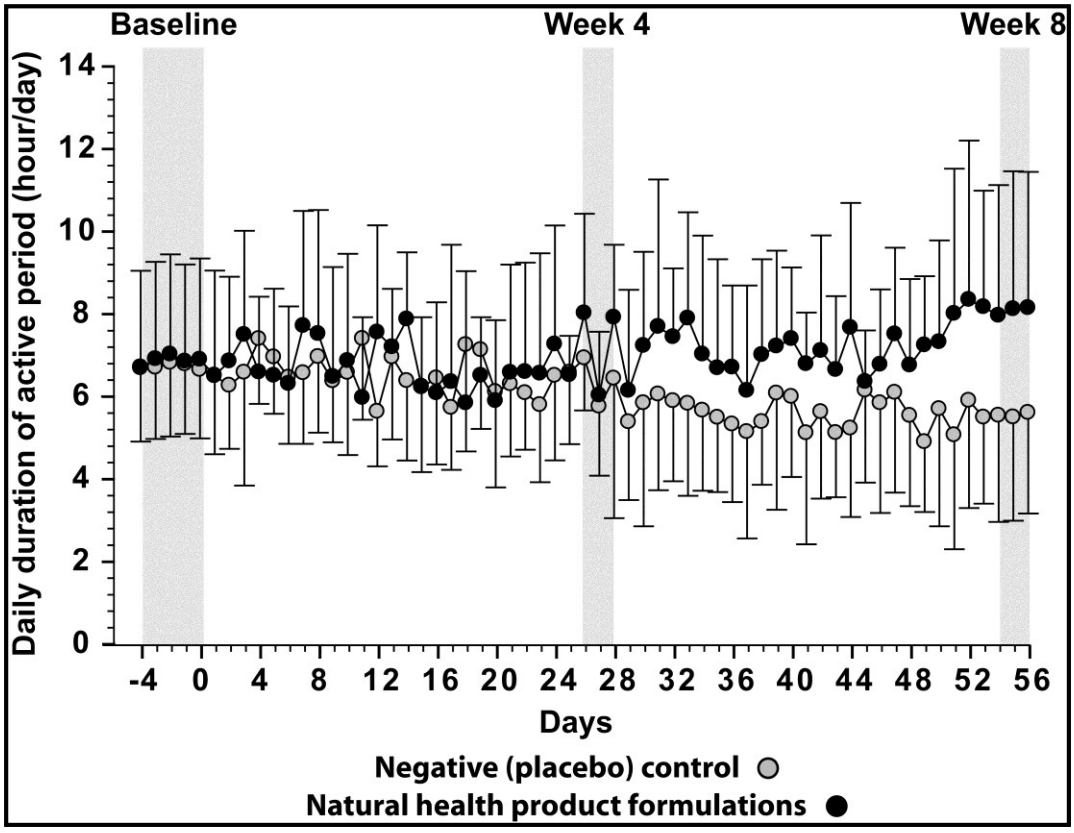

Figure 25. Locomotor activity recording

Temporal evolution of the locomotor activity recorded in dogs having received either natural health product formulations or a negative (placebo) control over 61-day duration. The daily duration of active period is expressed as mean (hour/day) with positive (natural health product formulations) or negative (placebo) standard deviation. Periods are Baseline (Day -4 to Day 0), Week 4 (Day 26 to Day 30) and Week 8 (Day 52 to Day 56) for the statistical analysis. For the natural health product formulations group, values at Week 8 were significantly higher than Baseline ($p<0.05$).

6.1.3.4.4 Case-specific outcome measure

The CSOM analysis revealed no significant period (p=0.053), group (p=0.960) and period×group (p=0.524) effect. The **Figure 26** presents the evolution of the CSOM recorded over the entire study duration.

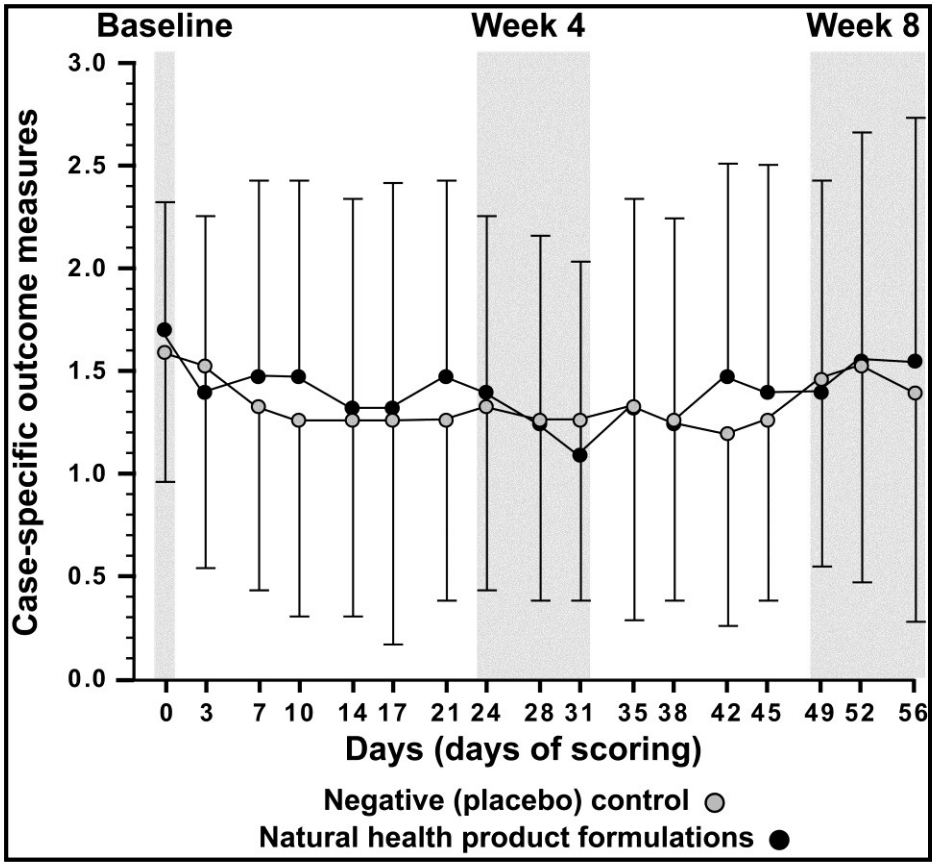

Figure 26. Case-specific outcome measures of disability

Temporal evolution of the case-specific outcome measures of disability (CSOM) recorded in dogs having received either natural health product formulations or a negative (placebo) control over eight weeks. Data are expressed as mean with positive (natural health products formulations) or negative (negative control) standard deviation. Periods are Baseline (Score on Day 0), Week 4 (Scores on Day 24, 28 and 31) and Week 8 (Scores on Day 49, 52 and 56) for the statistical analysis.

6.1.3.5 Discussion and conclusions

Current therapeutic approaches used to manage OA-afflicted patients remains largely palliative, NSAIDS being the first line of treatment (Bennell et al., 2012). The effect size reported for therapeutic modalities range from small to moderate (Bjordal et al., 2004; Zhang et al., 2007). Therefore, there is an opportunity for novel and effective therapeutics to alleviate pain for the OA-afflicted patient. As naturally-occurring models of OA have recently been proposed to accelerate the development of human therapeutics (Pelletier et al., 2010), and since canine OA models have a high translational value to human OA (Moreau et al., 2013), this randomized, double-blind, placebo-controlled trial was undertaken in the canine natural OA model to assess the efficacy of novel phytotherapeutics for human use. A recent systematic review concluded that NHPs had poor therapeutic potential for the treatment of companion animals affected by OA (Vandeweerd et al., 2012). This disappointing conclusion was largely based on the limited number of rigorous RCTs developed to challenge the proposed therapeutic efficacy of NHP. The quality and quantity of current research studies was also criticised for topical herbal medicines purported to alleviate the clinical signs of human OA, and the occurrence of adverse effects was common (Cameron and Chrubasik, 2013).The present trial was undertaken with the second intention to provide rigorous evidences regarding the therapeutic potential of medicinal herb-based NHP formulations to alleviate the clinical signs of canine OA, and to identify the occurrence of adverse effects with multi-NHP preparations.

According to the present trial, medicinal herb-based NHP formulations improved the functional ability in dogs afflicted by naturally-occurring OA to a higher degree than placebo-control animals. When given once daily, improvements were noted as early as four weeks after the initiation of the *alpha* formulation administration, and were even better when the *beta* formulation was given for an additional four-week duration.

The study primary endpoint was selected as the PVF measured using a force platform. Such an objective evaluation tool was previously used to measure the disability that characterised human OA patients as well as their response to treatment (Messier et al., 1992; Schnitzer et al., 1993; Gok et al., 2002; Detrembleur et al., 2005). Similarly, alterations from normality were detected in OA dogs based on the measurement of the PVF (Madore et al., 2007) while strong improvements in the pain-related limb disuse were reported for several therapeutic approaches including NSAIDS (Budsberg et al., 1999; Moreau et al., 2003), a dual inhibitor of cyclooxygenase and 5-lipoxygenase enzymes (Moreau et al., 2007), therapeutic diets (Roush et al., 2010; Moreau et al., 2012b; Rialland et al., 2013) and NHPs (Moreau et al., 2004; Hielm-Bjorkman et al., 2009; Moreau et al., 2012a).

The change over the initial condition [*i.e.*, 2.6 (2.1) %BW] provided by the medicinal herb-based NHP formulations is similar to common therapeutic approaches as recently reviewed (Moreau et al., 2012a). It outweighs the 95%

minimal detectable change (MDC_{95}), calculated as 2.0 %BW for PVF in canine OA (Moreau et al., 2013). The MDC_{95} can be interpreted as the change magnitude, below which there are more than 95% chances that the change has occurred as a result of measurement error (Kovacs et al., 2008). Outside this cut-off point (*i.e.*, lower than -2.0 or higher than 2.0 %BW), the change does reflect a real difference in the functional impairment toward worsening or improvement, in the canine natural OA model. Establishing such a cut-off point fulfils the requirement to define the magnitude of the measurement that corresponds to a clinically recognizable improvement in the individual animals, as previously criticised in a recent review (Sharkey, 2013).

The MDC_{95} can also serve as a responder criteria, similar to that developed for humans by the OARSI Standing Committee for Clinical Trials Response Criteria Initiative (Pham et al., 2004). According to **Figure 24**, 46% (6/13) of the medicinal herb-based NHPs treated dogs were positive responders while negative responders were absent. At the opposite, 36% (5/14) of placebo-control dogs had worsened their condition while 36% (5/14) were improved.

In the present study, statistical analyses revealed a significant difference between groups according to the changes in PVF values with a statistical power of 60%. The magnitude of the therapeutic benefits was consistent with a moderate effect size of 0.7 (95% confidence interval: 0.0 to 1.5). The effect size is recognized as a simple and straightforward index to quantify the effects of an intervention relative to a

comparator (Coe, 2012). However, effect sizes are not commonly reported in canine models of OA, which compromise comparisons among studies. Nevertheless, the effect size reported herein was similar to other therapeutic approaches including a therapeutic diet rich in omega-3 fatty acids from fish origin (Moreau et al., 2012b) as well as a plant extract from *Brachystemma calycinum* D don (Moreau et al., 2012a).

As previously demonstrated in this model of natural OA (Brown et al., 2010; Moreau et al., 2012a; Rialland et al., 2012; Rialland et al., 2013) the usefulness of the continuous monitoring of daily locomotor activity was sustained in the present study. After an eight-week period of treatment with the NHP formulations, the DDAP was increased, reaching more that 1.5 h/day of additional time spent on daily life activities. This finding is consistent with a recent review of experimental data aimed to determine the relationship between the limb function (as reflected by the measurement of the PVF) and the locomotor activity recording (Moreau et al., 2013). Hence, the effect of an additional 54 min/day of activity is expected to be mirrored confidently by an increase in PVF measurement exceeding the MDC_{95} (Moreau et al., 2013). As reported herein, the effects of the medicinal herb-based NHP formulations might have been translated into more active dogs, being able to rehabilitate their pain-related limb disuse towards a better muscular strength. This increase in limb use led to dogs more willing to accentuate their limb support by an average of 1.0 kg. These findings sustain the beneficial role of activity in OA dogs.

Nevertheless, the level of activity has to be low to moderate to avoid an exacerbation of lameness as reported after intense running (Beraud et al., 2010).

Unlike the objective measures of function, the CSOM did not document an improvement in NHP-treated dogs. The CSOM is a validated proxy method of assessment, which was shown to complement the information provided by the measurement of the PVF (Rialland et al., 2012; Rialland et al., 2013). Hence, the CSOM reflects the behavioural aspects of the OA disease affliction as perceived by the owner based on day-to-day environment and situation. The CSOM was used in the present study as an attempt to mirror the dog's quality of life over the eight weeks. This was done however without knowing the level of functional improvement required to be translated into a better quality of life. The present results suggest at first glance a need for more effective therapy based on owner perception, recognized as less sensitive and more prone to placebo response bias (Conzemius and Evans, 2012; Moreau et al., 2013). On the other hand, as OA is a lifelong disease, the limb impairment, which occurs over several years, may have compromised the sensitivity of the owner to detect an improvement in their dog. This is also supported by the relatively low value of CSOM at Baseline, compared to other similar population samples (Rialland et al., 2012; Rialland et al., 2013), inducing a risk of floor effect for CSOM masking the responsiveness to NHP treatment. Therefore, much time may be required by the owner to appreciate a better quality of life concomitantly to a functional improvement, as previously denoted in OA dogs after a 13-week treatment duration (Moreau et al., 2012b).

Our results indicate that treating with the medicinal herb-based NHPs did not result in a significant buccal mucosal bleeding time prolongation. This indicates that the platelet function was not affected by the treatment. Moreover, the NHP-treated dogs did not demonstrate clinically significant haematological or biochemical alterations when administered for eight weeks. This result is encouraging for promoting multi-NHP preparations, but would require further confirmation on larger sample size.

Several limitations to this clinical trial study need to be acknowledged. First, the study duration was eight weeks despite the chronic nature of OA. Second, the content and strength of the NHP capsules were based on empirical evidences (intern data files) suggesting anti-inflammatory and anti-nociceptive potential in rodent models of inflammation and pain. Whether or not the content and strength of the NHP capsule were optimal for dogs afflicted by naturally-occurring OA was unknown. Third, the design of the study did not allow conclusions about the respective potential of each NHP formulation (*i.e.*, *alpha versus beta* formulation). Therefore the efficacy of the medicinal herb-based NHP formulations should be considered as a whole therapeutic regimen involving *alpha* followed by the *beta* formulations. Finally, whether or not the improvement denoted in OA dogs is consistent with disease modifying effects is unknown and should be addressed. Of note licofelone, a dual inhibitor of cyclooxygenase and 5-lipoxygenase enzymes demonstrated similar functional improvement than the one observed with the NHP formulations in addition to a reduction in the progression of structural changes in

experimental dog OA model induced by CCL sectioning (Boileau et al., 2002; Moreau et al., 2006).

In summary, this RCT provided evidence of the efficacy of a medicinal herb-based NHP in alleviating the clinical signs of canine OA. The present findings provide relevant and new information about the potential of medicinal phytochemical compounds as a therapeutic modality for human OA. Such NHP appears also interesting for the management of canine OA as not only clear benefits were demonstrated on the function, but also this NHP mixture (with low grade dosage of each component) was not associated with any clinical toxicity.

6.1.3.6 Acknowledgements

Authors would like to acknowledge Ms Katherine Bernier and Ms Anne-André Mignault for their technical support. Authors are also grateful to Drs Christian Bollinger and Alexander O El-Warrak for their outstanding orthopaedic expertise. This study was funded in part by a grant from ArthroLab Inc., an ongoing New Opportunities Fund grant (#9483) and a Leader Opportunity Fund grant (#24601) from the Canada Foundation for Innovation for the pain/function equipment, a Discovery Grant (#327158-2008; #441651-2013) from the Natural Sciences and Engineering Research Council of Canada for the bio-analyses and salaries, and by the Osteoarthritis Chair of the University of Montreal Hospital Centre, Université de Montréal. Maxim Moreau received a doctoral scholarship from the Canadian

Institutes of Health Research – Strategic Training Program (MENTOR) and a doctoral scholarship from the Fonds de recherche du Québec-Santé.

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7 Modèle félin d'arthrose naturelle

L'arthrose prévaut également chez le chat domestique (*Felis domesticus*) (Lascelles, 2010). Chez cette espèce, l'usage de la force verticale maximale a permis de distinguer la présence d'arthrose au niveau des membres locomoteurs (Guillot *et al.*, 2012). La force verticale maximale s'inscrit également comme un moyen d'évaluer l'effet de thérapie contre l'arthrose chez cette espèce (**1.14 ci-dessus - Chapitre 1**) (Guillot *et al.*, 2013).

7.1 Article VI: Kinetic peak vertical force measurement in cats afflicted by coxarthritis: Data management and acquisition protocols

Cet article, publié dans *Research in Veterinary Science*, présente la force verticale maximale mesurée lors de l'analyse cinétique du mouvement locomoteur chez le chat arthrosique. Le but de cet article était de définir un protocole d'acquisition de la démarche et de gestion de données de force verticale maximale menant à une faible variance chez le chat arthrosique. Cet article s'intègre dans un effort de promouvoir la mesure de la force verticale maximale chez le modèle félin d'arthrose naturelle lors du développement de modalités thérapeutiques et ce, tant pour l'espèce féline que pour l'Homme.

M. Maxim Moreau a participé à l'acquisition des données, à l'analyse de ces dernières et a rédigé cet article présentement publié (*Research in Veterinary Science*, 95:219–224, 2013). L'article a par la suite été dûment révisé et bonifié par l'expertise de chacun des coauteurs.

Research in Veterinary Science
95:219–224, 2013

Kinetic peak vertical force measurement in cats afflicted by coxarthritis: Data management and acquisition protocols

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7.1.1 Abstract

The management of the peak vertical force (PVF) measurement need to be determined in coxarthritis cats. Six privately-owned coxarthritis cats were conditioned to trot across a floor mat-based plantar force measurement system. Hind limbs PVF was measured on level ground at day one (D1), D8, D42 and D84. Measurements were repeated after 10 minutes treadmill exercise (D1), trotting on an inclined (13°) plane (D42) and after stair climbing exercise (D84).

Test-retest reliability between D1 and D8 was good (intraclass coefficient of correlation of 0.8). Coefficients of dispersion (within-subject and between-subject) were <15% using the lowest hind limb PVF value. Only stair climbing exercise positively affected sample and effect size estimates.

To limit the dispersion of data, the measurement of PVF should be managed using the lowest hind limb PVF value. In addition, PVF should be measured following stair climbing to optimise sample and effect sizes and to preserve statistical power.

7.1.2 Introduction

Osteoarthritis (OA) is strikingly prevalent in cats and there is a strong suspicion that many afflicted animals would benefit from therapy to alleviate their chronic pain (Lascelles et al., 2010; Bennett et al., 2012a). However, the level of evidence for OA pain-relieving effect of therapeutics is actually insufficient, in part due to the challenge that represents the discrimination of symptomatic cats, as well as the absence of validated outcome measures applicable to clinical cases (Lascelles, 2010; Lascelles and Robertson, 2010; Bennett et al., 2012b).

Force plates are commonly used in clinical settings to measure ground reaction forces involved in the locomotion of dogs, but also horses (Barrey, 1999) and cows (Walker et al., 2010). As an objective outcome that reflects the limb function in dogs, the kinetic measurement of the peak of the vertically-oriented ground reaction force (PVF) has been used to monitor the therapeutic effects in canine OA of non-steroidal anti-inflammatory drugs,(Budsberg et al., 1999; Moreau et al., 2003; Moreau et al., 2007) complementary and alternative medicines (Moreau et al., 2012a; Moreau et al., 2012b; Rialland et al., 2012) as well as non-pharmacological approaches (Mlacnik et al., 2006; Marshall et al., 2010). The measurement of the PVF has been also used to characterize OA disease affliction (Madore et al., 2007) and to prospect responses to surgical procedures (van Klaveren et al., 2005).

The use of force platform in small animals (<20kg) such as cats compromises the recognition of ipsilateral foot strikes as multistep occurs over the surface of commonly used force platforms. The recent development of a floor mat-based plantar force measurement system has facilitated the acquisition and the description of the locomotion in cats. Hence, data from simultaneous, consecutive, and collateral foot strikes can be recorded together, providing a complete description of plantar force mapping and distribution among each limb during the stance phase of the stride. The recording area comprised into a thin walkway is sufficiently long to acquire several strides. Of note, cats require specific training and accommodation, as they are not so instinctively able to move in a natural manner when manipulated by leash, as are dogs and horses. Such a floor mat system has been used in domestic cats free of orthopaedic disease (Lascelles et al., 2007) as well as onychectomized cats (Romans et al., 2004; Romans et al., 2005). In addition, Guillot (2012) suggested recently that cats with coxarthritis present a lower PVF than disease-free cats (Guillot et al., 2012).

Further investigations are necessary to support the use of the PVF as a functional outcome in OA cats. Hence, it remains unknown how the measurement of PVF should be managed to optimise the outcome monitoring of OA-associated disability in cats. Peak vertical force data can be managed by different methods, including the use of the lowest limb value, the mean of paired (or all) limb values, or their difference (paired limb asymmetry index). Those methods should demonstrate low data dispersion and be sufficiently reliable to detect changes in a repeated

measurement design. Finally, the conditions of data acquisition should be explored. The effects of two different sessions of exercise, as well as the acquisition on an inclined plane should be interesting to be tested for their consequences on the kinetic assessment. The goal is to minimize the dispersion of data as an attempt to optimise sample and effect sizes and to preserve statistical power. Therefore, the objectives of this investigation were: (a) to examine the test-retest reliability of PVF acquired using a floor mat-based plantar force measurement system in freely trotting OA cats; (b) to define which method of data management yielded the lowest dispersion of PVF data; (c) to examine the effect of acquisition protocols on the dispersion of PVF data and (d) to present sample size and effect size estimations.

7.1.3 Material and methods

7.1.3.1 Cat selection and housing

The study was approved by the Institutional Animal Care and Use Committee (# Rech-1482) in accordance with the guidelines of the Canadian Council on Animal Care. Cats were long-term residents (more than two years) of a rescue shelter devoted to provide cares until the end of life of animals. In this condition, all cats of this community were considered privately-owned. The owners provided written informed consent before enrolling illegible cats. Six healthy, conditioned (vaccination, endo and exo-parasite controlled) adult cats (age ranging from 6-12 years) were selected based on normal physical, orthopaedic and neurological examinations, complete blood count and biochemistry panel, and urinalysis. The

presence of radiographic evidence of coxarthritis was a prerequisite for inclusion (see computed radiograph section). Stifles, lumbosacral, sacroiliac, shoulder and elbow joints had to be exempt of radiographic evidence of OA. Recruited cats were free of any pharmacological treatment (including anti-inflammatory drugs and natural health products) for at least three months prior to study initiation. Cats were individually acclimated for one week, and then housed together in a single dedicated room as described (Guillot et al., 2012).

7.1.3.2 Computed radiograph

Computed radiographs (Agfa CR-DX system; Toronto, ON, CAN) were obtained under the same sedative protocol as previously described (Guillot et al., 2012). Coxofemoral joint radiographs were assessed by a board-certified veterinary radiologist for the presence of osteophytosis, enthesiophytes and subchondral bone sclerosis using a diagnostic viewing station (Agfa IMPAX 6.0; Toronto, ON, CAN) as described (Guillot et al., 2012).

7.1.3.3 Chronology of events

Cats were progressively trained to trot across a floor mat system on level ground and to treadmill exercise three times a week for four weeks using positive reinforcement (treats and brushing). At day one (D1), PVF was measured before and immediately after the treadmill session. Then to test the repeatability of PVF measurement, this outcome was again measured at D8. At D14, cats were trained to walk on an inclined plane three times a week for four weeks. At D42, measurements were done on level ground and on inclined plane. At D56, cats were

trained to climb stairs three times a week for four weeks. After this training (D84), PVF was measured before and immediately after stair climbing exercise.

7.1.3.4 Peak vertical force measurement

Peak vertical force was acquired during locomotion using a floor mat-based plantar force measurement system (Walkway® with four Matscan® sensor 3150; Tekscan Inc, Boston, MA, USA) and managed using Research Foot® software v.5.71. The system provided a resolution of 1.4 sensels/cm^2. Dimension of the system was 1743 mm (length) x 369 mm (width) x 5 mm (thickness). Prior to data acquisition, the system was calibrated using a predefined weight after one second of latency in accordance with manufacturer recommendations. For each hind limb, the PVF was acquired under dynamic condition (constant velocity; 0.8 to 1.2 m/sec) monitored using a time frame set at a frequency of 44 Hz. A trial was considered valid when the entire floor mat was crossed by a cat moving undisturbed, consistently and in straight line. Only the first stride was considered. The right and left hind limb foot strikes from the first five valid trials were considered. The PVF was expressed as a percentage of body weight (%BW). The hind limb PVF data (right and left) were managed using three different methods: a) the lowest value (lowest), b) the difference between values (asymmetry), and c) the mean of values (average).

7.1.3.5 Treadmill exercise

Cats were submitted to treadmill exercise using a commercial treadmill (model 621T; Tempo Fitness, Cottage Grove, WI, USA). The treadmill was customized using transparent polycarbonate sheets to avert unwanted escaping. During the

treadmill session, cats were stimulated to complete a 10-minute period of treadmill activity with a belt speed at 0.4 m/sec. Belt speed was begun slower during training. Cats underwent PVF assessment within a delay of 60 sec after having completed the exercise.

7.1.3.6 Inclined plane

A force component perpendicular to the floor mat-based plantar force measurement system was referred to as the normal force. Peak normal force was acquired when cats where trotting on a 13° upslope inclined plane. A custom made wooden frame supported the floor mat-based plantar force measurement system. At the elevated end, a ramp was used allowing the cat to move away.

7.1.3.7 Stair climbing exercise

Cats were submitted to stair climbing using a 10 m long staircase running up/down twice, and then up. Characteristics of the rise and tread depth of stairs were 190 and 254 mm, respectively. The post-exercise measurement was performed within 60 sec of the final excursion up the stairs. Cats were handled with a leash by a veterinary technician ensuring full completion.

7.1.3.8 Statistical analysis

The coefficient of dispersion (CoD) is a relative, non-parametric and robust measure of dispersion used as an alternative to the parametric coefficient of variation (Esterman et al., 2005). The CoD was used to evaluate the uniformity of data using the following formula:

$$\frac{\Sigma|\text{xi} - \text{median}|/\text{sample size}}{\text{median}}$$

where x_i was the PVF value of a given trial (within-subject CoD) or was the mean of the five PVF values recorded for each trials (between-subject CoD). The mean (min; max) was presented as a measure of central tendency and the interval was representative of the data spread. Intraclass coefficient of correlation (ICC) was used to evaluate the test-retest reliability of the PVF (level ground) measured at D1 *versus* D8, at D1 *versus* D42, and at D1 *versus* D84. Sample size and effect size were estimated using means and standard deviation values obtained using a resampling (Bootstrap) technique. Hence, to improve the precision of the estimates (mean and standard deviation), 1000 resamples of the observed PVF measurement dataset, each of which obtained by random sampling with replacement from the original dataset, were obtained. Resamples were of equal size to the observed dataset (Adèr et al., 2008). Sample size and effect size were estimated before and after stair climbing exercise performed at D84. Sample sizes were estimated to achieve 80% power to detect a difference between the null hypothesis (absence of significant change) and the alternative hypothesis (5% change in PVF value) with a significant level of 5% using two-sided one-sample T-tests. Effect size for repeated

measurements, considering a proposed improvement of 5%, was calculated using the following formula:

$$\frac{\text{Change in PVF measurement (5\%)}}{\sigma\sqrt{2(1-\rho)}}$$

where ρ was the Pearson correlation coefficient (Morris and DeShon, 2002). Statistical power was estimated at the 5% level. The commercially available software packages SPSS 17.0 (SPSS, Chicago, IL, USA), PASS 2008 (Number Cruncher Statistical System, Kaysville, UT, USA) and XLSTAT (Addinsoft, New York, NY, USA) were used for the statistical analyses.

7.1.4 Results

7.1.4.1 Animals

All cats had radiographic sign of hip OA (coxarthritis). Mild bilateral disease was observed in four cats, unilateral affliction in two cats (one with mild and the other with moderate disease severity).

7.1.4.2 Test-retest reliability

Using ICC (95% interval), test-retest reliability (D1 *versus* D8) was 0.8 (0.2-0.9) for the lowest method, 0.5 (0.0-0.9) for the average method and 0.3 (0.0-0.8) for the asymmetry index. Six weeks later, test-retest reliability (D1 *versus* D42) was 0.6 (0.1-0.9) for the lowest method, 0.4 (0.0-0.8) for the average method and 0.3 (0.0-0.9) for the asymmetry index. When comparing D1 *versus* D84, test-retest reliability was 0.4 (0.0-0.9) for the lowest method, 0.3 (0.0-0.8) for the average method and 0.3 (0.0-0.9) for the asymmetry index.

7.1.4.3 Peak vertical force data management and acquisition protocols

Data for the PVF measured at D1 before and after treadmill exercise according to the three different methods of outcome management are summarized in **Tableau IX**. Before undergoing treadmill, within- and between-subject CoDs were <15% using the lowest and average methods while using asymmetry index, CoDs were >25%.

Tableau IX. Statistics for the peak vertical force measured before and after ten minutes of treadmill exercise in six cats with coxarthritis according to three methods of outcome management at D1.

	Methods of outcome management		
Before	**Lowest**	**Asymmetry index**	**Average**
Coefficient of dispersion			
Within-subject (%)	12.2	45.8	10.9
Between-subject (%)	10.0	28.6	6.9
Mean PVF (min; max) (%BW)	38.5 (34.0; 45.0)	11.7 (5.5; 15.9)	44.3 (40.3; 51.9)
After treadmill exercise			
Coefficient of dispersion			
Within-subject (%)	11.8	68.5	9.6
Between-subject (%)	12.0	15.1	10.8
Mean PVF (min; max) (%BW)	40.4 (30.4; 50.1)	8.6 (6.6; 11.9)	44.7 (34.9; 53.4)

PVF= Peak vertical force.

Lowest was the lowest values between the right and left hind limbs PVF values.

Asymmetry was the difference between the right and left hind limb PVF values.

Average was the mean of the right and left hind limbs PVF values.

Following treadmill exercise, the CoD was <15% for the lowest and the average methods. Larger CoD were observed for the asymmetry method. The lowest and average methods denoted an increase in mean PVF (1.9 and 0.4%BW, respectively), while a decrease was observed for the asymmetry index (-3.1%BW) after exercise. Lowest and average methods had larger intervals.

At D42, mean PVF and CoDs observed before upslope measurement were in accordance with those observed at D1 (**Table 10**). Conversely to level ground, larger intervals were denoted on inclined plane for the lowest and average methods. The CoDs were still <15% using the lowest and average methods and >25% for the asymmetry index. The mean PVF was decreased in the lowest (-0.9%BW), average (-1.3%BW) and asymmetry (-0.4%BW) methods.

Tableau X. Statistics for the forces measured before and when trotting on an inclined plane (13°) in six cats with coxarthritis according to three methods of outcome management at D42

	Methods of outcome management		
Before	**Lowest**	**Asymmetry index**	**Average**
Coefficient of dispersion			
Within-subject (%)	11.8	35.7	10.5
Between-subject (%)	10.9	25.3	10.2
Mean PVF (min; max) (%BW)	39.2 (33.8; 45.8)	7.4 (2.1; 11.1)	43.0 (39.3; 50.6)
Inclined plane			
Coefficient of dispersion			
Within-subject (%)	12.4	42.3	11.4
Between-subject (%)	12.6	29.5	12.8
Mean PNF (min; max) (%BW)	38.3 (25.0; 43.2)	7.0 (4.4; 12.1)	41.7 (29.2; 47.3)

PVF= Peak vertical force. PNF= Peak normal force.

Lowest was the lowest values between the right and left hind limbs PVF values.

Asymmetry index was the difference between the right and left hind limb PVF values.

Average was the mean of the right and left hind limbs PVF values.

Peak vertical force was measured at D84 before and after stair climbing (**Tableau XI**). The CoDs of the lowest and average methods were consistent with D1 and D42 measurements, while it was <10% after stair climbing exercise. The lowest and the average methods denoted an increase in mean PVF values (0.9 and 0.8%BW, respectively) and narrow intervals after stair climbing exercise. For the asymmetry index, there was a decrease in mean PVF (-0.4%BW), a larger interval and CoD >15% after exercise.

Tableau XI. Statistics for the peak vertical force measured before and after stair climbing in six cats with coxarthritis according to three methods of outcome management at D84

Before	Methods of outcome management		
	Lowest	Asymmetry index	Average
Coefficient of dispersion			
Within-subject (%)	7.5	41.1	6.5
Between-subject (%)	12.9	16.9	11.9
Mean PVF (min; max) (%BW)	39.1 (28.2; 47.1)	10.0 (5.3; 12.5)	44.0 (34.0; 50.7)
After stair climbing			
Coefficient of dispersion			
Within-subject (%)	7.1	51.6	5.7
Between-subject (%)	5.8	28.1	8.0
Mean PVF (min; max) (%BW)	40.0 (34.1; 44.0)	9.6 (6.1; 14.1)	44.8 (38.4; 49.0)

PVF= Peak vertical force.

Lowest was the lowest values between the right and left hind limbs PVF values.

Asymmetry was the difference between the right and left hind limb PVF values.

Average was the mean of the right and left hind limbs PVF values.

7.1.4.4 Sample size and effect size estimates

Conversely to the effect of treadmill exercise and upslope trotting, intervals of the mean PVF after stairs climbing were narrowed when the lowest and average methods were considered. The change in mean PVF measurement was also lower. Therefore, sample and effect sizes were analysed before exercise (D84) and in response to stair climbing (**Tableau XII**).

Tableau XII. Sample size and effect size consistent with a 5% change in peak vertical force measured in six cats with coxarthritis according to three methods of outcome management

	Change in PVF[a]; statistical power[b]; sample size; effect size[c]	
Methods	**Basal condition**	**After stairs climbing exercise**
Lowest	2.0% BW; 80%; 78; 0.5	2.0% BW; 80%; 19; 1.0
Asymetry	-0.5% BW; 80%; 218; 0.3	-0.5% BW; 80%; 289; 0.3
Average	2.2% BW; 80%; 50; 0.6	2.0% BW; 80%; 24; 0.9

a The change represented 5% of the PVF value measured either at basal condition or after stair climbing exercise. The change was oriented toward a decrease in limb impairment.

b Statistical power was estimated at the 5% level.

c Effect sizes were calculated using a Pearson correlation coefficient of 0.8 (Morris and DeShon, 2002).

PVF= Peak vertical force. Sample size and effect size were calculated using mean and SD values estimated from a 1000 resamplings.

Lowest was the lowest values between the right and left hind limbs.

Asymmetry was the difference between right and left hind limb values.

Average was the mean of right and left hind limbs

With regard to the before exercise values, the smallest sample size (n=50) was estimated using the average method for a change of 5%. The magnitude of a treatment effect (effect size) in these conditions was 0.6, consistent with an increase of 2.2%BW. After stair climbing, the smallest sample size was found using the lowest method while 19 cats provided an effect size of 1.0 (**Tableau XII**).

7.1.4.5 Discussion

A floor mat-based plantar force measurement system is a low profile walkway that facilitates the acquisition of multiple sequential foot strikes in cats (Romans et al., 2004; Romans et al., 2005; Lascelles et al., 2007; Robinson et al., 2007; Guillot et al., 2012). By the mean of this system, the current study provides further evidence to support the use of PVF measurement as a functional outcome in fully trained, privately-owned cats afflicted by coxarthritis.

Among the proposed methods of outcome management, lowest and average methods achieved the lowest dispersion of PVF data. The level of dispersion was in agreement with previous works performed in OA dogs using a common force plate (Madore et al., 2007). Those methods apparently limit between-subject disparity, giving an accurate measure of central tendency and the spread of data. Of note, high levels of within-group homogeneity ensure the preservation of statistical power, avoiding erroneous conclusion when groups are to be compared (Morris and DeShon, 2002).

Good test-retest reliability is desirable for outcomes that are not expected to change in a short delay. In the present study, good reliability was denoted with the lowest method when PVF was measured one week apart. The PVF is therefore measured with consistency over a short duration. Conversely, the PVF measurement seemed less reliable when recorded 6 and 12 weeks later. This was suggestive of an intrinsic modification in the condition of the OA cats. This phenomenon is referred to as the maturation effect (Dimitrov and Rumrill, 2003) and affects the test-retest reliability. Nevertheless, the mean differences in PVF measurements between test sessions were less than 1%BW.

Our study showed that 10 min of treadmill exercise increased the dispersion of PVF measurements managed with the lowest and average methods. As an explanation, we observed different degrees of activity response after treadmill sessions. This aspect may have precluded to impairment in the ability of cats to perform a constant ambulation throughout the subsequent measurement of PVF. Following treadmill exercise, changes toward normal gait (*i.e.* higher PVF and less asymmetry) were unexpected as exacerbation of hind limb lameness was reported in OA dogs following intense exercise (Beraud et al., 2010). Despite efforts addressed to ensure that velocity was thoroughly maintained, cats crossing faster the walkway may have contributed to the positive change in PVF measurement.

The biomechanics of incline slope walking have been investigated in cats. Hence, when traveling on an inclined plane, the cats need a distinct muscular pattern to move the centre of mass up the slope (Kaya et al., 2006). It was also reported that under this walking condition, cats increased their hind limb normal force and cranial shear force (Gregor et al., 2006). In the present study, cats moving uphill had larger data spread, similarly to those observed after a treadmill session. Nevertheless, the dispersion of data remained <15% for upslope measurement. Surprisingly, we observed that OA cats did not respond to upslope measurement by an increase in limb loading as it was expected from a previous experiment done in non OA cats (Gregor et al., 2006). The ability to generate higher hind limb normal force to travel up an inclined plane appears to be restricted in OA cats. This may suggest that an OA-related pain interfered with locomotion and favored gait compensatory mechanisms. Such alterations in the normal ambulation need to be further documented.

Stair climbing exercises improved the uniformity of the PVF measurements managed using the lowest and average methods. Climbing stairs requires a complex integration of sensory information and ability to generate forces to control body movement (Leitner et al., 2011). Higher coxofemoral joint motion to ascend and descend stairs was described in dogs (Millard et al., 2010; Durant et al., 2011). It is not known whether the motion of cats presents such a joint excursion, particularly when functional impairment secondary to OA occurred. Nevertheless, stair

climbing improved the uniformity of the PVF measurement reported herein, inducing trivial changes in mean values.

In a repeated measure single group design, the interest is in how the individual's performance changes over time in response to intervention. This design allows the same individual to be tracked across conditions, thereby introducing the notion of change. Before undergoing a large scale study, an estimation of sample size is necessary to have enough statistical power to reject the null hypothesis (absence of significant change) and should be carefully estimated based on preliminary data. Estimations require accurate standard deviation otherwise the result will be falsely estimated. Resampling methods improve the accuracy of the descriptive statistics in a manner to palliate for the distortion caused when data are not fully representative of the population (Adèr et al., 2008). Due to the low sample size in the present study, several resamplings (1000) were performed to give an accurate estimation of the mean and standard deviation of the PVF measurement. A change by 5% was proposed, that is consistent with the improvement generally observed in OA-afflicted dogs under non-steroidal anti-inflammatory drug treatment (Budsberg et al., 1999; Moreau et al., 2003; Moreau et al., 2007). Effect size was calculated in respect to the presence of dependency between sets of measurements (Morris and DeShon, 2002) that involved a Pearson correlation coefficient of 0.8. Such coefficient was expected in regard to the high level of correlation between the PVF measured at D1 and at D8 (Pearson correlation coefficient of 0.8).

As presented in **Tableau XII**, we estimated that a sample size of 50 coxarthritis cats should be required to distinguish a 5% change in PVF measurement before exercise using the average method, giving a moderate effect under the alternative hypothesis (Cohen, 1992). A considerable benefit was observed when using data acquired following stair climbing sessions. We estimated that a sample size of only 19 coxarthritis cats should be sufficient to distinguish for a 5% difference in PVF measurement, based on the lowest method. Moreover, the larger effect size observed in such conditions could be of importance in practice (Cohen, 1992). The conservative nature of the estimates presented herein should be carefully taken into account. Hence, lower sample size can be expected from a multivariate statistical model that uses covariates (such as individual velocity, stride length and duration, and number of trials to collect data) to optimise the detection of kinetic changes over time in cats with coxarthritis.

The present study has several limitations to be considered: a) the lack of statistical power related to the limited sample size and to the small magnitude of the effect did not allow statistical inference on the effect of exercise; b) the sampling rate for PVF measurement was lower than the one of a force platform (1000 Hz). Higher sampling rate would preclude to a better accuracy in the PVF measurement; c) the velocity was evaluated in a suboptimal manner. More recent software (Walkway Research® software v.7.0) computes the velocity of the subject crossing the walkway.

This study addressed the need for objective markers of OA pain-related disability by testing the potential of a floor mat-based plantar force measurement system to monitor the functional impairment associated with OA in cats. Our findings suggested favouring the lowest PVF as an outcome management after stair climbing exercise to optimise the detection of changes of clinical relevance in a small group of coxarthritis cats. The use of kinetic parameters under such conditions has the potential to monitor the effect of OA therapeutics purported to alleviate the clinical symptoms of feline OA.

7.1.4.6 Acknowledgments

Authors are grateful to the cat owners who kindly provided their animals for the study duration. The authors also wish to thank Dr Marc-André d'Anjou, DMV, DACVR for his radiograph expertise and all the veterinary technicians involved in this project for their technical skills. This study was supported by an Operating Grant from ArthroLab, Inc., the Morris Animal Foundation Grant (#D09FE-803A) for the pilot study "TOP-CAT: Tracking Osteoarthritis Pain in the CAT" (Troncy), a Leader Opportunity Fund Grant (#24601) from the Canada Foundation for Innovation (Troncy); and a Discovery Grant (#327158-2008) from the Natural Sciences and Engineering Research Council of Canada (Troncy). M. Maxim Moreau and Dr Martin Guillot received a doctoral scholarship from the Canadian Institutes of Health Research – Strategic Training Program (MENTOR). M. Maxim Moreau received a doctoral scholarship from the Fonds de recherche du Québec-Santé. Dr. Martin Guillot is the recipient of an Alexander Graham Bell Canada Graduate Scholarship for doctorate research.

7.1.4.7 References (Article VI)

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CHAPITRE 2. EXPÉRIMENTATIONS

La force de réaction au sol verticale maximale comme témoin d'effets fonctionnels et structuraux chez des modèles canins d'arthrose: Potentiel envers le développement thérapeutique

8 Modèle canin d'arthrose naturelle : La force verticale maximale et la détection d'effets thérapeutiques

8.1 Préambule

Des arthropathies développementales ou traumatiques (Johnston, 1997; Johnston, 2001; McLaughlin, 2001; Roush, 2001; Shearer, 2011) sont majoritairement responsables du désordre myo-arthro-squelettique que constitue l'arthrose. Dans la plupart des cas, le chien affligé d'arthrose démontre un dysfonctionnement locomoteur. Chez cette espèce, l'analyse cinétique des forces de réaction au sol est considérée comme une méthode de référence permettant de décrire objectivement la capacité fonctionnelle d'un membre locomoteur (Egger *et al.*, 2013). De cette analyse découle la mesure de la force verticale maximale.

Les modèles naturels d'arthrose ont été suggérés afin de promouvoir le développement thérapeutique d'agents anti-arthrosiques (Mogil, 2009; Poole *et al.*, 2010; Pertovaara, 2012; Vainio, 2012). Parmi ceux-ci, le modèle canin pourrait s'avérer être un choix pertinent (Vainio, 2012), particulièrement en regard de la

pathomécanique de l'arthrose chez cette espèce, de même qu'à la facilité d'évaluer le dysfonctionnement locomoteur.

En contexte d'essai clinique contrôlé, l'utilisation de la force verticale maximale comme critère primaire d'efficacité a permis d'obtenir des évidences cliniques probantes, telles qu'en témoignent de récentes révisons systématiques de la littérature (Aragon *et al.*, 2007; Sanderson *et al.*, 2009; Vandeweerd *et al.*, 2012). Fait intéressant, la force de réaction au sol verticale maximale fut utilisée comme critère primaire d'efficacité dans le mémoire de maîtrise du présent auteur intitulé : *Évaluations cliniques d'approches thérapeutiques pharmaceutiques contre l'ostéo-arthrose canine* (Moreau, 2002). Un regard critique est néanmoins posé au sujet de la force verticale maximale comme critère primaire d'efficacité thérapeutique. Ainsi, l'auteur d'un récent article (Sharkey, 2013) déplore l'absence d'un seuil limite au-delà duquel un changement se distingue de la marge d'erreur inhérente à la mesure de la force verticale maximale. Il y a donc absence d'un seuil permettant de distinguer un répondant lorsque la force verticale maximale est utilisée afin de refléter un changement au dysfonctionnement locomoteur.

Tenant compte de l'intérêt grandissant envers l'analyse télémétrique du mouvement locomoteur quotidien, cet auteur (Sharkey, 2013) suggère également d'explorer la relation entre le mouvement locomoteur et le degré de dysfonctionnement locomoteur représenté par la mesure de la force verticale maximale, chez le chien arthrosique.

8.2 Abrégé méthodologique et hypothèses

La force verticale maximale a été mesurée chez des chiens atteints d'arthrose naturelle (n=40), puis de nouveau après que ceux-ci aient reçu un contrôle négatif (placebo) pendant une durée de quatre semaines. Les hypothèses furent les suivantes :

- La force verticale maximale maximale démontre une excellente fidélité, telle que représentée par un coefficient de corrélation intra-classe supérieur à 0.9.
- L'erreur de mesure de la force verticale est faible, soit inférieure à 1% de poids corporel.
- La valeur du seuil de changement minimal détectable (à un intervalle de confiance de 95%) de la force verticale maximale est cohérente avec le gain communément observé lors d'essais cliniques contrôlés, soit supérieur à environ 2.5% de poids corporel (**Tableau VI**).

La force verticale maximale a été mesurée chez des chiens atteints d'arthrose naturelle (n=33), puis de nouveau après que ceux-ci aient reçu un contrôle négatif (placebo) pendant une durée de six semaines. L'analyse télémétrique du mouvement locomoteur a également été effectuée en continu pendant six semaines. Les hypothèses furent les suivantes :

- Le mouvement locomoteur effectué durant six semaines démontre un lien proportionnel significatif (seuil alpha de 5 %) avec la force verticale maximale enregistrée à la semaine six.

- Le changement entre le mouvement locomoteur initial et celui enregistré à la semaine six démontre un lien proportionnel significatif (seuil alpha de 5 %) avec le changement de la force verticale maximale.

9 Modèle canin d'arthrose par sectionnement chirurgical du ligament croisé crânial : Relations entre la force verticale maximale et les dommages structuraux

9.1 Préambule

Chez le modèle canin d'arthrose par sectionnement chirurgical du ligament croisé crânial, divers processus pathomécaniques associés à l'instabilité articulaire (Tashman *et al.*, 2004; Pozzi *et al.*, 2013) induisent des dommages qui miment l'arthrose chez l'humain (Visco *et al.*, 1996). Le dysfonctionnement locomoteur qui en résulte se fait ressentir par une force verticale maximale anormalement basse (O'Connor *et al.*, 1989). Bien que le degré de dysfonctionnement s'améliore avec le temps, la force verticale maximale demeure anormale et ce, quatre ans suivant le sectionnement (Budsberg, 2001).

Le lien entre le dysfonctionnement locomoteur et la sévérité des dommages structuraux est actuellement méconnue chez ce modèle. Une association positive entre le degré de dysfonctionnement locomoteur, représenté par la force verticale

maximale, et l'étendue des dommages au cartilage articulaire a toutefois été suggérée (Smith *et al.*, 2005). Cette relation pourrait signifier qu'en l'absence de ligament croisé crânial, la sollicitation du membre locomoteur serait délétère envers une structure articulaire dépourvue d'afférence nociceptive comme le cartilage. En assumant que cette hypothèse soit fondée, il serait possible de prédire l'étendue des dommages au cartilage en connaissant le degré de dysfonctionnement locomoteur chez ce modèle. La récente caractérisation des dommages structuraux, à l'aide d'imagerie par résonance magnétique, porte à investiguer le lien entre le dysfonctionnement locomoteur et la sévérité des différents dommages.

9.2 Abrégé méthodologique et hypothèses

La force verticale maximale a été mesurée chez des chiens sains (n=5), puis de nouveau à quatre, huit et vingt-six semaines suivant le sectionnement chirurgical du ligament croisé crânial. Au même moment, l'articulation du genou fut soumise à l'imagerie par résonance magnétique afin d'évaluer les atteintes au cartilage articulaire (volume et lésions), les lésions à l'os sous-chondral et aux ménisques, de même que la taille des ostéophytes et le degré d'effusion articulaire. Les hypothèses furent les suivantes :

- Les changements au niveau des dommages structuraux (lésions au cartilage et à l'os sous-chondral, taille des ostéophytes et le degré d'effusion articulaire) entre la semaine vingt-six et la semaine quatre démontrent un lien inversement proportionnel significatif (seuil alpha de 5 %) avec le changement de la force verticale maximale.

- Les changements au niveau des dommages structuraux (volume du cartilage et lésions aux ménisques) entre la semaine vingt-six et la semaine quatre démontrent un lien proportionnel significatif (seuil alpha de 5 %) avec le changement de la force verticale maximale.

La force verticale maximale a été mesurée chez des chiens (n=25) huit semaines après le sectionnement chirurgical du ligament croisé crânial. A ce moment, l'articulation du genou fut soumise à l'évaluation macroscopique de l'étendue des lésions au cartilage. L'hypothèse est la suivante :

- L'évaluation macroscopique de l'étendue des lésions au cartilage démontre un lien proportionnel significatif (seuil alpha de 5 %) avec la force verticale maximale.

10 Article VII. A posteriori comparison of natural and surgical destabilization models of canine osteoarthritis

M. Maxim Moreau a proposé ce travail, a participé à l'acquisition des données, à l'analyse de ces dernières et a rédigé cet article présentement publié (*BioMed Research International*, Volume 2013, Article ID 180453). M. Moreau a également effectué l'ensemble des travaux d'infographie. L'article a par la suite été dûment révisé et bonifié par l'expertise de chacun des coauteurs.

BioMed Research International
Volume 2013, Article ID 180453

A Posteriori Comparison of Natural and Surgical Destabilization Models of Canine Osteoarthritis

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10.1 Summary

For many years *Canis familiaris*, the domestic dog, has drawn particular interest as a model of osteoarthritis (OA).

Here, we optimized the dog model of experimental OA induced by cranial cruciate ligament sectioning. The usefulness of non-invasive complementary outcome measures, such as gait analysis for the limb function and magnetic resonance imaging for structural changes was demonstrated in this model. Relationships were established between the functional impairment and the severity of structural changes including the measurement of cartilage thinning.

In the dog model of naturally-occurring OA, excellent test-retest reliability was denoted for the measurement of the limb function. A criterion to identify clinically meaningful responders to therapy was determined for privately-owned dogs undergoing clinical trials. In addition, the recording of accelerometer-based duration of locomotor activity showed strong and complementary agreement with the biomechanical limb function.

The translation potential of these models to the human OA condition is underlined. A preclinical testing protocol, which combines the dog model of experimental OA induced by cranial cruciate ligament transection and the dog model of naturally-occurring OA offers the opportunity to further investigate the structural and

functional benefits of disease modifying strategies. Ultimately, a better prediction of outcomes for human clinical trials would be brought.

10.2 Introduction

Biomedical research is the broad area of science that investigates the biological processes and the causes of diseases mainly through experimentation and testing. Enticing this vision, the use of animal models is required to advance medical knowledge and overall health benefits. In the field of rheumatic diseases such as osteoarthritis (OA), animal models contribute to the understanding of the basic biology of OA and help to develop potent therapeutic approaches for the benefits of human medicine [1]. Unfortunately, a consensus regarding the ideal animal model for studying OA has not been established [2-4]. Actually, there is a need to optimize current models of OA and to propose avenues to enhance preclinical drug development.

The canine stifle is similar to a human's knee, sharing anatomical components and histological aspects [5]. To give a deep insight in the OA mechanisms, the dogs have been subjected to several approaches over the years to induce the structural changes of OA, including cartilage scarification (or groove model) [6], transarticular impact [7], tibial osteotomy [8] and meniscal lesions [9]. Another well described dog model of OA is the cranial (or anterior) cruciate ligament (CCL) transection. Surgical CCL transection (CCLT) alters the amount and distribution of biomechanical forces. Over days to months, the joint features structural changes

that mimic OA, including synovitis, osteophyte growth, cartilage depletion and bone marrow lesions (BMLs) development [10].

The conventional scientific outputs (*i.e.*, joint structural changes) of the experimental dog model of OA induced by CCLT has been recently coupled to peak vertical force (PVF) measurement using kinetic gait analysis to document concomitant potential benefits on the pain-related functional impairment [11-13]. The first aim of this study was to optimize the experimental CCLT-induced dog OA model by further exploring the translational relationship between the level of structural changes and the limb disability.

Developmental arthropathies and joint trauma predispose dog to structural changes of OA, which like in human beings lead to crippling pain and disability [14-16]. The potential of pharmaceutical as well as complementary and alternative medicines have been tested in different randomized controlled trials (RCTs) in naturally-occurring OA dogs using PVF as an outcome measure of pain-related functional impairment [17-20]. Naturally-occurring models of OA have been proposed even to accelerate the development of human therapeutics [10]. As a second aim, this study would characterize different outcome measures in a manner to optimize the use of naturally-occurring OA dogs in research and to improve the quality of RCT in this translational natural model.

10.3 Materials and Methods

10.3.1 Dog model of experimental OA

10.3.1.1 Specific research objectives

The evolution of the PVF measurement and its relationship with the progression of structural changes evaluated on magnetic resonance imaging (MRI) scans (*i.e.* cartilage volume loss, focal changes of the articular cartilage, BMLs, osteophytes, joint effusion size and meniscal lesions) was documented over a period of 26 weeks in CCL-deficient dogs. In addition, the relationship between PVF recording and the macroscopic measurements of cartilage thinning performed at eight weeks following CCLT was documented cross-sectionally. Such relationship served to determine the level of *in-vivo* structural changes to be predicted based on a given PVF measurement. To this end, data were selected from previous studies involving PVF measurement and structural changes on MRI (internal data, 2005) [21-23] and macroscopic measurement of cartilage thinning (internal data 2005, 2007) [11, 12] (**Figure 27A**).

All experiments were approved by the Institutional Animal Care and Use Committee in accordance with the guidelines of the Canadian Council on Animal Care. All dogs were acclimated, housed and then subjected to surgical CCLT of the right knee under pre-emptive (transdermal fentanyl 50 or 75 μg/h; Janssen Ortho, Markham, Ontario, Canada) and multi-modal (intra-articular block combined with opioid administration) analgesia as previously described [11]. Food was given once

daily and removed overnight. Body weight was monitored weekly and was kept constant throughout the study duration. Throughout the study, all dogs were actively exercised in exterior runs (1.35 m × 9.15 m) for a 2-hour period, 5 days a week, under the supervision of an animal care technician.

10.3.1.2 Peak vertical force measurement

Recognized as a reference method of functional outcome in dog [24-26], the PVF measurement was done at the trot (1.9-2.2 meter/second) using a floor mat-based plantar force measurement system (Walkway® with four Matscan® sensors 3150; Tekscan Inc, Boston, Massachusetts, USA), as previously described [11]. Data were acquired at four successive time points (**Figure 27A**). For the CCL-deficient hind limb, the first stride PVF was acquired. Data from the first five valid trials were averaged and expressed as percentage of body weight (%BW) and used to describe the change over time in PVF measurement.

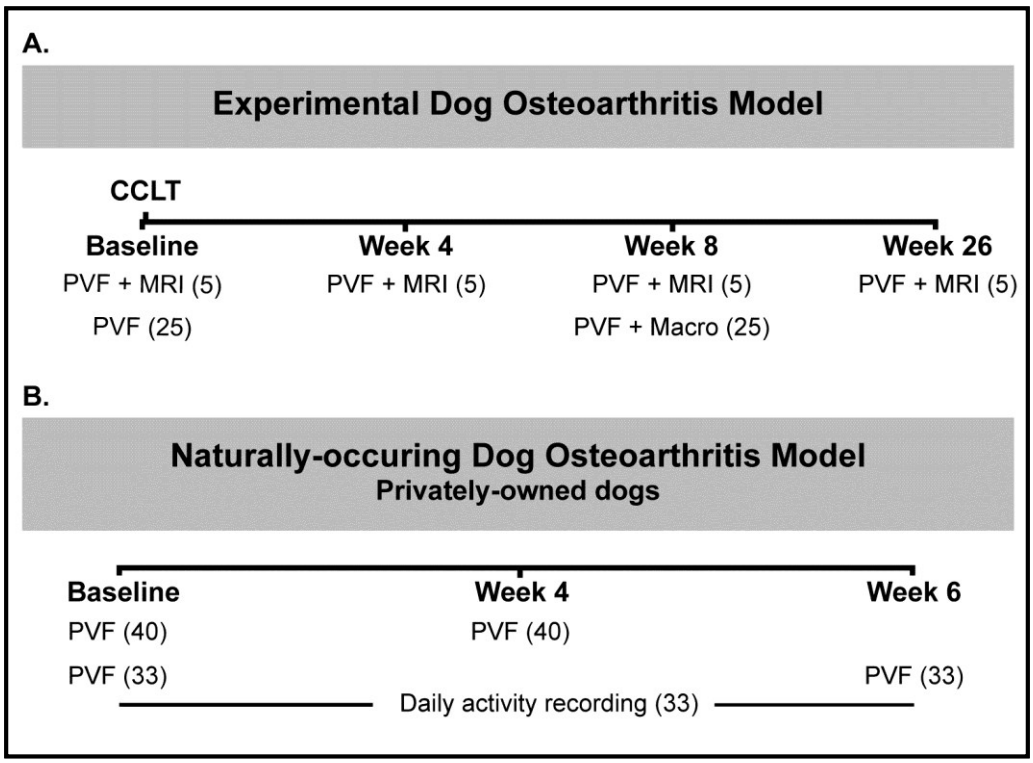

Figure 27. Schematic view of the data reported in A) the dog model of experimental osteoarthritis and B) the dog model of naturally-occurring osteoarthritis

PVF (peak vertical force), MRI (magnetic resonance imaging), macro (macroscopic structural measurement of cartilage thinning), CCLT (cranial cruciate ligament transection). Numbers of dogs are specified in parenthesis.

10.3.1.3 Magnetic resonance imaging

Structural changes were evaluated at four successive time points (**Figure 27A**) using MRI scans (Echospeed LX; General Electric Healthcare, Waukesha, Wisconsin, USA) and settings as previously described [21]. Previous publications detailed the quantification of cartilage volume (mm^3) [21] and the scoring system used for focal changes of the articular cartilage (0-4, maximum score of 44) [21],

BMLs (0-3, maximum score of 27) [23], osteophytes (0-3, maximum score of 45) [22], joint effusion size (0-3) [22], and meniscal lesions (0-3, maximum score of 6) [27]. Cartilage volume and structural changes were evaluated using the following sequences: 1) three-dimensional spoiled gradient recalled sequence (SPGR) with fat suppression, 2) T1-weighted three-dimensional fast gradient recalled echo (T1w-GRE) and 3) T2-weighted fast spin echo sequence with fat saturation (T2w-FS). Bone marrow lesions were scored independently in T1w-GRE and T2w-FS sequences as ill-defined areas of hypointensity or hyperintensity, respectively. Evaluation of cartilage volume and structural changes were for the entire (global) joint, except otherwise stated.

10.3.1.4 Macroscopic measurement of cartilage thinning

Cartilage thinning was quantified at eight weeks following CCLT (**Figure 27A**) using a dissecting microscope (Stereozoom; Bausch & Lomb, Rochester, New-York, USA) as previously described [28]. Macroscopic measurement of cartilage thinning (mm^2) was for the medial and lateral femoral condyles and medial and lateral tibial plateaus.

10.3.2 Dog model of naturally-occurring OA

10.3.2.1 Specific research objectives

The repeatability, standard error of measurement (SEM) and minimal detectable change (MDC) of PVF measured in privately-owned dogs affected by naturally-occurring OA were defined over a four-week period. Moreover, the PVF measurement was tested for its relationship with accelerometer-based duration of

daily locomotor activity. The goal of testing such relationship was to determine the level of PVF measurement exceeding the MDC to be predicted based on a change in daily locomotor activity, which would represent a practical outcome of animal welfare determinant at home [29]. To this end, data were selected from previous studies involving PVF measurement (40 placebo-treated dogs followed over four weeks) [17, 19, 30] or PVF measurement coupled to daily locomotor activity recording over six weeks (33 dogs, from which 14 were placebo-treated) (internal data, 2007) [17] (**Figure 27B**). All studies were approved by the Institutional Animal Care and Use Committee in accordance with the guidelines of the Canadian Council on Animal Care. All owners gave informed consent for their participation in each RCT.

10.3.2.2 Naturally-occurring OA dogs

Seventy-three privately-owned adult dogs weighing more than 20 kg having radiographic evidence of OA exclusively at the hip and/or stifle joints were considered, as previously described [17, 19, 30]. All dogs had OA-related hind limb disability according to orthopedic examinations and PVF measurements. Specific washout periods were respected for eventual OA treatment (including pharmaceuticals, natural health products and therapeutic diets).

10.3.2.3 Peak vertical force measurement

The PVF measurement was done at the trot (1.9-2.2 meter/second) using a force platform (Model OR6-6, Advanced Mechanical Technology Inc, Watertown,

Massachusetts, USA), as previously described [17, 19, 29, 30]. Measurements were done at different time points (**Figure 27B**). Averaged data from the first five valid trials were expressed as % BW. In each dog, the hind limb with the lowest PVF measurement was used for statistical analyses purpose.

10.3.2.4 Daily locomotor activity recording

Accelerometer-based daily locomotor activity recording was done using Actical® system (Bio-Lynx Scientific Equipment Inc., Montreal, Quebec, Canada) as previously described [17]. Collar-mounted accelerometers were worn by 33 dogs for six weeks, 24 hour/day (**Figure 27B**). The duration of motion was continuously monitored as counts every two minutes, giving 720 counts per day. Daily duration of active period was referred to the time spent (expressed in minutes) when the count exceeded 30 in term of intensity. This cut off value was based on internal data (2004) in comparison to video-analysis, and was previously used to discern movement in active (intensity > 30) from inactive (intensity < 30) period [17]. Data used were the area under the curve (AUC) which represents the integral of the daily duration of active period over six weeks and the mean of the first three days, and of the last seven days, which defined Baseline and week six data, respectively [31].

10.3.3 Statistical analyses

To describe the evolution of limb function in CCL-deficient dogs, a Friedman test was used using Dunn's tests for *post hoc* analyses. To describe the relationship between the limb function with MRI structural changes, data were analyzed with

Spearman correlation test and presented as Spearman coefficient (r_s). This coefficient shows by its magnitude the strength of the linear association. An r_s close to one (or minus one) indicates a strong positive (negative) linear correlation. To describe the relationship between the limb function (explanatory variable) with macroscopic measurement of cartilage thinning (response variable), data were analyzed with mixed linear model using studies as random effect. Random-effect models attempt to generalize findings beyond the included studies by assuming that the selected studies are random samples from a larger population. Such models incorporate a component of between-study variation into the uncertainty of the estimates [32]. The general equation of the linear regression was $y = mx + b$, where m refer to the slope and b to the y-intercept (*i.e.*, the value of y when x = zero). To describe the natural fluctuation in limb function of placebo-treated (negative control) privately-owned dogs, absolute reliability (test-retest) was calculated using intra-class coefficient of correlation (ICC) and related 95% confidence intervals (95% CI). Two-way random single measures model (ICC 2.1) was used. An ICC close to one indicates 'excellent' reliability [33]. The SEM quantifies the precision of individual PVF measurement and defines the boundaries around which a subject's value is expected to lie according to a given confident interval [34].

The SEM at 95% CI was calculated as follows:

$SEM = SD*\sqrt{1-ICC}$

where SD referred to the within-subject standard deviation [35].

The MDC in PVF measurement that can be recorded confidently (95% CI) between test sessions is referred as the MDC_{95} and was calculated as follows:

$MDC_{95} = SEM*1.96*\sqrt{2}$

The MDC_{95} can be interpreted as the magnitude of change, below which there is more than a 95% chance that change has occurred as a result of measurement error [36]. Outside this change, value does reflect a real alteration in the functional impairment toward improvement or worsening in privately-owned dog with naturally-occurring OA. To describe the relationship between the limb function (response variable) with daily locomotor activity recording (explanatory variable), data were analyzed with mixed linear model using study arms (placebo or tested agent) as fixed factor and studies as random factor. All analyses were performed with SPSS, version 20.0 (SPSS Inc., Chicago, Illinois, USA). Values are presented as mean (standard deviation). Significant level was set at $p<0.05$.

10.4 Results

10.4.1 Dog model of experimental OA

10.4.1.1 Measurement of the PVF

Peak vertical force measurement changed over time (p<0.003) following CCLT (**Figure 28**). Based on medians, there was a significant decrease at week four and at week eight when compared to Baseline. Then, PVF increased at week 26, reaching values significantly different than week four only. The individual changes over time involved different degrees of functional impairment characterized by a decrease in PVF measurement from Baseline reaching a nadir **(**at week four) followed by a phase of remission (from week four to week 26, **Figure 29**).

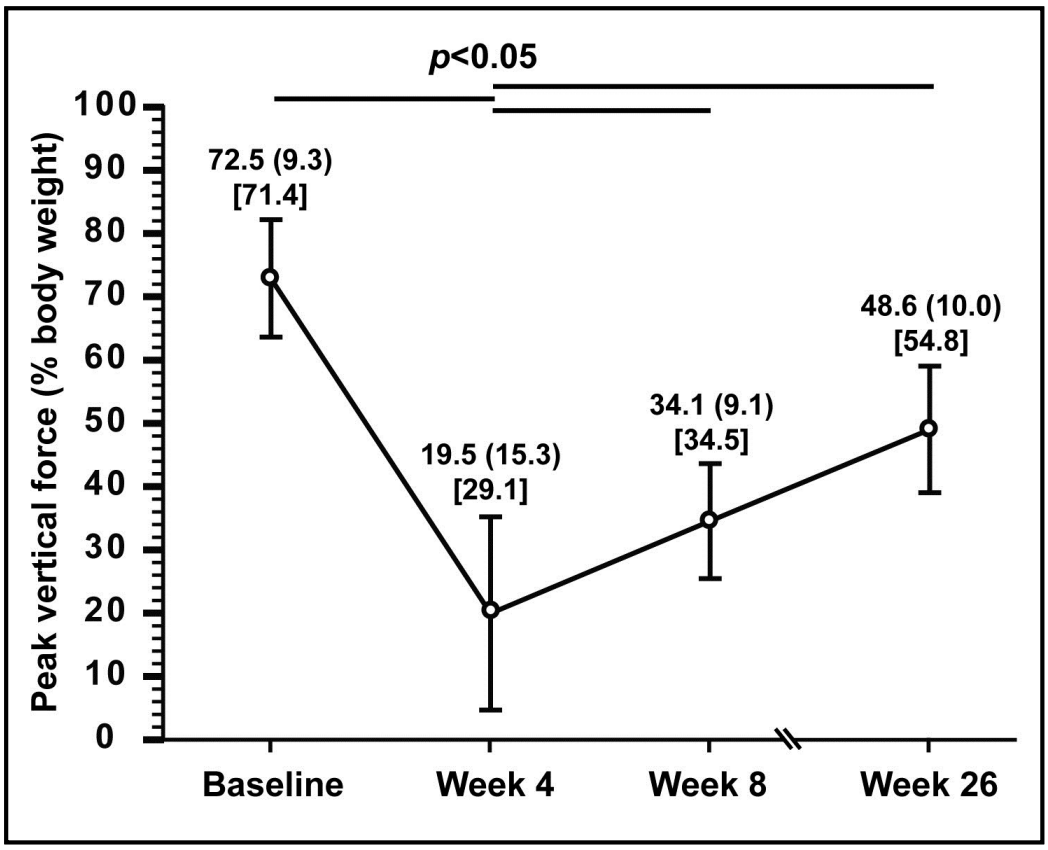

Figure 28. Averaged peak vertical force values measured before (Baseline) and four, eight and 26 weeks after cranial cruciate ligament transection in dogs

At each time point, group values are presented as mean (standard deviation) [median]. Dunn's test identified which medians were significantly different.

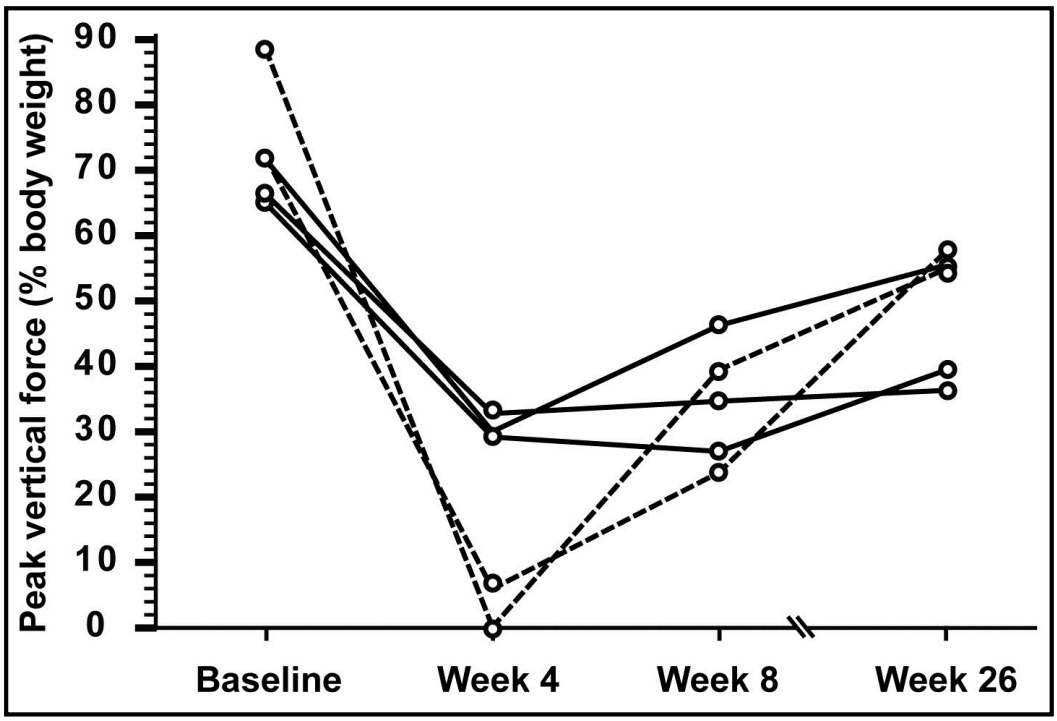

Figure 29. Individual peak vertical force values measured before (Baseline) and four, eight and 26 weeks after cranial cruciate ligament transection in dogs

Dotted lines identify dogs having the highest limb disability at week four and the highest levels of focal changes of the articular cartilage.

10.4.1.2 Relationship between PVF and structural changes on MRI

During the phase of functional impairment nadir **(**from Baseline to week four), the decrease in PVF measurement did not correlate in a significant manner with the development of structural changes as evaluated using MRI (**Tableau XIII**). Of note, the dogs having the more severe limb disability at week four (**Figure 29**) were those with the highest level of focal changes of the articular cartilage.

The increase in PVF measured during the phase of remission (from week four to week 26) correlated inversely with the score for osteophytes, joint effusion, hypointense BMLs **Figure 30**) and focal changes of the articular cartilage (**Tableau XIII**). These negative correlations mean an abrogated remission in the presence of severe chondral and subchondral lesions, and MRI-scored joint effusion.

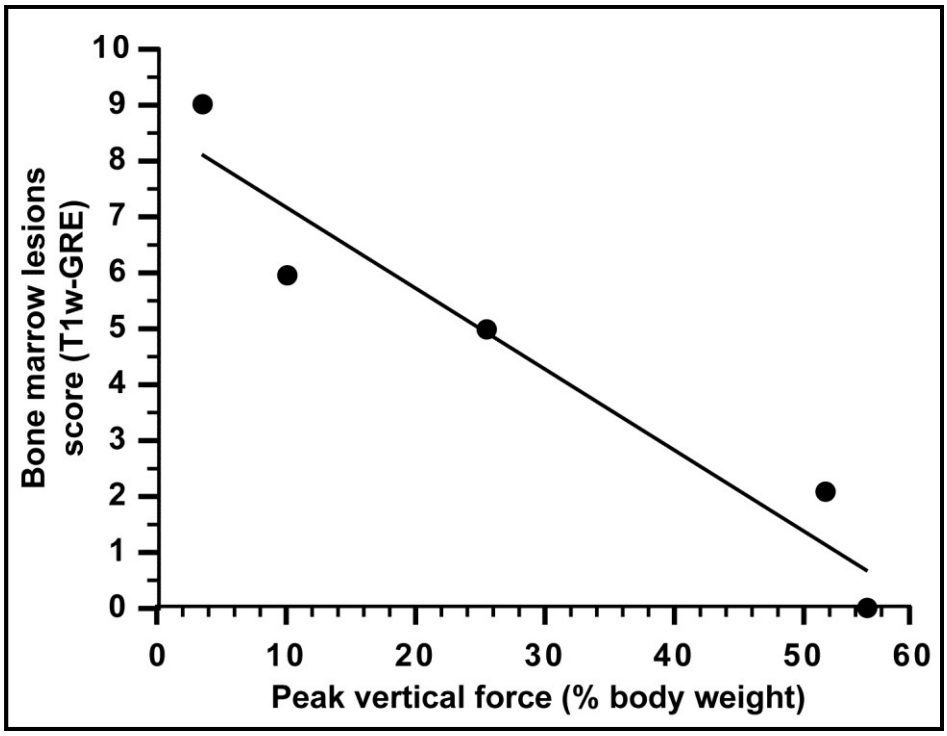

Figure 30. Significant correlation (r_s -0.99, $p<0.001$) for the differences of hypointense bone marrow lesions on T1-weighted three-dimensional fast gradient recalled echo images (T1w-GRE) scores during the remission phase (week 26 minus week four), with the concurrent difference in peak vertical force measurement

Linear regression trend is illustrated.

The measurement of PVF did not correlate with cartilage volume loss, hyperintense BMLs or meniscal tears. Only a trend was seen for a positive correlation with medial cartilage volume loss (the more PVF remission was, the more cartilage volume loss was) and medial tears score of the meniscus (**Tableau XIII**).

Tableau XIII. Correlation analyses of the change in peak vertical force measurement and magnetic resonance imaging over the different phases of functional impairment before (Baseline) and following cranial cruciate ligament transection in five dogs

				BMLs			
	Osteophytes	**Joint effusion**	**Focal Cartilage changes**	**T2w-FS**	**T1w-GRE**	**Meniscal tears**	**Cartilage Volume loss**
				Phase of functional impairment nadir			
r_s	-0.05	-0.26	-0.70	-0.70	-0.70	0.01	-0.40
p	NS	NS	NS	NS	NS	NS	NS
				Phase of remission			
r_s	-0.90	-0.95	-0.97	-0.70	-0.99	0.79	0.60
p	0.037	0.013	0.004	NS	<0.001	p=0.1	p=0.1

Non significant at 5% level (NS)

Spearman coefficients (r_s)

Probability value (p)

Bone marrow lesions (BMLs)

T1-weighted three-dimensional fast gradient recalled echo (T1w-GRE)

T2-weighted fast spin echo sequence with fat saturation (T2w-FS)

The changes in the phase of functional impairment nadir were calculated using week four values minus Baseline. The changes in the phase of remission were calculated using week 26 values minus week four.

10.4.1.3 Relationship with macroscopic measurement of cartilage thinning

Twenty-five dogs undergoing PVF measurement before and after CCLT were used. At Baseline, PVF measurement was 70.4 (10.9)% BW and was 26.6 (12.4)% BW and 33.9 (15.8)% BW at week four and eight, respectively. The PVF measured at week eight did not demonstrate significant relationship with cartilage thinning observed on the lateral condyle and plateau, and medial plateau (**Tableau XIV**). However, a significant relationship was observed with the severity of the thinning at the medial condyle, which means higher PVF value in the presence of more severe cartilage thinning. According to the regression parameters (**Tableau XIV**), for a group of CCL-deficient dogs weighing 25.0 (2.3) kg, a PVF measured at week eight of 33.9 (15.8)% BW is expected to correspond to an extent of cartilage thinning on the medial condyle of 27.3 mm2 [95% CI: 10.4-44.2].

Tableau XIV. Regression analyses between the recording of the peak vertical force and macroscopic measurement of cartilage thinning at eight weeks following cranial cruciate ligament transection in 25 dogs

Compartments			
Lateral condyle	**Lateral plateau**	**Medial condyle**	**Medial plateau**
NS	NS	p=0.002 m=0.8 [95% CI : 0.3-1.3] b=0.2 [95% CI : -25.7-26.0]	NS

Non significant at 5% level (NS)
Regression slope (m)
Regression y-intercept (b)
95% confidence intervals (95% CI)

10.4.2 Dog model of naturally-occurring OA

10.4.2.1 Characteristics of PVF measurement

Forty privately-owned dogs affected by OA who received a placebo (negative control) served to determine the test-retest reliability of PVF measurement over a period of four weeks (**Table 15**). Standard error of measurement was determined and served for the calculation of MDC_{95}. The MDC_{95} was consistent with an increase or a decline in the magnitude of 2.0% BW across this group of OA dogs. When expressed relatively to Baseline value, the MDC_{95} represented 3.6%. The **Figure 31** presents individual changes in PVF measured from Baseline to week four. According to the MDC_{95}, 22 dogs had clinically meaningful changes, which were positive in five dogs and negative in 17 others.

Tableau XV. Characteristics of peak vertical force measurement in 40 privately-owned dogs affected by naturally-occurring osteoarthritis

Baseline	Week 4	ICC [95% CI]	SEM	MDC_{95}
56.0% BW (7.5) [26.1-66.1]	54.5% BW (8.4) [23.6-64.9]	91 [80-95]	0.7% BW	2.0% BW

Values are presented as mean (standard deviation) [minimal value-maximal value]

Intra-class coefficient of correlation (ICC)

Standard error of measurement (SEM)

Minimal detectable change at the 95% confidence level (MDC_{95})

Percentage of body weight (% BW)

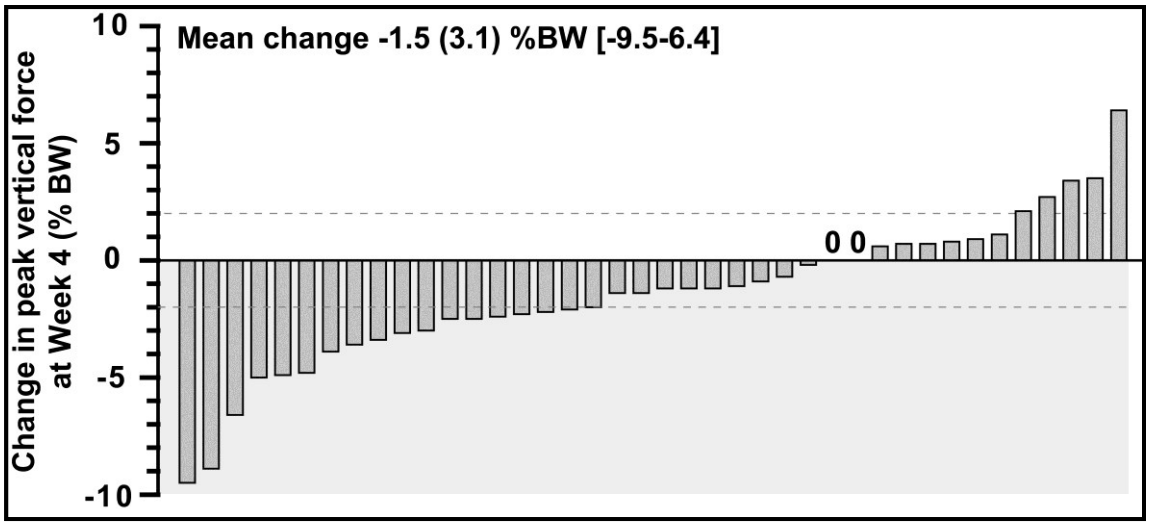

Figure 31. Individual changes in peak vertical force measured at week four in 40 privately-owned dogs receiving a placebo in randomized controlled trials

Changes were the difference between week four versus Baseline. Grey zone represent a decrease in peak vertical force measurement compared to Baseline. Dashed lines represent the MDC_{95}. Peak vertical force data are expressed in % BW (body weight) and presented as mean (standard deviation) [minimal value to maximal value].

10.4.2.2 Relationship with daily locomotor activity recording

Thirty-three privately-owned dogs affected by OA that had PVF measurement and daily locomotor activity recorded over a six-week period were used. The PVF measurement in OA dogs demonstrated a significant relationship with the integral (AUC) of the daily duration of active period recorded during the 6-week period (p=0.001), which means a higher PVF in the presence of higher locomotor activity. The change in the PVF measurement demonstrated a significant relationship with the change in daily duration of active period (p=0.003, m=0.03 [95% CI: 0.01-0.05], b=2.8 [95% CI: 0.4-5.1]) regardless of the study arms (*i.e.*, placebo or tested agents). According to the regression parameters, for an increase in daily duration of active period by 54 minutes in OA dogs, the change in PVF measurement was predicted to be 4.4% BW [95% CI: 2.1-6.8]. This by far exceeds the previously defined MDC_{95} (*i.e.*, 2.0% BW), meaning a significant positive effect in PVF measurement (limb function) related to the increase in locomotor activity.

10.5 Discussion

10.5.1 Dog model of experimental OA

The CCLT dog model of OA involves structural changes that mimic those encountered in human OA [2, 10, 37]. This model was further optimized keeping in mind the three Rs' principles of Replacement, Reduction and Refinement [38]. The present study demonstrated the usefulness of complementary outcome measures. Hence, PVF measurement, which echoes pain-related functional impairment, can be successfully combined to the common structural outcomes in the CCLT dog model

of OA. To maximize the information gained from CCL-deficient dogs, researchers can document in a non-invasive manner the pain-related disability, which comprises a phase of functional impairment with a nadir preceding a remission process. At the preclinical stage of drug development, such information has a clinically meaningful potential for disease modifying compounds proposed to confer pain and functional improvement in addition to structural benefits. As PVF measurement data were detailed, power and sample size can be determined *a priori*, again supporting the principle of reduction by providing statistical estimates. The principle of refinement is also addressed by documenting individual variability (per dog data) that occurs over an extended duration [39].

Anterior cruciate ligament (human counterpart of CCL) transection leads to rotational and translational changes that induce mechanical stresses on articular surfaces unaccustomed for such loading solicitation [40-44]. This phenomenon generates loss of tissue integrity, involving abnormal architecture and components known as structural changes. Using MRI, which is a non-invasive imaging technique, the present study supports the use of the CCLT dog model of OA for its potential to corroborate the relationship between structural changes and clinical signs observed in human. Hence, a recent systematic review indicated that OA knee pain is associated with BMLs and effusion/ synovitis [45]. In line with findings observed in humans [46, 47], severe limb impairment was denoted in dogs with the highest level of focal changes of the articular cartilage during the phase of limb disability (from Baseline to week four). When BMLs evolved minimally, these

manifestations were concomitant to lesser limb disability (**Figure 30**). Such benefits are in accordance with the report of Zhang [48] who observed a fluctuation of pain when BMLs were modulated. We also observed that CCL-deficient dogs had better limb functional remission when osteophyte growth and joint effusion size were minimal. These findings were suggestive of higher pain in the presence of osteophyte and effusion/ synovitis as reported in human [45, 49, 50]. In face of the present results, we propose to integrate the pain-related functional impairment to the presence of severe chondral and subchondral lesions. We suggest that a more unstable joint (*i.e.*, devoid of adaptive neuromuscular strategies to palliate for the excessive displacement) could be responsible for the more severe chondral and subchondral changes observed. As an attempt to restore limb function, marked expressions of secondary strategies, such as osteophytes growth and joint effusion, are suggested to develop for providing stability and cushioning, in a manner to minimize the deleterious knee joint load in CCL-deficient dogs.

Although we did not reach statistically significant levels, our findings are suggestive of a role of mechanical environment in cartilage volume loss and meniscus insult in CCL-deficient dogs. Hence, we found a trend to have more severe cartilage loss and meniscal tears in the medial part of the joint in dogs having recovered well their limb function (*i.e.*, with highest PVF measurement). In addition, the extent of medial cartilage thinning was greater in dogs having the highest limb function at eight weeks following CCLT. Those findings were in line with those of Smith [13], which reported a link between the level of knee joint

chondropathy and increasing limb function in this model. Furthermore, meniscal lesions have been linked with the progression of OA cartilage loss in humans [51, 52] while a strong relationship exists between high joint loading and meniscal lesions [53]. These findings are of major importance, not only because of their correspondence to findings in human knee OA, but also because the presence of meniscal lesions has an impact on the response to disease-modifying OA drug (DMOAD) treatment in human knee OA [46, 51], a finding that likely also applies to the CCLT dog model of OA.

Despite its burgeoning importance, translation of DMOAD therapies from the laboratory into clinical practice has slowed. Differences between the OA models studied preclinically and the disease evaluated in human clinical trials contribute to this failure [54]. First, a general concern is the use of quadruped animals as knee models for the bipedal human, particularly given their range of motion differences noted in a study comparing large animal (cow, sheep, goat, dog, pig and rabbit) to human cadaveric knees [55]. The disappearance of many of the observed differences in the cruciate and meniscal anatomy after normalization with the tibial plateau width suggested an overall conservation of relative size among species for the cruciates and menisci [55]. This anatomical and biomechanical analogy, while reviewing the different OA animal models, led Gregory [3] stating that the canine model is probably the closest to a gold-standard animal model for OA currently available. The present study adds the structure – function relationship translated from the dog CCLT model to the human pain OA condition.

Second, most animal models of OA induce disease through chemical insult, or surgical or mechanical disruption of joint biomechanics in young individuals rather than the spontaneous development of the disease. This instability-induced joint disease in animals best models the structural changes that develops in humans after an injurious event, known as post-traumatic OA [54]. The poor translational predictability to therapy response is particularly high with the rodent preclinical models. Studies in genetically modified mice suggest that post-traumatic OA has a distinct molecular pathophysiology compared with that of spontaneous OA, which might explain the poor translation from preclinical to clinical OA therapeutic trials [54]. On the contrary, molecular changes observed in a past study with the canine CCLT model suggest that dog cartilage responds to post-traumatic OA and degenerative conditions by regulating the same genes in a similar direction as that observed for chondrocytes in post-traumatic and late human OA [56]. Finally, the recent finding about the DMOAD effects of strontium ranelate [57] late in the CCLT dog model of OA being confirmed in a Phase III clinical trial in knee OA patients [58] is of the utmost importance in the context of this publication. Previously, many DMOADs have demonstrated efficacy in the dog OA model, including the matrix metalloproteinase inhibitor doxycycline [59, 60], the viscosupplementation *via* local hyaluronan [61, 62], the anti-resorptive agents such as bisphosphonate [11, 63] and calcitonin [64], the anti-inflammatory properties of diacerhein [65], licofelone [28] and NSAIDs (such as carprofen, *etc.*). All these products, but calcitonin (probably related to a deficient formulation) demonstrated

similar efficacy in human OA [51, 66-73]. To the best of authors' knowledge, no other preclinical animal OA model presents a better translational predictability record, partly because species differences with respect to the relative contribution of various mediators, receptors or enzymes to the pathology and xenobiotics metabolism are common.

In accordance with the three Rs' principles, the predictive character of the cartilage thinning based upon PVF measurement opens the idea of limiting the requirement of post-mortem analysis for future research aimed to gain insight in joint cartilage integrity in this model. Based on the regression parameters, the limb disability observed at eight weeks following CCLT predicted an extent of macroscopic lesions surface by 27.3 mm^2. This level of lesions represents 12% of the total surface of the medial condyle when based on MRI cartilage surface mapping in dogs of similar BW (ArthroVision, personal communication, 2013). As characterization of full-thickness cartilage thinning in end-stage OA in humans were shown to range between 10-23% at this joint compartment [74], the translational potential (macroscopical structural argument) of this model to human OA is further supported.

It should be pointed out that the statistical method used to correlate structural changes on MRI with PVF measurement does not pinpoint the sequence of events, and did not take into account the potential role of confounding factors, interrelationship and dependency. Findings of the pilot study reported herein will

help to promote future research of a more mechanistic (structure – function) approach based on a higher sample size. The complementary outcome measures proposed herein to optimize the use of the dog in OA research is not restricted to the CCLT model. Other experimental avenues should be explored for their potential to induce structural changes in close relationship with functional impairment.

10.5.2 Dog model of naturally-occurring OA

The recent interest in natural models of OA [10] put more emphasis on the need to improve the rigor of RCT using functional outcome measures, such as PVF, in naturally-occuring OA dogs. This study optimized the use of naturally-occurring OA dogs in research by characterizing the PVF measurement with regards to the high value of this outcome to address pain/ biomechanics-related joint alterations in the dog. Here test-retest PVF measurement values demonstrated excellent between-session reliability with an ICC of 91 [95% CI: 80-95] in placebo-treated dogs followed over a four-week period. The SEM provides an absolute index of reliability and refers to the precision of individual measurements. Determining magnitude of an intervention benefit is a critical methodological step in the design of a clinical trial. For the PVF, the MDC_{95} indicated that a change of at least 2.0% BW needs to occur to be confident, at the 95% level, that a change in PVF measurement reflects a real change and not a difference that is within what might be reasonably expected given the measurement error (noise). As PVF measurement characteristics were provided, such as standard deviation, SEM, and the MCD_{95}

such data should help researchers to estimate power and sample size, thus contributing to the principle of refinement.

Randomized controlled trials in naturally-occurring OA dogs usually focus on testing mean changes across groups of treated (test article) and control (placebo-treated) animals. This practice often obscures the individual change, which may be very informative in clinical studies [75]. Moreover, reporting the percentages of subjects who met the MDC_{95} requirements provides additional insightful interpretations other than considering only the overall mean change scores [76]. Accordingly, researchers have a tool to distinguish improved (or worsened) dogs by using the proposed MDC_{95} value as a cut-off. Of note, the level of 2.0% BW was in line with the improvement observed following therapeutic modalities in previous clinical RCT in OA dogs [17-20, 30, 77-80].

The results of the current study show that different levels of change in limb function reflected by PVF measurement were observed in privately-owned dogs afflicted by OA (**Figure 31**). Among the 40 dogs evaluated, 22 (55%) had clinically meaningful changes, which were positive (placebo effect) in five (12.5%) or negative (nocebo effect) in 17 (42.5%) dogs. The high proportion of dogs having a worsening of their condition contributes mainly to the overall decrease in PVF recording by -1.5 (3.1)% BW. A phenomenon known as the maturation effect may be suggested as being involved in changes exceeding the measurement error.

In a recent multicenter RCT in naturally-occurring OA, an arbitrary cut-off value (*i.e.*, 2.8% BW or ≥5% of Baseline measurement) was used to distinguish clinically meaningful responders from measurement error [81]. Of note, the global rate of responders reported according to this value was 20.7% whereas it was higher in our study (55%) by applying the MDC_{95} (calculated to be 2.0% BW or ≥3.6% of Baseline measurement). The latter finding is important, as applying the higher arbitrary cut-off value rather than the MDC_{95} proposed herein would lead to a high false negative rate of responders (being indeed considered as non-responders). This type II error overestimates the required sample size, and leads to an unnecessary high number of dogs affected by OA to be recruited in the RCT. It should be noted that the low placebo responder's rate (12.5% in the current study, 12.1% [81]) observed according to the objective PVF measurement again contributes to a judicious use of privately-owned dogs in RCT. This is a huge advantage compared to subjective assessment completed by either veterinarians or owners, for whom the placebo responder's rate was oscillating between 25 and 44.8% [81, 82].

As previously demonstrated in naturally-occurring OA dogs [17, 29, 31, 77] the usefulness of continuous monitoring of daily locomotor activity recording was sustained in the current study. Particularly, we denoted that continuous activity recording showed strong similarities with PVF measurement, being sensitive to functional improvement. This tool is therefore highly recommended to be used as a complement to punctual PVF measurement in a way to improve the detection of therapeutic benefits in OA dogs. In addition, the present results support the

relevance of naturally-occurring OA dogs for their potential to respond similarly to the human OA condition. This was illustrated in recent studies in which an anti-inflammatory drug, licofelone, was tested positively both in the dog model of experimental OA [28], naturally-occurring OA [30] as well as in a clinical Phase III study in patients with knee OA [51]. Similar concordance in efficacy was observed with doxycycline [59, 60, 71].

Hence, dogs with the most severe limb impairment were those with the lowest degree of daily activity. This was in line with findings in human OA reporting lower physical activity in more afflicted patients [83]. Present data also give a first impression of potential benefits of an increase by 54 minutes in daily life activity being mirrored confidently with an increase in PVF that exceeded the measurement error. This was recently supported in dogs with hip OA, showing a better condition when more than an hour of exercise was performed daily [84]. Human data are also in accordance with this finding as physical activity programs are supported to reduce pain, to improve physical performance, and to delay disability among persons with knee OA [85-87].

10.6 Conclusion

Biomedical research and testing often faces criticism and protestation against the use of dogs for research purposes. As for any animal experiments, the three Rs' principles must apply. In addition, findings from ideal animal models have to be rapidly translated to human characteristic with the ultimate hope to better predict

outcomes for human clinical trials. With this idea in mind, we present an optimization of the outcomes gained from the dog model of OA induced by CCLT. The relationship between structural changes and functional impairment denoted strong similarities with the human OA condition. This adds to the recognized anatomical and biomechanical, genomic, molecular, histological and macroscopical structural similarities to human OA, as well as to the access of yet validated and performing functional and imaging outcome measures, as reported in the present manuscript.

Regarding the dog model of naturally-occurring OA, the present analysis provides compelling evidence to better interpret complementary outcome measures assessing the OA condition: The PVF measurement data particularly is robust, precise and reliable for determining whether a change has taken place as a result of an intervention. Moreover, the data support the huge interest and applicability of monitoring the level of daily locomotor activity in clinical RCT with privately-owned OA dogs. Such natural model of OA in dog represents a spontaneous model of the disease, different and complementary to the post-traumatic OA model. At the difference of the standardized preclinical CCLT dog model, the conditions are close to those of a population pharmacological study integrating, in addition to the previously listed advantages, the genomics and environmental (such as the physical activity, the nutrition, *etc*.) influences of the disease.

Preclinical testing protocol combining the dog model of OA induced by CCLT and the dog model of naturally-occurring OA could better predict outcomes for human clinical trials in a close future, as is supported by the high translational pharmacological responsiveness.

10.7 Conflict of Interests

The authors declare that they have no conflict of interest in the research

10.8 Acknowledgements

Authors are grateful to Dr Jean-Pierre Raynauld, MD for his valuable and insightful comments throughout the writing process of the manuscript. M. Maxim Moreau received a doctoral scholarship from the Canadian Institutes of Health Research – Strategic Training Program (MENTOR) and a doctoral scholarship from the Fonds de recherche du Québec-Santé. This work was supported in part by an ongoing New Opportunities Fund grant (#9483) and a Leader Opportunity Fund Grant (#24601) from the Canada Foundation for Innovation (Eric Troncy) for the pain / function equipment, by the Osteoarthritis Chair of the University of Montreal Hospital Centre, Université de Montréal, and a Discovery Grant (#327158-2008) from the Natural Sciences and Engineering Research Council of Canada (Eric Troncy) for the bioanalyses and salaries.

10.9 References (Article VII)

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11 Modèle canin d'arthrose naturelle : Analyse de répondants selon la force verticale maximale

11.1 Préambule

En contexte d'essais cliniques contrôlés, l'utilisation de la force verticale maximale comme critère primaire d'efficacité a permis d'obtenir des évidences cliniques probantes, comme en témoignent de récentes révisions systématiques de la littérature (Aragon *et al.*, 2007; Sanderson *et al.*, 2009; Vandeweerd *et al.*, 2012). Généralement, les approches thérapeutiques sont des diètes thérapeutiques vétérinaires, des produits de santé naturels ou des AINS. Par contre, ce qui est actuellement méconnu, c'est le taux de répondants à diverses approches proposées face au dysfonctionnement locomoteur du chien arthrosique. Également, la relation entre la réponse thérapeutique et le degré de dysfonctionnement locomoteur initialement observé n'a jamais été explorée.

La valeur du seuil de changement minimal détectable (à un intervalle de confiance de 95 %) a été établie pour la mesure de la force verticale maximale chez le chien arthrosique (**10.4.2.1 ci-dessus - Chapitre 2**). Une analyse rétrospective est proposée afin de déterminer la présence d'un lien entre le taux de répondants et le niveau de dysfonctionnement locomoteur initial. La présence d'un lien entre le taux de répondants et le type de traitement administré (diètes thérapeutiques, produits de santé naturels, AINS ou contrôle négatif (placebo)) est également investiguée.

11.2 Hypothèses

Le taux de répondants, défini selon la valeur seuil du changement minimal détectable (à un intervalle de confiance de 95 %), démontre un lien statistiquement significatif avec le dysfonctionnement locomoteur initial. Plus spécifiquement, en présence d'un dysfonctionnement locomoteur plus faible, le taux de répondants est diminué.

Le taux de répondants démontre un lien statistiquement significatif avec le type de traitement administré, soit diètes thérapeutiques, produits de santé naturels, AINS ou contrôle négatif (placebo). Cependant, il n'y a pas de lien statistiquement significatif entre le taux de répondants et les approches thérapeutiques prises distinctivement.

11.3 Méthodologie

11.3.1 Essais cliniques sélectionnés

Les données de sept essais cliniques contrôlés et randomisés ont été considérées pour cette étude rétrospective. Les modalités thérapeutiques mises à l'essai ont été définies en trois groupes, soit diètes thérapeutiques, produits de santé naturels et AINS.

Essai clinique I : *Clinical evaluation of a nutraceutical, carprofen and meloxicam for the treatment of dogs with osteoarthritis* (Moreau *et al.*, 2003).

- **Modalité mise à l'essai :** Meloxicam (Metacam; Boerhinger Ingelheim)
- **Dosage :** 0.2 mg/kg le premier jour et par la suite 0.1 mg/kg/jour
- **Durée de l'essai : 60 jours**
- **Groupe thérapeutique :** AINS
- **Nombre de sujets sélectionnés :** 10 chiens

- **Modalité mise à l'essai :** Carprofen (Rimadyl; Pfizer) 2.2 mg/kg/jour
- **Groupe thérapeutique :** AINS
- **Nombre de sujets sélectionnés :** 12 chiens
- **Durée de l'essai :** 60 jours

- **Contrôle négatif (placebo) :** Excipient du Metacam
- **Nombre de sujets sélectionnés :** 10 chiens

Essai clinique II : *Clinical evaluation of a powder of quality elk velvet antler for the treatment of osteoarthrosis in dogs* (Moreau *et al.*, 2004).

- **Modalité mise à l'essai :** Poudre de velours de bois de cerf (*Cornu cervi)* (Cartiplex; Qeva Velvet Products)
- **Dosage :** 21 mg/kg/jour
- **Groupe thérapeutique :** Produit de santé naturel
- **Nombre de sujets sélectionnés :** 26 chiens
- **Durée de l'essai :** 60 jours

- **Contrôle négatif (placebo) :** Poudre inactive
- **Nombre de sujets sélectionnés :** 8 chiens

Essai clinique III : *Efficacy of licofelone in dogs with clinical osteoarthritis* (Moreau *et al.*, 2007)

- **Modalité mise à l'essai :** Licofelone (Merckle)
- **Dosage :** 2.2 mg/kg matin et soir
- **Groupe thérapeutique :** AINS
- **Nombre de sujets sélectionnés :** 13 chiens
- **Durée de l'essai :** 28 jours

- **Contrôle négatif (placebo) :** Fécule de mais
- **Nombre de sujets sélectionnés :** 16 chiens

Essai clinique IV: *Effect of a diet enriched with green-lipped mussel on pain behavior and functioning in dogs with clinical osteoarthritis* (Rialland *et al.*, 2013).

- **Modalité mise à l'essai :** Diète thérapeutique enrichie de moules vertes zébrées (Mobility Support JS; Medi-Cal/Royal Canin)
- **Dosage :** Selon les recommandations du manufacturier
- **Groupe thérapeutique :** Diète thérapeutique
- **Nombre de sujets sélectionnés :** 18 chiens
- **Durée de l'essai :** 60 jours

- **Contrôle négatif (placebo) :** Diète régulière (non-thérapeutique)
- **Nombre de sujets sélectionnés :** 18 chiens
- **Durée de l'essai :** 30 jours

Essai clinique V : *Effects of feeding a high omega-3 fatty acids diet in dogs with naturally occurring osteoarthritis* (6.1.1 au-dessus**6.1.1 au-dessus - Chapitre 1**).

- **Modalité mise à l'essai : Diète thérapeutique riche en acides gras oméga-3 (Joint mobility JM; Nestlé Purina PetCare Company)**
- **Dosage : Selon les recommandations du manufacturier**
- **Groupe thérapeutique : Diète thérapeutique**
- **Nombre de sujets sélectionnés : 14 chiens**
- **Durée de l'essai : 91 jours**

- **Contrôle négatif (placebo) : Diète régulière (sans acide gras oméga-3)**
- **Nombre de sujets sélectionnés : 12 chiens**
- **Durée de l'essai : 91 jours**

Essai clinique VI: *Brachystemma Calycinum D. Don effectively reduces the locomotor disability in dogs with naturally occurring osteoarthritis: A randomized placebo-controlled trial* (**6.1.2 au-dessus - Chapitre 1**).

- **Modalité mise à l'essai :** *Brachystemma calycinum* D. Don
- **Dosage :** 200 mg/kg/jour
- **Groupe thérapeutique :** Produit de santé naturel
- **Nombre de sujet sélectionnés :** 15 chiens
- **Durée de l'essai :** 42 jours

- **Contrôle négatif (placebo) :** Fécule de mais
- **Nombre de sujets sélectionnés :** 14 chiens
- **Durée de l'essai :** 42 jours

Essai clinique VII : *A medicinal herb-based natural health product improves the condition of a canine natural osteoarthritis model: A randomized placebo-controlled trial* (**6.1.2 au-dessus - Chapitre 1**).

- **Modalité mise à l'essai : Tableau VII**
- **Dosage : Tableau VII**
- **Groupe thérapeutique : Produit de santé naturel**
- **Nombre de sujets sélectionnés : 13 chiens**
- **Durée de l'essai : 56 jours**

- **Contrôle négatif (placebo) : Excipient**
- **Nombre de sujets sélectionnés : 14 chiens**
- **Durée de l'essai : 56 jours**

11.3.2 Sujets

Parmi l'ensemble des chiens répertoriés, uniquement ceux ayant des évidences d'arthrose exclusive au niveau des membres postérieurs ont été sélectionnés. La force verticale maximale devait être moindre que 66.0 % de poids corporel (**4.4.2.3.2 ci-dessus - Chapitre 1**)

11.3.3 Analyse de la démarche

La force verticale maximale fut enregistrée de manière standard pour l'ensemble des essais cliniques, soit à un trot variant entre 1.9 et 2.2 mètre par seconde. La force verticale maximale moyenne des cinq premiers essais valides a été utilisée.

11.3.4 Période de retrait

Lorsque c'était nécessaire, des périodes de retrait variant entre deux à seize semaines, devaient être respectées pour diverses modalités thérapeutiques contre l'arthrose.

11.3.5 Analyses statistiques

La valeur du seuil de changement minimal détectable (à un intervalle de confiance de 95 %) pour la mesure de la force verticale maximale a été utilisée. Cette valeur a été utilisée afin de qualifier les chiens de répondants (changement > 2.0 % de poids corporel), de non-répondants (changement entre 2.0 et -2.0 %) et de répondants-négatifs (changement < -2.0 % de poids corporel).

Pour les chiens traités, les données dichotomiques (répondants *versus* autres) ont été analysées à l'aide d'un modèle linéaire généralisé en considérant une distribution binaire des données. De manière similaire, les données dichotomiques (répondants-négatifs *versus* autres) des chiens ayant reçu un contrôle négatif (placebo) ont été analysées. La force verticale maximale au début de l'essai clinique de même que le type de traitement administré (diète thérapeutique, produit de santé naturel, AINS ou contrôle négatif) ont été ajoutés au modèle statistique. Pour l'ensemble des tests effectués, le facteur essai clinique a été considéré. Ce dernier s'est avéré non statistiquement significatif (données non présentées). Un seuil de signification (alpha) de 5 % a été utilisé pour l'ensemble des analyses.

11.4 Résultats

11.4.1 Taux de répondants

Pour l'ensemble des chiens traités (n=121), 62.8 % ont été considérés comme étant répondants contre 11.6% de répondants-négatifs (**Figure 32**). La force verticale maximale moyenne pour cet ensemble a été de 4.0% de poids corporel (minimum -19.3; maximum 23.0).

- **Groupe Diète thérapeutique** (n=32), 53.1 % des chiens ont été considérés comme étant répondants contre 21.9% de répondants-négatifs.
- **Groupe Produits de santé naturels** (n=54), 66.7 % des chiens ont été considérés comme étant répondants contre 7.4% de répondants-négatifs.
- **Groupe AINS** (n=35), 65.7 % des chiens ont été considérés comme étant répondants contre 8.6% de répondants-négatifs.

Pour l'ensemble des chiens ayant reçu un contrôle négatif (placebo, n=92), 34.8 % de ceux-ci ont été considérés comme étant répondants contre 33.7 % de répondants-négatifs (**Figure 33**). La force verticale maximale moyenne chez cet ensemble de chiens a été de -0.1 % de poids corporel (minimum -10.5; maximum 11.9).

- **Contrôles négatifs (Diète thérapeutique n=30**) 43.3 % des chiens ont été considérés comme étant répondants contre 26.7 % de répondants-négatifs.
- **Contrôles négatifs (Produits de santé naturels n=36)** 38.9 % des chiens ont été considérés comme étant répondants contre 36.1 % de répondants-négatifs.
- **Contrôles négatifs (AINS n=26)** 19.2 % des chiens ont été considérés comme étant répondants contre 38.5 % de répondants-négatifs.

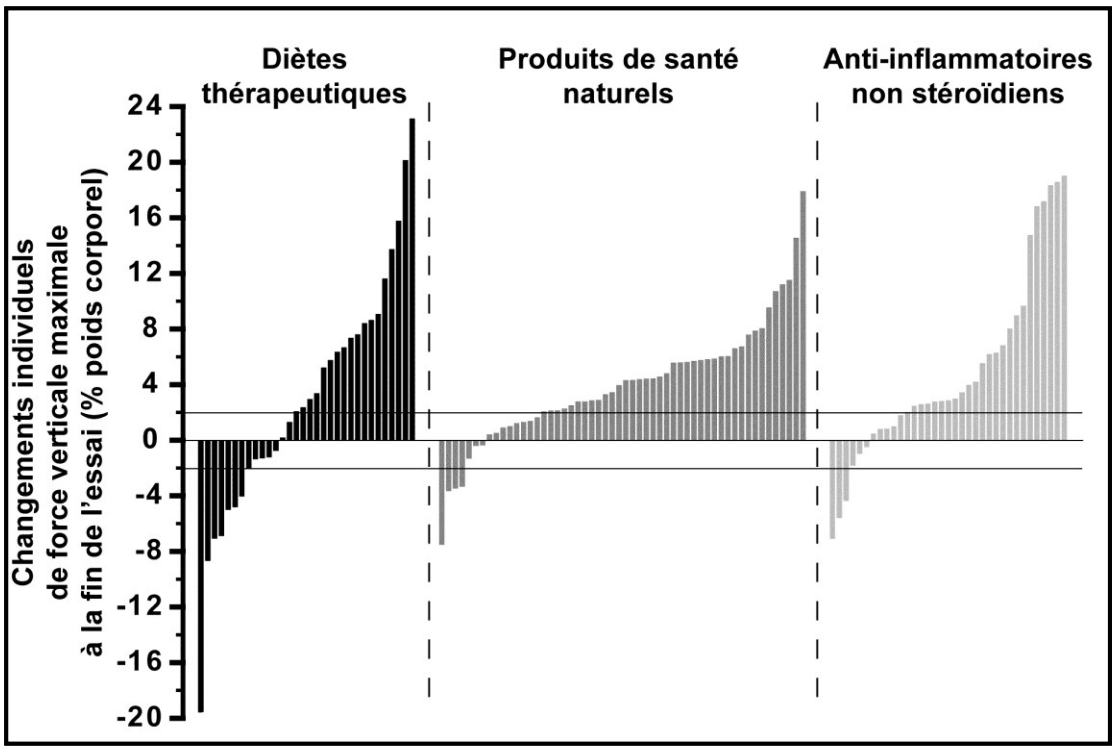

Figure 32. Changements individuels de force verticale maximale selon le groupe thérapeutique

Les changements individuels en force verticale maximale sont représentés pour les trois groupes thérapeutiques. La valeur du seuil de changement minimal détectable (à un intervalle de confiance de 95%), qui est de ± 2.0 % de poids corporel, est dénotée par un trait. Un chien ayant eu un changement de force verticale maximale supérieur à ce seuil a été considéré comme répondant. A l'inverse, en dessous de ce seuil, le chien a été considéré comme répondant-négatif.

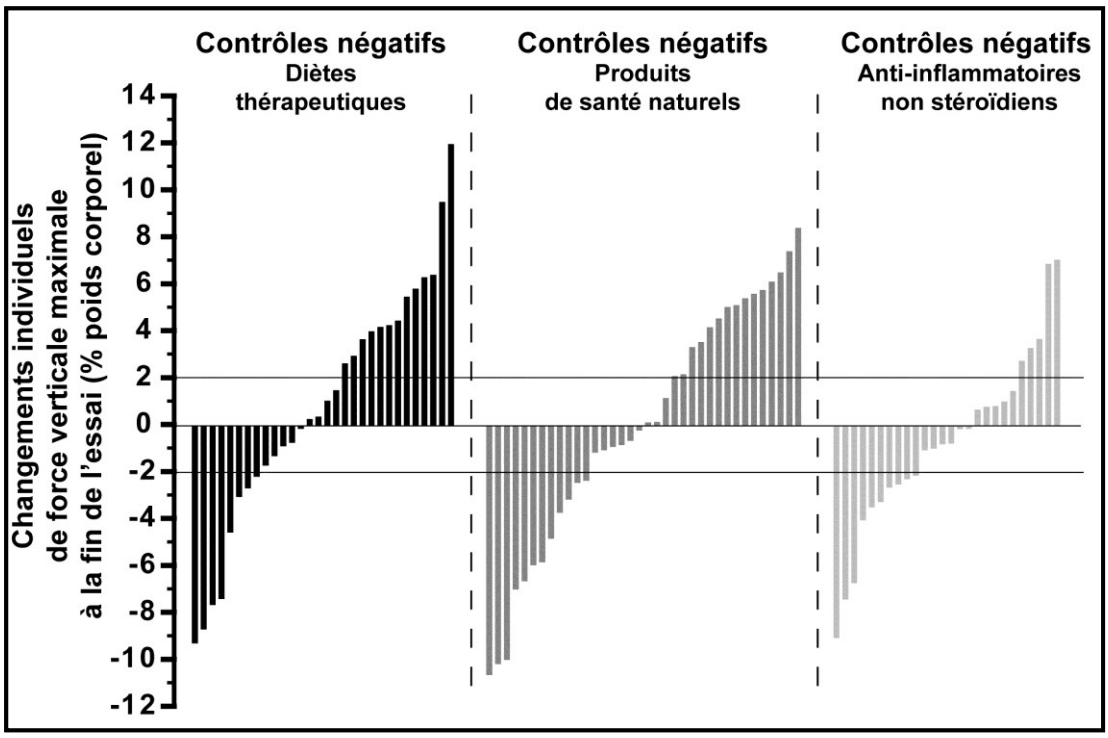

Figure 33. Changements individuels de force verticale maximale chez les chiens ayant reçu un contrôle négatif selon le groupe thérapeutique

Les changements individuels en force verticale maximale sont représentés pour les trois groupes thérapeutiques. La valeur du seuil de changement minimal détectable (à un intervalle de confiance de 95%), qui est de ± 2.0 % de poids corporel, est dénotée par un trait. Un chien ayant eu un changement de force verticale maximale supérieur à ce seuil a été considéré comme répondant. A l'inverse, en dessous de ce seuil, le chien a été considéré comme répondant-négatif.

11.4.2 Régression binomiale

Chez les chien traités, la relation entre les répondants (76/121) et la force verticale maximale initiale était significative (p<0.006). Ainsi, pour un gain de 1.0 % de poids corporel dans la valeur initiale de force verticale maximale, les chances de rencontrer un répondant étaient diminuées de 9.0 % (intervalle de confiance à 95 %; 2.0-16.0 %). Chez les chiens ayant reçu un contrôle négatif (placebo), la relation entre les répondants-négatifs et la force verticale maximale initiale n'était pas significative.

Le rapport de cotes sur l'ensemble des 213 chiens répertoriés (modalités thérapeutiques confondues *versus* contrôle négatif (placebo)) était de 2.8 (intervalle de confiance à 95 % 1.6-5.2). Donc, les chances d'être qualifié de répondants étaient 2.8 fois plus élevées sous l'effet d'une thérapie (diètes thérapeutiques, produits de santé naturels ou AINS) que celles d'un contrôle négatif (placebo). Cette relation entre taux de répondants et approches thérapeutiques (confondues) était statistiquement significative (p<0.001). Cependant, la relation entre le taux de répondants et les approches thérapeutiques prises distinctivement n'était pas significative au seuil statistique utilisé.

12 Modèle canin d'arthrose par sectionnement chirurgical du ligament croisé crânial : La force verticale maximale et la détection d'effets structuraux

12.1 Préambule

Le remodelage osseux est un facteur clé dans la physiopathologie de l'arthrose (Ding *et al.*, 2010b; Kwan Tat *et al.*, 2010; Burr *et al.*, 2012). Chez un modèle canin d'arthrose par sectionnement chirurgical du ligament croisé crânial, le remodelage excessif de l'os sous-chondral est notoire et implique une résorption osseuse anormale (Boyd *et al.*, 2002; Sniekers *et al.*, 2008). La préservation thérapeutique des structures osseuses sous-chondrales, *via* l'administration d'un agent anti-résorptif, pourrait donc avoir une répercussion positive au niveau des structures osseuses.

Chez ce modèle, la préservation thérapeutique du cartilage a démontré une répercussion positive envers le degré de dysfonctionnement locomoteur reflété par la mesure de la force verticale maximale (Annexe V). L'effet structurel présumé d'un agent anti-résorptif pourrait ainsi avoir également une répercussion positive envers le degré de dysfonctionnement locomoteur, tel que reflété par la mesure de la force verticale maximale.

12.2 Abrégé méthodologique et hypothèses

La force verticale maximale a été mesurée chez des chiens sains (n=32), puis de nouveau à quatre et huit semaines après le sectionnement chirurgical du ligament croisé crânial. Pendant huit semaines, les chiens ont reçu soit un agent anti-résorptif, soit un contrôle négatif. À la huitième semaine, l'articulation du genou fut soumise à l'évaluation qualitative des structures (cartilage et os sous-chondral) et quantitative de facteurs cataboliques et d'intervenants pro-inflammatoires. Il importe de préciser que l'étude présentée à la section suivante cadre dans une évaluation globale et exhaustive de l'effet d'un agent anti-résorptif à l'aide de différents critères d'efficacité. Dans cet ouvrage, uniquement la force verticale maximale sera considérée comme critère d'évaluation de la fonction. Les hypothèses furent les suivantes :

- Chez ce modèle, l'effet de l'agent anti-résorptif se fait ressentir par une préservation de la qualité des structures et une diminution de la quantité de facteurs cataboliques et d'intervenants pro-inflammatoires.
- Chez ce modèle, la qualité des structures et la diminution de la quantité de facteurs cataboliques et d'intervenants pro-inflammatoires se répercutent positivement sur le degré de dysfonctionnement locomoteur reflété par la mesure de la force verticale maximale.

13 Article VIII. Tiludronate treatment improves structural changes and symptoms of osteoarthritis in the canine anterior cruciate ligament model

M. Maxim Moreau a participé au design expérimental, à l'acquisition des données, à l'analyse de ces dernières et à la rédaction de cet article présentement publié (*Arthritis Research & Therapy*, 13:R98, 2011). M. Moreau a également participé aux travaux d'infographie. L'article a par la suite été dûment révisé et bonifié par l'expertise de chacun des coauteurs.

Arthritis Research & Therapy
13:R98, 2011

Tiludronate Treatment Improves Structural Changes and Symptoms of Osteoarthritis in the Canine Anterior Cruciate Ligament Model

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13.1 Abstract

Introduction: The aim of this prospective, randomized, controlled, double-blind study was to evaluate the effects of tiludronate (TLN), a bisphosphonate, on structural, biochemical and molecular changes and function in an experimental dog model of osteoarthritis (OA).

Methods: Baseline values were established the week preceding surgical transection of the right cranial/anterior cruciate ligament, with eight dogs serving as OA placebo controls and eight others receiving 4 TLN injections (2 mg/kg subcutaneously) at two-week intervals starting the day of surgery for 8 weeks. At baseline, week 4 and week 8, the functional outcome was evaluated using kinetic gait analysis, telemetered locomotor actimetry and video-automated behaviour capture. Pain impairment was assessed using a composite numerical rating scale (NRS), a visual analog scale, and electrodermal activity (EDA). At necropsy (week 8), macroscopic and histomorphological analyses of synovium, cartilage and subchondral bone of the femoral condyles and tibial plateaus were assessed. Immunohistochemistry of cartilage (MMP-1, MMP-13, and ADAMTS-5) and subchondral bone (cathepsin K) was performed. Synovial fluid was analyzed for inflammatory (PGE_2 and nitrite/nitrate levels) biomarkers. Statistical analyses (mixed and generalized linear models) were performed with an α-threshold of 0.05.

Results: A better functional outcome was observed in TLN dogs than OA placebo controls. Hence, TLN dogs had lower gait disability (p=0.04 at week 8) and NRS score (p=0.03, group effect), and demonstrated behaviours of painless condition

with the video-capture (p<0.04). Dogs treated with TLN demonstrated a trend toward improved actimetry and less pain according to EDA. Macroscopically, both groups had similar level of morphometric lesions, TLN-treated dogs having less joint effusion (p=0.01), reduced synovial fluid levels of PGE_2 (p=0.02), nitrites/nitrates (p=0.01), lower synovitis score (p<0.01) and a greater subchondral bone surface (p<0.01). Immunohistochemical staining revealed lower levels in TLN-treated dogs of MMP-13 (p=0.02), ADAMTS-5 (p=0.02) in cartilage and cathepsin K (p=0.02) in subchondral bone.

Conclusion: Tiludronate treatment demonstrated a positive effect on gait disability and joint symptoms. This is likely related to the positive influence of the treatment at improving some OA structural changes and reducing the synthesis of catabolic and inflammatory mediators.

13.2 Introduction

Osteoarthritis (OA) is among the most common musculoskeletal conditions [1]. This disease leads to functional disability and a reduced quality of life [2]. The abnormal biomechanics are believed to be among the major risk factors of disease progression and joint tissue damage [3].

Subchondral bone turnover is a well-defined component of OA [4]. The interactive process between articular cartilage and subchondral bone is complex and not yet fully understood. Yet, as these tissues are intimately related components of the joint, treatment to limit excessive bone remodelling is believed to have a possible positive effect on the global evolution of OA structural changes. Indeed, bone antiresorptive agents have been shown to limit the development of OA structural changes in a number of experimental models [5]. For instance, inhibition of bone remodelling by licofelone [6] and calcitonin [7] in the experimentally transected canine anterior/ cruciate ligament (ACL) model of OA was shown to reduce cartilage lesions. Similar evidence also emerged from the work done on oestrogen replacement therapy in ovariectomized monkeys [8].

Bisphosphonates (BPs) are a well-known class of molecules that contain two phosphonate groups attached to a single carbon atom, forming a “P-C-P” structure. The antiresorptive effects of these biochemical analogs of inorganic pyrophosphate have been demonstrated in skeletal diseases where excessive bone resorption is

present [9]. The anion of tiludronic acid (tiludronate, TLN) is a non-nitrogen-containing BP that acts on bone through mechanisms that involve induction of osteoclast apoptosis and prevention of extracellular degradation and of pro-inflammatory cytotrafficking [10], leading to decreased mineralized matrix resorption. This drug is recommended for skeletal disorders characterized by an increased and abnormal bone remodelling such as Paget's disease, and is currently the only BP approved in veterinary medicine to alleviate clinical signs of an OA condition in horses [11]. There is yet insufficient data to claim a potentially beneficial effect of TLN on the pathological changes encountered in OA.

A recent study demonstrated that pre-emptive chronic zoledronate (a nitrogenous BP) treatment increases bone mineral density, and is chondroprotective and analgesic in both chemical (mono-iodo-acetate, MIA) and surgical experimental models of painful joint degeneration in the rat [12]. The authors showed that osteoclast-mediated resorption of cartilage at the subchondral bone/cartilage interface is an early initiating event in the pathobiology of the MIA model as opposed to chondrocyte death and subsequent mechanical erosion of the articular surface. Pre-emptive zoledronate fully inhibited the subchondral bone/cartilage molecular cross-talk [4, 13] and/or the BP could have had a direct analgesic effect. This provided further rationale to test the potency of TLN at improving functional disability and structural changes in the canine ACL model of OA.

While BPs [14, 15] and other antiresorptive agents [5-7, 16] have shown promise, mostly structural effects (inhibition of cartilage degeneration [12, 14], prevention of osteophytes [14] and reduction in bone marker turnover [15]) in animal models of OA with pre-emptive treatment, clinical results in knee OA patients have been disappointing, *e.g.* with risedronate [17]. In OA, sclerosis of the subchondral bone is preceded by its resorption in the early phase [4, 13, 18]. This bone remodelling has also been characterized as bone marrow lesions (BML) on magnetic resonance imaging (MRI), and could also be perceived as an adaptation to changes in the biomechanics (maintaining intramedullary homeostasis [19]) or in an attempt to repair microdamages [18]. Therefore, bone remodelling has been associated with redistribution of mechanical stress [19], and the hypothesis has been advanced that to counteract it could prevent the repair of naturally occurring bone microdamage, thus increasing the susceptibility to crack initiation [20]. Moreover, in the ACL transection canine OA model, an experimental BP was demonstrated to be effective at reducing the turnover of cancellous subchondral bone, but ineffective at preventing osteophyte formation or pathologic changes of OA in the overlying cartilage [21]. A decrease in proteoglycan synthesis was rather observed, suggestive of impairment in the hypertrophic repair process. In contrast, chondroprotection was denoted in cruciate-deficient rats under BP treatment [12, 14] in parallel to a decrease in the expression of degradative enzymes [14] as well as of biochemical markers of cartilage degradation in human beings [17]. Also, in these studies stating a limited efficacy for BP treatment [17, 21], it has to be noted that one main limitation to inference was the relatively mild degree of OA in the control animals

[21] as well as the absence of OA radiographic signs progression in placebo subjects [21].

The ACL transection in dogs generates abnormal biomechanical forces and metabolic pathways that initiate structural changes on morphometry and histology [5], as well as on imaging [22, 23], mimicking those seen in naturally occurring OA. This model is additionally acknowledged to induce significant chronic gait disability and functional impairment [24, 25]. The canine ACL model of OA is valuable for assessing the evolution of functional outcomes in response to treatment [5]. In the present study, we hypothesized that the bone antiresorptive action of TLN might curb the development of structural and functional joint lesions associated with ACL transection. We used a set of complementary tools to relate pain and functional outcomes in parallel to joint structural, biochemical and molecular changes. This allowed the evaluation of the effect of TLN on limb loading, pain/stress sensation, activity level and behaviours related to canine experimental OA conditions.

13.3 Materials and methods

In this randomized, double-blind, placebo-controlled study with a parallel design (**Figure 34**), dogs were randomly allocated to two treatment groups of 8 dogs, stratified by body weight and gender. Investigators were blinded to group allocation, as well as treatment. The study protocol was approved by the institutional animal care and use committee (RECH-1268) and conducted in

accordance with the Canadian Council on Animal Care guidelines.

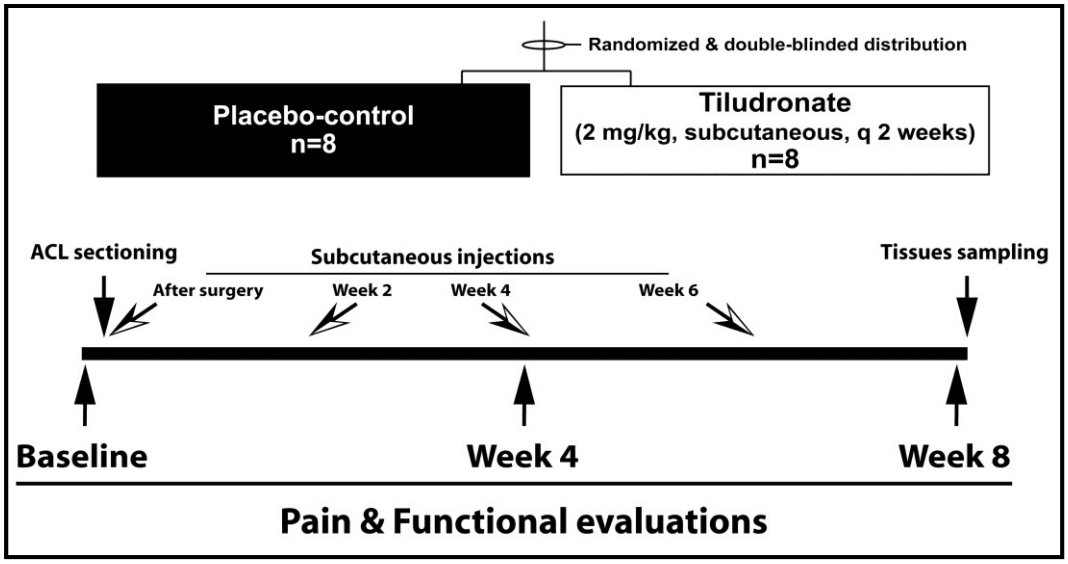

Figure 34. Schematic representation of the study design

13.3.1 Animals

Sixteen adult crossbred dogs (aged 2 to 3 years), with an average (SD) body weight of 26 (3.3) kg were used in this study. They were individually housed in galvanized steel cages (1 m [width] × 1.75 m [length] × 2.4 m [height]) fitted with automatic waterers. Dogs were included in the study after complete physical and musculoskeletal evaluation by a veterinarian, and haematological and biochemical analyses. Food (approximately 450 g Hill's Pet Nutrition Science Diet Canine Adult Original mixed with Harlan Teklab Global 27% Protein Dog Diet) was given once daily and removed overnight. Tap water (purified by filtration) was provided to the animals *ad libitum*.

13.3.2 Surgical transection of the anterior cruciate ligament (ACL)

After evaluation of baseline pain and functional outcome levels, all anaesthetized dogs were subjected to ACL transection of the right knee as previously described [26]. Under pre-emptive (transdermal fentanyl 50 or 75 μg/h, Duragesic®; Janssen Ortho, Markham, ON, Canada) and multimodal (intra-articular block combined with opioid administration) analgesia, the tibial edge was located with the thumb and index finger, followed by a medial sagittal skin incision (30 mm). The subcutaneous tissues were dissected, and a medial arthrotomy was performed, distal to the patella and parallel to the patellar ligament. A retractor was inserted to view the ACL to be sectioned, the completeness of which was verified by obtaining a large drawer motion in both flexion and extension. The capsule and the retinaculum were sutured in a simple continuous pattern. Bupivacaine (Marcaïne® 0.5%; Hospira, St-Laurent, QC, Canada) was injected (5 to 8 mL) in the capsule as an intra-articular block. Finally, the subcutaneous tissues were sutured, followed by intra-dermal and skin sutures.

13.3.3 Treatment

One group was treated with 2 mg/kg of disodium TLN dissolved in a mannitol solution (CEVA Santé Animale, Libourne, France). The OA control group received only the vehicle solution (CEVA Santé Animale). Both treatments (0.2 mL/kg) were injected subcutaneously (SC), starting on the day of ACL transection, and repeated every 2 weeks up to the end of the follow-up (total of 4 administrations).

The dose level of TLN was selected by the Sponsor based on preliminary studies in rats [27] and using an allometric scale-up to the weight of dogs.

13.3.4 Pain and functional evaluations

13.3.4.1 Gait analysis

In dogs, the use of a pressure-sensing walkway device acquires limb loading and is defined as a quantitative measurement of gait function [25]. Gait analysis was performed at baseline, week 4 and week 8 using the podobarometric recording device (Walkway® System; Tekscan Inc, Boston, MA, USA) [25, 28]. For the right (ACL-deficient) hind limb, the Peak Vertical Force (PVF) was acquired at a trotting gait velocity ranging from 1.9 to 2.2 meters/second. Velocity and acceleration (±0.5 meter per second^2) was ensured using a set of three photoelectric cells specially designed for this podobarometric device (LACIME; École de Technologie Supérieure, Montréal, QC, Canada). The gait acquisition window was 3 seconds with a sampling rate set at 44 hertz, producing a total of 132 frames. Raw PVF (Kg) data from the first 5 valid trials were obtained for each dog and later used for statistical purposes using body weight as a covariate [25]. Data were expressed as percentage of body weight (%BW).

13.3.4.2 Pain scoring systems

The lameness and pain of treated and control OA dogs were evaluated using previously developed scoring systems, and included a visual analog scale (VAS) [29] and a composite numerical rating scale (NRS) [30]. The pain scores were

obtained at baseline, week 4 and week 8 by the same technician [29] with a 100 mm VAS scale, coding from 0 (“no pain”) to 100 (“pain intensity could not be worse”). The composite NRS, which was scored by the same veterinarian throughout the study, includes the 7 following criteria: Global assessment (score 0–4); Evaluation of lameness while the dog is standing up (score 0–4), walking (score 0–4) and trotting (score 0–4); Willingness to hold up contralateral limb (score 0–4); Evaluation of response to palpation (score 0–4); Evaluation of response to flexion and extension (score 0–4). Inter- and intra-observer reliability of both VAS and NRS were tested and found to be highly satisfying (Spearman correlation, rho > 0.72, $p<0.001$).

13.3.4.3 Electrodermal activity (EDA)

Changes in skin conductance response (EDA) resulting from sympathetic neuronal activity [31] has recently been validated in the canine ACL model of OA as a measurement of stress or pain that is strongly associated with functional outcomes [30]. The EDA was recorded at baseline, week 4 and week 8 using a Pain Gauge® (PHIS, Inc., Dublin, OH, USA) system, which grades the signal intensity on a scale of 0 to 10, with 10 being the most painful. The device was placed for 2 seconds on the right palmar paw (dry and non-clipped).

13.3.4.4 Video-automated behaviour analysis

The computer-assisted behavioural analysis (The Observer v5.0.31; Noldus Information Technology, Inc., Leesburg, VA, USA) allowed the assessment of behavioural changes suggestive of a pain-related condition [32]. The capture of

behavioural changes in terms of body positions and motor activities allows a non-invasive monitoring of pain-related functional disability and discomfort. Recording was performed in the outdoor runs where the dogs exercised for two consecutive hours, at baseline and at week 8. The resulting ethogram included the following 8 classes of behaviour: location in the run, body position, facial expression, vocalization, tail position, self-care, motor activity and dog interaction. The analysis of behaviour occurrence was done following the manufacturer's recommendations.

13.3.4.5 Telemetered locomotor actimetry

Acceleration-based monitoring of frequency, intensity, and duration of physical activities is a valid objective tool to monitor pain-related functional disability [30, 33]. At baseline, week 4 and week 8, locomotor actimetry was monitored continuously for 24 hours with an electronic chip (Actical®; Bio-Lynx Scientific Equipment, Inc., Montreal, QC, Canada) that was placed inside a protective neck collar. During this time, all dogs followed the same daily routine to ensure consistency. The cumulative locomotor activity was recorded over two minutes, thus providing 720 measurements over 24 hours. The height of peaks for each recording was scaled in arbitrary units from 0 to ∞ to quantify the intensity of locomotor actimetry [30]. Comparison of actimetry with simultaneous video-automated recordings allowed the threshold to be set between active and inactive motions at 30 units. Data were then expressed as daily averaged total intensity (considering all counts, DATI), and daily averaged active intensity (considering

only counts higher than 30 in intensity as active counts, DAAI).

13.3.5 Macroscopic grading

At the end of the study, the dogs were euthanized under sedation with barbiturate overdose. The right knee of each dog was placed on crushed ice and dissected for quantification of gross morphological changes. Two independent observers who were blinded to treatment group allocation graded the findings with a consensual value [25, 26, 34].

13.3.5.1 Cartilage

Macroscopic lesion areas at the cartilage surface on the femoral condyles and tibial plateaus were measured (in mm^2) with an electronic digital calliper (Digimatic Caliper model No. 2071M; Mitutoyo Corporation, Kawasaki, Japan). The depth of erosion was graded with scores ranging between 0 (a normal surface) and 4 (erosion extending to the subchondral bone).

13.3.5.2 Osteophytes

When present, the degree of osteophyte formation was quantified by measuring the maximal width (mm) of the spurs on the medial and lateral femoral condyles as previously described [26]. For statistical purposes, data were evaluated separately for lateral and medial osteophytes and also summated for the entire condyles.

13.3.6 Histological grading of cartilage and synovial membrane

13.3.6.1 Cartilage

Full thickness cartilage sections were removed from the weight-bearing lesional areas of the femoral condyles and tibial plateaus allowing standardization of sampling [25]. Histological evaluation was performed on sagittal sections of cartilage from each femoral condyle and tibial plateau specimen [25, 26, 34]. After dissection, specimens were fixed in 4% buffered formalin and embedded in paraffin. Serial sections (5 μm) were stained with haematoxylin/Fast green and Safranin-O. The severity of cartilage pathology was graded by two independent observers using the OARSI histopathology scoring system [35]. For statistical purposes, data from both observers were considered for the lateral and medial part of the condyles and plateaus as well as for the entire joint.

13.3.6.2 Synovial membrane

Synovial membrane was removed and processed as described above, but stained with haematoxylin/eosin. Two independent observers evaluated two specimens. The severity of synovitis was graded on a scale (0-10) including four histological criteria as previously described [34]: synovial cell hyperplasia (scale 0-2), villous hyperplasia (0-3) and mononuclear (0-4) and polymorphonuclear (0-1) cell infiltration; 0 indicates normal structure. For statistical purposes, data from both observers and both specimens were considered.

13.3.7 Analysis of synovial fluid

At euthanasia, samples of synovial fluid were collected, measured, and then centrifuged and frozen (-80°C).

13.3.7.1 Prostaglandin (PG) E2 assay

The amount of PGE_2 (ng/knee) was determined using a commercially available Enzyme ImmunoAssay (Cayman Chemicals, Ann Arbor, MI, USA) according to the manufacturer's instructions, the limit of detection being 15 pg/mL. The concentration measurements were done in duplicate and the values averaged.

13.3.7.2 Nitrites and nitrates (NOx) assay

Nitrite and nitrate levels (nmol/knee) were determined by chemiluminescence [36] using a NO Analyzer (280i®, Sievers Instruments, Boulder, CO, USA), according to the manufacturer's instructions. Briefly, 0.025 mL of the supernatant was injected into the microreaction purge vessel of the analyzer. The purge vessel contained 5 mL of vanadium solution heated at 95°C. The instrument measures NOx on a gas-phase chemiluminescent reaction between NO and ozone. Each sample was analyzed in duplicate and values averaged.

13.3.8 Immunohistochemistry

Full thickness specimens from the tibial plateaus and femoral condyles were processed for immunohistochemical analysis, as previously described [6, 26, 34]. After the slides were incubated with a blocking serum (Vectastain ABC kit; Vector Laboratories, Inc., Burlingame, CA, USA) for 60 minutes, they were blotted and then overlaid with the primary antibody against the following: matrix

metalloproteinase (MMP)-1 (1/40 dilution, mouse monoclonal; Calbiochem ref. #444209; EMD Biosciences, Darmstadt, Germany), MMP-13 (1/6, goat polyclonal antibody; R&D Systems, Minneapolis, MN, USA), a disintegrin and metalloproteinase domain with thrombospondin motifs (ADAMTS)5 (1/50, rabbit polyclonal; Cedarlane ref. #CL1-ADAMTS5; Hornby, ON, Canada), and cathepsin K (1/200, goat polyclonal; Santa Cruz Biotechnology ref. #sc-6506; Santa Cruz, CA, USA) for 18 hours at 4°C in a humidified chamber.

Each slide was stained using the avidin-biotin complex method (Vectastain ABC kit), and incubated in the presence of the biotin-conjugated secondary antibody for 45 minutes at room temperature followed by the addition of the avidin-biotin-peroxidase complex for 45 minutes. The slides were counterstained with eosin. Determination of the staining specificity as well as the immunohistochemistry analysis (three fields from each specimen examined) was done as previously described [6, 26, 34]. Each section was examined under a light microscope (Leitz Orthoplan; Leica, Inc., St. Laurent, QC, Canada) and photographed using a CoolSNAP of Photometrics camera (Roper Scientific, Rochester, NY, USA).

The results are expressed as the percentage of cells staining positive for the antigen (MMP-1, -13 and ADAMTS5) in the upper zone of cartilage with the maximum value being 100%. Similarly, on decalcified specimens (see Histomorphometry section), the number of cathepsin K positive cells was quantified in the subchondral bone, as previously described [6]. For statistical purposes, data from all specimens

(tibial plateaus and femoral condyles) and all fields were considered. The data presented are the average of the three fields.

13.3.9 Histomorphometry

Specimens of full-thickness sections of the articular cartilage including the subchondral bone from the lesional area of the medial tibial plateau of all dogs were placed in 10% (vol/vol) formalin before being decalcified with 10% (vol/vol) formic acid in formalin for 12 hours and embedded in paraffin, as previously described [6].

13.3.9.1 Subchondral bone

Sections (5 μm) of each specimen were subjected to Fast green/Safranin-O staining. A Leitz Diaplan DMLS® microscope (Leica Microsystems, Wetzlar, Germany) connected to a personal computer (Pentium IV, using Image J software, v1.27; NIH, Bethesda, MD, USA and OSTEO II Image Analysis software; Bioquant, Nashville, TN, USA) was used to conduct the subchondral bone histomorphometry, which was performed as previously described [6, 26]. Measurement of the bone surface (mm^2), trabecular thickness (μm) and trabecular separation (mm) was done according to standard conventions [6].

13.3.9.2 Calcified cartilage

The calcified cartilage histomorphometry was done for each specimen, as previously described [6]. The surface (mm^2) of the calcified cartilage was calculated using the computerized program.

13.3.10 Statistical analysis

Linear mixed models for repeated measures were used to evaluate the effect of Time, Group and Time per Group interaction for PVF, EDA and actimetry recording using compound symmetry covariance structures. Trials (PVF) and dogs were random effects nested in treatment groups. At each time point, group's least squares means were compared with appropriate Tukey or Bonferroni adjustments. To evaluate the effect of Time, Group and Time per Group interaction on VAS and summated NRS, repeated-measures generalized linear models with generalized estimating equations were used, where data were assumed to distribute under the Log-Gamma, and the overdispersed Poisson probability functions, respectively. For the latter variable, the variance scale factor was estimated by Pearson's chi-square/Degree of freedom. Best working matrix was determined to be first-order autoregressive following the strategy proposed by Littell *et al*. [37].

For the video-automated behaviour analysis, the occurrence of each specific event was cumulated. Frequencies were then compared between TLN and placebo control groups using a negative binomial regression model with baseline occurrence as covariate. Values were expressed as changes in the frequency of a given behaviour according to the tested group.

Synovial fluid volume, levels of inflammatory factors, cellular ratios and structure measurements were tested between groups using linear mixed models. Where appropriate, specimen or fields were used as random effects nested in treatment

groups. Data presented as scores or counts were tested using a generalized linear model under the logistic polytomous distribution function using the proportional odds assumption, or the overdispersed Poisson probability functions when scores were summated. Where appropriate, specimen or observers were used as random effects nested in treatment groups. Alpha threshold for significance was set at 5%. Data are presented as mean (SD). Statistical analyses were done using SPSS Statistics software v17.0 (SPSS Inc, IL, USA) and SAS software, v9.1, (SAS Institute Inc, NC, USA).

13.4 Results

13.4.1 Pain and functional outcomes

The temporal evolution of PVF recording implied severe gait disability denoted by decreasing values over time ($p<0.01$) (**Figure 35**, **Tableau XVI**). Treatment interacted with the temporal recording of PVF ($p<0.01$). The TLN treatment over an 8 week duration provided reduction in the limb impairment compared to the placebo control over time ($p=0.05$), reaching PVF values 35% higher at week 8 ($p=0.04$).

Assessment provided by VAS and NRS (**Tableau XVI**) echoed the temporal evolution observed with gait analysis (PVF): After surgery, both methods detected a worsening of the dogs' condition. Assessment provided by VAS revealed an improvement from week 4 to week 8 ($p<0.01$) in both groups, without the presence of an interaction of treatment on VAS recording. Of note, TLN dogs tended to have lower VAS grades than controls ($p=0.06$). With respect to NRS, groups were not significantly improved from week 4 to week 8 and no interaction was denoted. However, there was a significant group effect ($p=0.03$) on the overall NRS recorded post surgery.

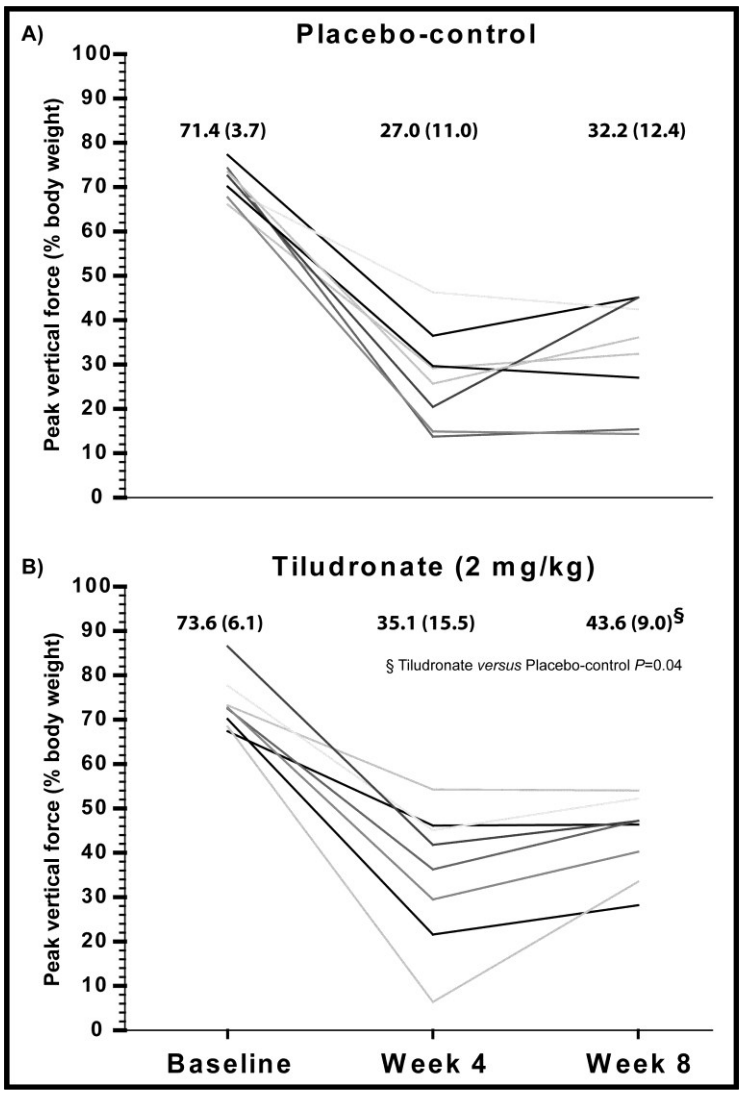

Figure 35. Kinetic gait analysis

Peak vertical force (mean ± standard deviation) recorded before (baseline) and 4 and 8 weeks after anterior cruciate ligament transection in dogs. Line plots representation includes respective values for A) Placebo-control and B) Tiludronate treated dogs. There was a significant time effect ($p<0.01$), a group effect ($p=0.05$) and a time per group interaction ($p<0.01$) with PVF value reaching 35% higher in tiludronate than in placebo-control at week 8 ($p=0.04$).

Tableau XVI. Pain and functional outcomes before and after anterior cruciate ligament transection in dogs

Evaluation methods/Groups	Time			P-values
	Baseline	Week 4	Week 8	
Function - kinetic gait analysis (%BW)				
Placebo-control	71.4 (3.7)	27 (11.0)	32.2 (12.4)	*<0.01
Tiludronate	73.6 (6.1)	35.1 (15.5)	43.6 (9.0)	§ = 0.05 ¶ <0.01
Pain - Visual analog scale (VAS, measurement)				
Placebo-control	0.0 (0.0)	37.6 (14.3)	26.8 (11.1)	*<0.01
Tiludronate	0.0 (0.0)	26.9 (18.9)	15.6 (9.2)	
Pain - Numerical rating scale (NRS, score)				
Placebo-control	0.0 (0.0)	19.4 (4.3)	18.3 (3.7)	
Tiludronate	0.0 (0.0)	15.5 (5.4)	15.0 (3.4)	§ = 0.03
Pain - Electrodermal activity (EDA, reading)				
Placebo-control	4.5 (2.5)	6.4 (2.5)	5.3 (2.4)	
Tiludronate	4.2 (2.7)	3.6 (2.5)	3.9 (2.4)	
Function - Telemetered actimetry recording (count)				
Daily averaged total intensity (DATI, no unit)				
Placebo-control	97.9 (41.4)	79.1 (22.7)	85.7 (35.8)	
Tiludronate	82.3 (25.4)	104.5 (44.6)	91.2 (33.1)	¶ = 0.04
Daily averaged active intensity (DAAI, no unit)				
Placebo-control	390.9 (101.3)	360.6 (73.9)	379.1 (127.1)	
Tiludronate	390.2 (82.9)	502.6 (145.7)	443.1 (117.1)	¶ = 0.04

Tiludronate was injected subcutaneously at 2 mg/kg, starting immediately on the day of ACL transection and repeated every two weeks for an eight-week follow-up. Placebo-control dogs received mannitol injection in a similar fashion.

Data presented are mean (SD).

Statistically significant Time effect (*), Group effect (§) and Time per Group interaction (¶)

According to EDA values (**Tableau XVI**), the level of pain/stress sensation recorded after surgery did not change over time in the TLN-treated dog group, whereas in the placebo control group, the maximal increase in EDA was noted at week 4. As a result, the interaction of treatment on the temporal evolution of EDA recording demonstrated a trend (p=0.07) without denoting group effect.

According to the video-automated analysis, there was a significant increase in the relative frequencies of two major body position behaviours suggestive of comfort for TLN dogs: Full weight bearing while standing with head down increased by a factor of 2.92 (p=0.03); Full weight bearing while standing and looking around

increased by a factor of 7.7 (p=0.03). Similarly, two motor activities (gait) were also more frequently observed in this group: Normal walking, factor of 5.22 (p=0.01); and normal trotting, factor of 6.34 (p=0.01).

The telemetered recording of DATI and DAAI denoted higher movement in TLN dogs following surgery (**Tableau XVI**). Conversely, placebo control dogs were less active than at baseline. This divergence led to the discernment of an interaction (p=0.04, DATI and DAAI) of treatment on the temporal evolution of telemetered actimetry, which resulted in higher DATI recording in TLN dogs when compared to placebo control at week 4 (p=0.05).

13.4.2 Synovial fluid

The amount of joint effusion in the TLN-treated dogs [6.7 (2.8) mL] was significantly less (p=0.01) than that found in the placebo control dogs [15.0 (7.6) mL]. The TLN-treated dogs also had lower levels of PGE_2 (p=0.02) [4.4 (3.6) ng/knee] and NOx (p=0.01) [306.2 (267.1) nmol/ knee] than those treated with placebo [11.7 (7.2) ng/knee, and 766.28 (379.0) nmol/knee, respectively].

13.4.3 Cartilage

13.4.3.1 Macroscopy

The severity of macroscopic lesions (depth and lesion surface) on the femoral condyles and tibial plateaus of TLN-treated dogs was not different from that observed in placebo control dogs (*data not shown*). The size of the osteophytes was

similar between groups, both on medial [TLN; 6.7 (2.6) mm, placebo control; 7.0 (1.9) mm] and lateral [TLN; 6.5 (1.4) mm, placebo control; 7.1 (2.1) mm] condyles.

13.4.3.2 Histology

Histological grading of cartilage lesions did not reveal significant difference between groups. The synovial membrane score of TLN dogs [total score of 6.8 (1.1)] was similar to the placebo control dogs [total score of 6.1 (1.1)]. The subset analyses revealed that the synovial lining cell score was significantly ($p<0.01$) less in TLN dogs [1.0 (0.0)] compared to placebo control [1.7 (0.6)].

13.4.4 Immunohistochemistry

The cartilage immunohistochemistry revealed a significant decrease in the percentage of cells staining positive in TLN-treated dogs compared to the placebo control (**Figure 36**) for MMP-13 [14.9 (2.5)% *vs.* 20.9 (4.2)%; $p=0.02$] and ADAMTS5 [16.9 (2.3)% *vs.* 22.2 (4.2)%; $p=0.02$]. The level of MMP-1 was similar in both groups (*data not shown*). Immunohistochemical analysis of the calcified cartilage revealed a slight decrease in the level of MMP-13 in TLN-treated dogs [19.7 (5.9)%] compared to control dogs [22.4 (2.3)%, NS]. In the subchondral bone, the cathepsin K expression was significantly lower in dogs that had received TLN compared to placebo [1.9 (0.6) *vs.* 2.7 (0.8), $p=0.02$] (**Figure 36**).

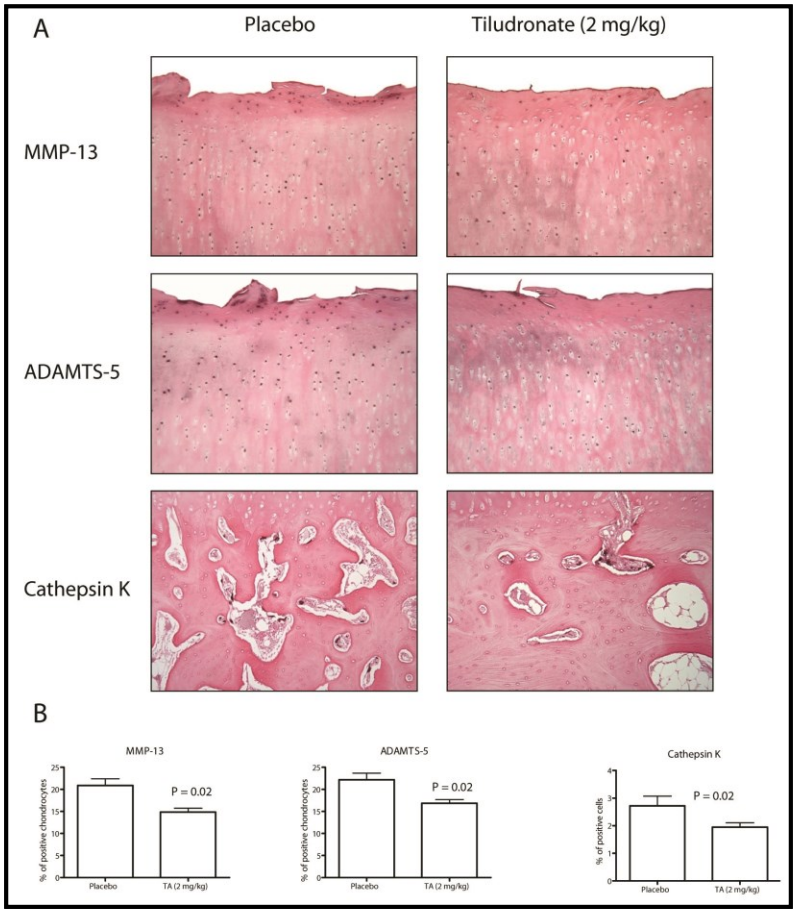

Figure 36. Immunohistochemistry

(A) Expression of matrix metalloproteinase 13 (MMP-13), ADAMTS5 and cathepsin K in representative sections of cartilage (MMP-13 and ADAMTS5) and subchondral bone (cathepsin K) from placebo-treated dogs with osteoarthritis (OA) and tiludronic acid (TA: 2 mg/kg/2 weeks)-treated dogs with OA. Positive cells are shown by dark brown staining (original magnification X100). (B) Levels of MMP-13, ADAMTS5 and cathepsin K as determined by immunostaining. The values are expressed as the mean ± SEM. P-values were calculated by using a generalized linear model under logistic polytomous distribution function using the proportional odds assumption.

13.4.5 Histomorphometry

The surface of the calcified cartilage demonstrated a trend (p=0.07) to be greater in the TLN-treated dogs compared to the placebo dogs (**Tableau XVII**, **Figure 37**). Dogs treated with TLN also had a significantly greater (p<0.01) subchondral bone surface and smaller trabecular separation compared to control dogs. Trabecular thickness was similar in both treatment groups. The values for TLN-treated dogs were similar to those previously reported for normal dogs [6].

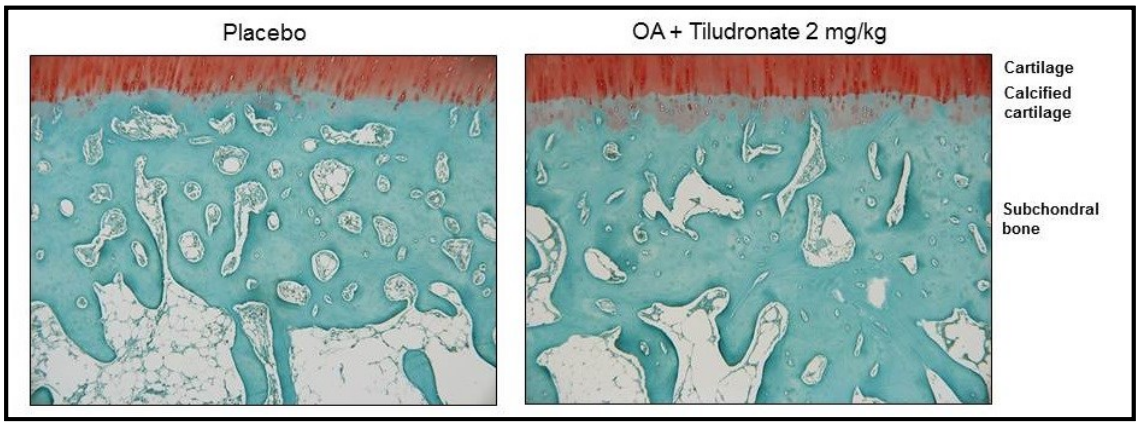

Figure 37. Histomorphometry

Representative histological sections of calcified cartilage and subchondral bone in osteoarthritic dogs that received either placebo (n=8) or treatment with tiludronate (n=8) 2 mg/kg/2 weeks. Specimens were selected from lesional areas of the tibial plateaus (Fast green/Safranin O staining, original magnification X63).

Tableau XVII. Histomorphometry of the calcified cartilage and subchondral bone 8 weeks after anterior cruciate ligament transection in dogs

	Calcified cartilage surface (10^{-2} mm^2)	Subchondral bone surface (10^{-2} mm^2)	Trabecular thickness (10^{-2} mm)	Trabecular separation (10^{-3} mm)
Placebo-control	16.9 (3.9)	75.1 (9.3)	8.9 (2.3)	86.0 (52.6)
Tiludronate	19.8 (2.3)	86.2 (5.1)*	10.3 (2.0)	45.5 (15.1)*

Tiludronate was injected subcutaneously at 2 mg/kg, starting immediately on the day of ACL transection and repeated every 2 weeks for an 8-week follow-up. Osteoarthritic placebo-treated dogs received mannitol injection in a similar fashion.

Data presented are mean (SD). * Statistically significant (p<0.01) compared to placebo-control.

13.5 Discussion

Studies in the dog ACL model have provided insight into OA mechanisms and pathophysiological pathways that surround the evolution of the degenerative process. The model was proven to be most useful at the preclinical stage of drug development for testing the abilities of new therapeutic modalities to limit or halt the disease development/progression [5]. In the present study, TLN was demonstrated to have a positive effect both on some of the structural changes and on pain/function. More particularly, TLN was found to decrease the production of catabolic enzymes, bone resorption, and synovial inflammation. These were associated with improved locomotion, reduced lameness and gait disability, and improved joint pain perception. These findings are in line with the report on a rat model of OA [12] showing that the analgesic effect of the nitrogenous BP

zoledronate is mediated by its antiresorptive action. In the current study, TLN promoted better function despite the presence of cartilage lesions, which were of similar extent in OA and control dogs. This finding suggests that cartilage integrity in a weight-bearing joint is not compulsory for functional improvement. Furthermore, even when the knee joint was exposed to additional mechanical constrains related to an increase in limb support (*i.e.* pain-relief), cartilage did not undergo excessive alteration and remained similar to control dogs.

This study demonstrated that TLN improves the functional disability following ACL transection, more specifically the limb impairment, allowing dogs to load their afflicted limb to a greater extent as demonstrated by the results of the PVF analysis. Dogs were also more active without showing evidence of severe lameness. The stress/pain sensation was also reduced by TLN treatment, as highlighted by the results of the EDA measurements, pain scoring and behaviours denoting changes in pain-related condition. Lameness improvement was found to be maximal at week 8 (PVF, NRS and video-automated analysis of body position and motor activities), after dogs had received full TLN treatment. However, at the intermediate time-point (week 4), the difference between TLN and placebo groups was greater than at week 8 for both EDA and telemetered actimetry. The beneficial effect of TLN therapy on pain/function was likely related to a combination of effects including its anti-inflammatory effect shown by a reduction in synovial effusion size, synovitis score, and level of inflammatory mediators, as well as its effect on the initiation of bone remodelling [12].

Transduction of noxious signals occurs through high-threshold receptors responding to a variety of thermal, chemical and mechanical stimuli, and defined as polymodal nociceptors. At the level of damage-sensing neurons, release of protons and high concentrations of adenosine triphosphate (ATP) act, respectively, on transient receptor potential (TRP) and acid-sensing (ASIC) ion channels, and on ionotropic ligand-gated purinergic (P2X) receptors to activate nociceptors [38]. During the early stages of inflammation, mediators such as PGs and bradykinin change the sensitivity of receptors and reduce the activation threshold for these conducting ion channels, which is the basis for peripheral sensitization.

This study provides interesting new findings on the ability of TLN to reduce the inflammatory changes in the OA synovium. Hence, TLN demonstrated a clear anti-inflammatory local effect while decreasing the joint effusion and synovitis (synovial lining cells). The analyses of synovial fluid confirmed the anti-inflammatory effects reflected by a decrease in the levels of PGE_2 and NOx, two well-known inflammatory mediators [39]. Inflammatory markers such as PGE_2 are known to be correlated with pain and functional disability in human OA [40] as well as in the canine ACL model of OA [41]. The high level of synovial fluid NOx found in this study is in line with our previous findings in this OA model, in which the inducible NOS was found to be increased [16, 34]. It is likely that NO contributed to the disability and perception of pain [42]. Moreover, the decrease in NOx levels by TLN treatment may have contributed to the structural protective effect of the drug as previously demonstrated in this OA model [16]. Tiludronate,

by inhibiting the ATP-dependent proton pumps located in the plasma membranes of the osteoclasts [43], can reduce the acidification of the bone matrix, which is the first step in the bone resorption process. In addition, TLN can disrupt adhesion of the osteoclast to the bone surface before bone resorption is initiated by modifying the phosphorylation of proteins of the cytoskeleton [44]. As well as decreasing pro-inflammatory cytotrafficking (cytokines and NO synthesis) [10], acidification and phosphorylation and, in consequence, activation of proteolytic enzymes, TLN can also inhibit activity of MMPs [45]. In the present study, TLN was found to decrease the expression of MMP-13 and ADAMTS5 in the cartilage and cathepsin K in the subchondral bone, which is in line with the findings of our previous study [6] and supports this hypothesis.

The anti-inflammatory effects of TLN are interesting, as they add to the most important recognized biological effect of BPs, *i.e.* the reduction in bone remodelling through the inhibition of osteoclastic activity. The pain and functional improvements observed under TLN therapy were present at week 4 and maintained at week 8. Therefore, it could be hypothesized that the actions of TLN on bone matrix and synovial inflammation would lead to a decrease in TRPV1, ASIC3, and P2X receptors activation, translating into diminished peripheral sensitization and subsequent benefits on pain sensation and mobility. Although BPs were developed for the treatment of pathologies associated with excessive bone resorption, several reports revealed that they were able to reduce the pain associated with different painful diseases. Direct analgesic effects of BPs have been largely acknowledged

using reflex algesimetric tests in mice after acute administration [46]. Clodronate and pamidronate presented anti-nociceptive dose-responses, comparing favourably to aspirin and morphine, and the central and peripheral analgesia did not appear to be mediated through opioid receptors [46]. Prolonged anti-nociceptive effects have also been demonstrated for clodronate, a non-nitrogenous BP, without inducing any behavioural side effects [47]. Such action has been proposed to explain the efficacy of zoledronate in experimental rat OA models [12]. Increased osteoclastic activity, as found in OA [4, 13, 17, 18], could contribute to neuronal excitation and pain, and therefore inhibition of bone resorption and of nociceptor activation through anti-acid, anti-inflammatory actions would be analgesic. This hypothesis maintains an interaction of TLN with the molecular subchondral bone/cartilage cross-talk.

Subchondral bone mineral density decreases as early as 1 month following ACL transection [48]. In this OA model, extensive remodelling leading to a reduction in subchondral bone plate thickness, bone surface, and trabecular thickness, increased trabecular separation and loss of bone density and anisotropic properties were also reported [6, 49-51]. In this study, we found that following TLN treatment a number of those changes were reduced and closer to the values found in normal dogs [6]. More specifically, the architecture of subchondral bone and calcified cartilage was better preserved. The anti-resorptive action of TLN may explain these findings.

The advance movement and duplication of the tidemark contributes to overall thinning of the articular cartilage, thickening of the calcified cartilage while altering the biomechanic of the joint [18, 52]. In ACL-deficient dogs, the calcified cartilage undergoes thinning and an advancement of the tidemark later in the OA process [6, 48]. However, duplication of the tidemark was previously reported following 12 weeks of BP treatment [21]. Whether or not TLN affects the endochondral ossification remains to be determined as change in the calcification front was not assessed in the present study.

The decrease in cathepsin K activity observed under TLN treatment is well in line with the known mode of action of the drug at reducing osteoclastic activity [9, 10]. Given that mechanical loading governs bone architecture and mass, the greater alteration in bone structure found in placebo-treated dogs may also be explained by the lower forces transmitted to the joint as a consequence of pain-related limb disuse. Whereas previous studies using BPs [14, 15] or other antiresorptive agents [5-7] focused on the beneficial structural effects in experimental OA models, the present study highlights that the antiresorptive effect of TLN was associated with an apparent absence of effect on cartilage lesions, but translated to beneficial analgesic and functional consequences. One must, however, take into account that the present study lasted only 8 weeks, which is an obvious limiting factor regarding the exploration of the chondroprotective effect of TLN.

A recent analysis using MRI confirmed the common belief that it is likely that changes in the subchondral bone (BML) predominate in relation to OA knee pain [2, 53]. Bone marrow lesions are also associated with cartilage lesions [54]. These results support the dynamics of bone/cartilage cross-talk, and the fact that TLN affected the molecular, nociceptive, and biomechanical bone/cartilage interface in the canine ACL model. In the dog ACL model, we have observed similar relationships between MRI structural and functional changes [22, 23, 55]. Such findings further support the translational nature of results obtained in the ACL dog model to human OA.

13.6 Conclusions

The use of the dog ACL model in association with the complete set of functional methods used in the present study represents a most useful tool for the monitoring of pain and joint function in OA. The present study brings into perspective a possible link between joint structural changes and functional outcomes. The level of joint inflammation is an important co-factor in generating pain-related functional disability. The preservation of bone integrity also likely plays a key role in functional outcome, being required to reduce the disability occurring in ACL-deficient dogs. This is supported by the fact that TLN treatment demonstrated a positive effect on gait disability and joint symptoms, while being associated with a better preservation of calcified cartilage and subchondral bone histomorphometry, as well as reducing the synthesis of catabolic and inflammatory mediators.

13.7 List of abbreviations

ACL = anterior/cranial cruciate ligament; ADAMTS = a disintegrin and metalloproteinase domain with thrombospondin motifs; ASIC = acid-sensing ion channel; ATP = adenosine triphosphate (ATP); BML = bone marrow lesions; BP = bisphosphonate; DAAI = daily averaged active intensity; DATI = daily averaged active intensity; EDA = electrodermal activity; MMP = matrix metalloproteinase; MRI = magnetic resonance imaging; NOx = nitrites and nitrates; NRS = numerical rating scale; OA = osteoarthritis; P2X = purinergic receptors; PGE2 = prostaglandin E2; PVF = peak vertical force; %BW = percentage of body weight; SC = subcutaneous; SD = standard deviation; TLN = Tiludronate; TRPV1 = transient receptor potential vanilloid 1; VAS = visual analog scale

13.8 Competing interests

The majority of authors declare that they have no competing interests, but Drs T Bertaim and D Thibaud are regular employees of CEVA, who supervised the study for this sponsor.

13.9 Authors' contributions

MM contributed to the study design, carried out the analysis and interpretation of data from the gait analysis, contributed to tissue sampling, performed the statistical analysis and drafted the manuscript. PR carried out the analysis and interpretation of data from the pain and functional evaluation, and participated in the manuscript drafting. JPP conceived the study, elaborated the design, interpreted the data, and

critically revised the manuscript. JMP conceived the study, elaborated the design, interpreted the data, and critically revised the manuscript. DL carried out the analysis and interpretation of data from the histomorphometry, and participated in the manuscript drafting. CB carried out the analysis and interpretation of data from the macroscopic and microscopic grading, and participated in the manuscript drafting. JC carried out the analysis and interpretation of data from the macroscopic and microscopic grading, contributed to the management of data from pain and functional evaluation, and participated in the manuscript drafting. DF contributed to the design, analysis and interpretation of data from video-automated behaviour recording, and participated in the manuscript drafting. BL contributed to the surgical process, pain and functional evaluation and critically revised the manuscript. JdC contributed to the statistical analysis and critically revised the manuscript. GB contributed to the statistical analysis, and participated in the manuscript drafting. DG carried out the nitrites and nitrates assays and contributed to the management and interpretation of data from the pain and functional evaluation and revised the manuscript. TB participated in the conception of the study, provided the test article and placebo, participated in the data interpretation and revised the manuscript. DT participated in the conception of the study, provided the test article and placebo, participated in the data interpretation and in the manuscript drafting. ET conceived the study, elaborated the design, managed the experiments, contributed to the pain and functional evaluation, collected the data, sampled tissues, interpreted the data, drafted and revised the manuscript, and

is responsibile for the integrity of the work as a whole. All authors read and approved the contents of this final version of the manuscript.

13.10 Acknowledgements

The authors thank Virginia Wallis for her assistance with the manuscript preparation. This study was supported in part by a grant from CEVA Santé Animale, Libourne, France, an ongoing New Opportunities Fund grant from the Canada Foundation for Innovation (#9483) for the pain/function equipment; a Discovery grant from the Natural Sciences and Engineering Research Council of Canada (#327158-2008) as operating fund for pain biomarkers, and by CRCHUM and the Osteoarthritis Chair, Université de Montréal.

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CHAPITRE 3. DISCUSSION GÉNÉRALE

14 Modèle canin d'arthrose naturelle

14.1 La force verticale maximale et la détection d'effets thérapeutiques lors d'essais cliniques contrôlés

Les expérimentations au sein de cet ouvrage (**8, 11 ci-dessus - Chapitre 2**) ont tenu compte du chien comme espèce modèle d'intérêt et ce, en contexte d'arthrose naturelle chez l'animal de compagnie. La force de réaction au sol verticale maximale est proposée comme témoin d'effets thérapeutiques lors d'essais cliniques contrôlés effectués chez ce modèle.

Afin de contribuer à valider l'usage de la force verticale maximale comme critère d'efficacité chez le modèle canin d'arthrose naturelle, il était impératif d'adresser certaines critiques (Sharkey, 2013) pouvant influencer l'interprétation de résultats provenant d'essais cliniques contrôlés. Les expérimentations (**8, 11 ci-dessus - Chapitre 2**) ont été entreprises avec la perspective de promouvoir le modèle canin d'arthrose naturelle. Ce modèle, couplé à la mesure de la force verticale maximale comme critère d'efficacité, pourrait ainsi permettre de développer des modalités thérapeutiques contre l'arthrose et ce, tant pour le chien qu'avant de pouvoir les transposer avec assurance chez l'Homme.

14.1.1 Retour sur les hypothèses

Les hypothèses préalablement émises (**8.2 ci-dessus - Chapitre 2**) ont été confirmées.

Lors d'un essai clinique contrôlé chez le chien arthrosique, la force verticale maximale démontre une excellente fidélité selon un coefficient de corrélation intra-classe de 91 [80-95] %. L'erreur de mesure de la force verticale maximale s'est avérée inférieure à 1.0 % de poids corporel, soit 0.7 % de poids corporel. Tenant compte de la fidélité et de l'erreur de mesure, le seuil du changement minimal détectable (à un intervalle de confiance de 95 %) de la force verticale maximale a été défini comme étant 2.0 % de poids corporel. Ce seuil demeure cohérent avec le gain de force verticale maximale communément observé lors d'essais cliniques contrôlés (**Tableau VI**).

Le mouvement locomoteur du chien arthrosique a démontré un lien proportionnel avec la mesure de la force verticale maximale. Également, le changement du niveau de mouvement locomoteur a démontré un lien proportionnel avec celui de la force verticale maximale.

14.1.2 Le changement minimal détectable (à un intervalle de confiance de 95 %) comme critère de discernement d'un effet thérapeutique

Bien que l'usage des forces de réaction au sol, particulièrement la force verticale maximale, soit couramment utilisé dans la littérature (Aragon *et al.*, 2007; Gillette *et al.*, 2008; Sanderson *et al.*, 2009), aucun seuil ne permettait à ce jour de distinguer un changement caractéristique d'une réponse. Par réponse, on entend un changement vers l'amélioration ou la détérioration du dysfonctionnement locomoteur. Par conséquent, l'absence d'un seuil limite au-delà duquel un changement se distingue de la marge d'erreur inhérente à la mesure de la force verticale maximale fut récemment critiqué (Sharkey, 2013).

Il est commun, dans la littérature, de qualifier la réponse d'un groupe de sujets (chiens arthrosiques) participant à un essai clinique contrôlé à l'aide de tests statistiques basés sur le rejet d'une hypothèse nulle précédemment émise (Aragon *et al.*, 2007; Sanderson *et al.*, 2009). Ces tests considèrent des comparaisons entre des indicatifs de tendance centrale (moyenne ou médiane) pour différents critères d'efficacité, en l'occurrence des mesures de force verticale maximale. Ces mesures peuvent être obtenues par un même groupe de sujets à différentes occasions (mesures répétées) ou par différents groupes de sujets (groupe contrôle négatif, groupe (s) test thérapeutique (s)). En général, l'hypothèse nulle sera l'absence d'une différence statistiquement significative entre les indicatifs de tendance centrale pour des mesures de force verticale maximale.

Le présent auteur émet, par contre, des limites aux approches statistiques qui comparent des indicatifs de tendance centrale afin de qualifier la réponse d'un groupe de sujets.

- La présence d'une taille d'échantillons considérables, soit par exemple le triple de ce que les analyses de puissance statistique suggèrent (**6.1.2.3.8 ci-dessus - Chapitre 1**), pourrait faciliter le rejet de l'hypothèse nulle. L'effet des données extrêmes sur la variance de la force verticale maximale serait ainsi réduit par la taille de l'échantillon. Ce principe est derrière la pertinence d'utiliser des procédures de ré-échantillonnage **(7.1.3.8 ci-dessus - Chapitre 1**) afin de déterminer une taille d'échantillons pour une puissance statistique donnée. Ceci signifie que, pour un même changement de force verticale maximale donné, la différence entre les mesures pourrait être statistiquement significative lorsque le groupe est grand.
- La présence de conditions expérimentales favorables pourrait faciliter le rejet de l'hypothèse nulle. Ceci signifie que, pour un même changement de force verticale maximale, la différence pourrait être statistiquement significative lorsque le groupe démontre une variance réduite pour des mesures de force verticale maximale.
- Le rejet de l'hypothèse nulle, dans un contexte expérimental précis, rend complexe l'interprétation plus large de résultats issus de différents essais cliniques. Ceci s'explique par le fait que la magnitude du changement, et donc la capacité de rejeter l'hypothèse nulle, est intimement reliée aux conditions expérimentales propres à chacun des essais cliniques contrôlés.

- Finalement le rejet de l'hypothèse nulle, pour un changement de force verticale maximale donné, ne permet pas de qualifier les données individuelles en termes de répondants. C'est la magnitude du changement d'un groupe qui est d'importance.

Il importe toutefois de préciser l'existence, au sein de la littérature, d'un seuil permettant de conclure à une réponse thérapeutique lors de l'augmentation de la force verticale maximale de 5 % chez un groupe de sujets (Vasseur *et al.*, 1995). Malheureusement, la démarche scientifique qui appuie ce seuil n'est pas connue. Par conséqquent, ce seuil semble signifier qu'un changement de force verticale maximale, qui excède 5 % de la mesure initiale, serait le reflet d'une réponse thérapeutique.

La relation proportionnelle entre le seuil proposé de 5 % (Vasseur *et al.*, 1995) et l'état initial de l'animal est, selon le présent auteur, une limite à son utilisation. Ainsi, un chien arthrosique ayant une force verticale maximale très faible (par exemple à 36 % de poids corporel) devra démontrer un changement qui excède 1.8 % de poids corporel afin d'être qualifié de répondant. Par contre, ce seuil sera plus élevé (2.8 % de poids corporel) pour un animal atteint d'un dysfonctionnement locomoteur qui est moindre (par exemple 56 % de poids corporel). A l'aide du seuil proposé, la qualification d'une réponse thérapeutique individuelle est donc assujettie à la variabilité du degré de dysfonctionnement initial plutôt qu'à l'erreur de mesure. De plus, ceci est contraire à nos résultats (**11.4.2 ci-dessus - Chapitre**

2) qui témoignent qu'un animal avec une force verticale maximale plus faible aura plus de chances d'être un répondant au traitement.

Récemment, il a été démontré que les chiens gonarthrosiques sont aux prises avec des degrés de dysfonctionnement locomoteur qui sont plus sévères que ceux atteints de coxarthrose (Madore *et al.*, 2007). Par conséquent, l'usage du seuil proposé de 5 % (Vasseur *et al.*, 1995) pourrait s'avérer trop robuste en compromettant l'identification d'une réponse thérapeutique chez des chiens atteints, par exemple, de coxarthrose.

Face à ces réticences, l'établissement d'un seuil limite au-delà duquel un changement se distingue de l'erreur de mesure était donc nécessaire. Lorsque les travaux de maîtrise du présent auteur (Moreau, 2002) furent présentés à des podiums internationaux, il était fréquent d'être confronté à la question suivante : quelle était la proportion des répondants? N'étant pas en mesure de définir ce qu'était un répondant, la réponse avancée n'était pas satisfaisante pour l'audience. Cette faille constitue la motivation première des expérimentations entreprises chez le modèle canin d'arthrose naturelle.

Le seuil limite au-delà duquel un changement se distingue de la marge d'erreur inhérente à la mesure de la force verticale maximale a été déterminé, contribuant ainsi à l'avancement des connaissances dans le domaine de l'évaluation clinique de modalités thérapeutiques contre l'arthrose canine. L'usage du changement minimal

détectable (à un intervalle de confiance de 95 %) est ainsi proposé comme critère de discernement d'une réponse thérapeutique basée sur l'erreur de mesure de la force verticale maximale.

14.1.3 Procédurier statistique lors de l'analyse de la force verticale maximale en contexte d'essais cliniques contrôlés

À ce jour, l'absence de procédurier fait en sorte que différentes comparaisons statistiques peuvent être effectuées afin de qualifier la réponse d'un groupe de sujets selon la force verticale maximale utilisée comme critère d'efficacité. Certaines sections (**6.1.1.3.6, 6.1.2.3.7 ci-dessus - Chapitre 1**) témoignent de cette réalité, ce qui rend complexe l'interprétation statistique. La détermination du changement minimal détectable (à un intervalle de confiance de 95 %) comme critère de discernement d'une réponse thérapeutique, porte le présent auteur à suggérer un procédurier statistique. En contexte d'essais cliniques contrôlés (groupe contrôle négatif *versus* groupe(s) test thérapeutique(s)), l'approche statistique suivante est proposée :

- Effectuer un test statistique selon l'hypothèse nulle que les mesures de force verticale maximale sont similaires entre les groupes à l'initiation de l'essai clinique. Dans le cas où il y aurait rejet de l'hypothèse nulle, le groupe ayant un degré de dysfonctionnement locomoteur plus sévère pourrait être favorisé en termes de réponse thérapeutique (**11.4.2 ci-dessus - Chapitre 2**).

- Effectuer un test statistique selon l'hypothèse nulle que la proportion de répondants, selon un changement minimal détectable à un intervalle de confiance de 95 %, est similaire entre les groupes.
- Effectuer un test statistique selon l'hypothèse nulle que les changements de force verticale maximale sont similaires entre les groupes.
- Appuyer la proportion de répondants avec l'indice de tendance centrale pour le changement de force verticale maximale. Appuyer également la proportion de répondants avec le résultat de la comparaison statistique.

- Effectuer un test statistique selon l'hypothèse nulle que la proportion de répondants-négatifs, selon un changement minimal détectable à un intervalle de confiance de 95 %, est similaire entre les groupes. Scruter les proportions attentivement. L'acceptation de l'hypothèse nulle peut tout de même suggérer l'exacerbation du dysfonctionnement locomoteur en cours d'essai.

14.2 Le mouvement locomoteur et la force verticale maximale

L'enregistrement du mouvement locomoteur comme critère d'efficacité est en émergence au sein d'essais cliniques contrôlés chez le chien arthrosique (Brown *et al.*, 2010; Wernham *et al.*, 2011). Il est désormais possible d'obtenir une estimation de la durée quotidienne d'activité du chien affligé d'un dysfonctionnement locomoteur associé à l'arthrose. En effet, il s'avère que ce dernier est en mouvement durant approximativement sept heures par jour, soit près d'un tiers de

la journée (**Tableau V**, **Tableau VIII**). L'inférence envers la population générale est difficile, compte tenu de la faible taille d'échantillon. Néanmoins, les expérimentations (**Tableau V**, **Tableau VIII**) suggèrent que les chiens arthrosiques pourraient être moins actifs, comme en témoignent de récentes investigations chez l'humain (Wallis *et al.*, 2013).

Le degré de mouvement locomoteur chez le chien arthrosique est assujetti d'une variance qui demeure à être expliquée. D'emblée, par contre, il est fort possible que l'espace accessible, l'environnement et l'interaction avec l'humain ou avec d'autres animaux soient des facteurs pouvant expliquer la variance dans le degré de mouvement locomoteur. Les expérimentations (**10.4.2.2 ci-dessus - Chapitre 2**) démontrent que, chez le chien arthrosique, le mouvement locomoteur effectué sur un période de six semaines est en lien avec le degré de dysfonctionnement locomoteur reflété par la force verticale maximale. Ainsi, l'animal arthrosique peu actif au quotidien sera celui ayant une faible force verticale maximale et donc, celui ayant un degré de dysfonctionnement locomoteur plus sévère. Au regard de ce lien, le mouvement locomoteur s'avère être un déterminant de la force verticale maximale. D'un regard mécanistique, le présent auteur suggère que la sollicitation musculaire soit accentuée en présence d'un degré moindre d'inconfort au niveau de l'articulation arthrosique. La capacité musculaire et le soulagement de l'inconfort pourraient donc avoir influencé la mesure subséquente de la force verticale maximale vers l'amélioration. Toutefois, il importe d'investiguer l'implication des différents muscles du membre locomoteur face aux forces de réaction au sol

produites lors du mouvement. L'identification, par électromyographie (Kaya *et al.*, 2006), des muscles responsables de la force verticale maximale chez le chien pourrait permettre de résoudre ce questionnement. L'induction expérimentale d'une paralysie des muscles locomoteurs (Yaraskavitch *et al.*, 2008), pourrait mener à reconnaître l'impact de l'atrophie musculaire envers le dysfonctionnement locomoteur. L'atrophie musculaire a été identifiée comme facteur contribuant au dysfonctionnement locomoteur chez l'humain arthrosique (Fisher *et al.*, 1997; Karamanidis *et al.*, 2014). L'implication de la capacité musculaire envers le dysfonctionnement locomoteur a également été mise en évidence lors d'un essai clinique contrôlé chez le chien arthrosique. Une réduction de l'atrophie a été observée en parallèle à un gain en force verticale maximale (Moreau *et al.*, 2004).

Des réponses thérapeutiques ont été observées (**Figure 19**, **Figure 24**) en se basant sur le changement minimal détectable (à un intervalle de confiance de 95 %) de force verticale maximale. En parallèle à ce critère primaire d'efficacité, l'impact au niveau du mouvement locomoteur s'est également fait ressentir, *via* des gains quotidiens en période active de plus de 60 minutes, soit environ 14 % (**Figure 20**, **Figure 25**). Une relation entre le changement au mouvement locomoteur et celui au niveau de la force verticale maximale était donc envisageable. Les expérimentations (**10.4.2.2 ci-dessus - Chapitre 2**) démontrent la présence d'un lien entre le changement au degré de mouvement locomoteur et le dysfonctionnement reflété par la force verticale maximale. Ainsi, le changement de force verticale maximale du chien arthrosique, établi sur une période de six

semaines, peut être expliqué en connaissant le changement concomitant au niveau du mouvement locomoteur. De cette relation, il est également possible d'estimer la répercussion de l'activité quotidienne envers le degré de dysfonctionnement locomoteur. Au regard de ces résultats, une durée additionnelle d'activité quotidienne, de l'ordre de 54 minutes, pourrait avoir une répercussion thérapeutique tangible envers le degré de dysfonctionnement locomoteur. L'amélioration pourrait excéder le changement minimal détectable (à un intervalle de confiance de 95 %).

14.3 L'activité quotidienne comme modalité thérapeutique chez le chien arthrosique

L'approche multimodale de l'arthrose canine considère, par définition, la combinaison de différentes modalités thérapeutiques et adresse des recommandations sur l'obésité et la sédentarité (Fox *et al.*, 2010). Les expérimentations (**10.4.2.2 ci-dessus - Chapitre 2**) soutiennent la pertinence de maintenir le chien arthrosique actif au quotidien. Pour le propriétaire de l'animal arthrosique et le clinicien, le fait de devoir augmenter l'activité de l'animal prend désormais une signification concrète. Ainsi, 54 minutes d'activité additionnelle au quotidien pourraient s'avérer bénéfiques. De plus, le soulagement de l'inconfort serait comparable à des modalités thérapeutiques reconnues, comme les antiinflammatoires non stéroïdiens, les diètes thérapeutiques ou les produits de santé naturels (**Tableau VI**).

Il importe de préciser que la durée additionnelle d'activité peut, cependant, s'avérer contraignante pour le quotidien du propriétaire, ce dernier percevant cette recommandation comme un fardeau, c'est-à-dire devoir effectuer une heure additionnelle d'activité par jour. Dans les faits, chaque minute additionnelle d'activité compte et contribue au cumul journalier. La durée additionnelle d'activité quotidienne suggérée est également en lien avec une récente étude utilisant l'appréciation subjective de l'activité et du dysfonctionnement locomoteur (Greene *et al.*, 2013). Ainsi, le degré de dysfonctionnement locomoteur démontra une amélioration tangible chez les chiens soumis à plus de 60 minutes d'exercice au quotidien. Il importe, par contre, de soumettre à l'investigation l'effet de l'augmentation de l'activité quotidienne en combinaison à l'administration de modalités thérapeutiques reconnues (**Tableau VI**). Une telle investigation supporterait davantage l'approche multimodale de l'arthrose canine.

Un point important n'a pas été souligné concernant le mouvement locomoteur. Actuellement, le degré d'activité quotidienne du chien sain dans un environnement domestique est inconnu. En l'absence d'une telle donnée, il s'avère donc difficile de positionner le gain d'activité obtenu ou à obtenir, comparativement à l'état normal de l'animal. La relation entre le mouvement locomoteur et le dysfonctionnement pourrait être valable si, et seulement si, l'animal est sédentaire et, par conséquent, démontre une faiblesse musculaire. L'effet thérapeutique pourrait plafonner, lorsque le chien atteint un degré normal d'activité quotidienne et qu'il possède une pleine capacité musculaire. Il importe donc d'investiguer l'effet

de l'augmentation du degré d'activité quotidienne chez le chien arthrosique sédentaire et également, chez celui idéalement actif.

L'impact à long terme de l'activité quotidienne soutenue demeure également à être investigué chez des chiens arthrosiques atteints d'une arthropathie développementale, comme la dysplasie de la hanche. Au-delà de l'effet bénéfique associé au gain en activité quotidienne, et possiblement à l'amélioration de la capacité musculaire, l'exacerbation de l'atteinte structurelle pourrait survenir en présence d'une sollicitation mécanique excessive. Le degré de sollicitation excessive pourait être amplifié par un changement locomoteur. Ainsi, une association délétère entre le mouvement et les dommages structuraux pourrait exister, comme le souligne une récente étude chez l'humain à l'aide d'imagerie par résonance magnétique (Doré *et al.*, 2013).

14.4 Le mouvement locomoteur comme source de biais potentiel envers la force verticale maximale

La pertinence d'enregistrer le mouvement locomoteur, dans un contexte d'essais cliniques contrôlés chez le chien arthrosique, a déjà été présentée (**6.1.2.4.5, 6.1.3.4.3 ci-dessus - Chapitre 1)**. En contraste avec la mesure de la force verticale maximale, l'enregistrement du mouvement locomoteur permet une évaluation en continu, et ce, sans altérer la routine de l'animal. En plus de l'usage du mouvement locomoteur à titre de critère d'efficacité, il s'avère également pertinent de proposer d'utiliser l'enregistrement du mouvement locomoteur en complément de la mesure

ponctuelle de la force verticale maximale. De la sorte, la variance dans la mesure de la force verticale maximale qui serait tributaire du degré de mouvement locomoteur pourrait être atténuée en ajustant cette mesure en fonction du mouvement.

La démonstration de la pertinence du mouvement locomoteur comme covariable de la mesure de la force verticale maximale requiert toutefois d'être démontrée. Néanmoins, il importe à l'investigateur de s'affranchir de toute disparité entre les différents groupes au regard du degré d'activité quotidienne en cours d'essai clinique. A cette fin, l'usage d'une échelle descriptive simple pourrait être un indicateur intéressant (Greene *et al.*, 2013).

14.5 Analyse de répondants selon la force verticale maximale

14.5.1 Retour sur les hypothèses

Les hypothèses préalablement émises (**11.2 ci-dessus - Chapitre 2**) ont été confirmées.

Le taux de répondants est en lien avec le dysfonctionnement locomoteur initial. Le taux de répondants est également en lien avec le type de traitement administré, soit diètes thérapeutiques, produits de santé naturels et AINS, comparativement à un contrôle négatif (placebo). Toutefois, il n'y a pas de lien statistiquement significatif entre le taux de répondants et les approches thérapeutiques prises distinctivement.

14.5.2 Le taux de répondants

À l'aide du changement minimal détectable (à un intervalle de confiance de 95 %) l'analyse rétrospective des répondants témoigne d'un taux de réponse de 62.8 %. Dans l'ensemble, une réponse favorable est à prévoir lors de l'administration de diètes thérapeutiques, de produits de santé naturels ou d'AINS. Plus spécifiquement, en découle une taille d'effet estimée de 0.7 et un nombre nécessaire à traiter (*number needed to treat*) estimé de 3.6. Ces deux paramètres sont légèrement favorables comparativement aux traitements actuels de l'arthrose chez l'humain (Kahan *et al.*, 2009; Zhang *et al.*, 2010a; Hochberg *et al.*, 2012). Cette disparité pourrait s'expliquer par le fait que le changement minimal détectable représente l'amélioration « biomécanique » du dysfonctionnement locomoteur, au-delà de l'erreur de mesure de la force verticale maximale. Il importe maintenant de mettre en relation le niveau de changement de force verticale maximale qui correspond à une amélioration qualifiée de « clinique » selon l'appréciation à l'aide d'un tiers. En établissant le seuil minime d'importance clinique (selon le clinicien) ou le seuil minimal d'importance à la qualité de vie (selon le propriétaire), il serait donc possible de s'affranchir de tout doute en ce qui concerne l'amélioration de la fonction considérée comme strictement « biomécanique ».

14.5.3 Relation binomiale

La relation entre le taux de répondants (76/121) et la force verticale maximale a démontré que, pour une augmentation de 1.0 % de poids corporel, les chances de rencontrer un répondant étaient diminuées de 9.0 %. Ceci suggère que le chien

affligé d'un déficit locomoteur sévère, est plus susceptible de démontrer une réponse thérapeutique qu'un animal moins sévèrement atteint. Cette relation vaut pour des modalités thérapeutiques qui font partie intégrante du contexte thérapeutique actuel. Chez l'humain, par contre, la relation entre le taux de répondants et certaines caractéristiques des sujets ne suggère pas d'emblée la présence de ce lien, mais nécessite de plus amples investigations (Eyles *et al.*, 2014).

14.6 Hiérarchisation de l'approche thérapeutique contre l'arthrose

De nombreuses discussions ont eu lieu avec les propriétaires de chiens arthrosiques durant les expérimentations de cet ouvrage. Le constat suivant peut être établi : il est commun chez le clinicien vétérinaire de hiérarchiser l'approche thérapeutique en fonction du degré d'atteinte de l'animal. Autrement dit, le chien affligé d'un dysfonctionnement locomoteur sévère sera celui dont les efforts thérapeutiques seront mis de l'avant promptement. A l'inverse, le chien qui démontre un léger dysfonctionnement locomoteur pourra, dans certains cas, ne bénéficier que de recommandations usuelles. Chez ce dernier, l'usage d'AINS pourrait même ne pas être considéré. Le clinicien opterait plutôt pour des produits de santé naturels, en l'occurrence le chlorhydrate de glucosamine avec ou sans sulfate de chondroïtine, afin de prévenir l'apparition d'inconforts plus conséquents. Malheureusement, dans la plupart des cas, cet agent est administré à des doses qui sont qualifiées de sous thérapeutiques (Maxim Moreau, données personnelles, 2012) selon une conversion

allométrique (FDA, 2005) de dosages ayant démontré des effets thérapeutiques chez l'humain (Reginster *et al.*, 2012; Zegels *et al.*, 2013).

Les expérimentations (**11 ci-dessus - Chapitre 2**) démontrent que le chien ayant un dysfonctionnement locomoteur moins sévère est celui le moins susceptible de démontrer une réponse thérapeutique. À la lueur des expérimentations, le défi pour le clinicien est donc à ce niveau : tenter de soulager l'inconfort de l'animal qui démontre un degré de dysfonctionnement subtil. Chez ce dernier, la combinaison de diverses approches thérapeutiques, comme par exemple une diète thérapeutique et un AINS, pourrait, dans ce cas, être une option thérapeutique à considerer. Toutefois, pour l'ensemble des chiens arthrosiques, il demeure pertinent de combiner l'activité quotidienne aux modalités thérapeutiques actuelles, comme le démontrent des évidences cliniques chez l'humain (Armagan *et al.*, 2014).

Le rapport de cote favorable sous l'effet d'une modalité thérapeutique (diètes thérapeutiques, produits de santé naturels ou AINS) comparativement à un contrôle négatif (placebo) souligne l'efficacité thérapeutique des modalités actuelles. Ce qui est intriguant, par contre, c'est la constatation que, les différentes modalités procurent généralement le même degré d'amélioration envers le dysfonctionnement locomoteur. Également, même en tenant compte du changement moyen soit 4.0 % de poids corporel, une disparité existe toujours comparativement à la force verticale maximale d'un chien sain. De ces constatations découlent les réflexions suivantes :

- L’efficacité globale des modalités thérapeutiques suggère le besoin de pallier de manière plus optimale à l’inconfort du chien arthrosique. Actuellement, le soulagement semble être partiel. La recherche et le développement de modalités thérapeutiques doivent être accentués, soit par des avenues novatrices ou par la combinaison de modalités thérapeutiques existantes comme le recommande l’approche multimodale de l’arthrose canine (Fox *et al.*, 2010).
- L’efficacité globale des modalités thérapeutiques semble atteindre un seuil plafond. Ce seuil pourrait suggérer que le dysfonctionnement locomoteur soit déterminé, principalement, par des phénomènes autres que ceux associés au ressenti douloureux. L’altération au dynamisme articulaire, en présence de dommages arthrosiques, pourrait donc être un déterminant majeur du dysfonctionnement locomoteur reflété par un niveau anormal de force verticale maximale. Chez l’humain, même lorsque le ressenti douloureux est soulagé, le dysfonctionnement locomoteur semble être récurrent (Henriksen *et al.*, 2006), ce qui suggère l’altération au dynamisme articulaire lors d’arthrose.

14.7 Effet nocebo selon la force verticale maximale

Le changement minimal détectable est proposé afin de distinguer une réponse qualifiée de thérapeutique et ce, en s’affranchissant de l’erreur de mesure. En cours d’essais cliniques contrôlés, il est également possible que l’exacerbation du dysfonctionnement locomoteur soit observée. Par exemple, l’atteinte traumatique

au ligament croisé crânial, qui est une cause d'exclusion très fréquente (**Figure 17**, **Figure 22**), est responsable d'un degré additionnel de dysfonctionnement locomoteur. En général, le propriétaire est à même d'identifier ce changement drastique de condition. Par conséquent, les investigateurs peuvent aisément retirer le sujet de l'essai en cours. Cependant, les changements plus subtils, eux, peuvent passer inaperçus. Le changement minimal détectable se propose également afin de cerner l'apparition de l'exacerbation du dysfonctionnement locomoteur en cours d'essais cliniques.

Bien qu'il soit commun de rapporter l'amélioration de la condition du chien arthrosique lors d'essais cliniques contrôlés, l'exacerbation de la condition ne semble pas, aux yeux du présent auteur, susciter suffisamment d'intérêt. En effet, un essai clinique (Imhoff *et al.*, 2011) a récemment rapporté une diminution de force verticale maximale de 3.8 % de poids corporel chez un groupe de chiens arthrosiques ayant reçu un produit de santé naturel. Ce changement au dysfonctionnement locomoteur excède largement le seuil minimal détectable actuellement proposé, qui est de 2.0 % de poids corporel. Les chiens ayant reçu un contrôle négatif ont également eu une diminution de force verticale maximale, soit de 2.2 % de poids corporel. Les investigateurs n'ont malheureusement pas présenté l'exacerbation de la condition comme étant une limite d'expérimentation et une source de biais potentiel envers l'interprétation des résultats. Il importe donc que les investigateurs documentent la présence de changements, qu'ils soient favorables ou non, afin d'éviter de biaiser l'interprétation des résultats. A titre d'exemple, il est

à entrevoir que la présence de répondants-négatifs, au sein du groupe contrôle négatif, puisse faciliter le rejet de l'hypothèse nulle en introduisant la présence d'un effet nocebo suite à l'exacerbation du dysfonctionnement locomoteur. L'exacerbation du dysfonctionnement locomoteur, au sein du groupe test thérapeutique, sera tout aussi nuisible en altérant la capacité de l'essai clinique à démontrer l'efficacité du traitement. Dans ce dernier cas, l'essai clinique pourrait même mener à la conclusion qu'un effet néfaste est issu de la substance mise à l'essai.

Les taux de répondants (**11.4.1 ci-dessus - Chapitre 2**) de même que la **Figure 33** ne suggèrent pas la présence d'un effet nocebo lorsque la force verticale maximale est utilisée comme critère d'efficacité en cours d'essais cliniques contrôlés. Les résultats suggèrent plutôt un effet net qui est presque nul, soit de 1.1 % au profit d'une réponse favorable. Cet effet nul considère la différence entre la proportion de répondants-négatifs (33.7 %, effet nocebo) et de répondants (34.8 %, effet placebo).

Les expérimentations (**10.4.2.2 ci-dessus - Chapitre 2**) permettent d'identifier la diminution de l'activité quotidienne comme étant une source potentielle de biais envers l'exacerbation du dysfonctionnement locomoteur chez le chien arthrosique. À ceci s'ajoutent le gain de poids (**4.4.2 ci-dessus - Chapitre 1**) et l'exercice intense (Annexe VI).

Ces facteurs doivent donc être considérés par l'investigateur afin de s'assurer de la validité des résultats qui découlent de l'usage de la force verticale maximale comme critère d'efficacité. Par contre, pour le clinicien, il importe de considérer la perte de poids corporel chez le chien arthrosique en surpoids et ce, en accord avec l'approche multimodale de l'arthrose canine (Fox *et al.*, 2010).

14.8 Effet placebo selon la force verticale maximale

14.8.1 Article IX. Does a placebo effect really occur in dogs afflicted by hip osteoarthritis as measured by force platform gait analysis?

Cet article de correspondance, publié dans *BMC Veterinary Research*, présente le point de vue des auteurs en réponse à un récent article (Malek *et al.*, 2012) mentionnant la présence d'un effet placebo lors d'un essai clinique contrôlé chez le chien arthrosique. Cet effet placebo était particulièrement basé sur la mesure de la force verticale maximale utilisée comme critère d'efficacité. Le but de cet article était d'émettre des arguments opposés aux conclusions énoncées, particulièrement en relevant des failles méthodologiques.

M. Maxim Moreau a rédigé cet article présentement publié (*BMC Veterinary Research* 9:260, 2013). L'article a par la suite été dûment révisé et bonifié par l'expertise de chacun des coauteurs.

BMC Veterinary Research
9:260, 2013

Does a Placebo Effect Really Occur in Dogs Afflicted by Hip Osteoarthritis as Measured by Force Platform Gait Analysis?

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14.8.1.1 Abstract

A recent study investigated the therapeutic response of dogs afflicted by hip osteoarthritis when evaluating therapeutic modalities compared to a negative (placebo) control group. Authors suggested a placebo effect based on peak vertical force measurement. In addition, small effect size for each of the tested therapeutics as well as the extremely large sample size needed (>450) to discern therapeutic efficacy using force platform gait analysis were reported. We wish to express our concerns regarding the eligibility criteria used to select the studied cohort, the small effect size, and the placebo effect reported in force platform gait analysis.

14.8.1.2 Correspondence

We would like to provide some comments on the recently published article on the clinical outcome measures in a randomized controlled trial (RCT) of canine osteoarthritis (OA) from the perspective of clinical investigators. The article investigated the therapeutic response of dogs afflicted by hip OA to three therapeutic modalities compared to a negative (placebo) control group [1]. The main goal of the study was to determine the effect size (ES) of key outcome measures and, secondly, to highlight, in such RCTs assessing different treatments, the interest of combining multiple (of different nature) outcome measures. Effect size emphasises the size of the difference rather than confounding this with sample size as in statistical significance. However, it is very rarely used in primary reports. Due to the unfamiliarity of using this test in such a context, we would like to call for caution before a conclusion can be drawn from this study.

As indicated in their Introduction, in a recent meta-analysis of RCTs in human OA, Zhang *et al*. [2] reported a placebo ES (the standard mean difference between baseline and endpoint) of 0.51 (95% CI 0.46 to 0.55). Effect sizes can be interpreted in terms of the percentiles or ranks at which two distributions overlap [3]. With an ES of 0.51, the probability that one could guess which group (naïve at baseline, or placebo at endpoint) a person was in based on their 'score' is around 60%, whereas an ES of 0.00 would logically provide a similar probability of 50%. This is, at least in our judgement, a more relative interpretation of a 'mild' ES (0.20 < ES < 0.80); a placebo ES of 0.51 gives only a 10% increased chance of determining the group of the examined person. Two other points need to be reported from the study of Zhang *et al*. [2]. They found placebo to be effective in all subjective outcomes (not just patient-associated [*e.g*. pain, stiffness, self-reported function] but also observer-centred [doctor global opinion]), but ineffective for almost all objective outcomes (*e.g*. quadriceps strength, knee circumference, range of movement, radiographic narrowing); the ES of placebo was twice as high in hand OA (0.80) compared to hip OA (0.37).

To reach their goal, Malek *et al.* [1] recruited dogs having hip OA in addition to concomitant musculoskeletal conditions (n = 27/49, 55% of included dogs). Several dogs (n = 7) had surgically altered hip or stifle joint structures including excision arthroplasty and total hip replacement. We believe that inclusion criteria should have been more restrictive to homogenise the studied cohort and to avoid any distinct gait pattern or joint biomechanical changes, thus preserving statistical

power and maximising ES. To avoid therapeutic plateau or positive bias in outcome measures, which could be particularly harmful to the ES, we also believe in the necessity of carefully defined wash-out periods for OA therapeutics including joint supplements or diets (n = 24/49) purported to improve afflicted dogs. Several peer-reviewed studies published in the last few years have respected the implementation of wash-out periods for sporadic non-steroidal anti-inflammatory drugs (NSAIDS), oral nutraceuticals, OA therapeutic diets, fatty acid supplements, continuous oral or injectable anti-inflammatory drugs (including both steroids and NSAIDs), or polysulfated glycosaminoglycan therapy [4-13].

Regarding the force platform gait analysis, the limb with the smallest F_z vector (later referred to as peak vertical force by Malek *et al.* [1]) was analysed, which does not necessarily mean that this parameter departed from normality. This was particularly critical considering the large heterogeneity in the cohort of recruited dogs. One would suggest, rather, a selection of subjects based on a predetermined variable threshold [4,6,9,14]. This threshold can be determined *a priori* with respect to normal values [13]. We would also like to suggest a selection of trials according to a velocity range limited to 0.3 m/s (*e.g.*1.6-1.9 or 1.9-2.2 m/s at the trot) [4,6-12,14] instead of a larger one that overlaps walking and trotting gait intervals (*e.g.* 1.3-1.9 m/s). For the measurement of pelvic gait parameters, conditions that predispose dogs to thoracic limb pain or functional abnormalities, such as elbow and shoulder OA, should be discarded to limit force redistribution to the pelvic limbs [11]. Furthermore, no documentation of the body weight was recorded at the

end of the study. It is believed that this point is of importance as changes in individual body weight can affect outcome measures in OA dogs [15].

Is it unclear why Malek *et al.* [1] interpreted the changes in falling slope observed in placebo-treated dogs as being "an undesired effect" and "due to pain" (see Tables 8 and 11) when the authors later concluded a placebo effect in dogs with hip OA after having denoted a mean (standard deviation, SD) change in peak vertical measurement of 2.8 (10.6)% body weight (Table 8). Without precise information on the stance phase duration, it is difficult to fully integrate falling slope changes. As gait parameters are intimately linked, it is strongly suggested to make *a priori* determination of the primary outcome to avoid any misleading interpretation.

We would like to express our disagreement with a sole representation of force platform gait analysis data as change in percentage relative to the initial condition. Rather, we suggest group central tendencies at each end-point as well as individual changes. Such representation could have been useful to demonstrate the regression to the mean phenomenon hypothesised by the authors [1] in an attempt to support a placebo effect in dogs with hip OA upon force platform gait analysis. Moreover, we believe that the accumulation of previously listed methodological short cuts could explain the large variability in kinetic (force platform) parameters observed in the study [1]. The variability of the results is reflected by the large SD values in kinetic parameters reported in Table 8.

As mentioned by Malek *et al.* [1], dogs showing a positive response to a negative control (placebo) were previously observed based on peak vertical [6,16], but globally the ground reaction forces did not demonstrate clinically meaningful changes under placebo and rather tend to be slightly negative [9,10,12,13]. This was confirmed by a recent meta-analysis including 40 OA dogs followed over 28 days [mean (SD) change −1.5 (3.1)% body weight with an intra-class coefficient of correlation of 91, and only 5 dogs presenting a real positive response for peak vertical force] [17] and a recent prospective study including 58 OA dogs over 42 days [18]. These latter studies reported a rate of positive responses around 10% using peak vertical force measurement.

Moreover, our concern about the negative interference of the methodology used by Malek *et al*. [1] with the kinetic measurement is supported by the absence of response to carprofen, which could be considered a positive control. Three previous publications reported positive response on peak vertical force in n = 36, n = 15 and n = 16, respectively, OA dogs treated with carprofen for 14 [16], 56 [13] and 60 [11] days. These findings could be related to a high proportion of neuropathic dogs in the recruited sample as mentioned by Malek *et al.* [1]. Hence, neuropathic pain is recognized as non-responsive to NSAIDS [19] and a component of a chronic painful condition such as osteoarthritis in dogs [20,21]. It is unfortunate that hyperalgesia/allodynia was not specifically tested in this population of dogs with the use, for instance, of von Frey anesthesiometer-induced paw withdrawal threshold, as recently reported in OA cats [22].

We are in agreement with the clinical relevance of reporting ES and the paramount importance of statistical power and sample size estimates to design fruitful RCTs. However, we encourage a thorough presentation of the method used for single group ES calculation [23] from which Malek *et al.* [1] derived their conclusions. The interest of this publication remains in the original report of a TRPV1 antagonist (ABT-116) and tramadol efficacy in canine OA. However, the poor ES reported for each of the tested therapeutics as well as the extremely large sample size needed (>450) to discern efficacy of force platform gait analysis, should be considered with scepticism. Furthermore, the data did not support placebo (or nocebo) effects in dogs with hip OA upon force platform gait analysis.

14.8.1.3 Abbreviations

RCT: Randomized controlled trial; OA: Osteoarthritis; ES: Effect size; NSAIDS: Non-steroidal anti-inflammatory drugs; SD: Standard deviation

14.8.1.4 Competing interests

The authors declare that they have no competing interests.

14.8.1.5 Authors' contributions

MM drafted the manuscript. BL, JPP and ET reviewed, commented and complemented the manuscript. All authors have read and approved the final manuscript.

14.8.1.6 Authors' information

MM is experienced in force platform gait analysis in canine and feline naturally-occurring and canine experimentally induced osteoarthritis.

BL, JPP and ET are full professors at the Université de Montréal, and present several decades of expertise in canine and feline osteoarthritis.

JPP and ET are heads of the Osteoarthritis Research Unit and GREPAQ, respectively.

14.8.1.7 Acknowledgements

Authors are grateful to Virginia Wallis for her assistance with the manuscript.

14.8.1.8 Author Response

14.8.1.8.1 Author name and address

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14.8.1.8.2 Response

Force-plate analysis-of-gait is one of several different outcome measures used in clinical trials studying client-owned dogs with osteoarthritis. Approaches to experimental design and statistical analysis of data in force-plate analysis-of-gait studies vary widely in the veterinary literature and often vary from the perspective of Moreau et al. For example, there are at least 10 different published velocity ranges used for force-plate analysis-of-gait in dogs, including a recent paper

published in the *Journal of Veterinary Medical Association* that used a velocity range of 1.3-2.1 m/s [24].

The main objective of our study was to develop a screening assay in client-owned dogs for analgesic activity of new compounds for the treatment of human and veterinary osteoarthritis. As such, the number of animals and the duration of the study were kept to a minimum. Other studies have often used larger group sizes and longer durations of treatment. In our study, effect size determinations were used to establish which of the measured variables could best detect differences among treatment groups. In undertaking a treatment experiment, trial design may be restrictive or model-based, such that a test article may show a treatment effect that is subsequently less evident in clinical practice. Trials that more closely model clinical practice represent a greater treatment challenge for a test article to overcome. Whilst homogenous cohorts are often used in experimental osteoarthritis studies, client-owned dogs represent a more heterogeneous, but more clinically relevant, population for longitudinal veterinary treatment studies. For example, lumbosacral abnormalities in dogs are very common and may not always have been identified in past osteoarthritis studies in client-owned dogs. Lumbosacral spondylosis is often an incidental finding in clinically normal dogs, but may also be associated with clinical signs. Neuropathic pain as a clinical syndrome is poorly recognized in dogs [19] and its association with lumbosacral spondylosis is unclear. The responsiveness of dogs in the present study to carprofen, as measured by the

Canine Brief Pain Inventory questionnaire [25], suggests that neuropathic pain was not an important phenomenon in our study.

Our study highlights the value of examining a combination of clinical outcome measures in clinically relevant longitudinal studies of client-owned dogs with osteoarthritis. Our study also highlights the need for better analgesic treatments for canine osteoarthritis in veterinary medicine. In this short-term study, habitual diet was maintained to minimize change in body weight over time. Whilst improvement in some gait analysis parameters was identified with placebo treatment in our study, these effects were not statistically significant. Some individual dogs showed an improvement in measured parameters with carprofen treatment, similar to a previous report, in which 19 of 34 dogs showed improvement in ground reaction forces with placebo treatment [16].

To summarize, the article by Moreau et al. contributes to discussion of force-plate analysis-of-gait as an outcome measure in longitudinal studies in client-owned dogs with osteoarthritis. Their commentary does not affect our conclusions regarding its importance in small screening studies.

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14.9 Validation de la force verticale maximale comme critère d'efficacité lors d'essais cliniques contrôlés chez le chien arthrosique

La validation est un concept qui s'enrichit à l'aide d'évidences démontrant un faible degré d'erreur de mesure (Higgins *et al.*, 2006). L'erreur de mesure est soit systématique, soit aléatoire (**Tableau I**). Les expérimentations (8**, 11 ci-dessus - Chapitre 2**) supportent davantage l'interprétation valide de résultats d'essais cliniques contrôlés chez le chien arthrosique, dont la force verticale maximale est utilisée comme critère d'efficacité.

Certains points présentés au **Tableau I** seront détaillés afin de supporter la sensibilité de la force verticale maximale envers la détection de changements au niveau du dysfonctionnement locomoteur chez le chien arthrosique. Ces changements sont en fonction de facteurs articulaires extrinsèques, comme le type de traitements administrés (diètes thérapeutiques, produits de santé naturels, AINS ou contrôle négatif (placebo)) et le mouvement locomoteur.

14.9.1 La validité de contenu

Selon certains manuels de référence, la force verticale maximale offre un reflet objectif de la capacité fonctionnelle d'un membre locomoteur chez le chien (Fox, 2009; Tobias *et al.*, 2012; Egger *et al.*, 2013; Fox, 2013). Également, le dysfonctionnement locomoteur que peut démontrer ce dernier en présence d'arthrose est un concept au contenu validé. Plus précisément, le membre affligé démontrera un degré de force verticale maximale qui est anormalement moindre. Ce qui demeure, par contre, à être davantage validé est la contribution du ressenti douloureux face au degré de dysfonctionnement locomoteur. Autrement dit, est-ce que le dysfonctionnent locomoteur que reflète la force verticale maximale est responsable du degré de douleur articulaire que ressent l'animal arthrosique ? Le contexte expérimental de douleur inflammatoire supporte, en partie, ce concept (**1.13.1 ci-dessus - Chapitre 1**). De plus amples investigations sont par contre nécessaires afin de valider le concept que la force verticale maximale, lorsque anormale, est le reflet du ressenti douloureux. À cet effet, il est proposé d'investiguer la persistance de l'anomalie de force verticale maximale sous analgésie periarticulaire multimodale.

14.9.2 La validité de critères

14.9.2.1 Relation convergente, divergente et prédictive

Les expérimentations (**10.4.2.2 ci-dessus - Chapitre 2**) démontrent la présence d'un lien causal entre la force verticale maximale et le mouvement locomoteur. Ces résultats supportent le concept de relation convergente et de relation prédictive.

Ainsi, ces critères d'évaluation partagent des points communs en ce qui a trait au concept d'intérêt, soit l'incapacité fonctionnelle du chien arthrosique. Par contre, la relation convergente serait davantage supportée en établissant une valeur seuil permettant de distinguer une augmentation du mouvement locomoteur qui excède l'erreur de mesure. Le taux de répondants, selon le critère de force verticale maximale, pourrait par la suite être comparé à celui concernant le mouvement locomoteur. L'établissement de ce seuil pourrait également permettre d'améliorer la capacité de prédire le changement de force verticale maximale en connaissant le mouvement locomoteur.

Une relation divergente a précédemment été démontrée entre la mesure de force verticale maximale du chien arthrosique comparativement à l'état normal (Madore *et al.*, 2007). La tendance centrale pour ce paramètre est donc distincte de la normale lorsqu'il y a présence d'arthrose au niveau d'un membre locomoteur. Toutefois, lorsque la force verticale maximale est utilisée comme critère d'efficacité, il importe à l'investigateur de sélectionner des sujets qui divergent suffisamment de la normale et ce, en tenant compte de la variance de la mesure. Chez des chiens arthrosiques ayant une force verticale maximale de plus de 66 % de poids corporel, la capacité à distinguer l'effet thérapeutique pourrait être compromise par l'absence d'anomalie initiale et donc, par la présence d'un niveau plafond. Le seuil proposé de 66 % (**6.1.1.3.3 ci-dessus - Chapitre 1)** de poids corporel correspond à la différence entre la moyenne d'un groupe de chien normaux et un écart type (Madore *et al.*, 2007). Ce seuil est proposé afin que l'investigateur

puisse être confiant que la force verticale maximale est anormalement basse. À ce seuil, le dysfonctionnement locomoteur que démontre l'animal sera également relevé, dans la grande majorité des cas, par un expert lors de l'examen visuel de la démarche (Bertrand Lussier et Maxim Moreau, données personnelles, 2008-2010).

14.9.3 La validité interne

La validité interne regroupe certains facteurs que l'investigateur tente de prévoir et de contrôler *a priori*, ou d'expliquer *a posteriori* en présence de résultats non anticipés (Higgins *et al.*, 2006).

14.9.3.1 Événements concurrents

Certains événements pourraient survenir de manière concurrentielle en cours d'essais cliniques et ainsi, biaiser l'interprétation des résultats. Par exemple, un gain de poids corporel pourrait compromettre le rejet de l'hypothèse nulle en limitant la sensibilité de la force verticale maximale à détecter un effet thérapeutique. Afin de préserver la validité interne, le changement de poids corporel doit donc être contrôlé par l'investigateur tout comme d'autres événements concurrents, tels que l'exercice intense (Annexe VI) et le degré d'activité quotidienne.

14.9.3.2 Effet maturation de la condition

En cours d'essais cliniques, il est très probable qu'un changement dans la condition arthrosique survienne et se répercute envers la force verticale maximale. Toutefois, les expérimentations démontrent que cette mesure est fidèle lorsque répétée à un intervalle de quatre semaines (**Tableau XV**). La force verticale maximale mesurée

sur un intervalle plus grand, soit de plus de huit semaines, semble également non affectée par l'effet maturation de la condition. Selon la **Figure 33**, le changement minimal détectable permet de distinguer un effet net qui est presque nul, soit de 1.1 %. Cet effet considère la différence entre la proportion de répondants-négatifs (33.7 %) et de répondants (34.8 %) chez les chiens ayant reçu un contrôle négatif (placebo). La maturation de la condition du chien arthrosique ne semble pas avoir eu un effet en faveur de l'exacerbation ou de l'amélioration de la condition.

14.9.3.3 Régression envers la moyenne

Chez les chiens ayant reçu un contrôle négatif (placebo) l'absence de lien statistiquement significatif, entre la force verticale maximale initiale et le taux de répondants (ou de répondant-négatif), ne supporte pas la présence du phénomène de régression envers la moyenne. La régression envers la moyenne aurait plutôt signifié soit un taux de répondants élevé lorsque la force verticale maximale était initialement très faible, soit un taux de répondants-négatifs élevé lorsque la force verticale maximale était initialement élevée. Toutefois, si le phénomène de régression envers la moyenne avait été présent, il est possible qu'il ait été limité par le phénomène concurrent de maturation de la condition.

14.9.3.4 Sélection des sujets

La fidélité de la force verticale maximale a été établie en considérant uniquement des chiens affligés de coxarthrose et/ou de gonathrose. Il incombe donc à l'investigateur de s'assurer d'un certain degré d'homogénéité dans la sélection des sujets avant d'entreprendre un essai clinique contrôlé. Il importe de préciser que le

changement minimal détectable a été déterminé chez des chiens affligés de coxarthrose et/ou de gonathrose. Sans autre expérimentation, il serait actuellement inopportun de transposer ce seuil aux chiens affligés d'arthrose aux membres antérieurs.

14.9.3.5 Attrition de sujets et égalité compensatoire des traitements

En cours d'essais cliniques, il est assez fréquent qu'il y ait attrition de sujets. La cause la plus fréquente d'attrition est l'exacerbation du dysfonctionnement locomoteur suite à l'atteinte au ligament croisé crânial (**Figure 17**, **Figure 22**). Le niveau d'attrition chez les sujets ayant reçu un contrôle négatif est généralement minime lors d'essais cliniques contrôlés (**Figure 17**, **Figure 22**). Par contre, l'attrition par désistement pourrait être favorisée par des suivis à plus long terme, voir plus de treize semaines, chez les sujets ayant reçu un contrôle négatif. Ce désistement pourrait être motivé sachant la présence d'un groupe contrôle négatif aléatoirement distribué.

La présence d'un groupe contrôle négatif pourrait ne pas être permise pour des considérations d'ordre éthique et de bien-être animal. L'usage du changement minimal détectable pourrait faire valoir sa pertinence. À cet égard, il est proposé que l'investigateur s'exempte de devoir requérir à des sujets sous contrôle négatif afin de tester l'hypothèse nulle que les proportions de répondants sont similaires entre les groupes. L'investigateur pourrait plutôt considérer le changement minimal détectable uniquement. La proportion de répondants observée serait par la suite

comparée à un taux de réponses hypothétiques basé sur des essais cliniques antérieurs.

14.9.4 La validité externe

14.9.4.1 Interaction circonstance *versus* traitement

La fidélité de la mesure de la force verticale maximale pourrait être affectée, par exemple, lors d'un essai clinique effectué en période estivale où les sujets sont plus propices à l'exercice intense ou à des traumatismes. La fidélité de la mesure de la force verticale maximale a été démontrée sur une période de quatre semaines et ce, sans égard à la saison. Il incombe cependant au chercheur de s'assurer que l'étude se déroule au même moment pour les sujets contrôle négatif (placebo) et pour ceux traités.

14.9.5 Validité des conclusions statistiques

14.9.5.1 Puissance statistique

Il importe à l'investigateur de déterminer *a priori* la puissance statistique en fonction des conditions expérimentales et de la taille d'effet escomptée. Ceci afin d'éviter une erreur statistique de type II, correspondant à l'absence de rejet d'une hypothèse nulle qui est fausse (c-à-d que l'on n'arrive pas à démontrer une différence entre les groupes qui existe pourtant). Les expérimentations (**Tableau XV**) démontrent que la variance de la mesure de la force verticale maximale est relativement faible. Par conséquent, le rejet de l'hypothèse nulle pourrait survenir

avec suffisamment de puissance statistique en fonction d'une taille d'échantillons acceptable, soit moins de vingt sujets par groupe expérimentaux.

14.9.5.2 Hétérogénéité des sujets

Les expérimentations (**Tableau XV**) démontrent que la variance de la mesure de la force verticale maximale est relativement faible chez des chiens atteints de gonarthrose et/ou de coxarthrose. Tenant compte que le membre antérieur génère, au trot, une force verticale maximale de l'ordre de 113 % de poids corporel (**Figure 2**), la présence de sujets hétérogènes pourrait augmenter la variance associée à l'indice de tendance centrale. Il est donc à éviter de regrouper, sans ajustement statistique, des chiens atteints d'arthrose aux membres postérieurs avec des chiens atteints aux membres antérieurs au sein d'un même essai clinique.

14.9.6 Stabilité

14.9.6.1 Coefficient de corrélation intra-classe (test-retest)

Les expérimentations (**Tableau XV**) démontrent que la force verticale maximale, mesurée chez le chien arthrosique est fidèle en contexte d'essais cliniques contrôlés et ce, selon un test moyen de corrélation intra-classe de 0.91. Également, le changement, qui représente 2.7 % de la mesure initiale, est en accord avec la littérature (Hudson *et al.*, 2004).

14.10 La force verticale maximale en contexte d'essais cliniques contrôlés : Conclusion

La force verticale maximale est un critère d'efficacité utilisé en contexte d'essais cliniques contrôlés chez le chien arthrosique. Les expérimentations présentées au sein de cet ouvrage contribuent à valider l'interprétation des résultats qui découlent de l'usage de ce critère lors d'essais cliniques contrôlés. En tenant compte de l'ensemble des points précédemment discutés, il est envisagé qu'un essai clinique contrôlé effectué chez le modèle canin d'arthrose naturelle contribue à la pratique d'une médicine vétérinaire basée sur des faits. Également ce modèle, couplé à la mesure de la force verticale maximale, atteint un degré supérieur de pertinence au regard de l'évaluation préclinique de modalités à vocation thérapeutique pour l'humain arthrosique. Pour le clinicien, les expérimentations de cet ouvrage supportent la pertinence d'appliquer une approche multimodale afin de soulager le chien arthrosique. Ainsi, la force verticale maximale a été le témoin des bienfaits de la perte de poids, de l'activité quotidienne et de modalités thérapeutiques reconnues, comme les anti-inflammatoires non stéroïdiens, les diètes thérapeutiques ou les produits de santé naturels.

Toutefois, il subsiste des doutes qu'en à la validité des résultats qui découlent de l'usage de la force verticale maximale comme critère d'évaluation fonctionnelle suite à la correction chirurgicale d'une rupture naturelle du ligament croisé crânial. L'opinion du présent auteur est exprimée sous forme d'une lettre adressée à l'Éditeur du *Journal of the American Veterinary Medical Association* (Annexe

VII). En somme, l'interprétation des résultats qui découlent de l'usage de la force verticale maximale ne serait actuellement valide que chez le chien arthrosique et donc, difficilement transposable à des contextes d'évaluation fonctionnelle de remissions postopératoires. Un processus de validation est suggéré en de tels contextes.

15 Modèle canin d'arthrose par sectionnement chirurgical du ligament croisé crânial

15.1 La force verticale maximale et le dysfonctionnement locomoteur

Le dysfonctionnement locomoteur, induit par le sectionnement chirurgical du ligament croisé crânial (**Figure 28**), est en accord avec des observations cliniques réalisées chez l'humain (Hasan *et al.*, 1991). Le dysfonctionnement est également en accord avec les niveaux anormaux de force verticale maximale précédemment rapportés chez ce modèle (O'Connor *et al.*, 1989). Selon la force verticale maximale, l'évolution du dysfonctionnement ne s'est pas avérée être rectiligne, mais plutôt bi-phasique. En effet, une phase d'incapacité sévère a été dénotée à quatre semaines, suivie d'une phase graduelle de rémission (**Figure 28**).

15.2 Le sectionnement chirurgical du ligament croisé crânial et l'altération à la dynamique de l'articulation

En l'absence du ligament croisé crânial, l'atteinte à la dynamique de l'articulation est délétère à l'état des structures articulaires (Tashman *et al.*, 2004; Andriacchi *et al.*, 2006), comme en témoigne l'évolution des dommages encourus chez ce modèle (Annexe II, III et IV). Chez l'humain, des contraintes mécaniques, topographiquement orientées vers des points de contact anormalement sollicités lors de la mise en charge de l'articulation, pourraient expliquer l'apparition des dommages (Andriacchi *et al.*, 2004; Andriacchi *et al.*, 2006; Andriacchi *et al.*, 2014). Le modèle canin d'arthrose par sectionnement chirurgical du ligament croisé crânial partage des similitudes avec cette condition traumatique observée chez l'humain (Pozzi *et al.*, 2013). Ainsi, les déplacements cranio-caudaux sont anormaux de huit à douze millimètres lors de la mise en charge du membre dépourvu du soutien que procure le ligament croisé crânial (Korvick *et al.*, 1994; Tashman *et al.*, 2004). Les mouvements articulaires, comme la rotation interne et l'adduction, sont également augmentés (Tashman *et al.*, 2004). La dynamique de l'articulation, en présence de ses altérations, pourrait expliquer l'apparition des dommages chez ce modèle, comme il en est le cas chez l'humain (Noyes *et al.*, 1992; Chehab *et al.*, 2014).

Lorsque le ligament croisé crânial est sectionné, les surfaces articulaires se compressent avec une plus grande vélocité, ce qui génère l'augmentation des contraintes de cisaillement (Anderst *et al.*, 2009). Ces phénomènes pourraient également contribuer aux changements structuraux observés chez ce modèle (Tashman *et al.*, 2004). Le rôle de l'activation désordonnée des muscles du membre locomoteur n'est pas à négliger, comme le démontre une récente étude chez l'humain (Noyes *et al.*, 1992; Rutherford *et al.*, 2013). Dans l'ensemble, les altérations à la dynamique articulaire, qui découlent de l'absence du ligament croisé crânial, seraient responsables de sollicitations mécaniques excessives et ainsi, de l'initiation de la perte d'intégrité au niveau des structures articulaires (Wu *et al.*, 2000; Desrochers *et al.*, 2012).

Le sectionnement chirurgical du ligament croisé crânial induit l'apparition de dommages structuraux (Annexes II, III et IV). Cependant, certaines évidences portent à entrevoir un rôle protecteur du système somatosensoriel, *via* l'afférence nociceptive périphérique (**Figure 9**). L'articulation dépourvue du ligament croisé crânial pourrait donc avoir été préservée de l'apparition de dommages encore plus sévères. L'exacerbation des dommages, habituellement rencontrés chez ce modèle, a été démontrée lors de l'ablation de l'afférence du membre lésé (O'Connor *et al.*, 1999). L'absence d'un report de forces vers le membre collatéral, au profit de la mise en charge accentuée du membre lésé, serait responsable de l'exacerbation des dommages. Dans ce contexte, le patron de mise en charge aux quatre membres a été précédemment décrit à l'aide de la force verticale maximale (Rumph *et al.*, 1995;

O'Connor *et al.*, 1999). Chez l'humain, l'adaptation musculo-squelettique, en réponse à l'afférence nociceptive périphérique du membre arthrosique, a été proposée comme étant responsable de l'apparition de lésions de sévérité moindre, soulignant un éventuel rôle protecteur du système somatosensoriel (Hurwitz *et al.*, 2000; Andriacchi *et al.*, 2014). Il est donc envisageable que l'afférence nociceptive périphérique et l'adaptation musculo-squelettique soient favorables à l'état des structures en l'absence du ligament croisé crânial chez le chien.

L'évolution individuelle de la force verticale maximale a démontré des profils distincts (**Figure 29**). Ces profils suggèrent que le degré de dysfonctionnement locomoteur soit modifié en fonction de facteurs articulaires intrinsèques.

15.3 La force verticale maximale et les dommages structuraux à l'aide d'imagerie par résonance magnétique

15.3.1 Retour sur les hypothèses

Les hypothèses préalablement émises **(9.2 ci-dessus - Chapitre 2)** ont été confirmées.

Les changements au niveau des dommages structuraux (lésions au cartilage et à l'os sous-chondral, taille des ostéophytes et le degré d'effusion articulaire) démontrent un lien inversement proportionnel avec le changement de la force verticale maximale. Les changements au niveau des dommages structuraux (volume du

cartilage et lésions aux ménisques) démontrent également un lien, cependant proportionnel, avec le changement de la force verticale maximale. Ce lien proportionnel n'était toutefois pas statistiquement significatif au seuil préalablement déterminé.

15.3.2 Les lésions focales au cartilage

Les expérimentations (**10.4.1.2 ci-dessus - Chapitre 2**) démontrent que l'évolution des lésions focales au cartilage est en lien avec la force verticale maximale. Plus précisément, la phase de rémission s'avère être compromise par la présence de dommages focaux au cartilage. Il est important de préciser que le cartilage articulaire est dépourvu d'afférence nociceptive périphérique et donc, de la capacité de générer un signal chimio-électrique neuronal. Cependant, une récente étude a mise en évidence les régions dénudées de cartilage comme étant source potentielle de douleur articulaire (Moisio *et al.*, 2009). L'exposition de l'os sous-chondral, doté d'afférences nociceptives périphériques, et les changements osseux en réponse aux contraintes mécaniques pourrait avoir compromis la rémission du dysfonctionnement locomoteur. Également, la libération d'intervenants pro-inflammatoires, de substances algogènes et de facteurs cataboliques en réponse à l'atteinte du cartilage, pourrait également être impliquée. Chez l'humain, un lien étroit existe entre les lésions focales au cartilage et la douleur articulaire (Baum *et al.*, 2012). Fait intéressant, les chiens affligés d'un dysfonctionnement sévère, quatre semaines suivant le sectionnement chirurgical du ligament croisé crânial, sont ceux ayant démontré l'atteinte focale au cartilage la plus sévère à ce moment.

15.3.3 Les lésions de la moelle osseuse

Chez l'humain, la présence de lésions de la moelle osseuse est associée au ressenti douloureux (Felson *et al.*, 2007; Lo *et al.*, 2009; Ahedi *et al.*, 2014), et rend l'articulation plus susceptible aux dommages cartilagineux lors de gonarthrose (Lim *et al.*, 2013). L'évolution des lésions de la moelle osseuse est en lien avec des facteurs associés à la sollicitation mécanique excessive, comme l'index de masse corporelle et l'activité intense (Foong *et al.*, 2014). Le degré de lésions au cartilage est également en lien avec l'évolution des lésions de la moelle osseuse (Felson *et al.*, 2007; Driban *et al.*, 2011).

Les expérimentations témoignent d'une meilleure rémission en présence d'un degré moindre de lésions de la moelle osseuse (**Figure 30**). Le degré de dysfonctionnement locomoteur serait donc en lien avec l'altération structurelle au niveau de l'os sous-chondral. Il est envisageable que l'afférence nociceptive périphérique ait été activée, provoquant l'adaptation du système myo-arthro-squelettique vis-à-vis de la mise en charge du membre. Par conséquent, la force verticale maximale serait modulée en fonction du ressenti douloureux découlant de la fluctuation des lésions de la moelle osseuse. Le lien entre la présence de lésions de la moelle osseuse et la rémission a été également observé chez l'humain suivant la rupture d'un ligament croisé antérieur (Johnson *et al.*, 2000). Les progrès technologiques permettent la quantification automatisée des lésions de la moelle osseuse (Ratzlaff *et al.*, 2013). La supériorité d'une telle technique automatisée, face à l'appréciation subjective des lésions, demeure à être déterminée.

15.3.4 Les ostéophytes

Chez l'humain sans évidence radiographique d'arthrose, les ostéophytes sont les lésions les plus détectées à l'aide d'imagerie par résonance magnétique (Guermazi *et al.*, 2012). Un rôle stabilisateur bénéfique, en présence d'une dynamique articulaire altéré, est suggéré (Goldring *et al.*, 2010) et ce, *via* la diminution des mouvements de translation antéropostérieure (Brage *et al.*, 1994). Bien que la présence d'invasions vasculaires et d'afférences nociceptives soit rapportée au niveau des ostéophytes (Suri *et al.*, 2007), le lien entre la présence d'ostéophytes et le ressenti douloureux n'est pas clairement défini (Sengupta *et al.*, 2006; Sowers *et al.*, 2011). Chez le modèle canin d'arthrose par sectionnement chirurgical du ligament croisé crânial, il a été précédemment suggéré que les ostéophytes puissent protéger le cartilage des sollicitations mécaniques excessives issues de l'instabilité articulaire (Marshall *et al.*, 1971; Olsson *et al.*, 1972; Menkes *et al.*, 2004; Anderst *et al.*, 2005).

Les expérimentations (**10.4.1.2 ci-dessus - Chapitre 2**) suggèrent que la rémission soit compromise chez les chiens ayant eu un degré élevé d'ostéophytes. Bien qu'aucune mesure de laxité n'ait été obtenue chez ces derniers, il est envisageable que la présence d'ostéophytes soit associée à un dysfonctionnement locomoteur élevé et ce, en dépit d'un rôle stabilisateur potentiel. Chez le modèle canin d'arthrose par sectionnement chirurgical du ligament croisé crânial, la surface de contact au sol a été proposée comme témoin du rôle fonctionnel bénéfique des ostéophytes (Moreau *et al.*, 2009). Une surface au sol maximisée pourrait être le

reflet d'une articulation plus stable, en dépit d'une force verticale maximale moindre. Les ostéophytes agiraient donc comme une mesure compensatoire face à l'altération à la dynamique articulaire. Cette mesure compensatoire pourrait limiter l'usage du membre et ainsi, préserver les structures en stabilisant l'articulation.

15.3.5 L'effusion articulaire

L'effusion articulaire est associée au ressenti douloureux chez l'humain arthrosique (Lo *et al.*, 2009) et est reconnue pour altérer la démarche (Rutherford *et al.*, 2012). Les expérimentations (**10.4.1.2 ci-dessus - Chapitre 2**) suggèrent que la présence d'effusion articulaire soit associée à un dysfonctionnement locomoteur élevé, alors que la résorption de l'effusion favorise la rémission fonctionelle. Lors d'effusion, il est envisageable que la distension de la membrane, *via* l'augmentation de la pression intra articulaire (Jayson *et al.*, 1970), active l'afférence nociceptive périphérique, ce qui compromettrait la mise en charge du membre lésé. La présence de substances algogènes (Bas *et al.*, 2014; Li *et al.*, 2014; Miller *et al.*, 2014; Wang *et al.*, 2014a), au sein du liquide synovial, pourrait également avoir été responsable du ressenti douloureux.

Récemment, le coussinet adipeux infra patellaire a été suggéré comme structure pouvant permettre de réduire l'impact délétère issu de la sollicitation mécanique excessive (Pan *et al.*, 2014). Dans cette perspective, l'effusion articulaire est proposée comme mesure compensatoire face à l'altération de la dynamique articulaire. L'effusion articulaire pourrait agir en amortissant les contraintes

mécaniques issues de la mise en charge de l'articulation. Chez le modèle canin d'arthrose par sectionnement chirurgical du ligament croisé crânial, nous rapellons que la surface de contact au sol a été proposée comme témoin du rôle fonctionnel bénéfique de l'effusion articulaire (Moreau *et al.*, 2009). L'effusion articulaire pourrait se répercuter sur la fonction du membre par une surface au sol maximisée, sous une faible force verticale maximale. Il y aurait donc modification des patrons de pression au sol. Cette hypothèse pourrait être testée à l'aide d'un modèle cadavérique de sectionnement chirurgical du ligament croisé crânial (Hagemeister *et al.*, 2010) couplé à l'analyse des patrons de pression avec, ou sans, effusion articulaire expérimentale (Pietrosimone *et al.*, 2014). L'augmentation de la surface de contact articulaire est proposée comme une méthode pour pallier à l'augmentation de la pression issue des forces articulaires externes (Clark *et al.*, 2002). Il est envisageable qu'il en soit ainsi pour la surface de contact au sol.

15.3.6 Le volume de cartilage et l'atteinte aux ménisques

Chez l'humain, la perte en volume du cartilage au genou démontre un lien avec l'intensité de l'exercice (Lu *et al.*, 2014) et une dynamique articulaire altérée (Bennell *et al.*, 2011), ce qui souligne l'impact délétère de la sollicitation mécanique excessive (Messier *et al.*, 2014). Il en découle une perturbation de l'homéostasie des chondrocytes et des cellules de l'os sous-chondral (Findlay *et al.*, 2014). À l'inverse, la réduction de la sollicitation excessive, *via* une perte de poids chez l'obèse, limite les effets délétères sur le volume du cartilage de la sollicitation mécanique excessive (Teichtahl *et al.*, 2014). Il importe de préciser que l'effet de

l'activité soutenue demeure bénéfique aux structures articulaires (Urquhart *et al.*, 2011).

Les expérimentations (**10.4.1.2 ci-dessus - Chapitre 2**) suggèrent que la mise en charge du membre, en présence d'instabilité, est délétère au cartilage articulaire. Les chiens qui se sont remis plus favorablement (au niveau du dysfonctionnement locomoteur) de la rupture du ligament croisé crânial ont eu tendance à démontrer davantage de perte en volume de cartilage au compartiment médial. Il est donc envisageable qu'en présence d'une dynamique articulaire altérée, les contraintes mécaniques soient accentuées par la mise en charge du membre, ce qui exacerberait les lésions. Indirectement, ceci met en évidence le rôle protecteur du système somatosensoriel. Ainsi, le soulagement du ressenti douloureux pourrait favoriser l'usage du membre et, par conséquent, amplifier les contraintes mécaniques issues de la dynamique de l'articulation (Briem *et al.*, 2009). Selon cette perspective, en l'absence du ligament croisé crânial ou en présence de *genu varum* (Foroughi et al., 2009), le soulagement du ressenti douloureux pourrait mener à davantage de perte en volume de cartilage. Il importe donc d'intégrer cette notion lors de la mise à l'essai de molécules aux propriétés analgésiques avec ou sans propriété structurelle.

Chez l'humain, l'atteinte au ménisque est un déterminant de la nécessité de requérir à des approches chirurgicales lors de gonarthrose (Raynauld *et al.*, 2011). Ce type d'atteinte est également un facteur de risque de dommages articulaires, particulièrement la perte de volume en cartilage (Berthiaume *et al.*, 2005) et les

lésions de la moelle osseuse (Lim *et al.*, 2013). Un lien est également démontré entre la dynamique articulaire altérée et l'apparition de lésions aux ménisques (Davies-Tuck *et al.*, 2008).

Les expérimentations (**10.4.1.2 ci-dessus - Chapitre 2**) suggèrent que la sollicitation du membre est délétère à l'intégrité du ménisque médial. Les chiens ont eu tendance à démontrer davantage de lésions au ménisque lors d'une rémission plus favorable. L'instabilité articulaire, en l'absence de ligament croisé crânial, pourrait s'avérer particulièrement délétère pour le compartiment médial. Le compartiment médial est le lieu ou l'altération à la dynamique articulaire se fait particulièrement ressentir et ce, *via* les contraintes mécaniques associées au moment d'adduction (Andriacchi *et al.*, 2004; Andriacchi *et al.*, 2006; Andriacchi *et al.*, 2014).

15.4 Mécanistique de la relation entre le dysfonctionnement locomoteur et les dommages structuraux

Face à l'établissement d'une relation entre le dysfonctionnement locomoteur et les dommages structuraux, l'approche mécanistique suivante est proposée :

La rupture du ligament croisé crânial altère la dynamique articulaire et, par conséquent, induit un dysfonctionnement locomoteur. L'altération de la dynamique articulaire favorise l'apparition d'ostéophytes et de lésions de la moelle osseuse.

Des lésions focales au cartilage et un dysfonctionnement locomoteur plus sévère surviennent en présence d'une dynamique articulaire plus altérée. La rémission du dysfonctionnement locomoteur est favorisée par un degré moindre de lésions de la moelle osseuse et d'effusion articulaire. Par conséquent, la perte de volume du cartilage et l'atteinte au ménisque sont amplifiées suite à la mise en charge de l'articulation.

La faiblesse de cette intégration mécanistique repose, en partie, sur l'absence de témoin du degré d'altérations à la dynamique articulaire. Actuellement, il importe d'intégrer la magnitude des forces externes du membre locomoteur au degré d'altérations observé chez le modèle d'arthrose par sectionnement chirurgical du ligament croisé crânial. Ainsi, pour un même niveau de force verticale maximale, l'altération à la dynamique pourrait amplifier les contraintes mécaniques et, par conséquent, exacerber l'ampleur des dommages articulaires. Cette intégration mécanistique est en accord avec une modélisation statistique complexe intégrant le dysfonctionnement locomoteur et les dommages structuraux observées chez ce modèle (del Castillo *et al.*, 2009). La modélisation statistique pourrait également bénéficier d'informations quantitatives sur l'altération à la dynamique articulaire.

15.5 La force verticale maximale et les dommages macroscopiques au cartilage

15.5.1 Retour sur les hypothèses

Les hypothèses préalablement émises **(9.2 ci-dessus - Chapitre 2)** ont été confirmées.

L'évaluation macroscopique de l'étendue des lésions au cartilage démontre un lien proportionnel avec la force verticale maximale. Ceci est en accord avec l'observation de Smith et collaborateurs (Smith *et al.*, 2005) qui suggère une association entre le degré de dysfonctionnement locomoteur, représenté par la force verticale maximale, et l'étendue des dommages cartilagineux (score de chondropathie). Il est donc envisageable que la force verticale maximale agisse en amplifiant la répercussion de l'altération à la dynamique articulaire envers le compartiment médial chez le chien dépourvu d'un ligament croisé crânial. La conformation du condyle fémoral, qui permet une plus grande surface de contact avec sa contrepartie tibiale, pourrait expliquer la présence de dommages accrus à ce niveau.

Le ligament croisé crânial procure la stabilité de l'articulation afin de restreindre les mouvements de translation antéropostérieure et de rotation (Korvick *et al.*, 1994; Anderst *et al.*, 2005; Andriacchi *et al.*, 2006; Anderst *et al.*, 2009). Lors d'instabilité articulaire, la médialisation du vecteur des forces de réaction au sol **(1.8 ci-dessus - Chapitre 1)**, pourrait expliquer l'apparition de lésions plus sévères en présence de forces verticales maximales plus élevées. La médialisation aurait pour conséquence

d'augmenter la distance entre le vecteur des forces de réaction au sol et le centre articulaire du genou (Reeves *et al.*, 2011; Hart *et al.*, 2013). Un bras de levier plus long signifierait l'augmentation du moment d'adduction, pour un rapport de force externe et interne équivalent. Chez l'humain, la laxité du genou est associée à la présence d'un moment d'adduction plus élevé (Chang *et al.*, 2004) alors qu'une articulation plus stable est associée à une perte moindre en cartilage (Dayal *et al.*, 2005). Le mouvement d'adduction est augmenté sur le modèle canin d'arthrose par sectionnement chirurgical du ligament croisé crânial (Tashman *et al.*, 2004).

Le système myo-arthro-squelettique est en étroite relation avec l'altération à la dynamique articulaire et les dommages structuraux. Ainsi, chez l'humain, la démarche peut être adaptée afin de compenser pour l'altération à la dynamique articulaire (Jenkyn *et al.*, 2008). Le quadriceps est responsable de contrecarrer un moment d'adduction excessif (Shelburne *et al.*, 2006). Par contre, la faiblesse de ce dernier est également un facteur de risque d'arthrose (Segal *et al.*, 2011) alors que la présence de dommages articulaires affectera sa réponse (Hurley *et al.*, 1997). Chez le modèle canin d'arthrose par sectionnement chirurgical du ligament croisé crânial, il est envisageable que le système myo-arthro-squelettique est en mesure de limiter l'étendue des dommages en reprogrammant l'activation musculaire lors de la locomotion. Cependant, lorsque cette procédure est effectuée chez le chat, la reprogrammation musculaire semble plus performante et ce, en procurant davantage de stabilité (Suter *et al.*, 1998).

Les évaluations macroscopiques du cartilage sont en accord avec la quantification du volume de cartilage précédemment rapportée à l'aide imagerie par résonance magnétique (**10.4.1.2 ci-dessus - Chapitre 2**). Les évaluations macroscopiques témoignent de l'effet délétère de la mise en charge du membre lors d'altérations au dynamisme de l'articulation.

15.6 La force verticale maximale et la plasticité du système somatosensoriel

La force verticale maximale témoigne de la capacité fonctionnelle d'un membre locomoteur chez le chien. En présence d'arthrose, cependant, le dysfonctionnement locomoteur qui en découle pourrait s'avérer être le reflet, partiel ou complet, d'un ressenti douloureux. Si tel est le cas, des changements inadaptés et persistants au niveau du système somatosensoriel seraient à l'origine du ressenti douloureux *via* la plasticité du système somatosensoriel nociceptif. Le remodelage nociceptif périphérique et central amènerait le chien arthrosique à démontrer un état d'hypersensibilité nociceptive issue d'une sensibilisation périphérique et/ou centrale.

Récemment, des tests quantitatifs sensoriels ont démontré un état d'hypersensibilité chez le chien en contexte d'arthrose naturelle associée à une rupture traumatique du ligament croisé crânial (Brydges *et al.*, 2012). Ce constat est en lien avec la présence d'hypersensibilité centrale, comme le rapporte une étude chez l'humain arthrosique (Kuni *et al.*, 2014). Cette dernière démontre également que le degré de

changement aux tests quantitatifs sensoriels est en relation avec l'altération fonctionnelle. À cet égard, la présence d'une relation entre la force verticale maximale et l'état d'hypersensibilité nociceptive (selon des tests quantitatifs sensoriels) est une hypothèse précédemment soulevée (Tomas *et al.*, 2014) qui demande à être supportée chez le chien affligé d'arthrose naturelle. La présence d'une force verticale maximale anormalement basse pourrait être issue d'afférences nociceptives, celles-ci ayant la capacité d'induire (allodynie) ou d'exacerber (hyperalgésie) le ressenti douloureux au niveau de l'articulation arthrosique. L'usage du seuil de changement minimal détectable (à un intervalle de confiance de 95 %) pourrait s'avérer utile afin de raffiner la prospection d'une possible relation entre le dysfonctionnement locomoteur et l'hypersensibilité nociceptive. Ceci éviterait de tenter de mettre en relation l'erreur de mesure de la force verticale maximale au profit d'une analyse de répondants/répondants-négatifs.

Sur le modèle canin d'arthrose par sectionnement chirurgical du ligament croisé crânial, la présence d'une sensibilisation périphérique et centrale a été démontré selon des tests quantitatifs sensoriels (Annexe VIII). Un lien a également été démontré entre le degré de sensibilisation (périphérique et centrale) et la force verticale maximale. Le chien démontrant un fort dysfonctionnement locomoteur était donc celui assujetti d'une hypersensibilité marquée. Le rôle de la sensibilisation périphérique et centrale envers le dysfonctionnement locomoteur est d'autant plus clair en tenant compte de l'amélioration du dysfonctionnement locomoteur sous l'effet d'un biphosphonate (le tiludronate) chez ce modèle (**13.4.1**

ci-dessus - Chapitre 2). Ainsi, l'augmentation de la force verticale maximale sous l'effet du tiludronate fut concomitante à une absence de sensibilisation (périphérique et centrale) et à des changements favorables en termes de neurotransmetteurs peptidiques spinaux (suggérant une sensibilisation centrale moindre) (Annexe VIII).

15.7 La force verticale maximale et la détection d'effets structuraux

15.7.1 Retour sur les hypothèses

Les hypothèses préalablement émises **(12.2 ci-dessus - Chapitre 2)** ont été confirmées.

La préservation de la qualité des structures et la diminution de la quantité de facteurs cataboliques et d'intervenants pro-inflammatoires résultent de l'administration d'un agent anti-résorptif, soit le tiludronate. La qualité des structures et la diminution de la quantité de facteurs cataboliques et d'intervenants pro-inflammatoires se répercutent positivement sur le degré de dysfonctionnement locomoteur reflété par la mesure de la force verticale maximale.

Les expérimentations précédentes (**10.4.1.3 ci-dessus - Chapitre 2**) ont suggéré que l'altération à la dynamique articulaire, en l'absence du ligament croisé crânial, soit néfaste pour le cartilage et aussi pour les structures sous-jacentes lors d'une force verticale maximale élevée. Ainsi, les lésions au cartilage et aux ménisques et la perte en volume de cartilage s'avèrent être plus prononcées lorsque la fonction

est meilleure. Dans cette perspective, ce modèle pourrait donc apparaitre limité, voir trop robuste, face à sa capacité à démontrer des effets structuro-modulateurs, en parallèle à un effet bénéfique à la fonction. Sous un effet thérapeutique, l'amélioration du dysfonctionnement locomoteur signifierait une dynamique articulaire plus nocive, ce qui limiterait la démonstration d'un effet structuro-modulateur.

Cette perspective s'avère toutefois fausse. En effet, les expérimentations suggèrent plutôt que, sous un effet thérapeutique, la force verticale maximale soit un témoin d'effets structuraux ayant un impact fonctionnel bénéfique chez le modèle canin d'arthrose par sectionnement chirurgical du ligament croisé crânial (**Figure 35**). Ceci est en accord avec une récente étude chez ce modèle (Annexe V), qui témoigne d'effets structuro-modulateurs au cartilage, en parallèle à des bénéfices envers la fonction du membre lésé. Lors de cette étude, le dysfonctionnement locomoteur reflété par la force verticale maximale, a été amélioré de 20 % comparativement à un contrôle négatif (placebo). Les résultats au présent ouvrage démontrent que l'amélioration de la fonction peut également découler d'un effet thérapeutique, *a priori*, au niveau de l'os sous-chondral (**Tableau XVI**). Les bénéfices escomptés sont de l'ordre de 35 % supérieurs au contrôle négatif (placebo) (**Figure 35**).

En assumant un effet purement analgésique de l'agent mis à l'essai (bisphosphonate ou autre agent), il est envisageable que la présence d'une force verticale maximale

élevée génère des dommages plus sévères. La force verticale maximale n'est pas seulement le reflet du niveau du dysfonctionnement locomoteur, mais signifie également la présence de forces externes qui contribuent à l'exacerbation des lésions en présence d'altérations à la dynamique articulaire. Le mécanisme du tiludronate semble plutôt impliquer un effet analgésique intimement lié à des actions structuro-modulatrices. L'action anti-résorptive du tiludronate pourrait agir en limitant la présence de protons H^+ qui découle de l'activité résorptive exagérée des ostéoclastes en présence d'une sollicitation mécanique excessive. La diminution de l'activation de récepteurs nociceptifs (**Figure 11**), sensibles à l'acidité du milieu, contribuerait à limiter l'afférence nociceptive, ce qui expliquerait la diminution de la sensibilisation périphérique et centrale observée sous l'effet du tiludronate (Annexe VIII). Ce mécanisme se répercuterait par l'amélioration du dysfonctionnement locomoteur, communément observé en l'absence du ligament croisé crânial.

Bien que la force verticale maximale ait été accentuée sous l'effet de l'agent mis à l'essai (tiludronate), les chiens ont démontré des lésions macroscopiques au cartilage qui étaient similaires au groupe contrôle (**Figure 37**). En présence d'altérations au dynamisme articulaire et d'une force verticale maximale élevée, il est envisageable que la répercussion néfaste est limitée si, et seulement si, l'agent mis à l'essai démontre également des effets structuro-modulateurs.

Chez le modèle canin d'arthrose par sectionnement chirurgical du ligament croisé crânial, il importe d'investiguer l'effet de l'analgésie face au dysfonctionnement locomoteur et aux dommages structuraux afin de mieux intégrer la relation proposée entre l'atteinte structurelle et la fonction du membre. L'inhibition des prostanoïdes ne peut toutefois pas être considérée comme une analgésie simple et ce, au regard des bénéfices structuraux observés chez ce modèle (Pelletier *et al.*, 1999).

Une étude récente a utilisé l'émission de radiofréquences pour induire la rupture du ligament croisé crânial chez le chien, dont l'apparition sur ce modèle est estimée à huit semaines (Steinera *et al.*, 2009). Dans ce contexte, un AINS de type COX-2 spécifique a été testé pour son efficacité fonctionnelle et structurelle. Cet agent a démontré sa supériorité fonctionnelle comparativement au contrôle négatif, uniquement à dix-huit semaines après la rupture. Aucun effet structuro-modulateur n'a été rapporté. Face à l'absence d'amélioration fonctionnelle avant dix-huit semaines (Steinera *et al.*, 2009), un regard critique a par conséquent été posé sur le modèle canin d'arthrose par sectionnement chirurgical du ligament croisé crânial (Kruger *et al.*, 2009). Il a été suggéré que l'instabilité articulaire soit l'unique cause du dysfonctionnement. Selon cette perspective, l'instabilité serait purement mécanique et l'intervention du ressenti douloureux serait secondaire, voire absente. Par conséquent, il ne serait pas pertinent d'utiliser le modèle canin d'arthrose par sectionnement chirurgical du ligament croisé crânial. La mesure de la force

verticale maximale ne serait pas un témoin du dysfonctionnement locomoteur mais bien un reflet du degré d'instabilité induite.

La démonstration d'un effet fonctionnel, secondaire à l'administration d'un biphosphonate, est en désaccord avec la critique précédemment adressée envers le sectionnement chirurgical du ligament croisé crânial (Kruger *et al.*, 2009). Les résultats supportent la pertinence d'utiliser la force verticale maximale comme critère d'évaluation fonctionnelle (**Tableau XVI**). L'instabilité, en l'absence du ligament croisé crânial, ne serait donc pas l'unique responsable du dysfonctionnement locomoteur. La présence d'inconfort articulaire, de même que le remodelage nociceptif périphérique et central (Annexe VIII), témoignent de la contribution du ressenti douloureux au dysfonctionnement locomoteur en l'absence du ligament croisé crânial.

Fait intéressant, dix-huit semaines après la rupture du ligament par émission de radiofréquences (Steinera *et al.*, 2009), les chiens, sous l'effet d'un AINS de type COX-2 spécifique, ont eu tendance à avoir des lésions accentuées au niveau du cartilage. Ce point supporte l'hypothèse du présent auteur qui suggère l'exacerbation des lésions au cartilage en présence d'une force verticale maximale élevée possiblement associée à un effet analgésique.

16 Conclusion générale

Actuellement, il n'existe pas de consensus en ce qui a trait au modèle le plus susceptible de répliquer avec justesse l'ensemble des intervenants biochimiques, cellulaires et mécaniques impliqués dans l'initiation et la progression de l'arthrose chez l'humain. Le caractère inférentiel des trouvailles précliniques vers l'Homme, issues des modèles actuels, pourrait donc bénéficier de plus amples investigations afin d'augmenter la portée des résultats qui en découlent. Le modèle à préconiser doit être en mesure de produire fidèlement ce qui sera observé ultérieurement en contexte d'essais cliniques, particulièrement au regard des effets envers les dommages structuraux et le dysfonctionnement locomoteur.

Au sein de cet ouvrage, le chien a été considéré comme espèce modèle d'intérêt pour l'ensemble des expérimentations réalisées en contexte d'arthrose naturelle et expérimentale. Afin de maximiser ces modèles d'arthrose, un effort a été adressé afin de présenter la force verticale maximale acquise lors de l'analyse cinétique de la locomotion, comme témoin d'effets fonctionnels et structuraux.

Les expérimentations valident davantage les résultats découlant d'essais cliniques utilisant la force verticale maximale comme critère d'efficacité fonctionnelle. Une répercussion sur la qualité des essais futurs est anticipée, ce qui s'avère bénéfique pour la science vétérinaire. Un essai clinique, dont la force verticale maximale est

utilisée comme critère primaire d'efficacité, procurera des évidences cliniques probantes qui sont nécessaires à la pratique d'une médecine basée sur des faits.

Les modèles naturels d'arthrose ont été proposés afin d'accélérer le développement d'agents thérapeutiques contre l'arthrose (Pelletier *et al.*, 2010). Le modèle canin d'arthrose naturelle, couplé à la mesure de la force verticale maximale, s'avère être un complément judicieux aux effets structuro-modulateurs observés en contexte préclinique. L'exemple du licofelone adhère à cette perspective. Cet agent est doté d'un dossier étoffé supportant la préservation des structures chez le modèle canin d'arthrose par sectionnement chirurgical du ligament croisé crânial (Boileau *et al.*, 2002; Pelletier *et al.*, 2004; Pelletier *et al.*, 2005; Moreau *et al.*, 2006). Le licofelone a également réussi à démontrer son efficacité à l'égard du dysfonctionnement locomoteur chez le modèle canin d'arthrose naturelle (Moreau *et al.*, 2007). Ce dernier a été par la suite en mesure de préserver l'état des structures et de réduire les symptômes de l'humain arthrosique (Raynauld *et al.*, 2009). Ceci souligne le caractère inférentiel des modèles canins d'arthrose vers l'Homme.

Le potentiel inférentiel des modèles canins vers l'Homme est également accentué suite aux expérimentations réalisées avec le ranélate de strontium. Cet agent, aux propriétés *a priori* anti-résorptives, a récemment démontré des effets structuro-modulateurs (Pelletier *et al.*, 2013b) de même qu'un taux de répondants jugé satisfaisant en cours d'essais cliniques chez l'humain gonarthrosique (Bruyere *et al.*, 2014). En contexte de développement préclinique, le potentiel structuro-modulateur

de cet agent avait été démontré sur le modèle canin d'arthrose par sectionnement chirurgical du ligament croisé crânial (Pelletier et al., 2013a). Un constat similaire s'adresse à l'extrait insaponifiable d'avocat et de soja. Ainsi, un récent essai clinique a démontré des effets structuro-modulateurs chez l'humain coxarthrosique avec ce produit de santé naturel (Maheu et al., 2014). Ceci confirme le potentiel thérapeutique précédemment observé sur le modèle canin d'arthrose par sectionnement chirurgical du ligament croisé crânial (Boileau et al., 2009). À cette liste non exhaustive s'ajoutent certaines évidences concernant la doxycycline (un type d'antibiotique) (Yu et al., 1992; Brandt et al., 2005) de même que la calcitonine aux propriétés *a priori* anti-résorptives (Manicourt et al., 1999; Karsdal et al., 2011) et le zoledronate (un biphosphonate) (Laslett *et al.*, 2012; Dearmin *et al.*, 2014). Il est toutefois important de préciser que la validation du potentiel inférentiel des modèles canins vers l'Homme dépend ultimement de la qualité des essais cliniques entrepris chez l'humain, un prérequis à l'obtention de méta-analyses de la littérature dont les conclusions seront justes envers l'agent étudié.

Les expérimentations chez le modèle canin d'arthrose expérimentale supportent la pertinence d'enregistrer le niveau de dysfonctionnement locomoteur, ce dernier étant en lien avec l'état des structures. À l'aide de la force verticale maximale comme critère fonctionnel, le modèle canin d'arthrose expérimentale se positionne favorablement envers le discernement d'effets structuraux ayant un impact bénéfique sur le dysfonctionnement du membre. En combinant l'analyse de la démarche à des investigations structurelles, l'investigateur peut s'affranchir de

doutes concernant la répercussion de l'effet structurel envers le soulagement de l'inconfort articulaire.

L'ensemble des travaux de recherche s'intègre dans une quête de conformité envers la règle des trois « R » qui consistent à Remplacer, Réduire et Raffiner l'utilisation d'animaux en recherche. Le chien est reconnu comme étant le meilleur ami de l'Homme. Il n'est pas rare que des gens perçoivent le chien comme un membre de la famille (Hasiwa *et al.*, 2011). La présence de modèles canins en recherche biomédicale fait donc face à des réticences au sein de la société. Il importe donc de tenter d'optimiser l'usage du chien, et de l'ensemble des animaux de recherche, par l'ajout d'investigations complémentaires et non invasives.

À l'égard de cet ouvrage, les constats suivants se conforment avec la règle des trois « R ».

- L'usage de la force verticale maximale, en complément à l'évaluation des structures articulaires, renseigne sur la fonction du membre dépourvu du ligament croisé crânial. Pour l'investigateur, il est ainsi possible de raffiner, et ultimement de réduire, l'utilisation du chien en recherche, ceci en estimant, avec une puissance statistique convenable, non seulement la taille d'échantillons requis, mais également la taille de l'effet fonctionnel pouvant être escompté.
- La relation entre l'état des structures et l'atteinte fonctionnelle supporte davantage l'usage de l'imagerie par résonance magnétique comme outil

d'évaluation. Pour l'investigateur, il est possible de raffiner l'utilisation du chien de recherche en favorisant une méthode d'évaluation exhaustive, et non invasive, de l'état des structures articulaires.

- L'établissement du seuil de changement minimal détectable (à un intervalle de confiance de 95 %), et du taux de répondants et de répondants-négatifs permet de faciliter l'élaboration d'un essai clinique contrôlé chez le chien arthrosique. Pour l'investigateur, il est encore une fois possible de réduire l'utilisation du chien en recherche en estimant la taille d'échantillons requis de même que l'effet fonctionnel pouvant être escompté. La puissance statistique pourrait également être préservée en limitant, en cours d'essais cliniques, les sources de biais de mesure de la force verticale maximale, comme l'exercice intense, le gain de poids corporel et le changement au niveau du mouvement locomoteur.

L'usage judicieux du chien en recherche est donc escompté à l'aide de la force verticale maximale comme témoin d'effets fonctionnels et structuraux, ce qui est conforme avec la règle des trois « R ». L'optimisation des modèles canins d'arthrose présentée au sein de cet ouvrage, pourrait contribuer à contrebalancer les considérations éthiques attenantes à l'usage du chien en recherche biomédicale.

Cet ouvrage suggère qu'une plateforme d'investigations précliniques, combinant le modèle canin d'arthrose par sectionnement chirurgical du ligament croisé crânial à un essai clinique chez le modèle naturel, est un moyen de distinguer la présence de bénéfices structuraux ayant un impact fonctionnel. Le potentiel inférentiel des modèles canin d'arthrose vers l'Homme serait ainsi favorisé en utilisant la force verticale maximale.

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Annexe I.

Plateforme d'enregistrement des forces de réaction au sol

Aux sections 4.4.2.3.1, 6.1.1.3.3, 6.1.2.3.4, 6.1.3.3.4 et 10.3.2.3 de cet ouvrage, la force verticale maximale a été mesurée à l'aide d'une plateforme d'enregistrement dont le modèle est illustré à la Figure 1.

Figure 1. Modèle OR6-6 fabriqué par Advanced Mechanical Technology, Inc (Waltham Street Watertown, Massachussetts, États-Unis).

La surface de contact du modèle OR6-6 est de 464 mm de long par 508 mm de large. À chaque coin, des jauges de contraintes (ou éléments transducteurs) sont présentes, pour une épaisseur totale de 83 mm. Sous l'effet d'une force, la déformation conséquente des jauges de contraintes induira un changement de résistance électrique. Ce changement affectera la tension électrique de sortie (voltage) de manière proportionnelle à la force appliquée.

L'arrangement des jauges de contraintes est sous un brevet américain (#4493220) détenu par Advanced Mechanical Technology, Inc. Le modèle OR6-6 génère trois sorties analogues qui correspondent aux forces orientées dans chacun des axes orthogonaux. Ce modèle génère également trois autres sorties qui correspondent aux moments de chacune des forces. La fréquence d'enregistrement des forces verticales est de 1000 hertz avec une capacité maximale de 4450 newton.

Tapis d'analyse podobarométrique

Aux sections 7.1.3, 10.3.1.2 et 13.3.4.1 de cet ouvrage, la force verticale maximale a été mesurée à l'aide d'un tapis d'analyse podobarométrique dont le modèle est illustré à la Figure 2.

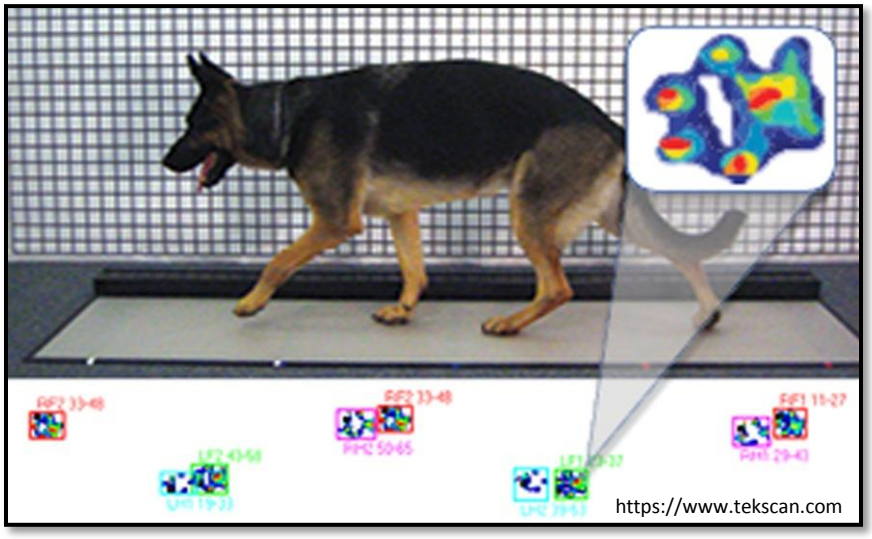

Figure 2. Modèle Walkway® fabriqué par Tekscan (Boston, Massachussetts, États-Unis).

Le modèle Walkway® permet d'enregistrer la force verticale de même que la surface de contact plantaire lors de multiples foulées. La dimension du modèle Walkway® est de 1743.5 mm de long par 368.8 mm de large. Le modèle Walkway® est en fait le jointement de quatre unités sensitives (modèle 3150) dont l'épaisseur est de 6 mm.

La mesure de la force verticale s'effectue grâce à un arrangement de 9152 éléments sensitifs (Figure 3), ce qui procure une résolution de 1,4 élément par centimètre carré. Les éléments sensitifs sont constitués de minces feuilles de polyester qui forment un treillis où s'entrecroisent perpendiculairement des électrodes conductrices (Figure 3). À chaque intersection, la résistance est déterminée comme étant maximale en l'absence d'une force. Sous l'effet d'une force, il y a augmentation de la conductance électrique. Les éléments sensitifs sont sous un brevet américain (5033291 A) détenu par Tekscan. Les composantes électroniques de ce système permirent d'enregistrer les forces verticales à une fréquence de 44 hertz avec une capacité maximale de 8,8 kg par centimètre carré.

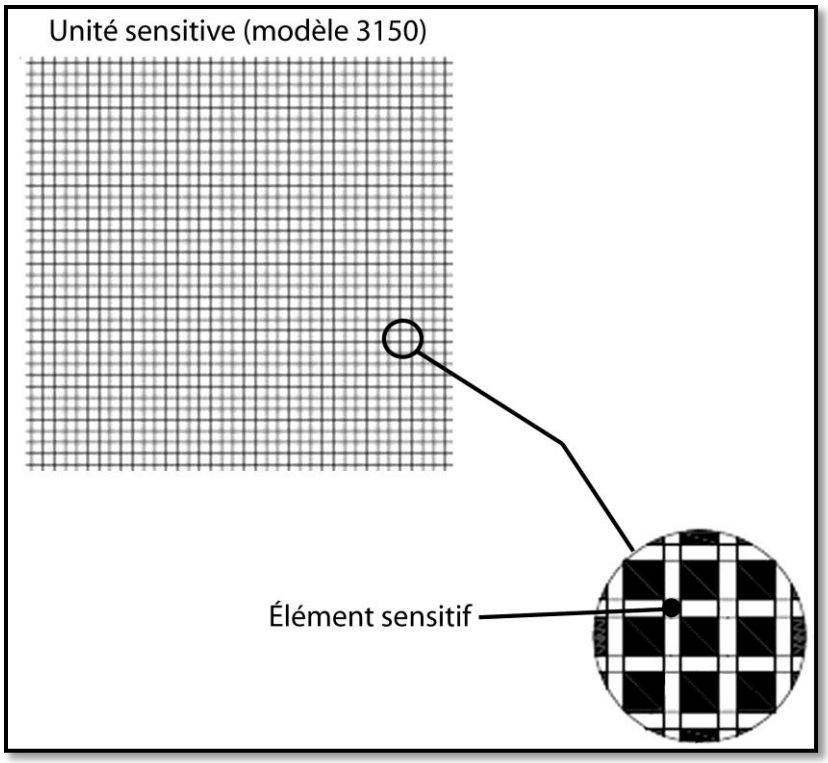

Figure 3. Schéma de l'unité sensitive (modèle 3150) et de l'élément sensitif (adapté de https://www.tekscan.com)

Annexe II.

Magnetic resonance imaging can accurately assess the long-term progression of knee structural changes in experimental dog osteoarthritis

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Accepted 2 October 2007
Published Online First 25 October 2007

ABSTRACT

Objectives: Osteoarthritis (OA) structural changes take place over decades in humans. MRI can provide precise and reliable information on the joint structure and changes over time. In this study, we investigated the reliability of quantitative MRI in assessing knee OA structural changes in the experimental anterior cruciate ligament (ACL) dog model of OA.

Methods: OA was surgically induced by transection of the ACL of the right knee in five dogs. High resolution three dimensional MRI using a 1.5 T magnet was performed at baseline, 4, 8 and 26 weeks post surgery. Cartilage volume/thickness, cartilage defects, trochlear osteophyte formation and subchondral bone lesion (hypersignal) were assessed on MRI images. Animals were killed 26 weeks post surgery and macroscopic evaluation was performed.

Results: There was a progressive and significant increase over time in the loss of knee cartilage volume, the cartilage defect and subchondral bone hypersignal. The trochlear osteophyte size also progressed over time. The greatest cartilage loss at 26 weeks was found on the tibial plateaus and in the medial compartment. There was a highly significant correlation between total knee cartilage volume loss or defect and subchondral bone hypersignal, and also a good correlation between the macroscopic and the MRI findings.

Conclusion: This study demonstrated that MRI is a useful technology to provide a non-invasive and reliable assessment of the joint structural changes during the development of OA in the ACL dog model. The combination of this OA model with MRI evaluation provides a promising tool for the evaluation of new disease-modifying osteoarthritis drugs (DMOADs).

Osteoarthritis (OA) is a chronic disease in which the evolution of joint structural changes takes place over a number of years if not decades in humans. Therefore, it is understandably difficult to precisely study the changes observed in the early stages of the disease. Animal models that can reproduce many of the morphological and molecular changes of OA have been used extensively to study the pathophysiology of the disease[1] and some have been very useful for testing the effects of drugs that may have the potential to modify the evolution of the disease (disease-modifying osteoarthritis drugs; DMOADs). Among these, the anterior cruciate ligament (ACL) model has been widely used.[2–7] Moreover, this model has been demonstrated in dogs followed over 54 months to be indeed one of progressive OA.[8] Interestingly, positive correlations were found between the DMOAD effects of three drugs, doxycycline,[2] licofelone,[6] and diacerhein[9] in this model, and those found in human knee and hip OA clinical trials.[10–12]

Recent developments in MRI technology used in the field of musculoskeletal research have created new possibilities by providing precise and reliable quantitative information on the joint structure as well as changes over time.[13 14] A number of longitudinal and cross-sectional clinical knee OA studies[15–26] have provided a large body of novel information about the sensitivity and reliability of quantitative MRI (qMRI) in such studies. Important new knowledge has been acquired on the topographical location and rate of the loss of cartilage in patients with knee OA.[26] Moreover, a number of risk factors that are leading causes of cartilage loss, such as gender (female), high body mass index (BMI), meniscal and subchondral bone lesions, and malalignment have been confirmed or identified.[16 17 19 20 22–25 27 28]

There is, to date, little information about the possible use of qMRI to assess knee structural changes occurring over time in the dog ACL model. Moreover, most of the reports so far have been mainly descriptive in nature.[29 30]

The aim of the present study was to evaluate the usefulness of qMRI to assess the OA knee structural changes in the dog ACL model over a period of 26 weeks and correlate the MRI findings with the macroscopic assessment of OA lesions. The changes in the cartilage volume/thickness were evaluated using quantitative technology, while the severity of cartilage lesions (cartilage defect), the osteophyte growth and the subchondral bone lesion (hypersignal) were evaluated using semi-quantitative scoring systems. The results of this study show that, through MRI technology, knee OA structural changes can be precisely assessed over time in this model and correlate well with the morphological changes.

MATERIALS AND METHODS

Experimental group

Five adult crossbred dogs (22–48 months old), weighing mean (SD) 25 (3) kg, were used. Surgical transection of the ACL of the right knee was performed under general anaesthesia as previously described[3 31] without any special procedure for haemostasis control after sectioning. Following surgery, the dogs were housed and they had free access to exercise in a large enclosure. All dogs

exercised in exterior runs for a 2-h period during the morning, 5 days a week (Monday to Friday). The study protocol was approved by the Institutional Ethics Committee.

MRI procedures

High-resolution 3-D MRI examinations of the right knee were performed before surgery (baseline) and post surgery (follow-up) at weeks 4, 8 and 26. The examinations were performed with a 1.5 T magnet (Echospeed LX; General Electric Healthcare, Waukesha, Wisconsin, USA) using a wrist coil, as previously described[18] with modifications. All examinations were standardised by using a dedicated device allowing the dogs to be placed in supine position with the right leg placed in the antenna at full extension. In addition, cushions were used to maintain the knee position and the internal rotation of the leg.

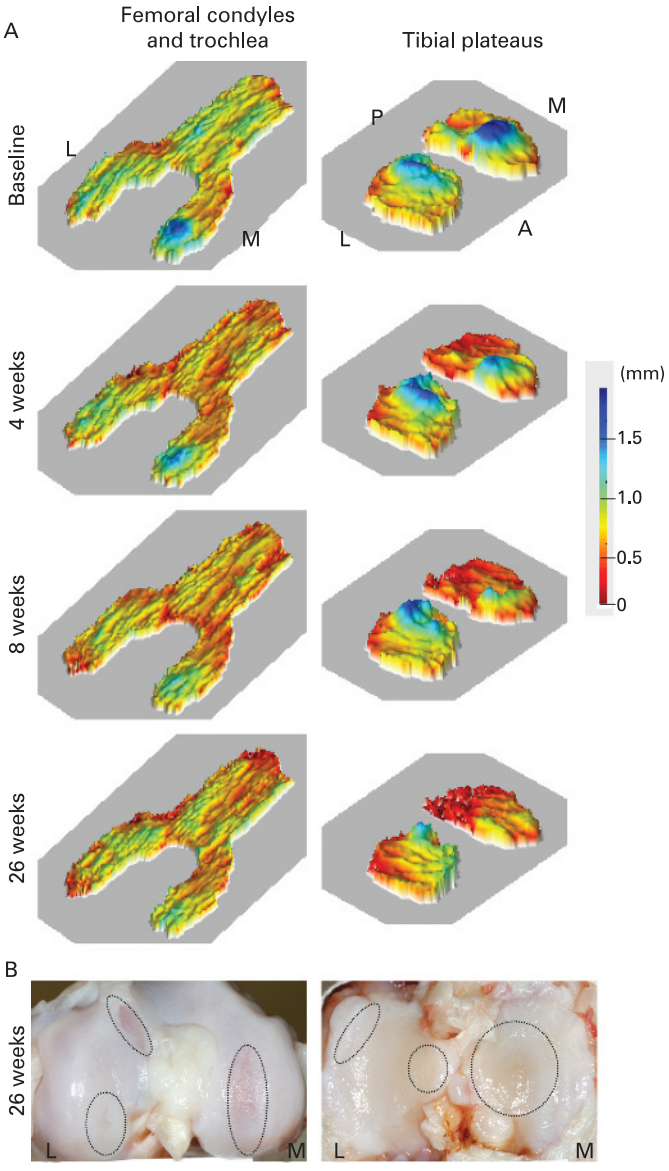

Figure 1 A. Unfolded 3-D cartilage thickness reconstruction colour map of femoral condyles and trochlea (left panel) and tibial plateaus (right panel) from baseline (intact cartilage) to 26 weeks after anterior cruciate ligament (ACL). The red colour represents areas with less cartilage. B. Macroscopic appearance of osteoarthritis (OA) cartilage 26 weeks post surgery. A, anterior; P, posterior; L, lateral; M, medial. Circles indicate areas of lesion.

MRI acquisition was performed under general volatile anaesthesia using isoflurane (Abbott Laboratories, Chicago, Illinois, USA; 2–2.5%) mixed with oxygen (20–40 ml/kg/min). Animals were appropriately premedicated with an intramuscular administration of buprenorphine (0.01 mg/kg), followed by an intravenous induction with propofol (4 mg/kg). Animals were orotracheally intubated and hydrated with physiologic saline (0.9% NaCl) for the duration of the acquisition. The dogs were monitored (heart and respiratory rates, non-invasive systemic blood pressure, pulsed oximetry) under the supervision of a veterinarian.

The MRI examination consisted of three fast imaging acquisition sequences of the operated (right) knee. The left knee was not evaluated. A sagittal 3-D spoiled gradient sequence (SPGR) with fat saturation (TR 42 ms, TE 6.6 ms, flip angle 20°, slice thickness 1 mm, matrix size 384, field of view 10 cm, number of excitations (NEX) 2) served for cartilage analysis (cartilage volume and defects); a sagittal 2-D fast spin echo sequence (FSE) with fat saturation (TR 3000 ms, TE 98 ms, slice thickness 2 mm, matrix size 384, field of view 10 cm, NEX 2, Flip angle 90°) for the subchondral bone lesions; and a coronal 3-D gradient recall sequence (GRE) without fat saturation (TR 11.4 ms, TE 3.15 ms, slice thickness 1.5 mm, matrix size 256, field of view 8 cm, NEX 3, flip angle 15°) for osteophytes. The total acquisition time was approximately 60 min.

Quantitative and semi-quantitative scoring systems

The images were analysed to assess cartilage volume, cartilage defects, bone marrow lesion (hypersignal) and osteophytes. The cartilage volume was assessed by quantitative measurement, the others by semi-quantitative scoring systems.

Cartilage volume

The quantitative measurement of the cartilage volume was performed on the 3-D SPGR images using Cartiscope (ArthroVision Inc., Montreal, Quebec, Canada) as previously described.[18 24 26] The cartilage volume of the entire (total) knee and five subregions was assessed: the trochlea, the lateral and medial femoral condyles, and the lateral and medial tibial plateaus. The patella was excluded from the evaluation. The limit between femoral condyles and trochlea was defined by the natural anatomical structures of the dog. Change in cartilage volume was calculated as the difference in cartilage volume (mm^3) over time compared to baseline, and expressed as a difference percentage (%) as previously described.[18]

Semi-quantitative scoring of cartilage defects and osteophytes

Cartilage defects were assessed in subregions of the knee, which included the anterior and posterior medial and lateral femoral condyles, the anterior, central and posterior medial and lateral tibial plateaus and the trochlea. For the osteophytes, the medial and lateral trochlea were evaluated separately.

The cartilage defects were scored on a scale of grades 0 to 4 as described previously:[20 25] 0, normal cartilage; 1, cartilage oedema with changes in cartilage shininess, texture and local thickening; 2, loss of less than 1/3 of thickness; 3, loss of less than 2/3 of thickness and; 4, loss of more than 2/3 up to complete cartilage loss with exposure of subchondral bone. The reading was performed for each of the defined subregions using SPGR acquisition. For each dog, the sum of the grade (score) and the mean of the individual grade were calculated. The osteophytes were identified on the trochlea as local bone outgrowths on

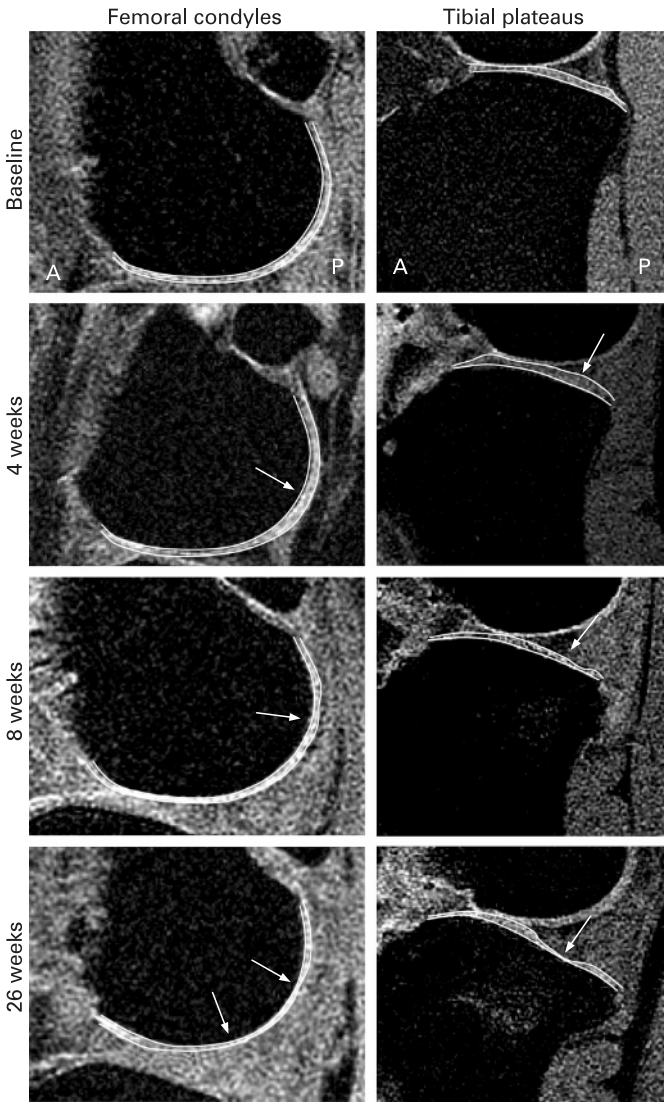

Figure 2 Representative sagittal spoiled gradient sequence (SPGR) images of dog knee from baseline (intact cartilage) to 26 weeks after anterior cruciate ligament (ACL) began showing cartilage defects on medial femoral condyles and tibial plateaus. Cartilage is intact (grade 0) at baseline. Arrows indicate the cartilage thickening (grade 1) 4 weeks post surgery, erosion of cartilage (grade 2) 8 weeks post surgery and loss of cartilage (grade 3–4) 26 weeks post surgery. Lines show cartilage/bone interface and cartilage surface. A, anterior; P, posterior.

coronal GRE MRI sections. The maximum width of osteophyte strips growing along the lateral and medial edges was measured. The final score was the mean of the osteophyte size measured on both edges.

Semi-quantitative scoring of the subchondral bone lesions (hypersignal)

Bone hypersignal was identified as brighter zones in the subchondral bone in fat saturated MRI. The assessed subregions include the medial and lateral condyles and plateaus, and trochlea. The lesion in each subregion was scored on a scale from 0 to 3, where 0 = normal bone, 1 = a hypersignal less than 1/3 of the surface of the subregion, 2 = a hypersignal less than 2/3 of the surface and 3 = a hypersignal greater than 2/3 of the surface as previously described.[13 24] The reading was performed for each of the defined subregions using SPGR MRI images. The size of lesion in the subregions was assessed on the sections where the hypersignal was the greatest. The final score for each dog was the sum of the grade (score) and the mean of the individual grade for the subregions: femoral condyles and trochlea, plateaus, compartments, and the entire knee (total).

Macroscopic grading

Immediately following the animal being killed at 26 weeks post surgery, the right knee of each dog was resected, placed on ice, dissected and examined for gross macroscopic morphologic changes, as previously described.[32]

The degree of osteophyte formation was graded by measuring the maximal width (mm) of the spur on the medial and lateral femoral trochlea using a digital calliper (Digimatic Caliper; Mitutoyo Corporation, Kawasaki, Japan). The two values recorded for each dog were considered separately for the statistical analysis.

The cartilage lesion surfaces on the medial and lateral femoral condyles, including the trochlea and the tibial plateaus, were graded separately as previously described.[3 6 32] The cartilage lesion areas were measured and expressed in mm^2. The final score consisted of the sum of the surface of each lesion present on the femoral condyles and trochlea or tibial plateaus. The total joint score consisted of the sum of scores obtained for condyles and trochlea, and plateaus.

The depth of each macroscopic lesion was also evaluated as previously described[3 6 32] using a grade from 0 to 4, where 0 represents intact cartilage and 4 the total loss of cartilage. The results are given as the mean of the grade of the lesions on the femoral condyles and tibial plateaus.

Histological grading

Histological evaluation was performed on sagittal sections of cartilage from the lesional areas of each femoral condyle and tibial plateau as previously described.[3 6 32] Specimens were dissected, fixed in TissuFix 2 (Laboratoires Gilles Chaput, Montreal, Quebec, Canada), and embedded in paraffin for histological evaluation. Serial sections (5 μm) were stained with Safranin-*O*. The severity of the OA lesions was graded on a scale of 0–29 by two independent observers blinded to the treatment, using the histological scale modified from Sakakibara *et al*.[33] This scale was used to evaluate the severity of OA lesions based on the loss of Safranin-*O* staining (scale 0–4), cellular changes (scale 0–12), structural changes (scale 0–10, where 0 = normal cartilage structure and 10 = complete disorganisation) and pannus formation (0–3). The final score was the mean score of the most severe histological changes within the cartilage lesions found on femoral condyles or tibial plateaus.

Statistical analysis

Data are presented as mean (SEM). Data were analysed with analysis of variance (ANOVA) for repeated measures, followed, if positive ($p<0.05$), by a Tukey multiple comparison test. A p value <0.05 was considered significant. Correlations between MRI parameters were established with Pearson test where a $p<0.05$ represents a statistically positive correlation.

RESULTS

Cartilage volume and defects

At baseline the cartilage volume (table 1) of the femoral condyles and trochlea was about twice that of the tibial plateaus. The cartilage volume in the medial and lateral compartments (ie, medial or lateral femoral condyles and tibial

Table 1 Cartilage volume and loss in osteoarthritic dogs

	Femoral condyles and trochlea	Tibial plateaus	Medial compartment	Lateral compartment	Total
Baseline:					
Volume (mm^3)	703.7 (41.7)	376.4 (30.9)	358.9 (22.0)	386.9 (29.8)	1080.0 (70.2)
4 weeks:					
Volume (mm^3)	599.3 (32.9)	318.0 (26.0)	294.8 (21.7)	350.6 (20.9)	917.2 (54.9)
Loss (%)	−14.7 (1.9)	−15.3 (2.2)	−18.1 (2.0)	−8.7 (3.0)	−14.9 (1.8)
Significance	p<0.05*	p<0.001*	p<0.01*		p<0.001*
8 weeks:					
Volume (mm^3)	620.2 (40.7)	274.7 (25.2)	269.0 (15.3)	344.5 (25.1)	894.9 (65.0)
Loss (%)	−11.6 (4.5)	−27.1 (2.3)	−24.6 (3.6)	−10.6 (3.4)	−17.0 (3.6)
Significance		p<0.001* p<0.01†	p<0.001*		p<0.001*
26 weeks:					
Volume (mm^3)	556.0 (53.8)	211.0 (11.8)	220.3 (16.0)	307.4 (29.6)	767.0 (63.9)
Loss (%)	−21.2 (5.6)	−43.2 (2.2)	−38.0 (4.9)	−20.6 (4.4)	−29.0 (3.5)
Significance	p<0.01*	p<0.001*‡§	p<0.001* p<0.01‡ p<0.05§	p<0.001* p<0.05‡	p<0.001* p<0.01‡§

The knee cartilage volume (mm^3) and loss calculated as percentage (%) from baseline were assessed as described in Materials and methods. Data (n = 5) are mean (SEM).
*p Value compared to baseline. †p Value for 8 weeks compared to 4 weeks. ‡p Value for 26 weeks compared to 4 weeks. §p Value for 26 weeks compared to 8 weeks.

plateaus) was about equal. Over time, a progressive loss of cartilage volume (%) was found on the femoral condyles/trochlea and tibial plateaus table 1, fig 1. These changes were detected as early as 4 weeks post surgery and became more pronounced over time. Of note was the presence of cartilage oedema detected at 4 weeks. A greater percentage of cartilage loss was found on the tibial plateaus, starting at 8 weeks, compared to the femoral condyles/trochlea. This was also observed at 26 weeks. The loss of cartilage volume (%) was more pronounced (about twice as much) on the medial than on the lateral compartment at all times post surgery (table 1).

Interestingly, the findings from MRI correlated very well with the macroscopic cartilage lesions observed at 26 weeks, the time when the animal was killed (fig 1B). Macroscopic evaluation revealed that the total surface cartilage lesion size was 186.1 (58.8) mm^2 for the femoral condyles and 147.4 (32.0) mm^2 for the tibial plateaus, while the mean grade of lesions was 2.3 (0.2) for the femoral condyles and 1.9 (0.2) for the tibial plateaus.

The histological evaluation of cartilage lesions gave a score of 18.2 (0.6) for the femoral condyles and 18.2 (0.8) for the tibial plateaus.

The findings from the 3-D reconstruction map of the cartilage thickness correlated well with the cartilage volume loss (table 1, fig 1A). As illustrated in fig 1A, cartilage thickness loss that was found to increase over time occurred mainly on the anterior and central weight bearing areas of the femoral condyles as well as on the trochlea, and the central and posterior portion of the tibial plateaus.

MRI cartilage defect data (table 2, fig 2) show that lesions were found as early as 4 weeks post surgery. At that time, lesions were seen more commonly on the medial condyles, the trochlea and plateaus. Data showed that the cartilage defect incidence, scores and grades increased progressively over time. By 26 weeks, lesions were present on all subregions including the lateral region, although still higher in the medial compartment (table 2). This is particularly true for the scores. Altogether, these findings indicate an increase in the number and, more particularly, the severity of defects over time in the different subregions of the knee.

Osteophytes

The trochlea was the site of significant osteophyte formation, which was found on the lateral and medial sides in all dogs as early as 4 weeks post surgery (2.4 (0.4) mm, $p<0.01$ compared to baseline). The mean size was much larger than those on the condyles and plateaus at all times (data not shown), and markedly increased over time (4.2 (0.9) mm, $p<0.001$, 8 weeks post surgery). At 26 weeks post surgery, the osteophyte size estimated by MRI (6.1 (0.7) mm, $p<0.001$) was somewhat smaller than that found during the macroscopic examination (8.6 (0.6) mm).

Subchondral bone hypersignal

Bone hypersignal was frequently observed in OA dogs with their score and grade increasing over time (table 3, fig 3). No more than one bone hypersignal per subregion was found in the OA knee. They were generally observed in the early stages of the disease on the medial femoral condyles and tibial plateaus, their grade being slightly greater on the plateaus. The lesions were seen mainly in the posterior section of the tibial plateaus and femoral condyles (fig 3). Over time, they were also found on the lateral regions of the femoral condyles, the tibial plateaus and the trochlea. However, as for the cartilage lesions, the bone hypersignal score and grade were more pronounced in the medial than in the lateral compartment.

Correlations between cartilage and subchondral bone parameters evaluated by MRI

Statistically significant correlations were found between the total cartilage volume loss ($r = -0.81$, $p<0.001$) or the total cartilage defects (score or grade) ($r = 0.75$, $p<0.001$) and total bone hypersignal (score or grade).

DISCUSSION

The present study provides new and original information about the evolution of knee structural changes in the ACL dog model of OA as assessed by MRI. It demonstrates the progressive nature of the OA changes and shows that cartilage and bone alterations can be accurately followed and quantified by MRI. Here, we showed by 3-D imaging reconstruction the progressive

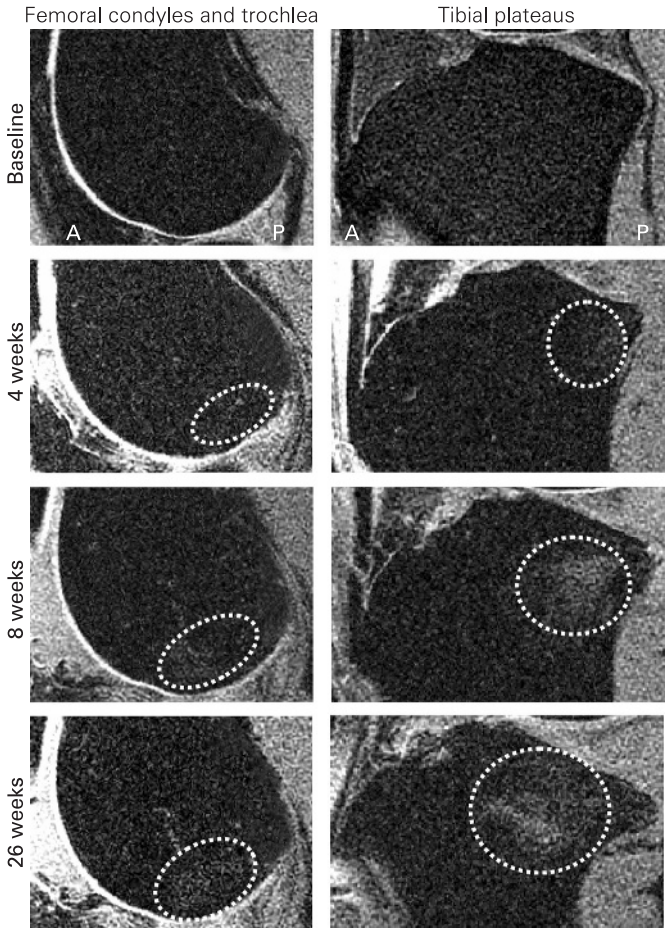

Figure 3 Representative sagittal spoiled gradient sequence (SPGR) images of femoral condyles and trochlea (left panel) and tibial plateaus (right panel) showing the evolution of subchondral bone hypersignal from baseline (no lesion) to 26 weeks post surgery. A, anterior; P, posterior; circles indicate the areas of lesions.

and focal nature of cartilage lesions and volume loss over time on femoral condyles and trochlea, and tibial plateaus. Interestingly, the loss of cartilage volume correlates well with the severity of cartilage defects. The data also demonstrated a predominance of cartilage loss on the tibial plateaus over the femoral condyles and trochlea together, and on the medial over the lateral compartment. The volume of cartilage on the condyles and trochlea being about twice that found on the plateaus combined with the fact that the surface of lesions was only slightly higher on the condyles and trochlea, explains the greatest percentage of cartilage loss on the plateaus. These findings are nicely illustrated in the 3-D cartilage thickness map (fig 1). These findings also correlate well with previous reports on the distribution and severity of cartilage lesions in this model,[3 4 6 31] as well as being in line with the findings of a recent study in patients with knee OA.[24]

This study also provides long-term data on the evolution of knee structural changes in the ACL dog model. The correlations between the MRI data, the 3-D reconstruction cartilage mapping and the macroscopic cartilage lesions found at 26 weeks are also interesting, and provide conclusive information on the accuracy and usefulness of MRI technology to assess the topographical distribution and severity of cartilage lesions in the different subregions of the knee. It also prevents possible errors in evaluating the progression of lesions that could affect the accuracy needed to precisely assess the evolution of OA cartilage changes (loss) over time. These results are well in line with the work performed by our group and others in patients with knee OA[14 15 20 26] as well as a recent study in the guinea pig spontaneous OA model.[34] The use of quantitative MRI with 3-D reconstruction has proven to be a very powerful tool to precisely locate and quantify the loss of cartilage volume/thickness in human knee OA. The results from previous studies on humans correspond to those of the present one in which there is a preferential location of cartilage lesions on the weight-bearing areas of the femoral condyles and tibial plateaus, with a predominance of cartilage loss in the medial compartment.[3 4 6 26]

One must, however, acknowledge the fact that studies performed using an animal model, including the present one, have some limitations imposed, first by the use of an experimental model and secondly by the fact that the results are obtained using a small number of animals. However, despite these limitations, the statistical results (p values) obtained in

Table 2 Cartilage defect score in osteoarthritic dogs

	Femoral condyles and trochlea	Tibial plateaus	Medial compartment	Lateral compartment	Total
4 weeks:					
Score	7.6 (1.3) (5:3:5)	6.8 (2.0) (5:5)	6.6 (1.4) (5:5)	5.0 (1.7) (3:5)	14.4 (3.1)
Significance	p<0.001*	p<0.01*	p<0.01*	p<0.05*	p<0.001*
Grade	1.5 (0.3)	1.1 (0.3)	1.3 (0.3)	1.0 (0.3)	1.3 (0.4)
Significance	p<0.001*	p<0.01*	p<0.01*	p<0.05*	p<0.001*
8 weeks:					
Score	9.2 (1.7) (5:3:5)	11.0 (1.7) (5:5)	10.2 (1.7) (5:5)	6.4 (1.9) (3:5)	20.2 (3.1)
Significance	p<0.001*	p<0.001*	p<0.001*	p<0.01*	p<0.001*
Grade	1.8 (0.3)	1.8 (0.3)	2.0 (0.3)	1.3 (0.4)	1.8 (0.3)
Significance	p<0.001*	p<0.001*	p<0.001*	p<0.01*	p<0.001*
26 weeks:					
Score	15.8 (1.0) (5:5:5)	19.8 (0.3) (5:5)	29.8 (1.2) (5:5)	14.0 (0.7) (5:5)	35.6 (1.3)
Significance	p<0.001*‡§	p<0.001*‡§	p<0.001*‡§	p<0.001*‡§	p<0.001*‡§
Grade	3.1 (0.2)	3.3 (0.1)	3.5 (0.1)	2.8 (0.1)	3.2 (0.1)
Significance	p<0.001*‡§	p<0.001*‡§	p<0.001*‡§	p<0.001*‡§	p<0.001*‡§

Cartilage defects were evaluated as described in Materials and methods. Data (n = 5) are the mean (SEM). The scores are expressed as the summation of grades, and the grades as an average of the grades obtained for each region. Incidence of the lesions in subregions is indicated in parentheses (number of dogs with lesions on medial/lateral femoral condyles/trochlea, medial/lateral tibial plateaus, or on femoral condyles/tibial plateaus in the compartments).
*p Value compared to baseline. †p Value for 8 weeks compared to 4 weeks. ‡p Value for 26 weeks compared to 4 weeks. §p Value for 26 weeks compared to 8 weeks.

Table 3 Subchondral bone hypersignal in osteoarthritic dogs

	Femoral condyles and trochleaTibial plateaus		Medial compartment	Lateral compartment	Total
4 weeks:					
Score	1.2 (0.8) (1:2:1)	1.8 (0.5) (4:0)	2.0 (0.6) (1:4)	0.6 (0.4) (2:0)	3.0 (1.0)
Significance		p<0.05*	p<0.01*		
Grade	0.4 (0.2)	0.9 (0.2)	1.0 (0.3)	0.3 (0.2)	0.6 (0.2)
Median (range)	0.0 (0.0–1.3)	1.0 (0.0–1.5)	1.0 (0.0–2.0)	0.0 (0.0–1.0)	0.4 (0.4–1.4)
8 weeks:					
Score	2.0 (1.1) (1:2:2)	2.8 (0.4) (5:1)	3.0 (0.5) (1:5)	1.0 (0.5) (2:1)	4.8 (1.1)
Significance		p<0.001*	p<0.001*		p<0.01*
Grade	0.6 (0.4)	1.4 (0.2)	1.5 (0.3)	0.5 (0.3)	0.9 (0.2)
Median (range)	0.3 (0.0–2.0)	1.5 (1.0–2.0)	1.5 (1.0–2.5)	0.5 (0.0–1.5)	0.8 (0.6–1.8)
26 weeks:					
Score	4.0 (1.2) (4:3:4)	4.6 (0.4) (5:5)	4.2 (0.4) (4:5)	2.4 (0.4) (3:5)	8.6 (1.2)
Significance	p<0.01*	p<0.001*‡	p<0.001*	p<0.001*	p<0.001*
		p<0.05§	p<0.01‡	p<0.01‡	p<0.01‡
				p<0.05§	p<0.05§
Grade	1.3 (0.4)	2.3 (0.2)	2.1 (0.2)	1.2 (0.2)	1.7 (0.2)
Median (range)	2.0 (0.0–2.0)	2.0 (2.0–3.0)	2.0 (1.5–2.5)	1.5 (0.5–1.5)	2.0 (0.8–2.2)

Subchondral bone hypersignal was identified as brighter zones in the subchondral bone in the fat saturated images, as described in Materials and methods. Data (n = 5) are mean (SEM). The scores are expressed as the summation of grades, and the grades as an average of the grade obtained for each region. Incidence of the lesions in the subregions is indicated in parentheses (number of dogs with lesions on medial/lateral femoral condyles/trochlea, medial/lateral tibial plateaus, or femoral condyles/tibial plateaus in the medial or lateral compartment). The median (range) indicates the median of the grade for each compartment and the spread of the grade (ie, minimum and maximum).
*p Value compared to baseline. †p Value for 8 weeks compared to 4 weeks. ‡p Value for 26 weeks compared to 4 weeks. §p Value for 26 weeks compared to 8 weeks.

the present study are good and the standard deviations quite small, supporting the reliability of the technology.

The semi-quantitative scoring system to assess the progression of cartilage lesions was found to correlate with the quantitative cartilage loss volume evaluated in the different subregions. Indeed, cartilage changes were detected very early in the disease process: they were found in almost all subregions at 4 weeks and progressed from a grade of approximately 1 to 1.5, indicating the presence of cartilage oedema and minimal loss of cartilage, to severe lesions with a grade of 2.8 to 3.6 by 26 weeks, indicating the presence of severe erosion, a finding corroborated by the macroscopic examination at the time the animal was killed. The finding of the progressive increase in the score and grade of lesions in the subregions is indicative of existing lesions worsening over time. This is in line with the data from quantitative MRI data, including the 3-D reconstruction map, as well as our previous observations[3 4 6 32] and that of Brandt *et al*[8] in this model. The MRI detection of cartilage oedema in the early lesions is also in line with the previous report of Calvo *et al*[35] in the rabbit meniscectomy model. The presence of cartilage oedema observed at 4 weeks indicates that the cartilage loss measured at that timepoint could have been somewhat underestimated.

Osteophytes are commonly found in the ACL dog model. They are often seen on the trochlear ridges where their size is greater than those on the femoral condyles or tibial plateaus, the latter being usually of a small size. There was a relatively good correlation between the incidence and size of trochlear osteophytes measured on MRI images at 4 and 8 weeks and macroscopic evaluations performed in previous studies.[3 4 6 32] Of note is that the trochlear osteophyte size evaluated by MRI at 26 weeks was slightly smaller than that evaluated by visual assessment. This could be explained by the fact that the osteophytes are generated by endochondral ossification, thus the cartilage covering the bone structure could not be seen on the MRI sequence used to measure the osteophytes.

Subchondral bone lesions, such as hypersignal (also called oedema) are integral components of the knee structural changes in OA.[14 17 22 24 28 36] Hypersignal has been linked to symptoms, particularly to pain, in human knee OA.[24 28 36] Recently, the increase in size of hypersignal was found to be correlated with the loss of cartilage over time in these patients.[28] The exact relationship between these two phenomena remains, however, to be further explored. To that effect, hypotheses have been postulated of a role of the subchondral bone in OA pathophysiology. Over and above biomechanical factors, the role of a number of biochemical pathways has been proposed.[37] It is interesting to note that the subchondral bone hypersignal identified in the ACL dog model was found to correspond, from a structural point of view, to areas of bone where there was an increased haematopoiesis, myxomatous transformation of the bone marrow and intertrabecular fibrosis without any signs of bone marrow oedema.[38] In the present study, we found a definite and progressive increase over time in the number of subregions with subchondral bone lesions as well as an increase in their sizes, and these lesions appeared early (4 weeks post surgery) in the evolution of the disease. The co-location of subchondral hypersignals with cartilage lesions was not explored for each dog. However, both of these structural changes were found to be more pronounced in the medial compartment, suggesting the likely relation between the two phenomena.

An interesting finding was a predominance of subchondral bone lesion incidence and size increase in the medial compartment, particularly in the tibial plateaus. This is well in line with previous reports in this model[3 5 6 32] and in patients with knee OA.[18 24 26 28] The statistically significant correlation between the bone hypersignal and the loss of cartilage volume and the severity of cartilage lesions agrees with the data in human knee OA studies.[17 26 28] These stress the important pathophysiological interrelationship between the subchondral bone remodelling and the development of OA cartilage lesions.

In summary, this study provides interesting and new data on the usefulness of MRI to assess the evolution of structural changes in experimental dog OA. It also provides information about the progressive nature of the structural changes in this model as well as its similarity to the natural human disease. The use of MRI in combination with the ACL dog model of OA

provides a very powerful tool for the evaluation of the effectiveness of new DMOADs in pre-clinical evaluation. Possible extrapolation of these data to humans will obviously need further exploration.

Funding: Supported in part by a grant from the CR-CHUM and the Osteoarthritis Chair of the University of Montreal.

Competing interests: None declared.

Ethics approval: The study protocol was approved by the Institutional Ethics Committee.

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Annexe III.

Osteoarthritis and Cartilage (2008) **16,** 1307–1311

doi:10.1016/j.joca.2008.03.022

Osteoarthritis and Cartilage

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Brief report
Temporal assessment of bone marrow lesions on magnetic resonance imaging in a canine model of knee osteoarthritis: impact of sequence selection

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Summary

Objective: To assess the evolution of bone marrow lesions (BMLs) in a canine model of knee osteoarthritis (OA) using three different magnetic resonance imaging (MRI) sequences.

Design: Three MRI sequences [coronal, T1-weighted three-dimensional fast gradient recalled echo (T1-GRE), sagittal fat-suppressed 3D spoiled gradient echo at a steady state (SPGR), and sagittal T2-weighted fast spin echo with fat saturation (T2-FS)] were performed at baseline, and at week 4, 8 and 26 in five dogs following transection of the anterior cruciate ligament. The same reader scored (0–3) subchondral BMLs twice, in blinded conditions, according to their extent in nine joint subregions, for all imaging sessions, and independently on the three MRI sequences. Correlation coefficients and Bland–Altman plots evaluated intra-reader repeatability. Readings scores were averaged and the nine subregions were summed to generate global BML scores.

Results: BMLs were most prevalent in the central and medial portions of the tibial plateau. Intra-reader repeatability was good to excellent for each sequence ($r_s = 0.87–0.97$; $P < 0.001$). Maximal intra-reader variability (24%) was reached on T2-FS and was associated to higher scores ($P < 0.05$). Global BML scores increased similarly on all three sequences until week 8 ($P < 0.05$). At week 26, score on T2-FS was decreased, being lower when compared to T1-GRE and SPGR ($P < 0.05$).

Conclusion: In this canine OA model, the extent of BMLs varies in time on different MRI sequences. Until the complex nature of these lesions is fully resolved, it is suggested that to accurately assess the size and extent of BMLs, a combination of different sequences should be used.

Key words: Bone marrow lesion, MRI, Osteoarthritis, Dog.

Introduction

Subchondral bone marrow lesions (BMLs), formerly known as bone marrow edema, represent an important component of osteoarthritis (OA) that is now routinely assessed with magnetic resonance imaging (MRI) whole-organ scoring systems[1–11]. Although the exact nature of BMLs needs further clarification, it has been stated that bone marrow and trabecular alterations that include fibrosis and necrosis are largely responsible for the signal changes in the human knee, rather than edema[12,13]. In dogs with advanced, experimental OA, infiltrates such as hematopoiesis, myzomatous transformation and fibrosis have been found in regions of BMLs[14,15].

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Received 26 March 2008; revision accepted 30 March 2008.

BMLs are most commonly assessed on fat-suppressed T2-weighted or intermediate-weighted (proton density) turbo or fast spin echo (FSE) sequences, or on short-tau inversion recovery (STIR), on which they appear as ill-defined hyperintensities that contrast with the hypointense marrow fat[1,6,9,12,14,16–19]. Other sequences such as T1-weighted FSE or gradient echo[12,14,17,20] and T2*-weighted gradient echo sequences[21] have also been used[12,14,17,20]. However, studies focusing on the impact of sequence selection on the appearance of BMLs are currently lacking.

The purpose of this study was to compare the manifestation and evolution of BMLs over a period of 26 weeks using three different MRI sequences in a group of dogs with experimentally induced knee OA.

Method

The study protocol was approved by the Institutional Ethical and Scientific Committee in accordance with the guidelines of the Canadian Council on

Animal Care. Five healthy, mature dogs (23.5 ± 2.3 kg) were selected, housed and exercised routinely as previously described[22]. Antibiotherapy, multimodal and pre-emptive analgesia, volatile anesthesia, right knee ACL surgical transection (under direct visualization)[23] and rehabilitation were done following standardized operating procedures taking place in our facilities[22,24–26].

The right knee was imaged with 1.5-T MRI (GE EchoSpeed LX, Milwaukee, WI, USA) prior to surgery (baseline) and at weeks 4, 8 and 26 postoperatively, using a phased-array, small-extremity coil with customized padding.

Coronal, T1-weighted three-dimensional fast gradient recalled echo (T1-GRE) images were obtained [slice thickness and gap (*s/g*) 1.5 mm/0 mm, time to echo (TE) 3.15 ms, repetition time (TR) 11.4 ms, flip angle (FA) 15°, number of acquisitions (NEX) = 2, field of view (FOV) 80 mm, matrix 256 × 256, pixel size = 0.32 mm]. Sagittal, fat-suppressed 3D spoiled gradient recalled acquisition at steady state (SPGR) images were also obtained (*s/g* 1.0 mm/0 mm, TR 42 ms, TE 6.6 ms, FA 20°, NEX = 2, FOV/matrix = 100 mm/384 × 384 or 110 mm/416 × 416 or 120 mm/448 × 448) depending on the size of the leg (pixel size = 0.26 mm), with a phase percent = 100%. Additionally, sagittal, T2-weighted fast spin echo sequences with fat saturation (T2-FS) were acquired (*s/g* 2 mm/0 mm, TR 3000 ms, TE 98 ms, FA 90°, FOV/matrix = 100 mm/384 × 256 pixel size = 0.26 × 0.39 mm). The total time required for patient set up and MRI evaluation was approximately 60 min.

MRI series acquired at week 4, 8 and 26 were scored individually and randomly under blinded conditions, twice, by a board-certified veterinary radiologist (MAD), using a diagnostic viewing station (Agfa IMPAX 6.0, Toronto, Canada).

BMLs were evaluated in nine subregions of the knee: patella, femoral trochlea, femoral condyles (medial and lateral), femoral intercondyla fossa, and tibial plateau (anterior, central, medial, and lateral). Hyperintense (SPGR and T2-FS) and hypointense (T1-GRE) BMLs were scored according to their extent in each of these subregions: 0 = none, 1 = less than 25% of the subregion volume, 2 = between 25 and 50%, 3 = more than 50% (Fig. 1). On T1-GRE images, subchondral signal voids consistent with bone sclerosis (calcified matrix)[20], were excluded.

For each knee as well as for each reading and each sequence, BML scores obtained in the nine anatomical subregions were summed to generate global BML scores. Spearman correlation coefficients (r_s) measured the association between readings and Bland–Altman plots assessed agreement and variability. Maximal variability was the range between confidence intervals (95%) of the difference between readings (score of 27 = 100%). Averaged reading scores were expressed in mean ± SD ($n = 5$). For each sequence, ANOVA for repeated measures followed by Tukey–Kramer multiple comparison tests compared BML scores overtime. Wilcoxon-signed rank tests compared sequences and delta-evolutions of scores. Significant level was set at 5% using statistical software (NCSS 2001, Kaysville, USA).

Results

A strong correlation was found between both readings for all three sequences ($r_s = 0.87$ on T2-FS, 0.92 on T1-GRE, and 0.97 on SPGR) ($P < 0.001$). Maximal inter-reading variability was 17% on T1-GRE, 13.2% on SPGR and 24% on T2-FS. The highest value of variability on T2-FS (24%) was measured in the dog that had the highest BML score at week 8. Nonetheless, intersequence variability was more pronounced, reaching 26.6% on T2-FS vs SPGR, 30.3% on T1-GRE vs SPGR, and 44.7% on T1-GRE vs T2-FS.

BMLs were observed on all follow-up imaging sessions in all dogs. BML scores were consistently higher in the medial and central portions of the tibial plateau, although BMLs were also commonly detected in the lateral femoral condyle, the femoral condylar fossa and trochlear groove (Table I).

A great variability was found between dogs in regard to BML scores at each imaging session as well as on the different MRI sequences. The distribution of the signal change also varied between sequences, even within a same affected subregion (Fig. 2). Nevertheless, BML scores increased between baseline and week 8 similarly on all three sequences. Between week 8 and 26, scores were relatively stable or slightly increased on T1-GRE; however, a trend to decrease was observed on SPGR and was significant for T2-FS (Fig. 3) ($P < 0.05$). In fact, at week 26, global BML

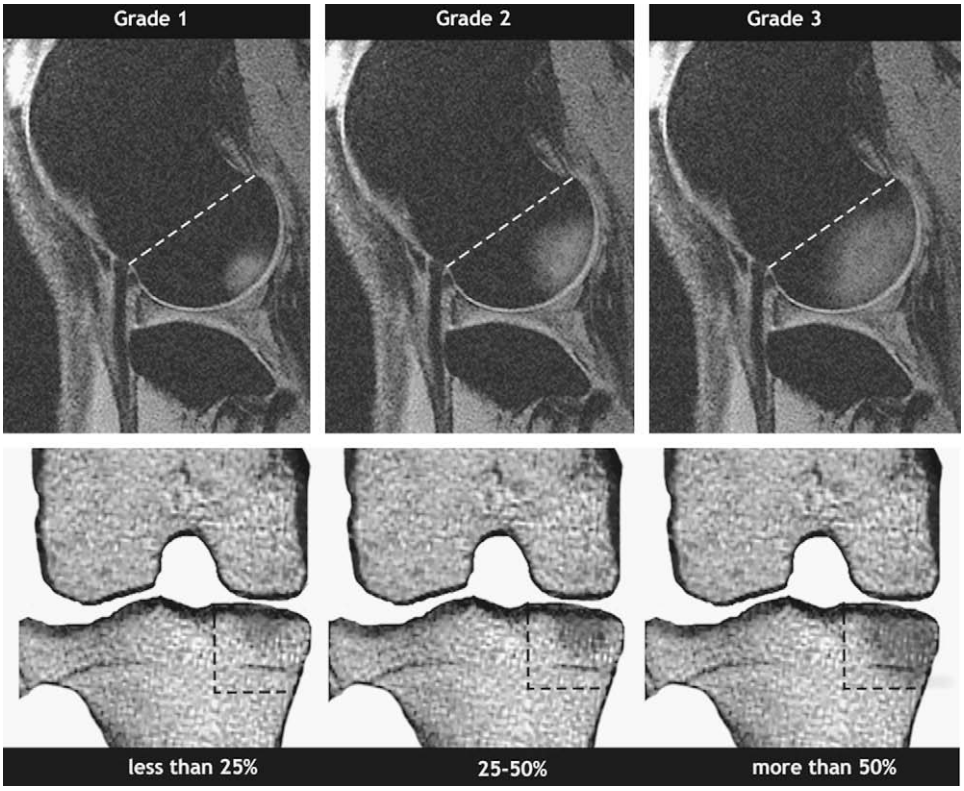

Fig. 1. Semi-quantitative grading scheme used to score BMLs in each subregion. Each BML was scored according to its extent in the volume of each subregion, individually on each of the three MRI sequences.

Table I
Temporal evolution of BML scores (mean ± SD) evaluated in nine subregions and globally for the whole knee joint in five dogs

Subregions		Baseline			Week 4			Week 8			Week 26		
		SPGR	T1-GRE	T2-FS	SPGR	T1-GRE	T2-FS	SPGR	T1-GRE	T2-FS	SPGR	T1-GRE	T2-FS
Patella		0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.9 ± 0.2	0.2 ± 0.4	0.9 ± 0.7	1.3 ± 0.4	0.8 ± 0.7	1.5 ± 0.9	1.2 ± 0.3	0.9 ± 1.2	0.9 ± 1.2
Femur	Medial condyle	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.4	0.2 ± 0.3	0.2 ± 0.4	0.8 ± 0.8	0.6 ± 0.5	0.8 ± 0.7	0.6 ± 0.6	0.5 ± 0.5	0.3 ± 0.4
	Lateral condyle	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.6 ± 0.9	0.7 ± 0.8	0.5 ± 0.7	0.6 ± 0.9	0.0 ± 0.0	0.7 ± 1.1	0.4 ± 0.5	0.4 ± 0.5	0.1 ± 0.2
	Trochlea	0.0 ± 0.0	0.2 ± 0.4	0.1 ± 0.2	0.3 ± 0.4	0.6 ± 0.7	0.4 ± 0.6	0.8 ± 1.0	1.2 ± 1.0	1.1 ± 1.1	0.5 ± 0.5	1.6 ± 0.9	0.5 ± 0.5
	Intercondylar fossa	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.5 ± 0.6	0.4 ± 0.4	0.6 ± 0.5	1.4 ± 0.9	0.8 ± 1.1	1.1 ± 0.8	1.1 ± 0.9	0.8 ± 0.9	0.7 ± 0.8
Tibial plateau	Medial	0.1 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	1.3 ± 1.0	1.3 ± 1.1	1.3 ± 1.0	1.8 ± 0.7	1.7 ± 1.1	2.2 ± 0.7	1.6 ± 1.1	1.9 ± 1.1	0.9 ± 1.0
	Lateral	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.2	0.0 ± 0.0	0.2 ± 0.3	0.3 ± 0.4	0.2 ± 0.4	0.1 ± 0.2	0.2 ± 0.3	0.0 ± 0.0
	Anterior	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.4 ± 0.5	0.4 ± 0.4	0.1 ± 0.2	0.6 ± 0.9	0.5 ± 0.3	0.5 ± 0.6	0.4 ± 0.5	0.5 ± 0.3	0.1 ± 0.2
	Central	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.0 ± 0.7	0.7 ± 0.4	0.7 ± 0.7	2.1 ± 0.9	1.9 ± 1.0	1.9 ± 0.6	1.2 ± 0.6	2.3 ± 0.8	1.2 ± 0.9
Global BML score*		0.1 ± 0.2	0.2 ± 0.4	0.1 ± 0.2	5.2 ± 3.9	4.6 ± 2.8	4.7 ± 4.5	9.6 ± 4.8	8.4 ± 4.4	10.0 ± 5.9	7.1 ± 3.4	9.1 ± 3.4	4.7 ± 3.3
Relative global BML score (%)†		0.4 ± 0.7	0.7 ± 1.5	0.4 ± 0.7	19.3 ± 14.4	17.0 ± 10.4	17.4 ± 16.7	35.6 ± 17.8	31.1 ± 16.3	37.0 ± 21.9	26.3 ± 12.6	33.7 ± 12.6	17.4 ± 12.2

BML scores (0–3) were averaged between readings and presented as mean ± SD.
*Global BML scores were the sum of the nine subregions.
†Global BML scores were expressed relatively to a maximal score of 27 (100%).

scores were significantly lower on T2-FS when compared to T1-GRE and SPGR ($P < 0.05$).

Discussion

The subchondral bone is recognized as an important structural component of disease progression in OA. Among changes to this element, subchondral BMLs have been linked to pain and cartilaginous loss in several human studies[4,8,19,27,28], justifying their assessment in disease-modifying OA drugs (DMOADs) studies. To our knowledge, this is the first report on the temporal behavior of BMLs graded on different MRI sequences.

It was found that BMLs in human OA are comprised of different zones that manifest different signal changes depending on the T-weighting and use of fat saturation techniques[12]. Our results also strongly suggest that different MRI sequences may variably enhance the visibility of different subchondral fluid or tissue infiltrates. While BMLs are now routinely included in the MRI evaluation of OA progression in human and animal studies, this new information appears crucial in regard to their assessment.

In the present study using the ACL canine OA model, BMLs were identified in several knee subregions, but most prevalent in the central and medial portions of the tibial plateau (Table I), correlating with previous studies using dogs[14,15]. Interestingly, the medial plateau shows a predilection for cartilage degeneration in this animal model[15,22,25,29,30].

There is growing evidence that BMLs are associated with limb misalignment, and that progressive BMLs occur mostly in regions of increased biomechanical loading[8,10,31]. Recently, a strong link was found between BMLs in the central tibial plateau and human ACL pathology[19]. Such link was also proposed in dogs with naturally-occurring ACL disease[32]. In dogs with transected ACL, as in our model, increased loading on the medial tibial plateau may occur as the result of joint instability, and a greater tension may arise at the insertion of intact ligaments, such as the posterior cruciate, explaining some of the BMLs observed.

The progression of BMLs over time is another source of debate. BMLs were previously found to be static or progressive in human knee OA[8]. In another study, although BML scores remained unchanged in most patients over a period of 3 months, an increase was found in 19%, while scores decreased in another 10% in this short period of time[6]. The dynamic behavior of these lesions was also found in other human studies that evaluated BMLs longitudinally[33,34]. Similarly, the present 6-month follow-up study using a canine model of OA brings evidence that BMLs are not a static phenomenon, and that the within-time BML evolution could largely vary with the MRI sequence used to quantify these lesions. Indeed, hyperintense BMLs scores were reduced on SPGR and especially on T2-FS at week 26, in comparison to week 8, following ACL transection. On the other hand, hypointense BML scores observed on T1-GRE images slightly progressed between week 8 and 26 (Fig. 3). The apparent discrepancy between these results leads to the following hypothesis: BMLs may initially correspond to an acute inflammatory response, edema, contusion or and/or necrosis, before being variably replaced by more permanent bone marrow remodeling changes such as fibrosis or myxomatous connective tissue[14] that can occur over time. Consequently, the MRI signal pattern of these initial (high-water content) and late (low-water content) phases would therefore be greatly influenced by sequence

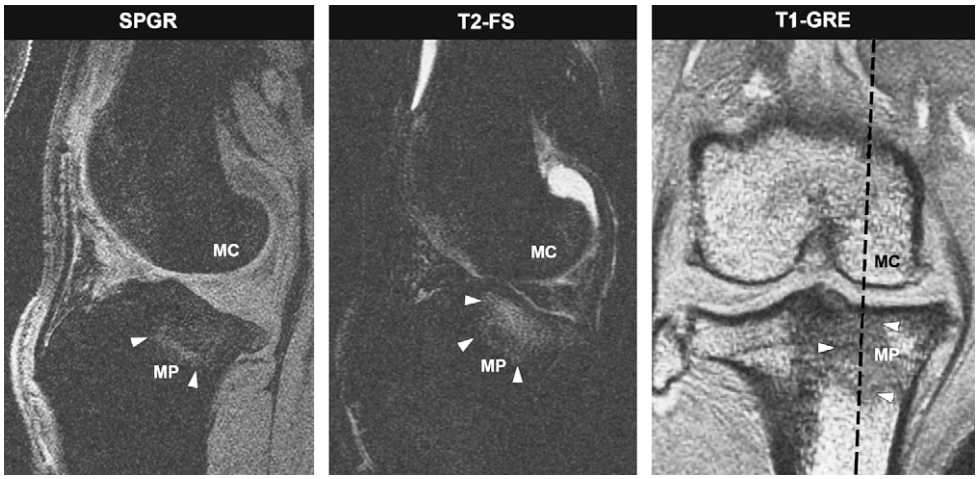

Fig. 2. Distribution of signal changes on three different MRI sequences. In this dog, the BML (arrowheads) affecting the medial portion (MP) of the tibial plateau differed between sequences. The lesion was more intense deeper in the subchondral bone marrow on SPGR vs closer to the subchondral plate on T2-FS. It was uniformly hypointense, superficially and deeply, on T1-GRE. Signal changes extended into the central portion of the tibial plateau, as seen on the dorsal T1-GRE image. The interrupted line on T1-GRE indicates slice localization for sagittal SPGR and T2-FS images.

parameters. Indeed, the heavily T2-weighted fast spin echo sequence with complete fat saturation used in our protocol probably enhanced the detectability of extra/intracellular fluids that present longer T2 relaxation times, thus resulting in hyperintense signals contrasting with the hypointense fat. This hyperintensity on T2-FS may be more closely linked to the initial and apparently transitional phase of BMLs. On fat-saturated SPGR sequence, the BML hyperintensity may also result from an increase in bone marrow fluid; however, on that sequence, tissues with magnetic resonance characteristics similar to cartilage demonstrate a higher signal. In this study, components of some BMLs were clearly more intense on SPGR when compared to T2-FS (Fig. 2), strengthening our hypothesis that these two sequences likely reflect different histological changes.

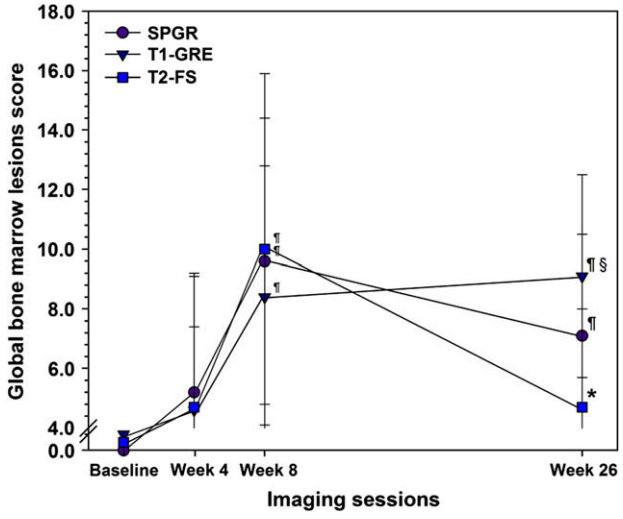

Fig. 3. Temporal evolution of global BML scores (mean ± SD) assessed on three MRI sequences in five dogs. Global BML scores where the average of both readings. Scores increased similarly on all three sequences between baseline and week 8, but progressed differently between week 8 and 26. *Global BML score significantly different compared to T1-GRE and SPGR. ¶Global BML score significantly different compared to baseline. §Global score significantly different compared to week 4.

Conversely, the BML hypointensity on T1-weighted non fat-saturated sequences, such as the T1-GRE sequence used in this study, results from a reduction in bone marrow fat content[35], regardless of the tissue taking place, as low- and high-water contents should both lead to a reduction in the signal intensity (Fig. 2). Furthermore, minimal changes in signal intensity may be more easily visible on that sequence that inherently presents a higher signal-to-noise ratio than fat-saturated sequences. It would therefore be logical to expect a progression of BML scores on that sequence in the late phase of this OA model.

The scoring system used in the present study was modified from published recommendations for human OA[2]. Although measures were repeatable, intra-reading variations were important in some instances, particularly in most severely affected joints and on T2-FS. BMLs were poorly marginated, heterogeneous and often extended into multiple subregions and even into the metaphysis, which made individual scoring challenging. Each sequence was evaluated independently and randomly in time, in order to limit reading biases. However, scoring BMLs on individual sequences but using all sequences (and different planes) would improve anatomical localization.

This study presents limitations that must be pointed out. Although we can speculate on the nature of some of the signal changes observed in our dogs, the corresponding temporal changes were not confirmed with histology. Additionally, our sample size was small. However, the MRI evaluations serially repeated over a 6-month period allowed to reach sufficient statistical power permitting data inference. Nonetheless, this study highlights the potential variable appearance and temporal behavior of BMLs on different MRI sequences. The capacity for these lesions, or at least some of their components, to rapidly change over time, in comparison to other OA features such as cartilage loss, makes BMLs interesting targets in the development and validation of DMOADs. Until the nature and

prognostic significance of these lesions are further clarified, it appears that BMLs should be evaluated independently on different MRI sequences in dogs with experimental OA. This important information could also apply to human clinical studies.

Conflict of interest

The authors have no conflict of interest.

Acknowledgments

The authors wish to thank Eric Norman Carmel, DVM, and Suzie Lachance, RT, for their technical assistance. This study was supported in part by a grant of the Centre Hospitalier de l'Université de Montréal – Research Center and the Osteoarthritis Chair of the Université de Montréal.

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Annexe IV.

Veterinary Surgery
37:166–177, 2008

Osteophytosis, Subchondral Bone Sclerosis, Joint Effusion and Soft Tissue Thickening in Canine Experimental Stifle Osteoarthritis: Comparison Between 1.5 T Magnetic Resonance Imaging and Computed Radiography

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Objective—To compare use of 1.5 T magnetic resonance imaging (MRI) and computed radiography (CR) for morphologic and temporal evaluation of osteophytosis, subchondral sclerosis, joint effusion, and synovial thickening in experimentally induced canine stifle osteoarthritis (OA).
Study Design—Prospective study.
Animals—Dogs (n = 8).
Methods—CR (mediolateral and caudocranial projections) and MRI (dorsal 3D T1-weighted gradient echo, sagittal 3D SPGR and T2-weighted fast spin echo with fat saturation) were performed at baseline (n = 8) and at week 4 (n = 5), week 8 (n = 8), and week 26 (n = 5) after cranial cruciate ligament transection. Osteophytosis, subchondral bone sclerosis, and joint effusion were scored on CR and MRI, and synovial thickening on MRI.
Results—MRI was more sensitive than CR for detection of osteophytosis and could better discriminate joint effusion from soft tissue thickening, although scores for these variables strongly correlated between modalities ($\rho = 0.94$ [osteophytosis] and 0.80 [effusion]; $P < .001$). Scores for subchondral bone sclerosis also correlated ($\rho = 0.54$, $P < .004$), although this variable may have been over interpreted on CR. Joint effusion and synovial thickening peaked at week 8, before partially regressing at week 26. Conversely, osteophytosis and sclerosis progressed semi-linearly over 26 weeks.
Conclusion—MRI is more sensitive than radiography in assessing onset and progression of osteophytosis in canine experimental stifle OA and provides enhanced discrimination between joint effusion and synovial thickening.
Clinical Relevance—MRI is as a more powerful imaging modality that should be increasingly used in animals to assess the joint related effects of disease-modifying OA drugs.

INTRODUCTION

RADIOGRAPHY IS routinely used to demonstrate or confirm the presence of osteoarthritis (OA). In dogs, stifle OA is most commonly associated with cranial cruciate ligament (CCL) damage, and results in well described radiographic signs.[1] Periarticular osteophytes and enthesiophytes, joint surface remodeling, subchondral bone sclerosis and cyst-formations, intra-articular mineralization, and joint effusion and soft tissue thickening

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Submitted June 2007; Accepted October 2007

0161-3499/08
doi:10.1111/j.1532-950X.2007.00363.x

can be recognized. Different radiographic scoring systems have been used to measure OA progression in dogs with naturally occurring CCL damage[2–4] and with experimental CCL transection.[5]

In humans, measurement of joint space narrowing on radiographs of weight-bearing hips and knees is most commonly used to assess OA progression.[6] Along with the assessment of pain and limb function, this radiographic measurement remains the reference method for evaluating efficacy of disease-modifying osteoarthritis drugs (DMOADs) in clinical trials.[6] Because of the difficulty in obtaining weight-bearing radiographs in animals, joint space width cannot be reliably measured,[2,5] unless a sophisticated system is used.[7] Consequently, osteophytosis is the most commonly used variable for assessment of OA progression in dogs.[2,8]

Magnetic resonance imaging (MRI) is increasingly used for diagnostic investigation of OA in humans, particularly for therapeutic research.[9–13] Cartilage thickness and volume measurements of the human knee have been validated,[14–16] replacing more invasive procedures like arthroscopy and cartilage biopsies. Non-cartilaginous features, such as joint effusion, synovitis, subchondral edema-like bone marrow lesions, meniscal and ligament disease, as well as osteophytes are also evaluated in human OA studies as part of semi-quantitative whole-organ MRI scoring systems.[14,17,18] Of MRI measures in humans, cartilage morphology, synovitis, and osteophytes appear more responsive to change when following patients with OA.[9]

The use of low-field (0.15–0.3 T)[19,20] and high-field (1–1.5 T)[21,22] MRI has been reported in dogs with experimentally induced stifle OA, revealing changes similar to human OA. A detailed, comparative description of joint alterations, such as osteophytosis and joint effusion, on radiographs and MRI using a canine model has not been reported. Thus, our purpose was to compare over 26 weeks, detection and evolution of osteophytes/enthesiophytes, subchondral bone sclerosis and synovitis (joint effusion and synovial thickening) with computed radiography (CR) and 1.5 T MRI, in a group of dogs with experimental stifle OA.

MATERIALS AND METHODS

This study represents part of a larger analysis of the role of high-field MRI in qualitative and quantitative assessment of experimental OA in dogs.

Dogs, Housing, and Surgical Procedure

The study protocol was approved by the Institutional Ethical and Scientific Committee in accordance with the guidelines of the Canadian Council on Animal Care. Eight skeletally mature, mongrel dogs (mean ± SD weight = 23.5 ± 2.3 kg) were selected based on normal physical examination, hematology, and serum biochemical profile results. During acclimation (7-day minimum) and follow-up, dogs were housed individually in galvanized-steel cages (1 m [width] × 1.75 m [length] × 2.4 m [height]) equipped with an automatic watering system providing ad libitum water. The animal room environment was controlled and recorded daily. Dogs were fed once daily with a standard certified commercial dog food.

All dogs were given the regular opportunity to exercise and socialize in an exterior run (1.35 m [width] × 9.15 m [length]) in accordance with the Canada Animal Welfare Act. Inherently to the development of reproducible lesions, all dogs were exercised in a run for 2 hours, 5 days a week.

Surgical transection of the right stifle CCL was performed in all dogs using the Pond-Nuki technique.[23] Antibiotics, multimodal and preemptive analgesia, volatile anesthesia, transection CCL surgery and postoperative rehabilitation followed standardized operating procedures for our facilities, as reported.[24–26]

Imaging Sequences and Scoring Variables

Mediolateral and caudocranial computed radiographic projections (Agfa CR QS system, Toronto, Canada) and 1.5 T MRI (GE EchoSpeed LX, Milwaukee, WI) series of the right stifle were obtained before (baseline, n = 8), and at week 4 (n = 5), week 8 (n = 8), and week 26 (n = 5) after surgery (day 0). For MRI, the stifle was positioned in a phased-array, small-extremity coil with customized padding. Stifle positioning within the coil was as consistent as possible to reproduce the position and orientation of voxels on all series obtained in a given dog.

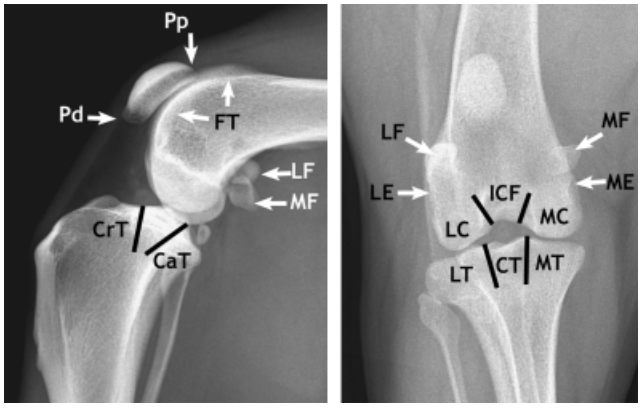

Fig 1. Anatomic compartments evaluated for osteophytosis/enthesiophytosis and subchondral sclerosis on computed radiographs. Femur: ICF, intercondylar fossa; FT, femoral trochlea; MC, medial condyle; ME, medial epicondyle; LC, lateral condyle; LE, lateral epicondyle. Fabellae: medial (MF) and lateral (LF). Tibial plateau: caudal (CaT), cranial (CrT), central (CT), medial (MT), and lateral (LT). Patella: proximal (Pp) and distal (Pd).

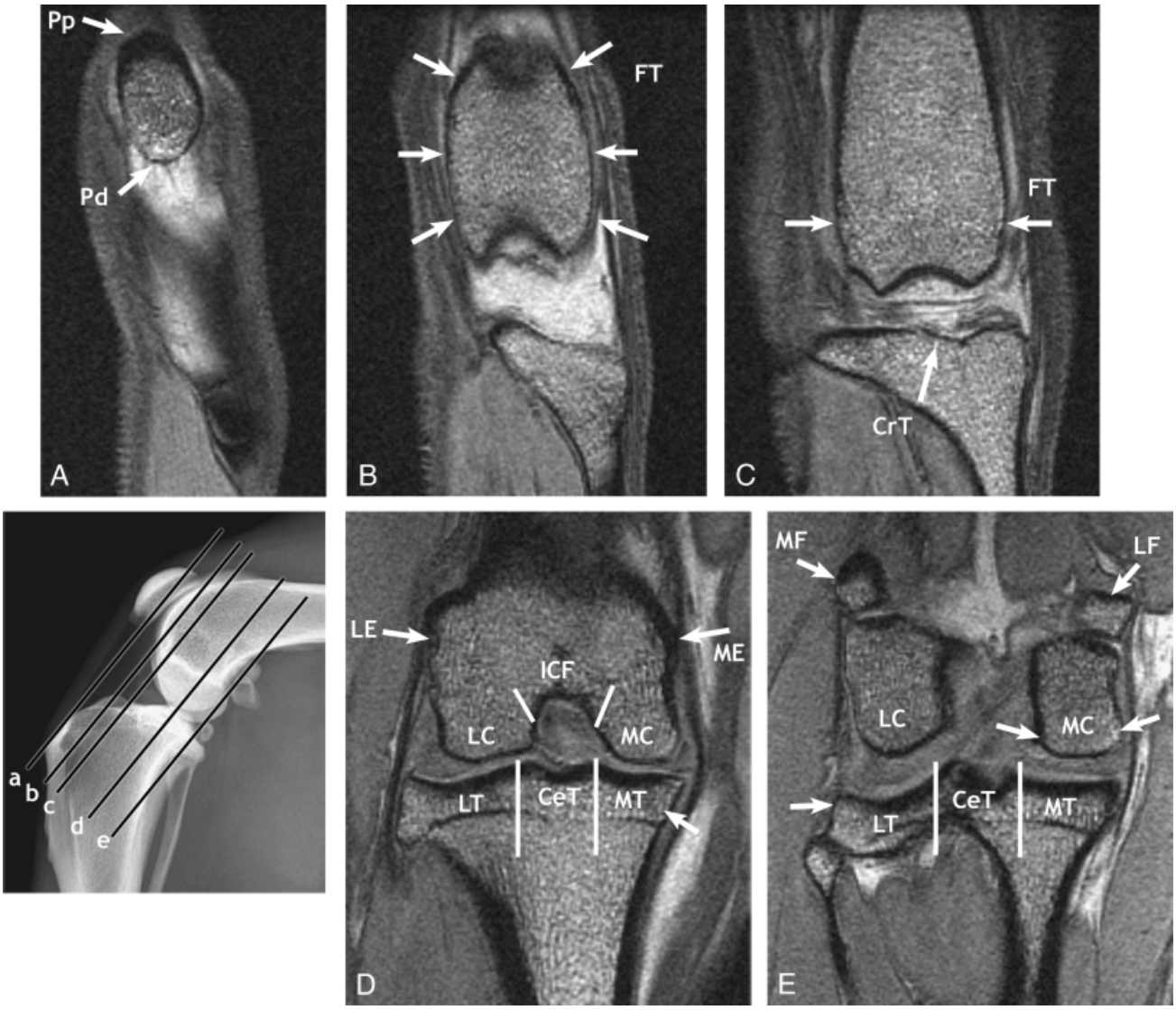

Fig 2. Anatomic compartments evaluated for osteophytosis/enthesiophytosis and subchondral sclerosis on dorsal T1-weighted gradient echo. Femur: ICF, intercondylar fossa; FT, femoral trochlea; MC, medial condyle; ME, medial epicondyle; LC, lateral condyle; LE, lateral epicondyle. Fabellae: medial (MF) and lateral (LF). Tibial plateau: caudal (CaT), cranial (CrT), central (CT), medial (MT), and lateral (LT). Patella: proximal (Pp) and distal (Pd).

Dorsal, T1-weighted three-dimensional (T1w) fast gradient recalled echo (GRE) images were obtained (slice thickness and gap [s/g] 1.5 mm, time to echo [TE] 3.15 ms, repetition time [TR] 11.4 ms, flip angle [FA] 15°, number of acquisitions [NEX] = 2, field of view [FOV] 80 mm, matrix 256 × 256, pixel size = 0.32 mm). Sagittal, fat suppressed T2*-weighted 3D spoiled gradient recalled acquisition at steady state (SPGR) images were also obtained (s/g 1.0/0 mm, TR 42 ms, TE 6,6 ms, FA 20°, NEX = 2, FOV/matrix = 100/384 × 384 or 110/416 × 416 or 120/448 × 448 mm depending on the size of the leg [pixel size = 0.26 mm], with a phase percent = 100%). Additionally, sagittal, T2-weighted fast spin echo sequences with fat saturation (T2w-FS) were acquired (s/g 2/0 mm, TR 3000 ms, TE 98 ms, FA 90°, FOV/matrix = 100/384 × 256 mm [pixel size = 0.26 × 0.39 mm]). The total time required for patient set up and MRI evaluation was ~ 60 minutes.

CR and MR images were reviewed for quality and scored independently using a diagnostic viewing station (Agfa IMPAX 6.0, Toronto, Canada). Series acquired at week 4, 8, and 26 were scored randomly under blinded conditions by a board-certified veterinary radiologist (M.A.D.), with only access to baseline series as control.

Osteophytes and enthesiophytes were similarly scored (0–3) on radiographs and MR images (T1w-GRE and SPGR sequences) according to their size at 15 specific locations: patella (proximal, distal); femoral trochlea and intercondylar fossa; femoral condyles and epicondyles (medial, lateral); tibial plateau (medial, lateral, cranial, caudal, central); fabellae

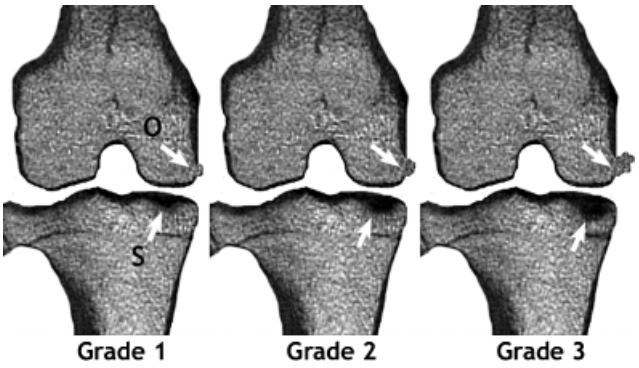

Fig 3. Schematic representation of grades 1–3 osteophytes (O) and subchondral sclerosis (S) as assessed on dorsal T1w-GRE planes.

(medial and lateral); Figs 1–3. Subchondral sclerosis was scored on radiographs and on T1w-GRE images according to its extent (0 = none, 1 = $\leq$ 25% of the area, 2 = 25–50%, 3 = > 50%) at all locations except for the patella, fabellae, and caudal medial plateau. On MR images, bone sclerosis was recognized as a signal void in the subchondral bone silhouetting with the cortex (signal void), in comparison to the hyperintense bone marrow.

Osseous structural changes (osteophytes, enthesiophytes, and sclerosis) were primarily assessed using the T1w-GRE sequence. The evaluation of osteophytes and enthesiophytes growing proximally and distally on the patella, as well as caudally on the tibial plateau, was complemented by using the sagittal SPGR sequence.

Joint effusion was scored (0–3) on lateral radiographs and sagittal T2w-FS images with fat saturation. On the same sequence, the presence of intra-articular soft tissue thickening attributed to synovial hyperplasia was graded according to its severity (0–3). This soft tissue proliferation was recognized as a tissue of moderate signal intensity protruding into the hyperintense synovial fluid.

All dogs were euthanatized at 26 weeks with an intravenous injection of pentobarbital. The proximal end of the right tibia and the distal end of the femur were removed. Dissection of the joint was followed by aspiration of synovial fluid and visualization of the different aspects of cartilage and synovial membrane. The cartilage lesions on the femoral condyles and tibial plateaus were graded separately by 2 independent observers,[24–26] confirming the presence of OA in all affected joints (data not reported here). Macroscopic or microscopic measurement of osteophytes in all joint compartments was not performed. Histologic analysis was performed on cartilage, synovial membrane, and subchondral bone specimens (data not presented here).

Statistical Analysis

For each anatomic compartment, descriptive statistics were provided. The mean was used to provide an overview of the dog's scores recorded at a given imaging session together with standard deviation to reflect inter-dog variation. The global score was the sum of all anatomic compartments scores. To compare scores between the 4 imaging sessions (baseline, weeks 4, 8, and 26), ANOVA for repeated measures were considered, using time as a within factor. When a significant difference was observed, contrasts between all pairs of imaging session's scores were highlighted using Tukey–Kramer multiple comparison tests. Only 5 dogs were considered to capture a within time effect. Comparisons between scores obtained from the imaging modalities (CR and MR images) were done using Wilcoxon's signed-rank tests. Spearman's correlation statistics were used to measure the degree of association between scores obtained with CR and MRI. As a complement to correlation testing, differences in scores (CR−MRI) were plotted against time to highlight their magnitude then tested using Wilcoxon's rank-sum test. Negative values represented lower CR scores. Scores recorded at each imaging session were considered independent. To maximize statistical power, the scores of the 8 dogs were considered at baseline and at week 8. Significance level was set at 5% using statistical software (NCSS 2001, Kaysville, UT).

RESULTS

Osteophytosis and Subchondral Sclerosis

CR and MRI scores for osteophytosis (and enthesiophytosis) and subchondral sclerosis are listed in Tables 1 and 2, respectively, for individual anatomic compartments as well as globally for each joint. In all dogs, osteophytes were detected at 4, 8, and 26 weeks after CCL transection on both CR and MRI images (Figs 4 and 5). Statistically, there was a significant difference within imaging sessions for the global osteophyte scores recorded on both imaging techniques ($P < .001$, Table 1). For each modality, scores at weeks 8 and 26 were higher than those at week 4 and at baseline. Scores at week 26 were also increased comparatively to week 8. MRI global osteophyte scores were grossly higher than CR scores at all imaging sessions, although this was statistically significant only at week 4 ($P = .042$) and at week 8 ($P = .028$). Probability value for week 26 was marginally non-significant at $P = .062$ according to a statistical power of 81%. Early osteophytes were typically hyperintense to the adjacent bone cortex that appeared as a signal void. Several grades 1 and 2 osteophytes were detected only on MRI (Fig 4). Not only osteophyte scores with MRI were consistently higher than with CR, this difference appeared to increase over time (Fig 6), reaching significant level at week 4 (-5 ± 2.3, $P < .004$), week 8 (-3.9 ± 3.6, $P < .023$), and week 26 (-8 ± 4.6, $P < .003$) when compared with baseline (-0.5 ± 1.1). Nevertheless, CR and MRI global osteophyte scores remained strongly correlated (Spearman's correlation coefficient 0.94, $P < .001$).

Table 1. Scores of Osteophytosis/Enthesiophytosis on Computed Radiography (CR) and Magnetic Resonance Imaging (MRI) in Dogs with Experimental Stifle Osteoarthritis

	Osteophytosis/Enthesiophytosis Score (Mean ± SD)							
	Baseline (8 Dogs)		Week 4 (5 Dogs)		Week 8 (8 Dogs)		Week 26 (5 Dogs)	
Anatomic compartments	CR	MRI	CR	MRI	CR	MRI	CR	MRI
Patella								
Proximal	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.4	0.0 ± 0.0	0.7 ± 0.5	0.6 ± 0.5	1.4 ± 0.5	1.0 ± 0.7
Distal	0.0 ± 0.0	0.0 ± 0.0	0.4 ± 0.5	0.6 ± 0.5	1.2 ± 0.9	1.1 ± 0.8	1.8 ± 0.8	2.2 ± 0.4
Femur								
Medial condyle	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.6 ± 0.5	0.4 ± 0.5	1.0 ± 0.5*	1.2 ± 0.8	2.4 ± 0.9
Medial epicondyle	0.1 ± 0.3	0.0 ± 0.0	0.4 ± 0.5	0.2 ± 0.4	0.5 ± 0.5	0.1 ± 0.3	0.6 ± 0.5	1.0 ± 0.7
Lateral condyle	0.1 ± 0.3	0.2 ± 0.5	0.4 ± 0.5	1.0 ± 0.7	1.0 ± 0.5	1.5 ± 0.9	2.0 ± 0.0	2.8 ± 0.4*
Lateral epicondyle	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.4	0.2 ± 0.7	0.5 ± 0.5	0.8 ± 1.1	1.2 ± 0.4
Intercondylar fossa	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.6 ± 0.5	0.1 ± 0.3	0.9 ± 0.3*	0.0 ± 0.0	1.2 ± 0.4*
Femoral trochlea	0.0 ± 0.0	0.1 ± 0.3	0.4 ± 0.5	0.6 ± 0.5	1.1 ± 0.3	1.1 ± 0.6	1.8 ± 0.4	2.6 ± 0.5*
Tibia plateau								
Medial	0.0 ± 0.0	0.1 ± 0.3	0.4 ± 0.9	0.8 ± 0.8	0.9 ± 1.0	1.4 ± 0.7*	2.0 ± 0.7	2.6 ± 0.5
Caudal	0.0 ± 0.0	0.1 ± 0.3	0.2 ± 0.4	1.4 ± 0.5*	1.4 ± 0.7	1.9 ± 0.6	2.6 ± 0.5	2.8 ± 0.4
Lateral	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.4	0.4 ± 0.5	0.6 ± 0.7	0.7 ± 0.5	1.8 ± 0.8	1.8 ± 0.4
Cranial	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.4	0.4 ± 0.5	0.4 ± 0.5	1.2 ± 0.8	1.0 ± 0.7
Central	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.4	0.0 ± 0.0	0.9 ± 0.3*	0.2 ± 0.4	1.8 ± 1.3
Fabellae								
Medial	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.4	0.4 ± 0.5	0.6 ± 0.5	0.7 ± 0.5	1.2 ± 0.4	1.6 ± 1.1
Lateral	0.0 ± 0.0	0.1 ± 0.3	0.0 ± 0.0	0.6 ± 0.5	0.2 ± 0.5	0.5 ± 0.5	1.2 ± 0.8	1.8 ± 0.8
Global score	0.2 ± 0.7	0.7 ± 1.4	2.8 ± 2.6	7.8 ± 2.7*	9.5 ± 4.2	13.4 ± 4.7*	19.8 ± 5.9	27.8 ± 7.2
Global score (5 dogs)	0.4 ± 0.9	1.2 ± 1.6	2.8 ± 2.6	7.8 ± 2.7	10.2 ± 5.2†‡	15.4 ± 3.2†‡	19.8 ± 5.9†‡§	27.8 ± 7.2†‡§

*MRI score significantly different compared with CR. To compare the global scores overtime, values from the 5 dogs that were imaged at all times were considered.

†Global score significantly different compared with baseline.

‡Global score significantly different compared with week 4.

§Global score significantly different compared with week 8. Significance was set at the 5% level.

Individually, scores for osteophytosis using MRI were significantly higher than for CR at week 4 at the level of the caudal tibial plateau ($P = .034$). Scores were also higher at week 8 at the level of the medial femoral condyle ($P = .047$), intercondylar fossa ($P = .014$) and medial ($P = .045$) and central tibial plateau ($P = .008$), and at

Table 2. Scores of Subchondral Sclerosis on Computed Radiography (CR) and Magnetic Resonance Imaging (MRI) in Dogs with Experimental Stifle Osteoarthritis

	Sclerosis Score (Mean ± SD)							
	Baseline (8 Dogs)		Week 4 (5 Dogs)		Week 8 (8 Dogs)		Week 26 (5 Dogs)	
Anatomic compartments	CR	MRI	CR	MRI	CR	MRI	CR	MRI
Femur								
Medial condyle	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.4	0.0 ± 0.0	0.4 ± 0.7	0.0 ± 0.0	0.2 ± 0.4	0.4 ± 0.5
Lateral condyle	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.5	0.0 ± 0.0	0.4 ± 0.5	0.0 ± 0.0
Intercondylar fossa	0.0 ± 0.0	0.0 ± 0.0	0.4 ± 0.9	0.0 ± 0.0	0.4 ± 0.7	0.5 ± 0.7	0.0 ± 0.0	1.0 ± 0.7
Femoral trochlea	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.6 ± 1.3	0.0 ± 0.0
Tibial plateau								
Medial	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.4	0.0 ± 0.0	0.2 ± 0.7	0.2 ± 0.7	0.2 ± 0.4	0.2 ± 0.4
Lateral	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Cranial	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.5 ± 0.7	0.1 ± 0.3	1.8 ± 0.8	0.4 ± 0.5
Central	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.4	0.0 ± 0.0	0.5 ± 0.7	0.2 ± 0.5	1.0 ± 0.0	1.2 ± 0.4
Global score	0.0 ± 0.0	0.0 ± 0.0	1.0 ± 1.7	0.0 ± 0.0	2.2 ± 2.5	1.1 ± 1.2	4.2 ± 2.2	3.2 ± 0.8
Global score (5 dogs)	0.0 ± 0.0	0.0 ± 0.0	1.0 ± 1.7	0.0 ± 0.0	2.8 ± 2.8†	1.4 ± 1.3	4.2 ± 2.2†‡	3.2 ± 0.8†‡§

*MRI score significantly different compared with CR. To compare the global scores overtime, values from the 5 dogs that were imaged at all times were considered.

†Global score significantly different compared with baseline.

‡Global score significantly different compared with week 4.

§Global score significantly different compared with week 8. Significance was set at the 5% level.

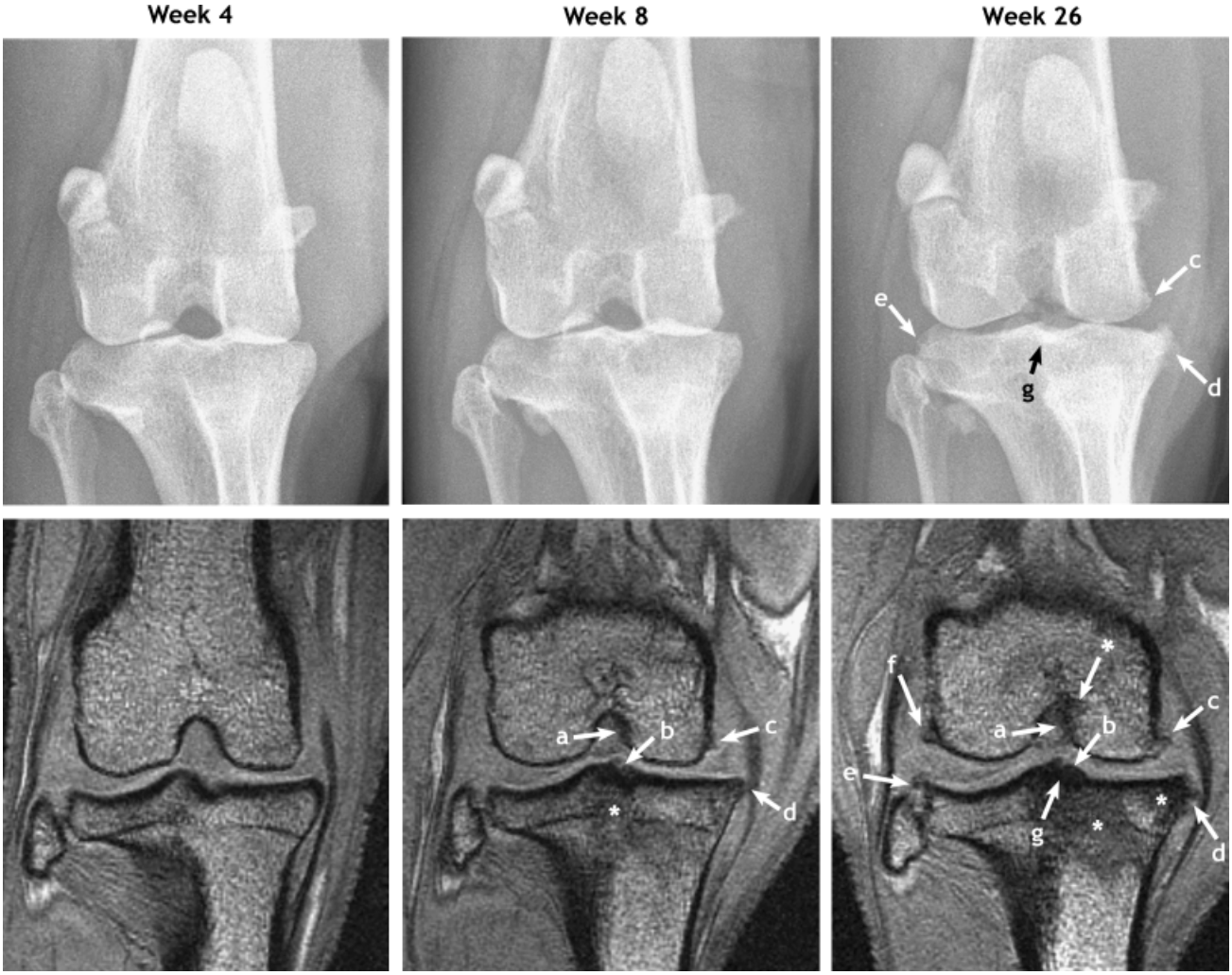

Fig 4. Corresponding computed radiographs (CR) and dorsal T1-weighted gradient-echo magnetic resonance imaging (MRI) obtained in the same dog at week 4, 8, and 26 after cranial cruciate ligament transection. Grade 1 osteophytosis is observed on MRI images at 8 weeks, involving the intercondylar fossa (a), the intercondylar eminence (b), the medial femoral condyle (c), and the medial margin of the tibial plateau (d). Some of these osteophytes (c, d) have progressed from a grade 1 to a grade 2 at 26 weeks, on CR and MRI, and a grade 1 osteophyte is now observed at the level of the lateral tibial plateau (e). Additionally, a grade 1 osteophyte is observed on MRI and not on CR at the lateral margin of the lateral femoral condyle (f). Also, note the presence of grade 1 enthesiophytes on fabellae on CR images at weeks 8 and 26. These were also observed on MR planes obtained at that level (not shown). At 8 and 26 weeks, progressive subchondral hypointensities (*) consistent with bone marrow lesions are also observed on MRI at the level of the central and medial tibial plateau. Grade 1 sclerosis (g), which appears as a signal void confluent with the subchondral plate on MRI, was identified at the level of the central tibial plateau at 26 weeks.

week 26 at the level of the lateral femoral condyle ($P = .045$), intercondylar fossa ($P = .033$) and femoral trochlea ($P = .045$). Although not statistically significant, scores for osteophytosis on MRI were also equal or higher for all other anatomic compartments at 26 weeks, except for the proximal portion of the patella and for the cranial portion of the tibial plateau.

CR and MRI global scores for subchondral sclerosis differed significantly within imaging sessions ($P < .006$, Table 2). At week 26, CR sclerosis score were higher than those recorded at baseline and at week 4, whereas the score at week 8 was also higher than baseline. For MRI, sclerosis score at week 26 were higher than those recorded at previous imaging sessions. Unlike osteophyte scores, MRI global scores for sclerosis were lower with MRI compared with CR scores on all post-transection imaging sessions, although this was not statistically significant. A correlation between global scores for sclerosis was found between CR and MRI (Spearman's correlation coefficient 0.54, $P < .004$).

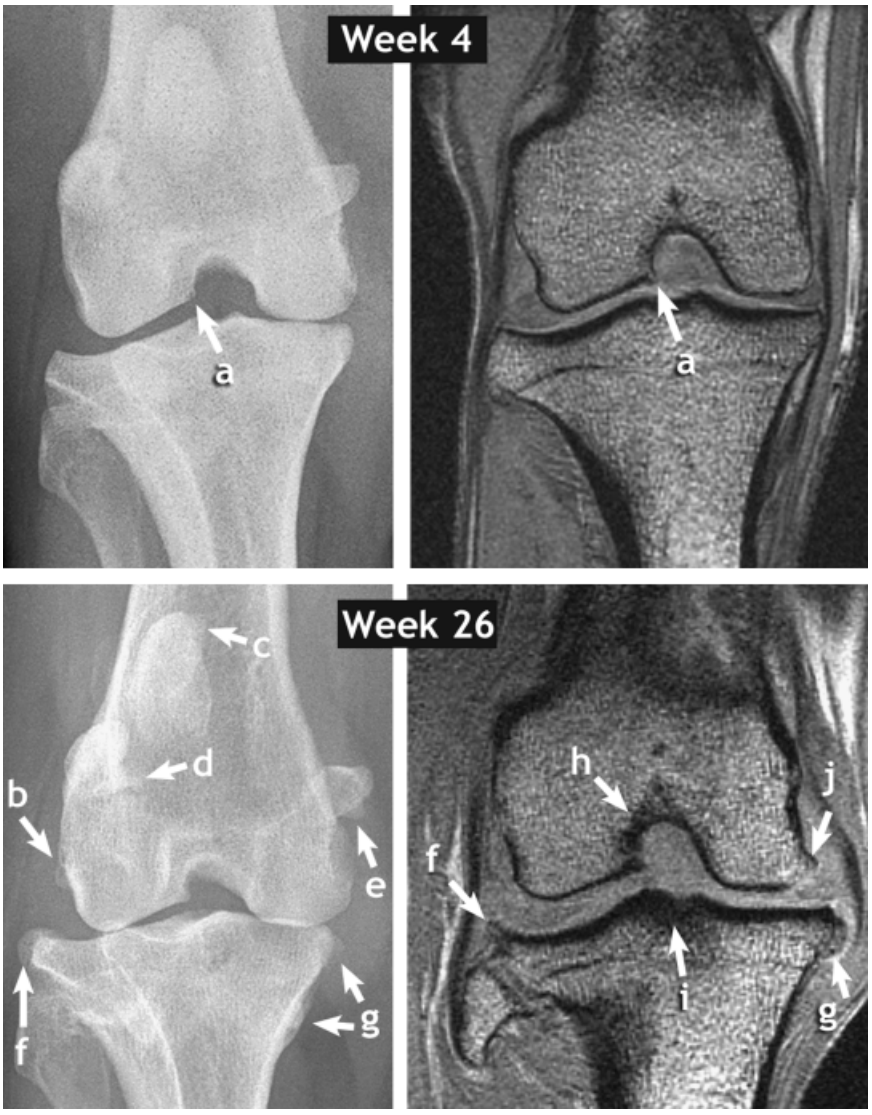

Fig 5. Corresponding craniocaudal computed radiographs (CR) and dorsal T1-weighted gradient-echo magnetic resonance imaging (MRI) obtained in the same dog at weeks 4 and 26 after cranial cruciate ligament transection. Grade 1 osteophytosis is observed at week 4 at the medial margin of the lateral femoral condyle (a). At week 26, grades 1 and 2 osteophytes (b–g) are observed on CR. These were also observed on different MRI planes. A grade 1 osteophyte is also apparent on the medial margin of the medial femoral condyle (j) on MRI. Additionally, subchondral hypointensities partially confluent to the subchondral bone plates and interpreted as bone sclerosis are noted on MRI at the level of the intercondylar fossa (h) and intercondylar eminence (i).

Synovitis and Joint Effusion

Joint effusion was detected with CR and MRI on all follow-up image acquisitions (weeks 4, 8, and 26; 18/18 occasions = 100%; Table 3). The distribution of the increased synovial fluid was more easily assessed with MRI (Fig 7) as was differentiation between effusion and soft tissue thickening (Fig 7). Extensions of the synovial fluid into the suprapatellar recess, proximally, and around the long digital extensor tendon, distally, were commonly observed (Fig 8). With severe (grade 3) effusion, the synovial fluid often extended beyond the imaged field of view, particularly proximally.

CR and MRI scores for joint effusion strongly and significantly correlated (Spearman's correlation coefficient 0.80, $P < .001$). Within imaging sessions, CR and MRI scores for joint effusion were significantly different ($P < .001$). Global effusion scores were increased at all post-surgical sessions when compared with baseline, with a small regression in score at week 26 (Table 3, Figs 7 and 9).

Synovial thickening was also observed with MRI on all follow-up image acquisitions (weeks 4, 8, and 26; 18/18

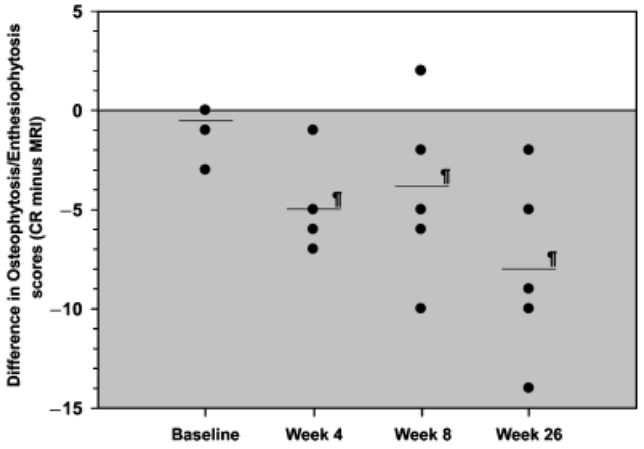

Fig 6. Graphical representation of the mean difference in osteophytosis/enthesiophytosis scores (computed radiography [CR]−magnetic resonance imaging [MRI]) against imaging sessions. Gray zone show negative values representing lower CR scores. Lines show the mean difference for a given session whereas dots indicate values for individual dogs. Dots representing similar values in different dogs are superimposed. ¶Mean values significantly different compared with baseline.

occasions = 100%; Table 3, Fig 7). Similarly to joint effusion, MRI scores for synovial thickening differed significantly within imaging sessions ($P < .001$) reaching higher values on all post-surgical sessions when compared with baseline (Fig 9). Again, slight reduction in score was observed at week 26 without reaching statistical significance.

DISCUSSION

Although radiography remains important in the diagnostic investigation of OA, technological advances now allow high-resolution MR images to be obtained, providing greater anatomic detail of cartilage and non-cartilaginous structures, including bone.[27] Hence, MRI is now recognized as the only diagnostic tool that allows evaluating the joint as a "whole organ."[28] The use of MRI has been reported in dogs with experimental OA[19–22]; however, factors such as field strength, coil configuration and sequence protocol were limited. Voxel dimensions as low as $0.26 \times 0.26 \times 1$ mm were obtained with acceptable signal-to-noise ratio and acquisition time with our system.

Osteophytes (and enthesiophytes) represent a classic feature of OA and are routinely evaluated in human clinical trials in the assessment of disease progression.[9,17] In our canine model of OA, a significant correlation (Spearman's correlation coefficient 0.94, $P < .001$) was found between global osteophytosis scores using CR and MRI modalities.

Radiographic detection of articular osteophytes and sclerosis is limited by several factors, such as morphologic distortion, geometric magnification, and superimposition of overlying structures.[18] Regions like the intercondyloid area cannot be easily evaluated in dogs because of the orientation of the radiographic beam on a caudocranial projection.[4] Use of CR with sharpening filters increases bone detail and may enhance the conspicuity of lesions like osteophytosis.[29]

The tomographic nature of MRI is an important aspect that facilitates detection of osteophytes.[11,17] In a large-scale human study, 72% of men and 67% of women with normal knee radiographs had osteophytes detected on MRI.[30] Similar to human knees, we found that that MRI is more sensitive for detection of osteophytes in canine stifles with experimental OA. In fact, osteophytes were detected at more locations on MRI at week 4, when compared with radiographs, and global scores were significantly higher on MRI at weeks 4, 8, and 26. The statistically marginally non-significant difference observed at week 26 can be explained by a statistical type II error, with regards of the differences observed at weeks 4 and 8, the P-value of .062 and the statistical power of 81%. Therefore, we conclude that a significant difference is also expected at week 26 between global osteophytes scores with CR and MRI.

A T1w-GRE sequence without fat saturation was chosen to assess all osseous changes, because it allows clear demarcation between cortical bone that normally

Table 3. Scores for Stifle Joint Effusion and Synovial Soft Tissue Thickening, and Synovial Joint Width Measurement on Computed Radiography (CR) and Magnetic Resonance Imaging (MRI)

	Joint Effusion and Soft Tissue Thickening Scores (Mean ± SD)							
	Baseline (8 Dogs)		Week 4 (5 Dogs)		Week 8 (8 Dogs)		Week 26 (5 Dogs)	
	CR	MRI	CR	MRI	CR	MRI	CR	MRI
Joint effusion	0.1 ± 0.3	0.1 ± 0.3	2.4 ± 0.5	2.4 ± 0.5	2.9 ± 0.3	2.6 ± 0.5	2.5 ± 0.5	2.4 ± 0.9
Joint effusion (5 dogs)	0.2 ± 0.4	0.2 ± 0.4	2.4 ± 0.5†	2.4 ± 0.5†	2.8 ± 0.4†	2.8 ± 0.4†	2.5 ± 0.5†	2.4 ± 0.9†
Soft tissue thickening (5 dogs)	—	0.2 ± 0.4	—	1.8 ± 0.8†	—	2.4 ± 0.5†	—	1.8 ± 0.8†

*MRI score significantly different compared with CR. To compare the global scores overtime, only values from the 5 dogs that were imaged at all times were considered.

†Global score significantly different compared with baseline. Significance was set at the 5% level.

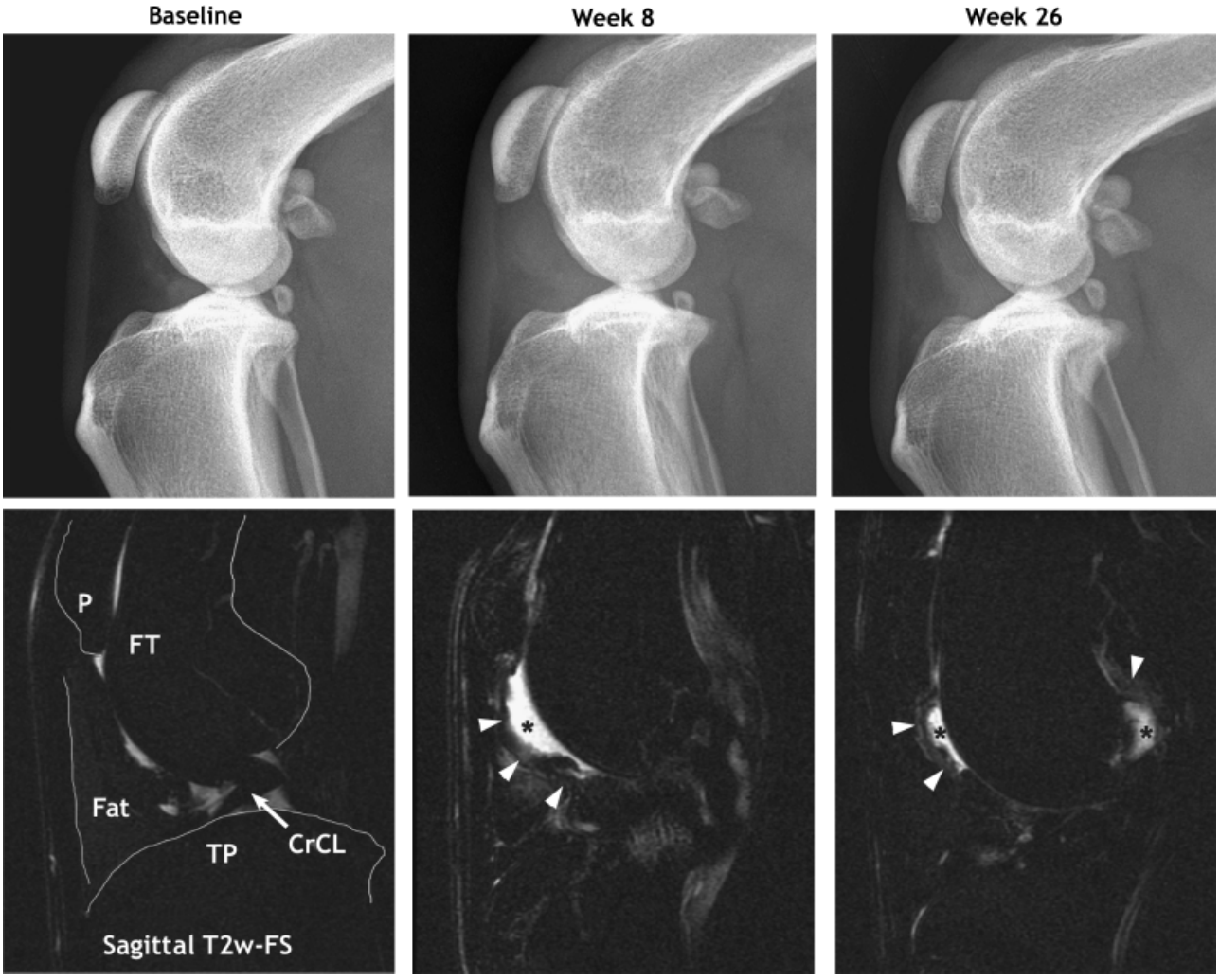

Fig 7. Stifle joint effusion and soft tissue thickening sequentially observed on lateral computed radiographs (CR) and sagittal T2-weighted fast spin echo magnetic resonance imaging (MRI). Some of the structure margins are outlined on baseline MRI. In this dog, the stifle joint swelling observed on CR increases between baseline (grade 0) and week 8 (grade 3), before regressing at week 26 (grade 2). The volume of hyperintense fluid (*) on MRI follows the same pattern. Synovial soft tissue thickening (arrowheads) appears as an irregular band of tissue of mild signal intensity at the periphery of the joint fluid. In this dog, the overall score of synovial thickening was static between weeks 8 and 26. P, patella; FT, femoral trochlea; TP, tibial plateau; CrCL, cranial cruciate ligament.

appears as a signal void, and bone marrow and articular soft tissue structures, which are both hyperintense on this sequence. A dorsal imaging plane was chosen because most osteophytes grow laterally or medially, and are therefore well outlined on this plane. However, evaluation of osteophytes growing proximally and distally on the patella, as well as caudally on the tibial plateau, was limited using this plane only, but could be adequately seen on sagittal SPGR images, which is another commonly used sequence for cartilage assessment. Although the addition of sagittal and transverse T1w-GRE sequences would have been optimal, these were not added so as to limit imaging time.

On MRI, most of these osteophytes were marginally hyperintense to cortical bone (signal void), consistent with cartilage and incomplete mineralization (Figs 4 and 5).[31] This may in part explain their lower detection on radiographs. Additionally, as expected, the detection of osteophytes involving the center of the stifle joint, i.e. femoral intercondylar fossa, central tibial plateau, and femoral condyles (intra-axial margin), was enhanced with MRI. Furthermore, not only MRI scores of osteophytosis were consistently higher than for CR, this difference even progressed temporally (Fig 6). The higher sensitivity of MRI to detect onset and progression of osteophytosis represents a significant advantage that justifies its use

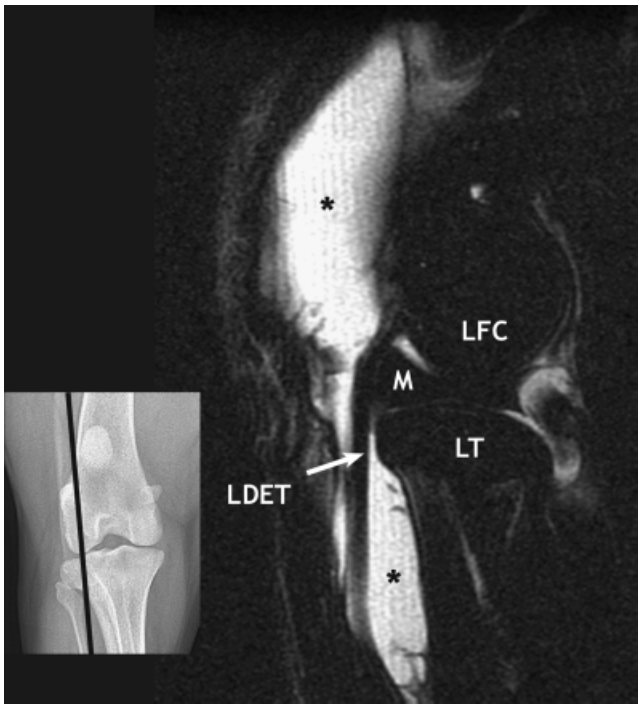

Fig 8. Severe joint effusion as seen on a sagittal magnetic resonance imaging plane (T2-weighted fast spin echo with fat saturation) obtained along the lateral portion of the stifle. The hyperintense fluid (*) extends proximally as well as distally around the tendon of the long digital extensor (LDET). LFC, lateral femoral condyle; M, lateral meniscus; LT, lateral tibial plateau. The severity and distribution of the effusion changed in time in many dogs.

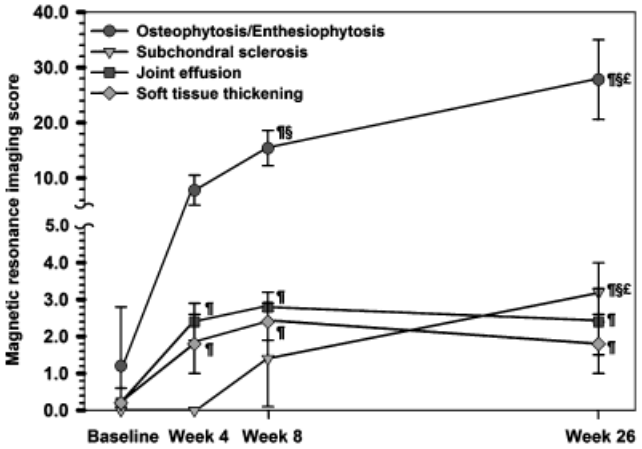

Fig 9. Temporal evolution of osteophytosis/enthesiophytosis, subchondral sclerosis, synovial effusion and soft tissue thickening scores on magnetic resonance imaging. Data is expressed as mean ± SD of global scores recorded in 5 dogs. ¶Score significantly different compared with baseline. §Score significantly different compared with week 4. £Score significantly different compared with week 8.

over radiographs for longitudinal assessment of experimental OA in dogs.

In a previous study on OA progression in dogs with CCL damage, intra- and inter-observer agreement was considered acceptable for features such as global score of OA, effusion, and osteophytosis, but not for subchondral sclerosis.[2] The lack of accuracy for the detection of subchondral sclerosis on radiographs has long been known in human medicine.[32,33] We found that correlation between scores for sclerosis using these 2 modalities was lower than for osteophytosis (Spearman's correlation coefficient 0.54, $P < .004$). A trend for higher scores of subchondral sclerosis was found with CR when compared with MRI. This may in part be explained by the difficulty in distinguishing true subchondral sclerosis from overlying periarticular osteophytosis on radiographs. Also, bone sclerosis was identified on MRI as a signal void that was perfectly confluent with the cortex, i.e. consistent with a fully calcified matrix.[11] It is possible that other subchondral bone marrow hypointensities observed on T1w sequences (Fig 4) may in part represent osteosclerosis[22] and contribute to subchondral bone radio-opacity, which would have resulted in underestimation of MRI scores for that variable.

Synovial effusion and inflammation represent an important source of pain in human OA[34]; however, this has not yet been well documented in dogs. The fluid and soft tissue components of synovitis, which silhouette as soft tissue opacity on radiographs, can be distinctly evaluated with MRI but not with CR. Joint effusion is best detected on fat-suppressed proton density or T2-weighted FSE sequences.[18,35] Semi-automatic volume analysis can also be performed, allowing a more objective assessment of joint effusion. However, large effusions usually extend proximally into the suprapatellar recess, which is beyond the proximal limit of the typical FOV used for knee imaging, imposing a significant limitation to the quantification of effusion volume.[36] In our study, large effusions were observed to extend not only proximally, but also distally around the tendon of the long digital extensor tendon (Fig 8). Gadolinium-enhanced MRI is usually recommended to assess synovitis as it allows better delineation of the synovium, although optimized, non-gadolinium sequences with fat suppression can also be used.[17] Indeed, routine non-gadolinium MRI imaging was shown to be highly accurate (95%) in detecting synovitis in human knees, when compared with arthroscopy.[35]

Joint effusion and synovial thickening were observed consistently in our dogs on all follow-up image acquisitions. Interestingly, both features progressed rapidly in the first 4 weeks, and remained stable or partially regressed between weeks 8 and 26. This regression was not significant; however, the small number of dogs in our

study may have resulted in a type II error. It is reasonable to believe that the progressive joint stabilization that occurs as a result of periarticular fibrosis may be linked to the regression of joint effusion and synovitis.

The presence of small osteophytes and excessive joint fluid in 2 separate dogs at baseline may have indicated a pre-existing subclinical pathology in these joints, or even a normal variant that may have been over interpreted. However, the magnitude of these changes was not considered significant in our study.

Although our study offers new information, there were limitations. Our sample size was small and inter- and intraobserver variability in the assessment of all joints changes was not evaluated. Although this variability was previously found to be acceptable for the radiographic assessment of OA in dogs,[2] the variability in the evaluation of all osseous and soft-tissue structural changes in this model of OA has not been investigated for MRI. In effort to increase the sensitivity of the scoring system, CR and MR images were randomly read using the baseline for comparison, as previously proposed for human clinical trials[37]; however, this may have resulted in a biased overestimation of the score of some of the variables. Finally, necropsy scoring of osteophytosis was not performed on all joint compartments, preventing a correlation with CR and MRI scores at 26 weeks. Although technically challenging, osteophyte bi- or tri-dimensional measurement would have strengthened the value of these results. On the other hand, our study was designed to assess in vivo the progression of osseous features of OA using non invasive techniques in a minimum number of dogs, which did not allow macroscopic measurements of osseous changes at all times. It would have been interesting to validate our scoring system for joint effusion and synovial thickening using arthroscopy at all times.

Radiography has been used for many decades for assessment of OA progression; however, MRI offers a greater ability to directly and non-invasively assess joint morphology and discriminate all articular tissues, including bone, synovium, and cartilage. MRI is now recognized as a more powerful technique that should likely be used increasingly in animals in the search for DMOADs.

ACKNOWLEDGMENTS

The authors wish to thank Eric Norman Carmel, DVM, Suzie Lachance, RT, Geneviève Rouleau, RT, and Anne-Marie Martel, RT, for their technical assistance.

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Annexe V.

Oral treatment with a *Brachystemma calycinum D don* plant extract reduces disease symptoms and the development of cartilage lesions in experimental dog osteoarthritis: inhibition of protease-activated receptor 2

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► Additional data are published online only. To view these files please visit the journal online (http://ard.bmj.com)

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Accepted 9 June 2009

ABSTRACT

Objective The aims of this study were to evaluate the effect of oral treatment with a whole plant extract of *Brachystemma calycinum D don* (BCD) on the development of osteoarthritic lesions and symptoms in the experimental dog anterior cruciate ligament (ACL) transection model and to document its mechanism of action.

Methods Osteoarthritis was induced by sectioning the ACL of the right knee in crossbred dogs. There were two experimental groups (n=6–7 dogs/group): placebo and BCD extract (200 mg/kg per day) given orally for 8 weeks. Macroscopic and histopathological evaluation of cartilage lesions and immunohistochemical analysis of cartilage to assess levels of inducible nitric oxide synthase (iNOS), matrix metalloprotease 13 (MMP-13) and protease activated receptor 2 (PAR-2) were done. A gait analysis of dogs was performed.

Results Treatment with BCD reduced the severity (depth) ($p=0.04$) and histopathological score ($p<0.02$) of osteoarthritis cartilage lesions. BCD treatment also significantly reduced the osteoarthritis chondrocyte level of key inflammatory and catabolic factors (iNOS, $p=0.009$ and MMP-13, $p=0.003$) as well as the level of PAR-2 ($p=0.03$). Dogs treated with BCD showed a significant improvement in peak vertical force measured at 8 weeks ($p<0.05$).

Conclusions Treatment with BCD extract exerts a positive effect on the prevention of cartilage lesions induced by joint instability, and improves joint function. This effect was associated with the inhibition of major catabolic and inflammatory mediators. This study is the first to demonstrate that a therapeutic intervention that can inhibit PAR-2 is associated with a disease-modifying osteoarthritis effect.

Osteoarthritis has been the focus of a number of comprehensive studies that have led to a better understanding of key pathophysiological pathways implicated in the disease process.[1 2] Cartilage lesions are induced by the excess synthesis of a number of proteases and catabolic factors that degrade the extracellular matrix, such as matrix metalloproteases (MMP), a disintegrin and MMP domain with thrombospondin motifs ADAMTS and other oxidative products such as nitric oxide (NO).[2]

The available treatments for osteoarthritis are mainly symptomatic in nature.[3 4] Many potential disease-modifying osteoarthritis drugs have been tested in different animal models[5 6] as well as in humans.[7–9] Several have failed because of significant systemic toxicity or a lack of efficacy.

There is an obvious need for treatment aimed at stopping the progression of the disease to be safe, because patients will need to be treated chronically for decades. This is an important reason for considering alternative treatments over conventional drugs and agents. Botanical medicinal products or nutraceuticals used for the treatment of osteoarthritis have been demonstrated in general to have better tolerability than classic drugs.[8] *Brachystemma calycinum D don* (BCD) is an indigenous plant of southwestern China (the Himalayas). It is classically used in traditional Chinese medicine to relieve rheumatic pain, numb limbs and aching.[10] Very little has been reported so far on BCD outside of the plant systematic and taxonomic literature.[11] The plant extract that has been partly characterised indicates that it contains a number of new cyclic peptides and alkyloids[12 13] Of the new alkyloids isolated, brachystemidines F and G were shown to have an inhibitory effect on lymphocyte B and T proliferation.[13]

A whole plant extract of BCD (J&L powder, Vita Green Pharmaceutical (HK) Limited, Hong Kong) has been tested to assess its effects on osteoarthritis and inflammatory processes. Preliminary clinical data indicate positive effects of BCD on knee osteoarthritis symptoms including joint swelling and pain (Vita Green internal files). Its analgesic and anti-inflammatory properties were also tested in a number of experimental models (Vita Green internal files). BCD was shown to be an effective anti-inflammatory agent by reducing in a dose-dependent manner the joint swelling observed in the carrageenan-induced arthritis rat model. At 300 mg/kg its effect was found to be comparable to ibuprofen (100 mg/kg) (supplemental file 1, available online only). In the adjuvant-induced arthritis rat model, BCD (3 g/kg) was able to reduce significantly the rear metatarsus swelling as well as serum tumour necrosis factor alpha and IL-2 levels (supplemental file 2, available online only).

The aim of the present study was to evaluate the potential of BCD to protect against the development of osteoarthritis in the dog anterior cruciate

ligament (ACL) experimental model, explore its mechanism of action, and evaluate its effect on joint function.

MATERIALS AND METHODS

BCD extract preparation

The production of BCD extract comprises the drying of the *B calycinum* raw herb, acidification by soaking in rice vinegar, heating the acid-soaked *B calycinum* to dryness and decocting it in distilled water, repeating the decoction, combining the filtrates from these two decoctions, concentrating the filtrates and freeze-drying to form a powder extract. The identity of the extract is ascertained by comparing prominent components of the herb *B calycinum* with those of the powder extract by thin-layer chromatography and high-pressure liquid chromatography analysis.

Experimental group

Thirteen adult crossbred dogs (2–3 years old), each weighing 20–25 kg, were used in this study. All aspects of animal surgery and care were as previously described.[14]

The osteoarthritis dogs were randomly divided into two experimental groups to which the animal care personnel were blinded. Group 1 (n=6) received placebo treatment (encapsulated starch) and group 2 (n=7) received BCD (J&L powder, Vita Green Pharmaceutical) orally once a day, every day including weekends, at a dosage of 200 mg/kg per day. The dose of BCD used was established based on the dose administered in humans (120 mg/kg) and according to the recent US Food and Drug Administration guidelines (guidance for industry and reviewers, http://www.fda.gov/cber/gdlns/dose.htm). Drug treatment was initiated after surgery and continued until the dogs were killed 8 weeks later. The study protocol was approved by the institutional ethics committee and conducted according to the Canadian Council for Animal Care guidelines.

Gait acquisition procedures

Gait analysis was performed as previously described in this osteoarthritis model[15] and in a dog osteoarthritis clinical trial assessing the effects of drug therapies[16 17] using a pedobarometric recording device (Matscan System, Tekscan Inc, Boston, Massachusetts, USA). The use of this kinematic and kinetic analysis has previously been validated as a useful tool in veterinary medicine, particularly in dogs.[18]

For the osteoarthritis-induced hind limb, the peak vertical force (PVF) and associated contact area were acquired at a trotting gait velocity ranging from 1.9 to 2.2 m/s. Velocity and acceleration (±0.5 m/s^2) were ensured using a set of three photoelectric cells specially designed for this pedobarometric device (LACIME, École de technologie supérieure de l'Université du Québec, Montréal, QC, Canada). The gait acquisition window was 3 s, with a sampling rate set at 44 Hz, producing a total of 132 frames. The first five valid trials were obtained for each dog and then averaged to characterise the dog profile at this time. Data are expressed for PVF in percentage of body weight (%BW) and contact area in square centimetres (cm^2).

Macroscopic grading

After killing, the right knee of each dog was dissected on ice and examined for gross morphological changes, as previously described,[19] by two independent observers (CB, FP) blinded to the treatment groups.

Each macroscopic cartilage lesion size (mm^2) was measured and graded (0–4) as previously described.[19]

Histological grading

Cartilage sections were removed from the weight-bearing lesional areas of the femoral condyles and tibial plateaus in accordance with the Osteoarthritis Research Society International (OARSI) guidelines.[20] Histological evaluation was performed on sagittal sections of cartilage removed from each femoral condyle and tibial plateau specimen as previously described.[21] Two independent observers (CB, JC) blinded to the treatment graded the severity (consensus score) of the osteoarthritis lesions on each cartilage section, which was divided into three subregions as previously described[21] and scored on a scale of 0–29 modified from Sakakibara *et al*.[22] The final score (0–87) corresponds to the sum of the scores for the three subregions.

Immunohistomorphometry

Cartilage specimens from condyles and plateaus were processed for immunohistochemical analysis as previously described.[21 23] The primary antibodies used were inducible nitric oxide synthase (iNOS; 1/200, rabbit polyclonal, ref #SC-650; Santa Cruz Biotechnology Inc, Santa Cruz, California, USA), MMP-13 (1/6, goat polyclonal antibody; R&D Systems, Minneapolis, Minnesota, USA) and proteinase activated receptor 2 (PAR-2; 1/50, mouse monoclonal antibody; Zymed Laboratories, Invitrogen Corporation, Burlington, Ontario, Canada). Appropriate controls were carried out.[21 23] Quantification of staining was performed on the superficial and deep zones of cartilage as previously described.[14 21 23]

The final score, which was a consensus between the two blinded readers (CB, FP), was expressed as the percentage of chondrocytes staining positive for the antigen (cell score) with the maximum score being 100%. For statistical analysis, the data from the condyles and plateaus were considered separately. Only the data from the superficial zones of cartilage are presented, as the chondrocyte staining was negligible in the deep zones.

Statistical analysis

To compare PVF values recorded overtime, the Friedman test and a posteriori Wilcoxon signed-rank sum test were used. The changes in PVF values were compared between groups using the Wilcoxon rank test.

Macroscopic and histological data are expressed as mean±SEM. Immunohistochemical and histomorphometric results are expressed as the median (range). Statistical analysis, was performed using the Mann–Whitney U test. p Values of 0.05 or less were considered significant.

RESULTS

Safety

All dogs completed the study and the treatment was well tolerated. There was no significant change in the weight of the dogs or evidence of any meaningful side-effects during the conduct of the study.

Gait analysis

After ACL transection, the dogs had a significant decrease in PVF values for both groups at week 4 and week 8 ($p<0.05$) compared with baseline (table 1). Both groups had statistically similar losses of PVF values at week 4, followed by different levels of remission at week 8 (table 1).

In particular, BCD-treated dogs had a significant improvement in PVF value from week 4 to week 8 ($p<0.05$). Analyses done on the contact area provided fairly similar results (data not shown).

Macroscopic evaluation of cartilage lesions

Cartilage

Lesions were assessed for size and depth in both groups. When the BCD treatment group was compared with the placebo group, there was a trend for a decrease in the size of the lesions on both femoral condyles (36.1±5.2 mm^2 vs 43.6±5.8 mm^2, BCD vs placebo) and tibial plateaus (64.7±4.4 mm^2 vs 74.5±6.2 mm^2). Moreover, BCD treatment significantly reduced the depth of the lesions on the femoral condyles (1.6±0.2 vs 2.3±0.2, p=0.04) and, to a lesser extent, on the tibial plateaus (2.0±0.1 vs 2.3±0.2).

Histological evaluation of cartilage lesions

The severity of osteoarthritis lesions (table 2, figure 1) was found to be significantly reduced in the BCD group compared with placebo on both femoral condyles and tibial plateaus (p=0.02) for the total histological score, as well as for the structural and cellularity changes.

Immunohistochemistry of cartilage

The chondrocytes staining positive for iNOS expression were found predominantly in the superficial zones of osteoarthritis articular cartilage in both groups (figure 2). The level of iNOS expression in the BCD treatment group was significantly lower ($p<0.009$) compared with placebo (figure 2).

MMP-13 was also detected preferentially in chondrocytes of the superficial zones of cartilage (figure 3). BCD treatment significantly reduced the MMP-13 levels ($p<0.003$) in these zones. Similar findings were observed with PAR-2 (figure 4), in which treatment with BCD significantly ($p<0.03$) reduced the level of the receptor.

Table 1 Peak vertical force acquired at the trot for the osteoarthritis-induced hind limb

Group	Dogs (n)	Peak vertical force (%BW)		
		Baseline	Week 4	Week 8
Placebo	6	58.2±6.3	22.4±10.7*	29.6±13.5*
BCD	7	62.3±6.3	19.0±13.7*	35.8±17.1*,†

Values are expressed in percentage of body weight (%BW) and presented as mean±SD.
*Compared with baseline ($p<0.05$) or †with week 4 ($p<0.05$).
BCD, *Brachystemma calycinum D don*.

DISCUSSION

This study demonstrated that BCD treatment can prevent the development of osteoarthritis lesions produced by instability and improve the gait of dogs in the ACL model. The effect of the BCD treatment could be related to its action on the inflammatory and catabolic pathways, as iNOS and MMP-13 are key mediators of osteoarthritis development.

Indeed, disease-modifying osteoarthritis drug effects have been demonstrated in preclinical studies with drugs that can inhibit iNOS and/or MMP-13 expression. A selective inhibitor of iNOS was shown to reduce the progression of osteoarthritis in the model used in the present study[24] by the inhibition of major catabolic pathways.[23 25] A similar effect was observed with a perixosome proliferator-activated receptor gamma agonist, pioglitazone[26] and a dual inhibitor of both cyclooxygenase and 5-lipoxegenase, licofelone[27] and a calcium antagonist was shown to reduce osteoarthritis development by inhibiting both iNOS and MMP-13.[21] These studies support the fact that the combined inhibition of iNOS and MMP-13 can be associated with the reduction in osteoarthritis development in this dog osteoarthritis model. We also explored the effect of the treatment on other key mediators of osteoarthritis, such as MMP-1, ADAMTS-4 and ADAMTS-5. No effect was found (data not shown), which again supports the hypothesis that the combined reduction in iNOS and MMP-13 would be sufficient to reduce the progression of cartilage lesions.

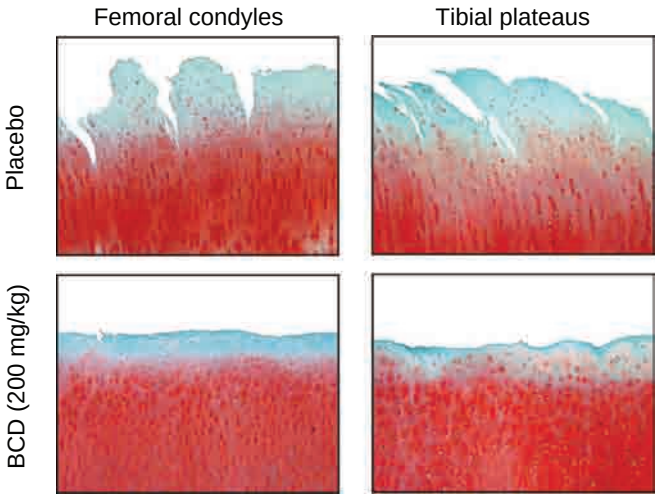

Figure 1 Representative histological sections of osteoarthritic articular cartilage from the femoral condyles and tibial plateaus of placebo-treated and *Brachystemma calycinum D don* (BCD)-treated dogs at 8 weeks after surgery. Safranin-O staining (original magnification ×100).

Table 2 Histological scores of lesions on the femoral condyles and tibial plateaus of placebo and BCD-treated dogs

	Structure (0–30)	Cellularity		Safranin-O staining (0–12)	Pannus (0–9)	Total (0–87)
		Tangential (0–6)	Transitional (0–30)			
Femoral condyles						
Placebo	20.0±1.2	5.6±0.2	15.7±0.9	4.5±0.5	0.8±0.4	46.7±2.5
BCD 200 mg/kg	17.1±1.1 (p=0.04)	5.0±0.2	12.7±0.7 (p=0.01)	3.8±0.5	0.5±0.3	39.1±2.3 (p=0.02)
Tibial plateaus						
Placebo	19.3±1.2	2.4±0.3	15.1±0.7	4.8±0.3	2.1±0.3	49.7±2.5
BCD 200 mg/kg	15.2±0.7 (p=0.01)	4.3±0.2 (p=0.01)	13.3±0.5	4.8±0.3	1.4±0.3	39.2±1.5 (p=0.02)

The scores were determined as described in the Materials and Methods section. The final score corresponds to the sum of the scores obtained for the three subregions. Data are mean±SEM and were analysed using Mann–Whitney two-tailed U test. $p<0.05$ versus placebo is considered significant.
BCD, *Brachystemma calycinum D don*.

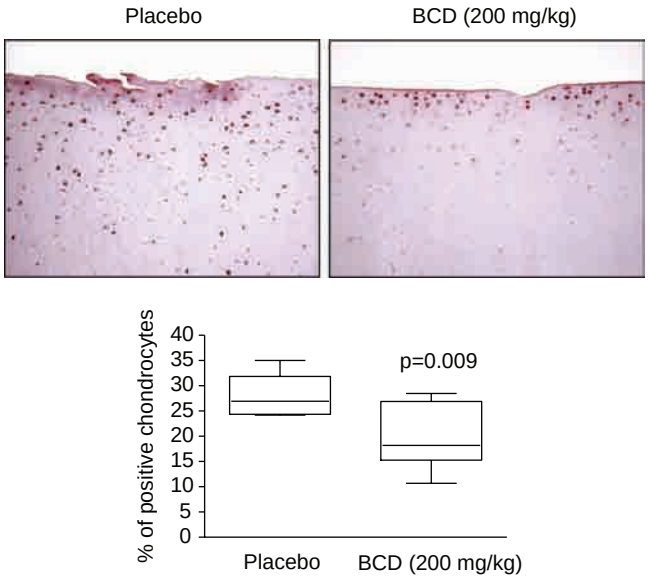

Figure 2 Representative section of the superficial zones of articular cartilage from placebo and *Brachystemma calycinum D don* (BCD)-treated dogs showing induciblep nitric oxide synthase immunostaining (original magnification ×100). Morphometric analysis data are presented as box plot and were analysed using Mann–Whitney two-tailed U test. $p<0.05$ is considered significant.

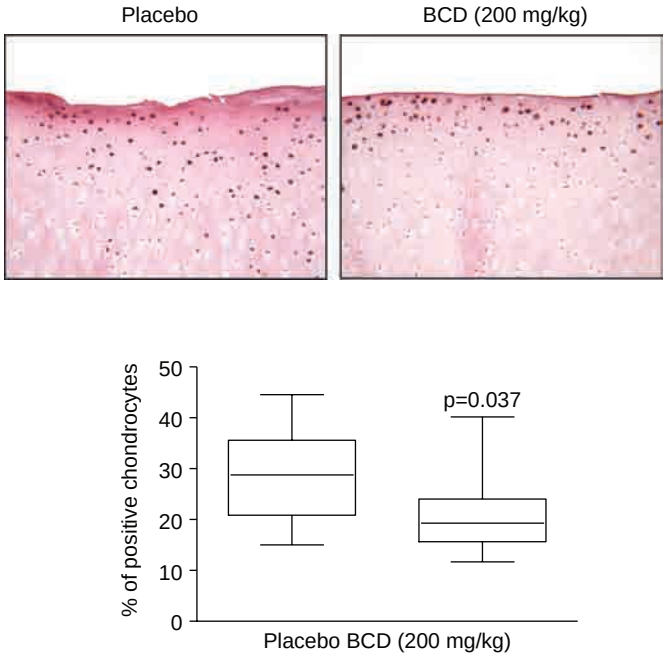

Figure 4 Representative section of the superficial zones of articular cartilage from placebo and *Brachystemma calycinum D don* (BCD)-treated dogs showing protease activated receptor 2 immunostaining (original magnification ×100). Morphometric analysis data are presented as box plot and were analysed using Mann–Whitney two-tailed U test. $p<0.05$ is considered significant.

It is of note that in this osteoarthritis model some negative results from therapeutic interventions have also previously been observed with hyaluronic acid[28] and naproxen,[29] which showed no effect on osteoarthritis lesions and aspirin, which caused an increase in cartilage degradation.[30]

PAR-2 is a member of the PAR family.[31–33] It is activated by proteolytic cleavage, mainly by serine proteases, which generates a

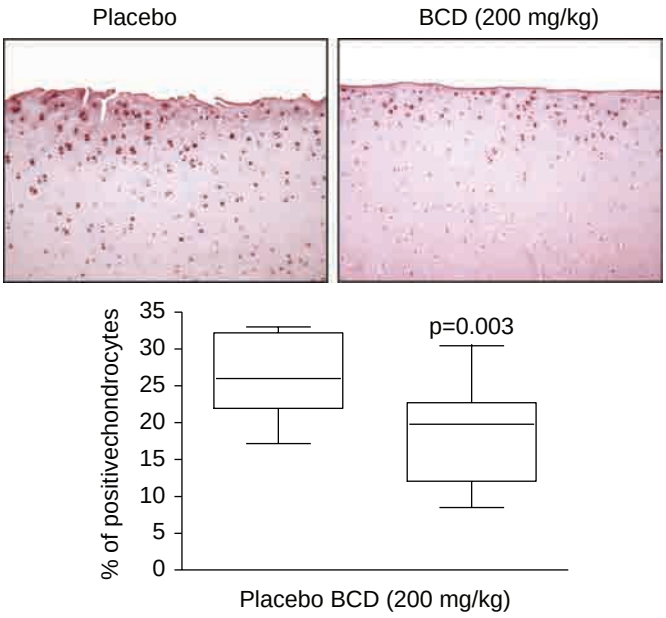

Figure 3 Representative section of the superficial zones of articular cartilage from placebo and *Brachystemma calycinum D don* (BCD)-treated dogs showing matrix metalloproteinase 13 immunostaining (original magnification ×100). Morphometric analysis data are presented as box plot and were analysed using Mann–Whitney two-tailed U test. $p<0.05$ is considered significant.

tethered ligand that autoactivates the receptor.[32 34 35] This receptor has been found to be involved in the inflammatory reaction in the rat hindpaw model[36] and in murine models of arthritis.[37] The proteases that could be involved in the activation of PAR-2 in situ in osteoarthritis cartilage are under investigation. This information is of importance as the activation of PAR-2 would probably contribute to the activation of inflammatory and catabolic pathways, such as iNOS and MMP-13. Recent studies[38 39] have demonstrated that PAR-2 is overexpressed in osteoarthritis articular chondrocytes and is involved in the signalling of a number of inflammatory and catabolic pathways involved in the disease.[39] Moreover, in these cells, PAR-2 activation by its specific agonist peptide SLIGKV-NH_2 was found to increase the level of MMP-13 expression significantly.[39] In the present study, BCD treatment was found to reduce both MMP-13 and PAR-2 levels in osteoarthritis cartilage chondrocytes. A link between these two findings is possible based on our recent findings that PAR-2 are key receptors involved in the mitogen-activated protein kinases p38 and p42/44 activation in osteoarthritis chondrocytes, two pathways known to be involved in the regulation of MMP-13 expression/synthesis.

In addition to the above findings, BCD treatment was also found to reduce the level of iNOS in the osteoarthritis chondrocytes. Several studies have demonstrated an interesting link between PAR-2 and iNOS. The activation of both PAR-1 and PAR-2 could induce the production of NO.[40] Moreover, a link between the increase in PAR-2 and iNOS messenger RNA levels was found in a rat model of esophagitis.[41] It has also been shown that NO can condition PAR-2 activation,[42] and that the inhibition of NO synthase could block the activation of PAR-2 by either trypsin (one specific activator) or by the specific agonist peptide.[43] Moreover, a NO synthase inhibitor was shown to abolish PAR-2 activation in a cutaneous model of inflammation in vivo in humans and mice.[44] It is therefore possible that an interactive link exists between iNOS and PAR-2 that can work

both ways, NO acting in a positive feedback loop in the activation of PAR-2.

The results of the present study suggest a number of hypotheses with regard to the link between PAR-2, iNOS and MMP-13. In osteoarthritis, iNOS could promote the activation and/or upregulation of PAR-2 that could increase the level of MMP-13. In turn, PAR-2 activation could further increase the iNOS level through a positive feedback loop. Therefore, the upregulation and/or activation of PAR-2 could be responsible for the increase in both iNOS and MMP-13 levels. The exact mode of action of BCD treatment on the PAR-2/iNOS/MMP-13 pathways remains unknown. However, as the treatment was effective at reducing the iNOS level in osteoarthritis chondrocytes and, therefore, likely also the production of NO, it is possible that such action was capable of secondarily inducing a decrease in the level/activation of PAR-2, which led to a reduction in the MMP-13 level. These hypotheses merit further investigation.

This study has a number of limitations. The study duration was relatively short, with only one dose of the drug having been tested. However, care was taken to ensure that the dosage selected was what is believed to be within the therapeutic range in accordance with the information obtained for clinical evaluation of BCD in osteoarthritis patients. Moreover, and as previously mentioned, an in-depth analysis of the mode of action of BCD on the osteoarthritis pathophysiological pathways needs to be performed.

CONCLUSION

This study demonstrated a promising effect of BCD on the prevention of osteoarthritis cartilage lesion development induced by joint instability. The treatment seems to impact the catabolic pathways of osteoarthritis by inhibiting PAR-2. This study is the first to demonstrate the effect of a treatment in situ on these receptors.

Acknowledgements The authors are grateful to Santa Fiori and Virginia Wallis for their assistance in the manuscript preparation.

Funding This study was funded in part by a grant from Vita Green Pharmaceutical (HK) Limited. Vita Green Pharmaceutical (HK) Limited participated in the decision to submit the manuscript for publication but was not involved in the study design, acquisition, analysis and interpretation of data, or the writing of the manuscript.

Competing interests JMP and JPP are consultants for Vita Green Pharmaceutical (HK) Limited.

Ethics approval The study protocol was approved by the institutional ethics committee and conducted according to the Canadian Council for Animal Care guidelines.

Provenance and peer review Not commissioned; externally peer reviewed.

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Annexe VI.

Effect of exercise on kinetic gait analysis of dogs afflicted by osteoarthritis

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Summary

Objective: To evaluate the effects of moderate exercise on kinetic gait analysis using a force platform in dogs with hindlimb lameness due to osteoarthritis (OA).

Methods: Ten control dogs (Control) and 10 dogs presented with chronic and stable hindlimb lameness (OA) were recruited. Dogs were subjected to force platform gait analysis to determine baseline data. They were thereafter trotted for a distance of 1.2 km on a short leash, lead by the same handler at a gait convenient for each of them (ranging from slow to fast trot), after which the gait analysis was immediately repeated to determine post-exercise values. Peak and impulse of the vertical and braking / propulsion forces were analysed using a linear model for repeated measures and Bonferroni sequential correction.

Results: In the Control group, the differences between baseline and post-exercise data were not significant. Conversely, post-exercise peak ($p = 0.020$) and impulse ($p = 0.009$) values of the vertical force, as well as the peak of the propulsion force ($p = 0.009$) values were significantly lower than baseline in the OA group.

Clinical relevance: This study demonstrates the significant effect of a moderate amount of exercise in exacerbating hindlimb lameness in dogs clinically afflicted by OA. It is suggested that: 1) exercise should be considered as a potential factor of variation in future force platform gait analyses and an effort should be made to limit bias in data recording; and 2) an exercise-based protocol could be added to the standard force platform gait analysis to potentially increase its sensitivity in the detection of lame dogs.

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Vet Comp Orthop Traumatol 2010; 23: 87–92
doi:10.3415/VCOT-09-06-0068
Received: June 25, 2009
Accepted: November 14, 2009
Pre-published online: February 11, 2010

Introduction

Force platform gait analysis is an objective, quantitative, non-invasive and reliable method of characterising ground reaction forces during locomotion, and has become an accepted technique for accurate evaluation of limb function in humans and animals (1–3). Over the last decade, the use of this kinetic tool has increased in veterinary medicine. For instance, it is now being used to identify pathological gaits and quantify the efficacy of various therapies. Force platform gait analysis is also being used to evaluate the effects of various pathologies on limb function and numerous medical and surgical treatments in orthopaedic diseases (such as hip dysplasia, cruciate ligament disease, and elbow dysplasia), and neurology (degenerative lumbosacral stenosis) (4–22). It has also been used as a 'gold standard' for validating many subjective rating systems or questionnaires (23–26).

However, the collection of kinetic data with a force platform must be carefully controlled. Indeed, several factors are known to affect ground reaction forces, such as inter-day testing, habituation of the dog to the 'runway', use of different handlers, large changes in velocity or stance time, large acceleration or deceleration changes and varying starting distances (27–33). It is the responsibility of the investigator to maintain as much consistency as possible so as to allow for an analysis of non-biased data. Therefore, factors of variation have to be 1) identified, 2) controlled within the same study and 3) standardised in multicentric studies.

To the authors' knowledge, the effect of exercise on force-platform data has never been evaluated in dogs. Furthermore, as physical activity could potentially modulate limb function, exercise could be another potential factor of variation, especially in dogs afflicted by osteoarthritis (OA) (6,34). The hypothesis of this study was that exercise would affect to a significant manner the vertical, braking, and propulsion forces recorded in dogs afflicted by OA.

Therefore, to test our hypothesis, we designed a prospective controlled study using force plate gate analysis to evaluate the effect of a moderate exercise programme in dogs with lameness secondary to OA and in a control group of normal dogs.

Materials and methods

Inclusion and exclusion criteria

Dogs of any breed that weighed more than 20 kg and that were older than 12 months

were eligible for inclusion in our study. Ten dogs with hindlimb lameness (OA group), which had been chronic and stable for a minimum of six months as reported by the owners, were recruited. The lameness had to be secondary to tarsal,stifle or coxofemoral joint(s) OA as evidenced by the orthopaedic examination and radiographic evaluation (subchondral bone sclerosis, bone remodelling or osteophytes or enthesiophytes). Dogs with cranial cruciate ligament disease were admitted if they had been diagnosed more than one year previously, were without surgical correction, and also without stifle instability (drawer sign) when examined. Dogs had to meet the following withdrawal times: four weeks for oral non-steroidal anti-inflammatory drugs, 12 weeks for oral corticosteroids, and six weeks for oral nutraceuticals. Dog that had received injectable corticosteroids or an injectable formulation of polysulphated glycosaminoglycans were excluded. Dogs with neurological, immune-mediated or other musculoskeletal pathologies (including any forelimb pathology), and those that had undergone orthopaedic surgery or arthrocentesis within one year were also excluded.

Ten clinically normal dogs (Control group), as determined by the owner and the medical history (including orthopaedic examination, neurological examination, and radiographic evaluation of both elbows, hips and stifles) were also recruited.

Study protocol

The research protocol was approved by the Institutional Animal Care Committee for the Faculty of Veterinary Medicine of the Université de Montréal. An informed consent form was completed by the owner before study initiation. The gait analysis was performed to record baseline data and to allocate dogs to their respective groups. Based on previously reported data (35), the absence (Control group) or presence (OA group) of hindlimb lameness was confirmed. All dogs were thereafter trotted for a distance of 1.2 km on a short leash, lead by the same handler (R.B.) at a gait convenient for each dog (ranging from slow to fast trot). Immediately after, another gait analysis was performed to record post-exercise data.

Table 1 Characteristics of sound (Control) and lame (OA) dogs.

Group	Breed	Sex	Age (years)	Weight (kg)	Osteoarthritis location	Side of lameness
Control	Giant Schnauzer	NM	2.5	31.8		
Control	Rottweiler	NM	6.0	49.1		
Control	Mixed breed	SF	2.0	25.2		
Control	Mixed breed	SF	3.5	30.6		
Control	Mixed breed	NM	2.0	32.7		
Control	Mixed breed	SF	3.5	36.8		
Control	Labrador Retriever	SF	2.0	26.5		
Control	Weimaraner	F	1.5	25.2		
Control	Weimaraner	NM	1.5	29.3		
Control	Bouvier des Flandres	M	2.5	43.4		
OA	Mixed breed	SF	10.0	26.9	Right tarsus	Right
OA	Mixed breed	SF	2.0	39.8	Left hip	Left
OA	Labrador Retriever	M	3.5	31.0	Both hips	Right
OA	Cane Corso	NM	1.5	66.4	Right hip	Right
OA	Golden Retriever	NM	5.5	28.1	Both hips, right stifle	Right
OA	Labrador Retriever	NM	13.0	28.9	Both hips	Left
OA	Mixed breed	SM	6.0	31.8	Left hip	Left

Key: M = entire male, NM = neutered male, F = entire female, SF = spayed female.

Force platform gait analysis

The gait analysis was performed using a single, permanently mounted, biomechanical force platform[a] levelled with the floor. The platform was interfaced with a dedicated computer using software[b] specially designed for the acquisition, numerical conversion and storage of data. All the dogs were led at a trot by the same person with a short leash without traction. A valid trial was determined by a single strike of the ipsilateral limbs on the force platform, at a velocity between 1.9 and 2.2 m/sec and an acceleration of ± 0.5 m/s^2, as measured by three photoelectric cells[c]. Gait analysis recorded the peak vertical force (PVF) and the vertical impulse (VI), as well as the braking and propulsion peaks (respectively BP and PP), and the braking and propulsion impulses (respectively BI and PI). For the studied hindlimb (right or left), data of the first five valid trials were recorded and then normalised relative to the dog's body weight (% BW) for statistical analysis. When bilateral hindlimb lameness was present, the more severely affected limb was selected for evaluation based on the PVF. In the Control group, one hindlimb was randomly selected for analysis.

Statistical analysis

Statistical analysis was performed with commercially-available software[d]. A linear model for repeated measures with group (Control versus OA) as a between-subject factor and exercise status (baseline versus post-exercise) as a within-subject factor

[a] Model OR6–6: Advanced Mechanical Technology Inc, Watertown, MA, USA
[b] Vetforce: Sharon Software, Dewitt, MI, USA
[c] MEK92-PAD: Sircon Controls, Mississauga, ON, Canada
[d] SAS® system version 9.1: SAS, Cary, N.C., USA

was used for each dependent variable. The Bonferroni sequential correction procedure (Holm's method) was applied to the contrasts used in each model. Specifically, a comparison was made between the groups at each time period, and between the two time periods in each group. For each dog, data from the five valid trials (pre- and post-exercise) were pooled for subsequent analysis. The family-wise alpha level was set at 0.05. Data are reported as mean ± standard deviation.

Results

The Control group was composed of 10 dogs of various breeds, 2.0 ± 1.5 years of age, and with a mean weight of 31.2 ± 8.0 kg. The OA group was composed of 10 dogs of various breed. Three were excluded because they were neither able to comfortably perform the exercise, nor able to perform five valid force platform trials (even with incomplete exercise). The remaining seven dogs were 5.0 ± 3.0 years of age and weighed 36.0 ± 11.0 kg, which was not significantly different from the Control group (unequal variance t-test, respectively $p = 0.09$ and $p = 0.61$). Four dogs had right hindlimb lameness and three had left hindlimb lameness secondary to the OA of the hip, stifle, or tarsus (▶Table 1) (1, 6).

At baseline, the two groups were significantly different regarding PVF ($p = 0.003$), VI ($p = 0.002$) and PP ($p = 0.010$). However, there was not any difference between braking components and PI.

In the Control group, the mean velocity of the dogs was 1.99 m/s pre-exercise and 2 m/s post-exercise; mean acceleration was 0 m/s^2 pre-exercise and 0.03 m/s^2 post-exercise. The effect of exercise on vertical ($p > 0.2$), braking ($p > 0.03$) and propulsion ($p > 0.1$) components was not significant (▶Fig. 1 and 2, Table 2). An average of 10.6 trials pre-exercise and 9.7 trials post-exercise were necessary to obtain five valid trials.

In the OA group, the mean velocity of the dogs was 1.9 m/s pre- and post-exercise; mean acceleration of dogs was –0.13 m/s^2 pre-exercise and –0.08 m/s^2 post-exercise. Within and between groups, comparison did not reveal any significant difference for velocity ($p = 0.17$) or acceleration ($p = 0.06$). Following exercise, dogs in the OA group had lower PVF (63 ± 5.3 % BW vs. 65.3 ± 4.5 % BW, $p = 0.020$), VI (8.7 ± 0.7 % BW x sec vs. 9.1 ± 0.8 % BW x sec, $p = 0.009$) and PP (3.6 ± 1.1 % BW vs. 4.3 ± 1.4 % BW, $p = 0.020$) than at baseline, while BP was higher (3.6 ± 0.9 % BW vs. 2.6 ± 1.1% BW, $p = 0.003$) (▶Fig. 1 and 2, Table 2). Braking and propulsion impulses were not affected by exercise (▶Fig. 1 and 2, Table 2). An average of 9.7 trials pre-exercise and 8.3 trials post-exercise were necessary to obtain five valid trials. Within and between groups comparison did not reveal any significant difference ($p = 0.31$).

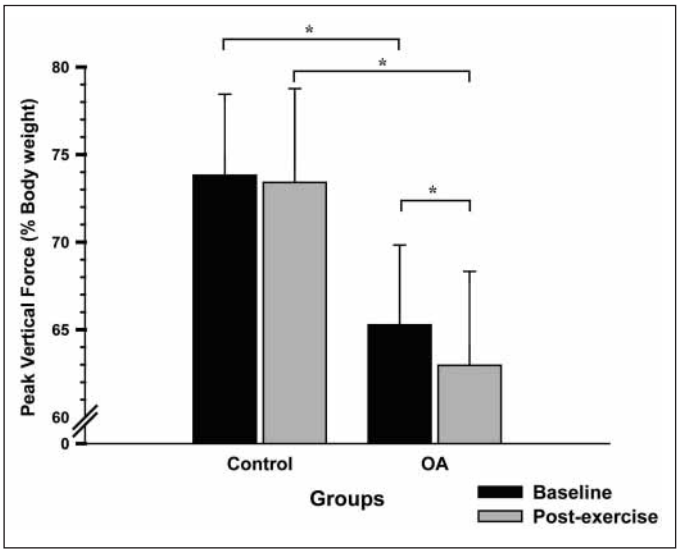

Fig. 1 Effect of exercise on peak vertical force in sound (Control) and lame (OA) dogs. (*indicates significant difference)

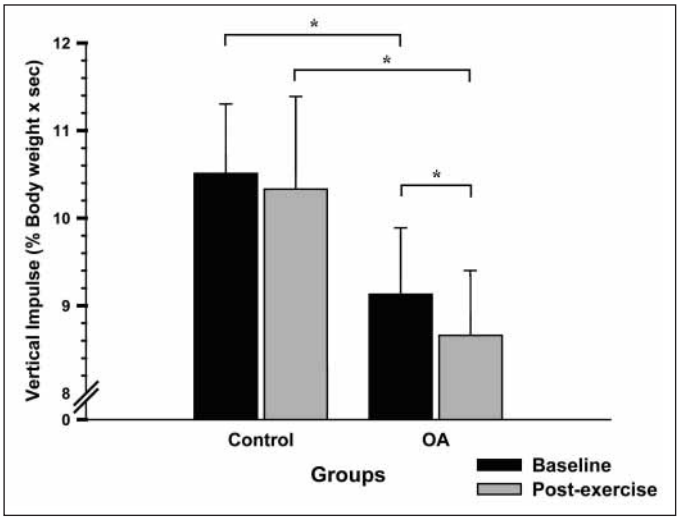

Fig. 2 Effect of exercise on Vertical Impulse in sound (Control) and lame (OA) dogs. (*indicates significant difference)

Discussion

The results demonstrate that exercise may cause a significant deterioration of limb function in dogs showing hindlimb lameness secondary to OA. In Control dogs, vertical forces recorded at baseline were comparable to those previously reported, thus validating their use in the present study (35–37). Although the baseline PVF and VI values recorded in OA dogs were slightly higher than those reported previously, they were significantly lower than the Control group, confirming a significant lameness (6, 35, 37). Baseline PP, but not PI or braking components in the OA group were sig-

Table 2 Effect of exercise on the peak and impulse of the braking and propulsion forces in sound (Control) and lame (OA) dogs.

	Control group		OA group	
	Baseline	**Post-exercise**	**Baseline**	**Post-exercise**
Braking peak (% body weight)	2.1 ± 1	2.7 ± 1.3	2.6 ± 1.1	3.6 ± 0.9*
Braking impulse (% body weight x sec)	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.2 ± 0.1
Propulsion peak (% body weight)	5.8 ± 0.8	5.5 ± 1.2	4.3 ± 1.4	3.6 ± 1.1*
Propulsion impulse (% body weight x sec)	0.5 ± 0.1	0.5 ± 0.1	0.4 ± 0.15	0.3 ± 0.11

Data are reported as mean ± SD.
* Significant difference from baseline (within group comparison)

nificantly lower than the Control group. As most dogs in this study were affected by hip OA, this corroborates with previous reports stating that propulsion forces tend to be more affected than braking forces in dogs with coxarthrosis (11, 13, 14, 35). The decrease in PP in the OA group after exercise could be used as a 'signature' of hip function worsening, as reported by Madore et al., and is probably correlated to an exacerbation of the hip kinematic alterations previously described in dysplastic dogs (e.g. greater dynamic coxofemoral joint angular acceleration in the middle through the end of the stance phase) (35, 38). The increase in braking peak after exercise could also be explained by an exacerbation of the hip kinematic alterations, e.g. greater dynamic coxofemoral joint angular deceleration from the end of the stance phase to the early swing phase, and from the middle to the end of the swing phase (38). Evans et al. previously stated that the vertical average falling slope (combined with PVF) was optimal for discriminating sound and lame Labradors (25). However, dogs were walked over the force platform rather than trotted. Although no conclusion can therefore be drawn about the value of these data in kinetic evaluation of lameness in trotting dogs, we did not observe any significant effect of exercise on vertical falling slope of dogs from either of the groups (unreported data).

We demonstrated that moderate exercise causes a significant deterioration of hindlimb function in dogs with pre-existing lameness secondary to OA. Although this effect is significant for lame dogs, it is important to ask how this observation translates to clinical practice. Comparing our results with those reported in the literature, the recorded decrease in PVF of 2.3 % BW is of similar magnitude to the improvement commonly observed in clinical studies evaluating the effect of licofelone and etodolac (6, 9).

The significant effect of exercise on the limb function of lame dogs has two implications. First, we suggest that exercise be considered a factor of bias and be standardised in future studies using force platform gait analysis. It would also be necessary to avoid a large difference in the number of trials on the force plate between studied dogs. Indeed, in order to validate five trials, some dogs will need two or three times more passages than others, and would therefore receive more exercise. Although 10 to 20 passages in the force plate corridor will hardly attain 1.2 km, it could still potentially induce a significant worsening of the lameness. However, this assumption would have to be verified. Moreover, this precaution should be emphasised with the newly described use of treadmill-mounted force plate, with which it would be easy to over-exercise dogs before obtaining valid trials (39–41). Secondly, we suggest that an exercise-based protocol be added to standard force plate analysis. The objective would be to mimic the daily exercise levels of tested dogs, and hopefully increase the sensitivity of the force platform analysis by exacerbating gait abnormalities. This protocol could therefore be integrated in the outcome measurement of different treatments of orthopaedic and neurological canine disorders. This concept concurs with the result of a study by Voss et al. who reported that force platform gait analysis at a trot was much more sensitive than at a walk for low-grade hindlimb lameness, but not for severe lameness (37, 42).

The exclusion of three dogs from our study because of an inability to complete the exercise stresses the fact that an exercise-based protocol would be too demanding for some dogs, especially those exhibiting severe lameness, as reported by Evans et al. (42). However, we think that this protocol could still be used in these dogs: evaluation of the outcome might thus be semi-quantitative by comparing the number of dogs able to complete the exercise, rather than quantitatively comparing ground reaction forces.

Our study sustained some limitations. The small number of dogs in our study decreased our statistical power and may have hindered the detection of an actual impact of exercise in normal dogs. We calculated *a posteriori* that, to show a significant difference, 80% of the time (with a family-wise p value of 0.05) between pre- and post-exercise PVF and VI respectively, 200 dogs would be needed for PVF and 35 dogs for VI. The exclusion of three dogs in the OA group might have created a bias in our analysis. As these dogs were not able to complete five valid post-exercise trials because of a significant exacerbation of their lameness, their exclusion most likely skewed our results toward a less important pre/post-exercise difference than really existed. Indeed, we performed a 'virtual' statistical analysis by replacing the missing data from these three dogs by those from the 'worst-case-scenario' OA dog. Results were similar to those mentioned although the difference between pre- and post-exercise data, and therefore significance, was increased ($p = 0.0006$ and 0.0004 for PVF and VI respectively). Therefore we felt that it was more appropriate to proceed that way instead of adding three additional dogs, which could also be seen as a potential bias. Ideally, a group of dogs with OA, but not exercised and passed twice on the force

plate would be needed to evaluate intra-day variability and allow for a stronger conclusion. However, from a practical point of view, this raises a major problem: passing these dogs for the pre-exercise trials on the force plate would exercise them and thus negate the value of the subsequent post-exercise trials and thus the value of that group as a purely negative control. The Bonferroni sequential method was used; however, it is less conservative than the original Bonferroni method, and there is an increased type I error rate. In order to standardise our population, we only included dogs with hindlimb lameness due to OA. However, OA varied in location and degree between dogs, and could have lead to different gait alterations as reported by Madore et al. in a study comparing dogs with hip or stifle OA (35). Ideally, sub-groups would have been made according to these factors; however, doing so with our low number of dogs would have dramatically lowered our statistical power. Moreover, it is well recognised that the degree of OA does not correlate with limb function, as reported by Gordon for stifle OA (34). Every dog underwent the same trotting distance, but the total effort varied as each dog determined its own gait. Ideally, dogs would have been trotted on a treadmill at one set speed for a set distance. However, we were concerned that it would possibly make some dogs uncomfortable and unable to complete the exercise. Finally, we focused on a kinetic evaluation of limb function without addressing the extent, speed, and direction of joint movements.

In this study, we have demonstrated the significant effect of moderate exercise (1.2 km trot) in exacerbating pre-existing hindlimb lameness secondary to OA in dogs. Therefore, exercise should be considered as a potential bias in study recording force platform data. An exercise-based protocol coupled to gait analysis appears interesting because it may increase sensitivity in the detection of lameness.

Acknowledgements

We would like to thank Guy Beauchamp for the statistical analysis, and Katherine Bernier and Anne Andrée Mignault for their valuable help in the kinetic analyses. Financial support was provided by the Fonds du Centenaire de la Faculté de Médecine Vétérinaire de l'Université de Montréal and by Boehringer-Ingelheim of Canada.

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Annexe VII.

Letters to the Editor

Call for antimicrobial stewardship policy

The recent publication of a study[1] that mentions the inappropriate administration of fluoroquinolones for management of otitis media in cattle highlights the importance of legal and ethical use of antimicrobials in all species. The subsequent editorial[2] calling for judicious use of these products in all species is a timely reminder in light of growing pressures on veterinary antimicrobial use as a result of concerns related to antimicrobial resistance. In 2005, the American College of Veterinary Internal Medicine published its consensus statement on the use of antimicrobials in veterinary species.[3] These recommendations remain valid today, and we believe that standardized protocols for the empirical use of antimicrobials in common case scenarios play an essential role in good antimicrobial stewardship. Such protocols are widely used in human health care,[4] but few veterinary organizations have adopted them, although they may have prevented the inappropriate use of enrofloxacin in the reported study.[1] An important feature of such protocols include the categorization of antimicrobials as primary, secondary, or tertiary, such that drugs classified as critically important for human health are used only in exceptional circumstances. The World Health Organization currently classifies third- and fourth-generation cephalosporins, fluoroquinolones, and macrolides as critically important agents for human health.[5]

In 2013, the *Equine Veterinary Journal* became, to our knowledge, the first journal worldwide to include an antimicrobial stewardship policy in its author guidelines.[6] This policy does not limit the publication of innovative research involving antimicrobials but requires any study that uses or evaluates agents classified as critically important to include a discussion of the importance of these antimicrobials to human health. The policy is similar to the policy that research be conducted in an appropriate, ethical manner and should lead to the development of research studies that consider the importance of antimicrobial resistance during the study design process. We encourage the *JAVMA* to adopt a similar policy regarding antimicrobial stewardship to ensure that published studies are not used as evidence of inappropriate use of antimicrobials by the veterinary profession.

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1. Stanton AL, Kelton DF, LeBlanc SJ, et al. Effects of tulathromycin on incidence of various diseases and growth of young heifers. *J Am Vet Med Assoc* 2013;243:267–276.
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Methods in a study of cranial cruciate ligament disease in dogs

In a recently published study[1] of outcomes 1 year after tibial plateau leveling osteotomy (TPLO) or lateral fabellar suture stabilization (LFS) in dogs with cranial cruciate ligament (CCL) disease, the authors concluded that "kinematic and owner satisfaction results indicated dogs that underwent TPLO had better outcomes than those that underwent LFS." We acknowledge the contribution this study makes, but we are concerned about some methodological issues.

First, the study did not include a control group of dogs that did not undergo surgical treatment. A previous study[2] of dogs with experimentally transected CCLs found that peak vertical force (PVF) at a trot improved over a period of 12 months without surgical stabilization of the knee joint, plateauing at approximately 60% of body weight. Thus, we believe monitoring of dogs treated without surgery is mandatory for sound interpretation of the results of surgical stabilization.

Second, we believe that statistical approaches for evaluating some

Instructions for Writing a Letter to the Editor

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Letters containing defamatory, libelous, or malicious statements will not be published, nor will letters representing attacks on or attempts to demean veterinary societies or their committees or agencies. Viewpoints expressed in published letters are those of the letter writers and do not necessarily represent the opinions or policies of the AVMA.

of the data may have been inappropriate. The PVF data were correctly identified as a repeated-measures variable, but incorrectly tested with an ANOVA method that assumes normally distributed data with no autocorrelation and requires identical group sizes and homogeneous variances among groups and across times.[3] Examination of Figures 1 and 2 in the report suggests that the groups were unbalanced and that the PVF data were not normally distributed, were autocorrelated, and were heteroscedastic. The discrete owner satisfaction scores (rated on a scale from 1 to 10) were not analyzed with a repeated-measures model, even when dichotomized (< 9 vs ≥ 9), which would have allowed the use of a generalized linear model with a logistic link function and a generalized estimating equation to account for autocorrelation. The goniometry, thigh circumference, and Canine Brief Pain Inventory data were not analyzed with repeated-measures, generalized, linear mixed models. Instead, the authors performed multiple paired *t* tests without controlling the overall type I error rate and, in the case of the Canine Brief Pain Inventory data, neglecting the requirement that the *t* test be used for continuous data. In addition, none of these tests included covariates such as body weight or velocity, and the rationale for recoding satisfaction rates and the criteria for pairing animals were not discussed by the authors.

Third, and of particular concern to us, dogs still demonstrated clear functional impairment after surgical correction, with mean PVF < 50% of body weight at a trot and < 38% of body weight at a walk for both groups 12 months after surgery. Such low PVFs are a hallmark of osteoarthritic lameness in dogs.[4,5]

With these concerns in mind, the benefits of surgical correction for dogs with CCL disease remain unclear. Given the study design, it cannot be determined what effect surgical stabilization, regardless of whether TPLO or LFS was performed, had on the outcome. The clinical importance of the claimed difference between the two methods of surgical correction is doubtful, and we cannot determine whether either had any significant beneficial effect.

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1. Gordon-Evans WJ, Griffon DJ, Bubb C, et al. Comparison of lateral fabellar suture and tibial plateau leveling osteotomy techniques for treatment of dogs with cranial cruciate ligament disease. *J Am Vet Med Assoc* 2013;243:675–680.
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The authors respond:

The authors appreciate the comments from Dr. Moreau et al. However, the specific research question addressed in our study was which surgical procedure—tibial plateau leveling osteotomy or lateral fabellar suture stabilization—would be better in dogs with cranial cruciate ligament disease, and the materials and methods were designed to answer that question. No other questions were addressed, and we did not specifically undertake to determine whether these procedures are better than nonsurgical treatment or whether either procedure would allow a return to normal function or decrease the progression of osteoarthritis.

We stand by our statistical methods, which were provided by our statistician and conformed to standard practice.

Although it would have been nice if our study solved all of the questions regarding cranial cruciate ligament disease in dogs, we only provided evidence toward one.

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AVMA governance changes

I agree with the opinion from Goldman et al[1] that the present federated structure of the AVMA House of Delegates (HOD) provides satisfactory professional representation and does not need radical overhaul. However, I do not agree with the assertion that "much more would be lost in terms of cohesiveness, the shared wisdom of our collective experience, and intraprofessional unity" than would be gained through the new governance structure. The veterinary profession urgently needs improved mutual understanding and support among its various sectors, each of which has some unique characteristics that are often poorly understood by others.

Effectively serving society in the future will depend on educational reforms that require a dynamic partnership between the academic community and the rest of the veterinary profession. We are unlikely to solve the profession's knotty problems unless we are moving together. A few years ago, Dr. Ole Nielsen, Dr. James Bellamy, and I urged the creation of a coalition[2] that would include representatives from the AVMA, the Canadian Veterinary Medical Association, state and provincial licensing authorities, and the Association of American Veterinary Medical Colleges, and I suggest that it is time to revive this idea. The number of representatives from each organization should be strictly limited so as to facilitate efficiency and control costs, and the coalition should be given the necessary freedom to use fair compromise in finding solutions to the profession's most pressing complex problems. In such a small close-knit group, members would be obliged to lower their voices and refrain from finger-pointing. I believe this idea merits consideration.

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1. Goldman AL, DeWitt W, Scheftel J, et al. AVMA governance changes (lett). *J Am Vet Med Assoc* 2013;243:1100.

2. Eyre P, Nielsen NO, Bellamy JEC. Serving society first: a time for change in veterinary medicine. *J Am Vet Med Assoc* 2004;225:40–41.

I appreciate the efforts of the Task Force on Governance and Member Participation in examining the governance structure of the AVMA but find myself in basic agreement with the delegates who recently submitted a letter[1] in opposition to the proposed changes.

I served in the AVMA House of Delegates as both an alternate and a delegate representing a state association, and I agree that there are flaws, inequities, and inconsistencies in the present AVMA system of governance. Over the course of time, however, it has served the profession well. I worry that the changes proposed in the report of the Task Force on Governance and Member Participation would essentially eliminate the important role that state and other allied organizations play in AVMA affairs and that the proposed Veterinary Resources Committee would have too much power to shape the future of our profession. In addition, considering the number of advisory committees and task forces envisioned and the complex voting procedures required under the proposed plan, it seems doubtful that substantial cost savings would result.

In past years, changes to the AVMA bylaws have been made to accommodate societal and professional changes. Perhaps, the current AVMA governance structure could be improved with relatively minor changes to the bylaws, such as granting the Executive Board explicit fiduciary power and increased management responsibility and policy authority.

As the political philosopher Russell Kirk once wrote, "Sudden departing from tradition, however abstractly rational, may sweep away much that is good together with a little that is bad."[2]

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1. Goldman AL, De Witt W, Scheftel J, et al. AVMA governance changes (lett). *J Am Vet Med Assoc* 2013;243:1100.
2. Kirk R. *Enemies of the permanent things*. New Rochelle, NY: Arlington House, 1969;181.

Urethral obstruction and age of cats

I read the recent retrospective study[1] on factors associated with recurrent obstruction in cats treated medically for urethral obstruction and wondered whether the authors knew how old the cats were when they were neutered. Others have suggested that certain medical problems may be prevented in dogs and cats by neutering them after physical maturity (ie, > 1 year of age).

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1. Eisenberg, BW, Waldrop JE, Allen SE, et al. Evaluation of risk factors associated with recurrent obstruction in cats treated medically for urethral obstruction. *J Am Vet Med Assoc* 2013;243:1140–1146.

Veterinary education and the profession's future

The recent commentary[1] concerning workforce dynamics by the Association of American Veterinary Medical Colleges (AAVMC) is timely, considering ongoing concerns about veterinarian supply versus demand. However, I ultimately found the commentary disappointing and thought that it did not contain any new ideas or recommendations for strategic changes. It has been shown that 70% of organizations fail to implement necessary changes despite overwhelming evidence of need,[2] and I worry that the veterinary education community is in that group. I believe that to enable the veterinary profession to adapt to new economic conditions, including the emphasis on one health, the colleges of veterinary medicine will have to train veterinarians with extensive socioeconomic and environmental science skills, in addition to clinical skills.

For many years, I have been urging more system-wide thinking within the profession,[3] and I believe that these discussions should be about veterinary education as a whole. The fact that the colleges of veterinary medicine are autonomous institutions makes such discussions a challenge, but I believe that the veterinary profession will improve if the colleges work closely together under AAVMC leadership.

The veterinary profession needs to change its conservative culture. If it cannot do so promptly, it will risk becoming a minor player in environmental and health sciences (one health), which would be tragic given its unique strengths in comparative medicine. Shall we, in good conscience, sidestep our societal obligations once again?

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Equality of opportunity in veterinary medicine

Dr. Andrew T. Maccabe's recent letter to the editor[1] raises some interesting questions regarding the issue of diversity in veterinary medicine. I am encouraged by efforts to present veterinary medicine as an interesting career option to high school and undergraduate college students, especially in communities where it would seem to be an infrequently chosen profession. As well, efforts to discuss diversity at the level of the profession are helpful, so long as these discussions are not limited to veterinary educators and are inclusive of practitioners.

That said, ongoing research into the effects of diversity, or the lack thereof, on the veterinary profession needs to be continued, as it seems to me that the positive and negative effects of efforts to enhance diversity are still unclear. Of course, no one with an interest in veterinary medicine should be dissuaded from pursuing such a career; quite to the contrary, such interest should be encouraged. However, we should not be pursuing diversity simply for the sake of diversity itself, and educators need to constantly guard against the temptation to use quotas as a tool to achieve diversity.

We want the brightest and the best, regardless of personal

background or interests outside of veterinary medicine, and the criteria used to determine whether a particular individual would make a good veterinary school candidate should not be adjusted to favor applicants of an underrepresented group solely on the basis of their being a part of such a group.

I applaud efforts not only to "gather data that helps us better understand where we are and where we could be,"[1] but also to better understand where we should be. Just as in practicing veterinary medicine, simply because we can do something doesn't mean we should.

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1. Maccabe AT. Diversity in veterinary medicine (lett). *J Am Vet Med Assoc* 2013;243:1241.

Broadening our veterinary clientele

The plan by the Partners for Healthy Pets, of which the AVMA is a major partner, to run advertisements promoting preventive healthcare visits for dogs and cats[1] is certainly a worthwhile one. My concern as a practice owner, however, is that the focus is too narrow. The targeted demographic (women 30 to 40 years of age with yearly household incomes exceeding $75,000 who have a veterinary relationship, yet are not seeking regular preventive visits for their pets) describes many of my clients, and I would certainly like to see them and their pets more often, at least annually. But, I'm hopeful that this campaign can be expanded to reach those socioeconomic groups that do not ever seek veterinary care. As a profession, we seek a more diverse workforce and there is no surer way to accomplish this than by broadening our veterinary clientele. For most veterinarians, being a pet owner and client was likely the most common introduction to the profession, and without this relationship, veterinary medicine would have been an unlikely career choice. Also, greater inclusiveness fosters the goals of both economic improvement for veterinarians and improved health and welfare for all pets. The long-term health of the veterinary medical community is stronger if there is a more broadly based relationship between veterinarians and clients and their pets.

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1. Ads to promote visits to veterinarians. *J Am Vet Med Assoc* 2013;243:1084.

Annexe VIII.

PAIN® 155 (2014) 2071–2079

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Association between sensitisation and pain-related behaviours in an experimental canine model of osteoarthritis

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Sponsorships or competing interests that may be relevant to content are disclosed at the end of this article.

ARTICLE INFO

Article history:
Received 20 December 2013
Received in revised form 3 June 2014
Accepted 21 July 2014

Keywords:
Dog surgical model of osteoarthritis
Spinal biomarkers
Electrical quantitative sensory testing
Peripheral and central sensitisation
Physiological and behavioural outcome measures
Bisphosphonate
Tiludronate

ABSTRACT

Evaluation of nociceptive sensitisation in canine osteoarthritis studies has been poorly reported, or even related to other clinical symptoms. In 16 dogs, peak vertical force (PVF), subjective pain assessment using 3 scales, sympathetic stress response with electrodermal activity (EDA) measurement, and behavioural changes with video analysis and telemetered motor activity were quantified at baseline (D-7), and 28 and 56 days post transection of the cranial cruciate ligament. As markers of central sensitisation, selected spinal cord biomarkers (substance P and transthyretin) were quantified at D56. Electrical withdrawal thresholds on the stifle and the tail were measured as indicative of peripheral and central quantitative sensory testing (QST) sensitisation, respectively. The effects of vehicle administration (n = 8) were compared with tiludronate (2 mg/kg subcutaneously, q2week, starting at D0) administration. Generalized estimated equations tested the association between the behavioural and physiological methods and QST sensitisation, and therefore the sensitivity of the methods for detecting treatment efficacy. Compared to tiludronate, at D56, vehicle-treated dogs had increased spinal substance P ($P = 0.01$), concomitant decreased transthyretin ($P = 0.02$), and (compared to baseline) demonstrated peripheral and central QST sensitisation, which was not present for tiludronate. Only PVF, the spontaneous behaviour "walking with full weight-bearing," and EDA were associated with occurrence of QST sensitisation and indicated significant tiludronate analgesic efficacy after inclusion of central QST sensitisation as a predictor variable in the statistical model. This study establishes the strong interest to implement QST as a predictor of canine osteoarthritis pain symptoms explained by pain sensitisation.

1. Introduction

Developmental arthropathies and joint trauma predispose dogs to the structural changes of osteoarthritis (OA), which, as in humans, leads to crippling pain and disability [29,30]. Animal experiments have produced a wealth of information on pain, but this knowledge has failed to yield new analgesics for use in humans [13,41].

Biomechanical, histological, and macroscopic structural [40], genomic, and molecular [28] similarities were reported between human posttraumatic OA [27] and the dog surgical cranial cruciate ligament transection (CCLT) model of OA, as well as a structure–function (pain) relationship translated from the dog CCLT model to the human OA pain condition: Similar to reports in human OA patients [56,60,61], pain in CCLT-dog model, indirectly assessed by kinetics, evolved with bone marrow lesions modulation, as well as progression of osteophyte, effusion/synovitis, and meniscal insults [33]. At variance with the preclinical rodent models, the dog CCLT model of OA offers an available translational predictability in therapy response with regard to the natural OA disease both in dogs [33] and in humans [31]. For example, structural and functional benefits of disease-modifying strategies have been translated with strontium ranelate from the dog CCLT model [36] to the human OA patient [44]. This was also the case for doxycycline, local hyaluronan, bisphosphonates, diacerhein, licofelone, and

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http://dx.doi.org/10.1016/j.pain.2014.07.017
0304-3959/

other nonsteroidal antiinflammatory drugs (for references, see [33]).

Evaluation of nociceptive sensitisation in canine OA has been poorly reported, and has not been related to other clinical symptoms [23]. Most information on quantitative sensory testing (QST) is based on rodent models and published data on humans. Recently, QST studies performed on naturally occurring OA in cats [19] and dogs [10,32] yielded the first evaluation of central processing changes in companion animals. Interestingly, whereas most of the OA cats responded favourably to meloxicam, a nonsteroidal antiinflammatory drug, those classified as allodynic on the basis of QST did not [19]. Moreover, in an experimental visceral pain model in cows, central sensitisation assessed with mechanical QST was associated with behavioural responses and changes in spinal pain proteomics [47].

Interestingly, tiludronate, a bisphosphonate known to inhibit bone resorption, demonstrated a decrease in structural changes [37] and antiinflammatory activity in the canine CCLT pain model [34]. Altogether, preventive administration of tiludronate might influence centralised pain concurrently to OA symptoms in the CCLT model.

The study aimed to evaluate how centralised pain occurs and is related to OA pain symptoms by comparison between tiludronate- and vehicle-treated CCLT dogs. The first objective evaluated centralised pain as assessed by the changes in spinal pain proteomics as well as in electrical QST, while comparing both groups. Our second objective investigated the relationship between the behavioural (gait analysis, visual analogue scale [VAS], the Standardized Technician Arthritis Pain Scale [STAPS], the Standardized Veterinarian Arthritis Pain Scale [SVAPS], and video analysis) and physiological (electrodermal activity [EDA] and motor activity) outcome measures related to OA pain symptoms with the centralised pain factor outcomes (spinal neuropeptides, peripheral and central QST measurements). If an association was observed, the third objective was to assess a modelisation of tiludronate treatment effect, including, or not, a centralised pain factor, for the concerned behavioural and physiological methods.

2. Methods

2.1. Dogs and experimental design

The experiments were performed according to the Canadian Council on Animal Care guidelines and were approved (RECH-1268) by the Institutional Animal Care and Use Committee of the Faculty of Veterinary Medicine, University of Montreal.

Sixteen adult crossbred dogs (aged 2 to 3.5 years), 26 (3.4) kg, originating from an external source (Laka [1994], St-Basile-le-Grand, QC, Canada) and housed in ArthroLab Inc. (St-Basile-le-Grand, QC, Canada) facilities, were used for the study. Before inclusion, health status evaluation included clinical examination, haematological and serum biochemical analyses, and radiography. The dogs were housed individually in a controlled room environment. Food was given once daily and removed overnight. Tap water (purified by filtration) was available to the animals ad libitum. The dogs were freely exercised in groups of 2 to 3 for 2 hours every weekday. During a 3-week period of acclimatization, the dogs were trained to trot over the floor mat-based plantar force measurement system.

In this randomized, double-blind, placebo-controlled study with a parallel design, the pain assessment was done at day (D)-7 (baseline), before being repeated at D28 and D56, while a standardized CCLT surgery was performed at D0 in dogs under general anaesthesia [5,34]. Observer #1 performed live assessments in the following sequential order: VAS-1, STAPS, and EDA. Observer #2 performed both live assessments (VAS-2 and SVAPS) and the video capture and analysis of the spontaneous behaviours. Both observers were unaware of the timeframe of the study and blinded to the treatment group where dogs were assigned.

Dogs were randomly divided into 2 treatment groups using a block randomization approach. Each group was equally divided with respect to sex and body weight of dogs. One group (n = 8 dogs) received 2 mg/kg disodium tiludronic acid dissolved in a mannitol solution (CEVA Santé Animale, Libourne, France). The negative control (n = 8) received only the mannitol solution (CEVA Santé Animale). Treatments (0.2 mL/kg) were administered subcutaneously at D0, D14, D28, and D42. At the end of the experiment (D56), all the dogs were euthanized with an overdose of intravenous (i.v.) sodium pentobarbital (Euthanyl, Bimeda-MTC Animal Health Inc., Cambridge, ON, Canada). The spinal cord of each dog was immediately collected, frozen (−60°C, methanol:hexane 50:50 on dry ice), and stored at −80°C pending analysis.

2.2. Anaesthesia and CCLT surgery

Preoperative analgesia was comprised of transdermal fentanyl (50 or 75 μg/h, Duragesic; Janssen Ortho, Markham, ON, Canada) applied 12 hours prior to surgery. Intramuscular meperidine (5 mg/kg Meperidine HCl 10%, Baxter Healthcare Corporation, Toronto, ON, Canada), acepromazine maleate (0.05 mg/kg, Atravet 1%, Boehringer-Ingelheim, Burlington, ON, Canada), and glycopyrrolate (0.01 mg/kg Robinul 0.2 mg/mL, Baxter Healthcare Corporation, Toronto, ON, Canada) were administered as premedication. Anaesthesia was induced with i.v. propofol (6 mg/kg, PropoFlo 1%, Abbott Animal Health, North Chicago, IL, USA) and was maintained with isoflurane (Aerrane, Baxter Healthcare Corporation, Mississauga, ON, Canada) via a coaxial circuit with oxygen flow set at 20-200 mL/kg/min and isoflurane vaporizer set at 2-3%. Surgery was performed on the right hind limb. The surgical method was previously described [5]. Briefly, a medial stifle arthrotomy was performed, distal to the patella and parallel to the patellar ligament to allow visual CCLT. Intraarticular bupivacaine (5-8 mL, Marcaïne 0.5%, Hospira, St-Laurent, QC, Canada) was injected as sutures of the capsule and the retinaculum were completed.

2.3. Centralised pain evaluation

2.3.1. Selected spinal cord neuropeptides

Each frozen spinal cord section from the lumbar area (L3 to L7 section) was weighed and homogenized using a Tissue-Tearor following the addition of phosphate-buffered saline solution 0.01 M at a ratio of 1:5 (v/v) and protease inhibitor cocktail (n° P8340, Sigma-Aldrich, Oakville, ON, Canada) at the same ratio. The samples were sonicated for 20 minutes and 150 μL of the homogenate was mixed with 150 μL of acetonitrile to remove larger proteins. The samples were then vortexed and centrifuged at 12,000 *g* for 10 minutes. The supernatant (150 μL) was transferred into an injection vial, and then spiked with 150 μL of the internal standard solution. Vials were capped and vortexed at high speed prior to analysis. Selected spinal cord peptides, substance P (SP), and transthyretin (TTR), were identified based on full-scan tandem mass spectrometry (MS/MS) spectra, and quantification was performed in selected reaction monitoring, and enzyme-linked immunosorbent assay, respectively.

Acetic anhydride 99.5% and ammonium bicarbonate were obtained from Sigma Aldrich, Inc. (St. Louis, MO, USA). Substance P was purchased from Phoenix Pharmaceutical (Belmont, CA, USA). Acetonitrile was purchased from Thermo Fisher Scientific (Bridgewater, NJ, USA) and trifluoroacetic acid, formic acid, and ammonium hydroxide 28.0-30.0% were purchased from J.T. Baker (Phillipsburg, NJ, USA). Standard solutions were prepared as previously described [35].

The high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) system included a Thermo Surveyor autosampler, a Thermo Surveyor MS pump, and a Thermo Advantage Quadrupole Ion Trap Mass Spectrometer (San Jose, CA, USA). Data were acquired and analysed with Xcalibur 1.4 (San Jose, CA, USA), and regression analyses were performed with PRISM (version 5.0d) GraphPad software (La Jolla, CA, USA) using the nonlinear curve-fitting module with an estimation of the goodness-of-fit. The calibration curves were constructed from the peak-area ratios of SP and corresponding analogue acetylated SP used as internal standard. The reaction was performed as previously described [12] and the bioanalytical selected reaction-monitoring method as previously published [35].

The TTR peptide identification was performed as previously described [47] by HPLC-MS/MS following sodium dodecyl sulfate polyacrylamide gel electrophoresis separation and tryptic digestion. Spinal cord homogenates were filtrated using Amicon Ultra 50K filters (Millipore, Bioscience, MA, USA). Quantitative analysis of canine TTR was performed in filtrate using an enzyme-linked immunosorbent assay (Cusabio Biotech Co., Ltd, Newark, DE, USA) kit.

2.3.2. Electrical QST sensitisation

As originally the therapeutic target of tiludronate was not expected to be antiinflammatory, but bone remodelling, a mechanical QST, looked less appropriate than an electrical QST to demonstrate a treatment effect on central sensitisation. Moreover, we expected the electrical stimulation to embrace all types (ie, Aβ, Aδ, and C) as well as peptidergic (or not) nerve fibres at the level of the periosteum, as they have been recognised as playing an important role in controlling bone-associated nociception [18].

Electrical QST threshold was quantified following a ramped cutaneous electrical stimulus applied to the dogs at 2 different sites to test, respectively, peripheral and central sensitisation. Bipolar stimulation electrodes (stainless steel, joined together by an isolated piece of plastic) were placed on the shaved skin at a mapped point around the scar of the stifle (QST-stifle) and on a marked point of the tail (QST-tail). Ramped stimulation currents were delivered by a continuous current stimulus isolation unit controlled by a stimulator (Grass Stimulator S8CR, Grass Instruments Company, Quincy, MA, USA). Stimuli (duration of 1 second, frequency of 1 Hz) were started from a baseline current at 8 volts, with increases by 2 volts. A cut-off value was set at 40 volts. An observer indicated painful electrical current thresholds at the time when a clear behavioural change in the dogs was observed, thus terminating the stimulation. One reading was collected for each site. Skin was checked afterwards for detection of any injury secondary to the electrical stimulus.

2.4. Behavioural data outcomes (gait analysis, VAS, STAPS, SVAPS, and video analysis)

2.4.1. Gait analysis

In dogs, the use of a floor mat-based plantar force measurement system acquires limb loading and is defined as a quantitative measurement of gait function. For the right (CCL-deficient) hind limb, peak vertical force (PVF) was acquired while the dogs applied the limb on the Walkway System (Tekscan, Inc., Boston, MA, USA) at a trotting velocity ranging from 1.9 to 2.2 meters/second. Velocity and acceleration (±0.5 meter per second^2) were ensured using a set of 3 photoelectric cells specially designed for this podobarometric device (LACIME; École de Technologie Supérieure, Montréal, QC, Canada). The gait acquisition window was 3 seconds, with a sampling rate set at 44 hertz, producing a total of 132 frames. Raw PVF (kg) data from the first 5 valid trials were obtained for each dog and later used for statistical purposes using body weight (BW) as a covariate [5]. Data were expressed as percentage of BW (%BW), as previously described [34].

2.4.2. Other behavioural data outcomes (VAS, STAPS, SVAPS, and video analysis)

The lameness and pain of treated and control OA dogs were evaluated additionally using previously developed scoring systems comprised of a continuous VAS of 100 mm range between the "no pain" and the "severest pain" boundaries [42], and 2 composite numerical rating scales (STAPS and SVAPS; Appendices 1 and 2), which were completed by, respectively, a technician and a veterinarian observer.

The video capture of behavioural changes in terms of body position and motor activities allows a noninvasive monitoring of pain-related functional disability and discomfort. Recording was performed at baseline and at week 8 only in the outdoor runs where the dogs were allowed to exercise. As previously described [45], the observer blinded to group assignment quantified the 4 following spontaneous behavioural items: "Stand full, head down" (dog was standing with full weight bearing of the operated stifle, while it had its head down), "Stand full, look around" (dog was standing with full weight bearing of the operated stifle, while it was looking around), "Walk full" (dog was walking with full weight bearing of the operated stifle), and "Trot full" (dog was trotting with full weight bearing of the operated stifle). The videos were watched in a randomized order. For each video, the items were quantified by means of a computer-compatible time coding (The Observer XT, Noldus Information Technology, Tracksys Ltd., Nottingham, UK). The occurrence rate of each item recorded during the 2-hour period was used for further analysis.

2.5. Physiological data outcomes (EDA, motor activity)

2.5.1. EDA

Changes in skin conductance response (EDA) resulting from sympathetic neuronal activity [52] has recently been validated in the canine CCLT model of OA as a measurement of stress or pain that is strongly associated with functional outcomes [34]. The Pain Gauge (PHIS, Inc., Dublin, OH, USA) device was applied to the right palmar paw (dry and nonclipped) of the dogs until the reading was recorded. Measurements ranged between 0.1 (the lowest pain and stress) and 9.9 (the highest pain and stress). Three readings were collected and averaged for thorough analysis.

2.5.2. Motor activity

Dog motor activity was recorded via an electronic chip placed on a neck collar (Actical; Bio-Lynx Scientific Equipment, Inc., Montreal, QC, Canada). Monitoring was performed during 24 hours with an epoch length of 2 minutes. The intensity of motor activity (no unit) was recorded for each count. The 24-hour period was divided into the 2 following periods: a daylight period from 6:00 am to 5:58 pm, and a night period for the remaining time. Exploratory analysis of the data showed that the intensities of motor activity during the night period were of zero value for every dog. Therefore, only motor activity recorded during the daytime period was used. We used the median value of the intensities of motor activity recorded during the daylight period. Motor activity recording was reliable and has been validated for monitoring pain-related functional disability in canine OA [9,46].

2.6. Statistical analysis

2.6.1. Treatment effect in centralised pain (spinal pain neuropeptides and electrical QST sensitisation)

2.6.1.1. Spinal cord neuropeptides. Welch's *t*-test with exact probabilities was conducted to compare (2-sided) the spinal

concentrations of SP and TTR between the 2 treatment conditions (vehicle and tiludronate) at D56.

2.6.1.2. Electrical QST sensitisation. The generalized estimation equation (GEE) for repeated measures was applied to QST-stifle and QST-tail [1]. The data distribution followed a Poisson distribution. Exchangeable correlation structure was used to account for repeated observations from data on the same dog over time. The independent variables were the treatment condition, time, and interaction between time and treatment condition. The examination of model-data agreement and quality of distributional assumptions were performed in a full residual analysis.

2.6.2. Relationship between centralised pain data outcomes and both the behavioural and physiological data outcomes

The inclusion of variables (spinal neuropeptides, QST-stifle, QST-tail) as an additional factor of dependent variable (PVF, pain scores, video analysis, EDA, and motor activity) aimed to evaluate the relationship between variables.

Regardless of distribution of the data and details on aspects surrounding the model-fitting processes, the general approach aimed at addressing each dependent variable as a function of the fixed effects of time, treatment, time-per-treatment interaction, and factor following a stepwise process, without and with inclusion of each significant factor. Thorough residual analyses were performed to check all distributional assumptions and to verify model adequacy. Models were performed using all pain outcomes (gait analysis, VAS-1, VAS-2, STAPS, SVAPS, and video analysis, EDA, and motor activity).

2.6.2.1. Pain scales. We first verified the reliability of the pain scales. Interobserver reliability was tested in calculating intraclass coefficient [ICC (A,1)] between the VAS-1 and VAS-2 by use of a 2-way fixed-effects model for the repeated-measures analysis of variance. Spearman rank-correlation tests (ρ) provided intraobserver reliability for the technician and veterinarian observers: respectively, VAS-1 score was correlated with the STAPS item #1 score, and VAS-2 score was correlated with the SVAPS item #1 score at both D28 and D56, such as the intraobserver reliability was performed only when dogs felt pain. The nonparametric correlation was chosen because it fit the best to the distribution of the data, and it was calculated using a paired data set where each "pair" was 2 different measurements made for a single unit. Cronbach α was calculated to estimate the internal consistency of both STAPS and SVAPS. In this study, data reliability was considered acceptable when ICC was higher than 0.80, ρ higher than 0.70, and Cronbach α higher than 0.70 [54].

Repeated-measures generalized linear models with GEE were used for VAS, STAPS, and SVAPS where data were assumed to distribute under the over-dispersed Poisson probability functions. The variance scale factor was estimated by Pearson's χ^2/degree of freedom. Best working matrix was determined to be first-order autoregressive following the strategy proposed by Littell et al. [26].

2.6.2.2. Gait analysis, EDA, and motor activity. A linear mixed-model approach for repeated measures [26] was applied to PVF, EDA, and motor activity intensity. We analysed the data using the restricted maximum likelihood as a method of estimation, with a compound symmetry within-subject covariance matrix for fixed and random effects, and the denominator degrees of freedom were estimated by the Kenward-Roger method. Intercept and trials (for PVF and EDA) were included as random effects. A logarithm transformation was applied to improve model fit. Baseline data were used as covariate for motor activity because groups were significantly different at D-7. We applied a compound symmetry covariance structure, based on goodness-of-fit criteria (Akaike information criterion and Bayesian information criterion).

2.6.2.3. Video-automated behavioural analysis. The occurrence of each specific event was cumulated. Frequencies were then compared between groups using a negative binomial regression model with baseline occurrence as covariate. Values were expressed as changes in the frequency of a given behaviour according to the tested group.

2.6.3. Sensitivity of the behavioural and physiological outcomes to detect a treatment effect over time including, or not including, a centralised pain evaluation as factor

Finally, if an association between the behavioural or physiological outcomes was significant with a centralised pain factor variable, we then evaluated the treatment effect while integrating (or not) the centralised pain factor variable in the statistical model. For these models, the least squares means and differences of estimated least squares means (DLSM) ± SE are presented.

Statistical analyses were carried out using SAS software (version 9.2, SAS Institute Inc, Cary, NC, USA). The α level of significance was set at 2-sided 5%. Bonferroni correction for multiple comparisons was applied when necessary. The adjusted *P*-values (P_{adj}) are multiplied by the number of multiple comparisons (7 comparisons for both QST-tail and QST-stifle alone, 2 comparisons for PFV, and EDA when factors are included).

3. Results

3.1. Spinal cord neuropeptides

At D56, the tiludronate-treated dogs had different spinal cord concentrations of SP (Fig. 1A) and TTR (Fig. 1B) than those in the

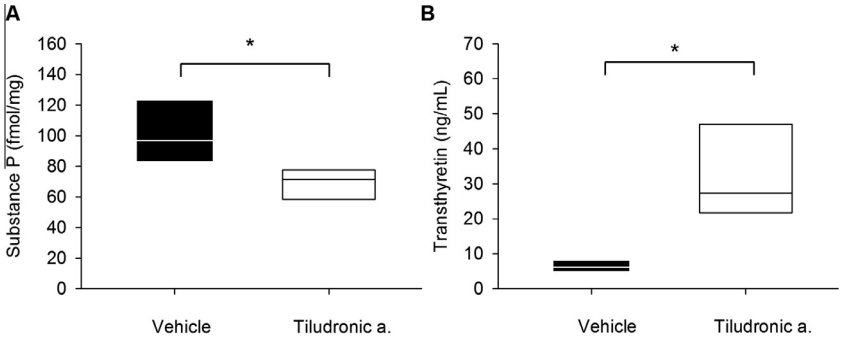

Fig. 1. Effects of vehicle and tiludronic acid (2 mg/kg subcutaneously, q2week, starting at D0) on the concentration of (A) substance P and (B) transthyretin in the spinal cord. The results are presented in box plots with medians and interquartile ranges. A star indicates significantly different values between treatment conditions with 2-sided Welch's *t*-test at D56, $P < 0.05$.

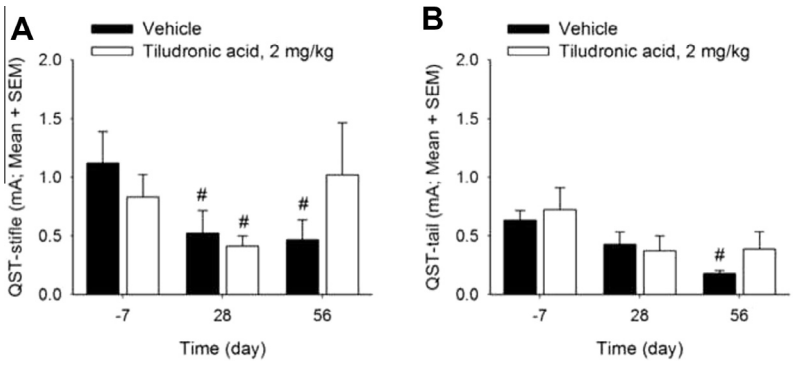

Fig. 2. Effects of vehicle and tiludronic acid (2 mg/kg subcutaneously, q2week, starting at D0) on the electrical quantitative sensory testing (QST) when stimulus was applied on (A) the stifle (QST-stifle)[a]; and (B) the tail (QST-tail)[b]. The results are expressed as the mean + SEM. # indicates significantly different QST values over time for the same treatment condition, $P_{adj} < 0.05$. [a]Wald statistics for type 3 generalized estimation equation (GEE) analysis for time ($\chi^2 = 13.1$, $P = 0.001$), time × treatment interaction ($\chi^2 = 6.4$, $P = 0.03$), and treatment ($\chi^2 = 0.1$, $P = 0.78$). [b]Wald statistics for type 3 GEE analysis for time ($\chi^2 = 1.89$, $P < 0.0001$), time × treatment interaction ($\chi^2 = 5.9$, $P = 0.05$), and treatment ($\chi^2 = 1.8$, $P = 0.19$).

vehicle-treated dogs, both differences showing statistical significance ($P = 0.01$, and $P = 0.02$, respectively).

3.2. Electrical QST sensitisation

The analysis of QST-stifle revealed that for the vehicle-treated dogs, it decreased progressively from D-7 to D56, but the tiludronate-treated dogs only had decreased values at D28 (Fig. 2A). These differences resulted in statistically significant effects for time ($P = 0.001$) and time × treatment interaction ($P = 0.03$), but not for treatment ($P = 0.78$).

The analysis of QST-tail also revealed significant effects for time ($P < 0.0001$) and time × treatment interaction ($P = 0.05$), but not for treatment ($P = 0.39$). Figure 2B reveals a shallower decrease pattern for QST-tail in the vehicle-treated dogs compared to QST-stifle, but significance was reached at D56 compared to both D-7 (DLSM = −1.37 ± 0.13, $P < 0.0001$) and D28 (DLSM = −0.87 ± 0.26, $P = 0.0008$). In contrast, the QST-tail variation recorded in the tiludronate-treated dogs did not reach statistical significance.

3.3. Relationship between factors and both the behavioural and physiological evaluations

3.3.1. Reliability of the pain scales

The VAS reached a high reliability, both between (ICC [A,1] = 0.92) and within ($\rho \geqslant 0.86$ at D28, and $\rho \geqslant 0.72$ at D56; $P < 0.001$ in all cases) observers. The internal consistency was acceptable for SVAPS (Cronbach $\alpha = 0.85$), but low for STAPS (Cronbach $\alpha = 0.55$). Therefore, only SVAPS was retained for further analyses to investigate its association with the factors.

3.3.2. Relationship between the factor QST-stifle and both the behavioural and physiological evaluations

The multivariate GEE regression analysis indicated that both PVF (Slope [95% confidence] = 0.019 [0.007-0.030], $P = 0.001$) and "Walk full" (0.15 [0.01-0.28], $P = 0.03$) increased with QST-stifle. Motor activity decreased with increasing QST-stifle (−0.62 [−1.24 to −0.01], $P = 0.05$). From these 3 outcomes, only the GEE regression analysis of "Walk full" yielded a significant time × treatment interaction ($P = 0.01$), not PVF or motor activity. There was no significant relationship between VAS-1, VAS-2, SVAPS, EDA, "Stand full, head down," "Stand full, look around," or "Trot full" and QST-stifle.

3.3.3. Relationship between the factor QST-tail and both the behavioural and physiological evaluations

The multivariate GEE regression analysis indicated that PVF increased with QST-tail (0.011 [0.001-0.022], $P = 0.03$). The following outcomes were all negatively associated with QST-tail: VAS-1 (−0.29 [−0.48 to −0.10], $P = 0.002$), VAS-2 (−0.25 [−0.43 to −0.07], $P = 0.006$), SVAPS (−0.19 [−0.27 to −0.10], $P < 0.0001$), EDA (−0.09 [−0.18 to −0.01], $P = 0.02$), "Walk full" (−0.05 [−0.09 to −0.02], $P = 0.002$), and motor activity (−1.09 [−1.85 to −0.33], $P = 0.005$). The GEE models also yielded a significant time × treatment interaction for PVF ($P = 0.05$), VAS-1 ($P = 0.03$), EDA ($P = 0.001$), and "Walk full" ($P = 0.004$). There was no relationship between QST-tail and "Stand full, head down," "Stand full, look around," or "Trot full" measurements.

3.3.4. Relationship between the spinal cord biomarkers and both the behavioural and physiological evaluations

Only SP was positively associated with EDA (0.014 [0.01-0.028], $P = 0.05$) and VAS-2 (0.01 [0.001-0.020], $P = 0.05$). No other significant relationship was observed.

3.4. Sensitivity of the behavioural and physiological evaluations to detect a treatment effect

In the statistical model that excludes the factors (Table 1), only PVF and the video-analysis behaviours demonstrated a significant treatment effect of tiludronate in comparison to the vehicle-placebo at D56. No treatment difference was observed at D28.

The results of the mixed model with QST-stifle as factor showed that only "Walk full" differed between groups. At D56, its DLSM estimates were higher in the tiludronate-treated dogs than in the vehicle-treated dogs (DLSM = 0.79 ± 0.39, $P_{adj} = 0.04$) (Fig. 3A).

The results of the mixed model with QST-tail as factor showed that PVF, EDA, and "Walk full" differed between groups. There was no significant difference between treatment conditions for VAS-1, VAS-2, SVAPS, and motor activity ($P_{adj} > 0.06$) (data not shown). However, when QST-tail was introduced in the model, the DLSM estimates of PVF were higher in tiludronate- than in vehicle-treated dogs (Fig. 3B), both at D28 (0.06 ± 0.02, $P_{adj} = 0.04$) and D56 (0.06 ± 0.02, $P_{adj} = 0.04$). For EDA at D28 (Fig. 3C), the DLSM estimates were lower for tiludronate- than for vehicle-treated dogs (−2.98 ± 0.70, $P_{adj} < 0.001$). At D56 (Fig. 3D), the DLSM estimates of "Walk full" were higher in tiludronate- than in vehicle-treated dogs (1.01 ± 0.44, $P_{adj} = 0.02$).

4. Discussion

The goal of this project was to assess centralised pain and the relationships between pain sensitisation and evaluation of OA symptoms in tiludronate- and vehicle-treated CCLT dogs. The novel findings of this study were that preventive and repeated

Table 1
Treatment effect without including the predictors.

Measures[a]		Treatment	Time	Time × treatment	Group difference Differences in time × treatment least squares means ± SEM[b]
PVF	F	3.56	686.35	4.72	Tiludronate > placebo at D56 0.59 ± 0.24
	P	0.08	**<0.0001**	**0.01**	P_{adj} = 0.03
VAS 1	F	1.30	2.85	1.77	
	P	0.26	0.09	0.18	
VAS 2	F	3.69	13.56	0.60	
	P	0.05	**0.0002**	0.44	
SVAPS	F	3.77	0.01	0.14	
	P	0.05	0.99	0.70	
Stand full, head down	χ^2	0.95	35.15	3.68	Tiludronate > placebo at D56 1.07 ± 0.49
	P	0.33	**<0.0001**	**0.05**	P_{adj} = 0.03
Stand full, look around	χ^2	0.31	18.04	0.68	
	P	0.58	**<0.0001**	0.41	
Walk full	χ^2	3.47	81.46	7.88	Tiludronate > placebo at D56 1.65 ± 0.53
	P	0.06	**<0.0001**	**0.005**	P_{adj} = 0.002
Trot full	χ^2	3.08	96.37	7.43	Tiludronate > placebo at D56 1.84 ± 0.58
	P	0.08	**<0.0001**	**0.006**	P_{adj} = 0.002
EDA	F	0.99	2.16	3.03	
	P	0.38	0.15	0.06	
Motor activity*	F	0.26	14.74	2.36	
	P	0.60	0.066	0.30	

[a] Measures were the following: peak vertical force (PVF); visual analogue scale of Observer 1 (VAS-1); visual analogue scale of Observer 2 (VAS-2); Standardized Veterinarian Arthritis Pain Scale (SVAPS); Electrodermal activity (EDA); the 4 spontaneous behaviours: "Walk full" (dog was walking with full weight bearing of the operated limb), "Stand full, head down" (dog was standing with full weight bearing of the operated limb while dog had its head down), "Stand full, look around" (dog was standing with full weight bearing of the operated limb while dog was looking around), and "Trot full" (dog was trotting with full weight bearing of the operated limb); and Motor activity. Data present the results of the tests (F or the χ^2 tests), and the *P*-value (*P*) of the type 3 fixed effects for time, treatment condition, and the interaction between time and treatment condition (time × treatment). Boldface highlights the significant type 3 fixed effects.

[b] SEM, and the adjusted *P*-value (P_{adj}) of the post hoc analysis.

* Values recorded at D-7 were used as covariates in the model. Significant group differences are presented by the differences in time × treatment least squares means.

administration of tiludronate reduced pronociceptive SP and concurrently increased TTR release at the spinal cord level when compared to placebo. There was no statistically significant difference between groups to support a treatment effect, but in contrast to vehicle-treated dogs, the tiludronate-treated dogs presented no peripheral (measured with QST-stifle) or central (measured with QST-tail) sensitisation 56 days post-CCLT when compared to D-7. First, these results support the development of peripheral and central sensitisation in the CCLT dog model at D28 and 56, after altered biomechanical stability and inflammation occurred. The pain sensitisation reported herein corroborates the reported findings in surgically induced OA in dogs [10] and rats [6], as well as in naturally occurring OA in cats [19]. Secondly, although bisphosphonates have not been reported to provide central analgesic activity, preventive tiludronate modulated onset of pain sensitisation and the release of spinal neuropeptides such as SP and TTR. The bone remodelling and bone loss are active components of the OA disease process [11], the antiresorptive activity of tiludronate might have contributed to decreasing the severity of posttraumatic OA process at an early stage in this CCLT model [34].

We demonstrated a strong relationship between behavioural and physiological data outcomes with the centralised pain factor outcomes, provided by measurement of spinal neuropeptides and peripheral and central QST sensitisation. This suggests that this research, encompassing statistical modalities, permitted the identification of validated surrogates for pain assessment in an OA dog model that are related to pain sensitisation. In addition, these statistical models will not necessarily produce different results compared with simpler methods. However, they tend to yield more accurate results, thereby increasing the likelihood that the findings might be replicable in future studies, or successfully translated to other species.

4.1. Sensitisation

As previously described in arthritic rats [16,22], the concentration of spinal SP, a pain biomarker, increased at 56 days post CCLT. Increased spinal SP partly supports more central sensitisation caused by the CCLT surgery in vehicle-treated dogs than in tiludronate-treated dogs. Higher SP was concomitant with a lower concentration of TTR at the spinal level in the vehicle-treated dogs when compared to tiludronate-treated dogs. Interestingly, concentration of TTR decreased in human patients suffering from rheumatoid arthritis [25], whereas cerebrospinal fluid (CSF) TTR downregulation (compared to baseline values) demonstrated an ability to predict the responsiveness to analgesics in a visceral experimental pain model in cows [47]. In the latter, large-volume CSF sampling allowed for a high-throughput screening of pain proteome. For all pain outcome measures tested in this experiment, the CSF TTR demonstrated the highest specificity and sensitivity [47]. It is thus conceivable that the inflammatory OA pain decreased the spinal concentration of TTR, a decrease modulated by the antiinflammatory activity of tiludronate.

Tiludronate-treated dogs presented some peripheral sensitisation at D28, but no more at D56. A partial but sustained antihyperalgesic effect of tiludronate was also demonstrated in the model of complete Freund adjuvant monoarthritis in rats [3]. Interestingly, meloxicam tested in this model was not very efficient against peripheral sensitisation [3], a result similar to this observed in OA cats [19]. Vehicle-, but not tiludronate-treated dogs presented a significant central sensitisation with measurement of QST-tail at D56 post CCLT. Tiludronate had too significant an effect at D56 on the release of spinal neuropeptides (lower SP, and higher TTR) compared to the vehicle group.

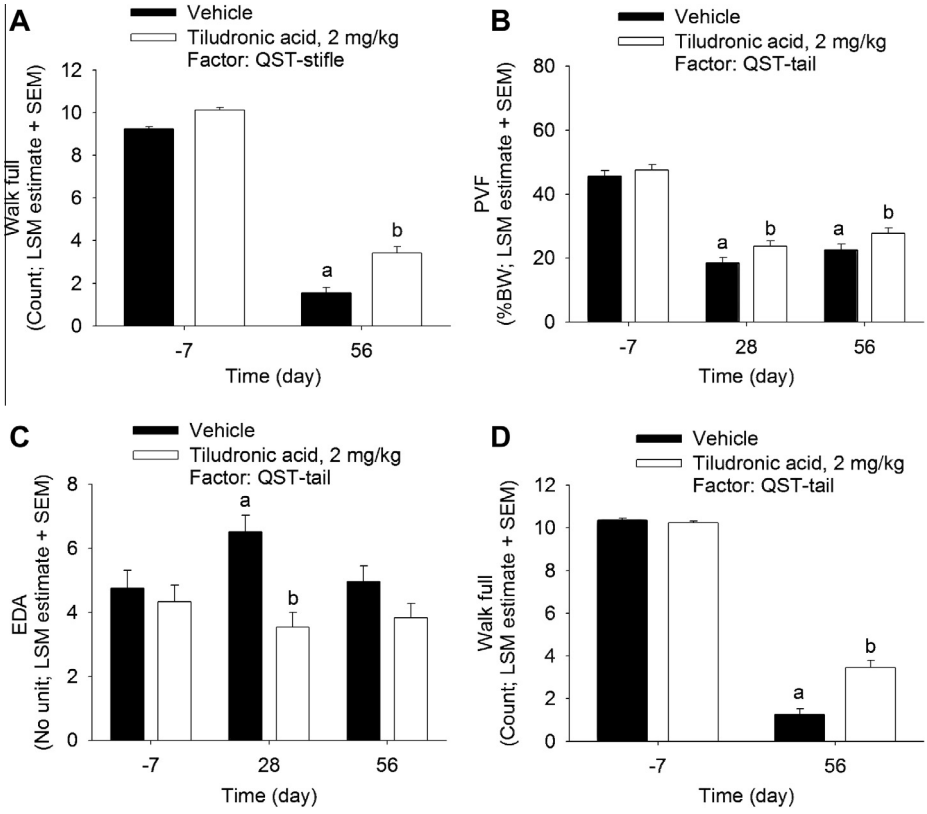

Fig. 3. Histogram of the least squares means (LSM) estimates + SEM derived from generalized linear analyses of: (A) "Walk full" when quantitative sensory testing applied on the stifle (QST-stifle) was a factor[a]; (B) Peak vertical force (PVF) when QST applied on the tail (QST-tail) was factor[b]; (C) Electrodermal activity when QST-tail was a factor[c]; (D) "Walk full" when QST-tail was a factor[d]. Different letters indicate significantly different values between vehicle-treated dogs and tiludronate-treated dogs (tiludronic acid, 2 mg/kg subcutaneously, q2week, starting at D0), $P_{adj} < 0.05$. [a]Type 3 tests of fixed effects for time (F value = 93.3, $P < 0.0001$), time × treatment interaction (F value = 6.0, $P = 0.01$), and treatment (F value = 2.8, $P = 0.09$). At D56, DLSM estimates were higher in the tiludronate-treated dogs than in the vehicle-treated dogs (DLSM = 0.79 ± 0.39, $P_{adj} = 0.04$). [b]Type 3 tests of fixed effects for time (F value = 515.3, $P < 0.0001$), time × treatment interaction (F value = 3.0, $P = 0.04$), and treatment (F value = 3.1, $P = 0.05$). The DLSM estimates of PVF were higher in tiludronate- than in vehicle-treated dogs, both at D28 (0.06 ± 0.02, $P_{adj} = 0.04$) and D56 (0.06 ± 0.02, $P_{adj} = 0.04$). [c]Type 3 tests of fixed effects for time (F value = 5.1, $P = 0.07$), time × treatment interaction (F value = 19.9, $P < 0.0001$), and treatment (F value = 6.7, $P = 0.009$). At D28, DLSM estimates were lower for tiludronate- than for vehicle-treated dogs (−2.98 ± 0.70, $P_{adj} < 0.001$). [d]Type 3 tests of fixed effects for time (F value = 88.3, $P < 0.0001$), time × treatment interaction (F value = 8.4, $P = 0.004$), and treatment (F value = 3.3, $P = 0.04$). At D56, DLSM estimates of "Walk full" were higher in tiludronate- than in vehicle-treated dogs (1.01 ± 0.44, $P_{adj} = 0.02$).

It is possible that a peripheral neuronal firing at 28 days post CCLT in tiludronate-treated dogs might be responsible for the peripheral sensitisation observed at this time point. This neuronal firing was not subsequently transduced in a central sensitisation. This is supported by the electrophysiological studies demonstrating that the neuronal excitability in OA rodent models was caused by both activation of knee joint-afferent fibres and increased dorsal horn neuronal activity [21,43,50,51]. Zoledronate, another bisphosphonate, reduced histological joint degradation in an monosodium iodoacetate rodent model [53,59], and its inhibition of subchondral bone lesions alleviated both joint pain and central nociceptive activation [24,59]. Tiludronate administration at an early stage of the experimental OA process would support a delayed effect of bisphosphonate on occurring central pain at 56 days post CCLT, when compared to placebo.

Even if mechanical-pressure QST seems a more physiologically relevant noxious stimulus method in inflammatory joint models and is responsive to antiinflammatory drugs [55], we opted for a broader electrical QST, as the degree of central sensitisation was poorly described in this experimental OA model and with the hypothesis that electrical QST would be more adapted to demonstrate a potential treatment effect of tiludronate to this outcome.

4.2. Relationship between centralised pain data outcomes and both the behavioural and physiological data outcomes

Behavioural change is a realistic measure of pain because it is a desired endpoint for assessing pain. If STAPS demonstrated a relative lack of reliability, the other reliable pain scales (VAS-1, VAS-2, and SVAPS) were positively associated with central sensitisation, and VAS-2 was correlated with the release of SP at D56. These results showed that dogs with decreased QST-tail presented higher pain scores and suggest concurrent validation for these different outcomes. Nevertheless, these above measures did not demonstrate a treatment effect. The result emphasizes that subjective VAS or numerical rating scales were not accurate, confirming the failure already observed in pain studies in OA dogs [20,46,58].

In the present study, the methods PVF, "Walk full," and motor activity were associated with both peripheral and central sensitisation. These results corroborated the unwillingness to move the surgically induced OA limb [7,34] and the increased sensitisation after CCLT surgery [10] in dogs. Nevertheless, the interpretation of these relationships was not straightforward. First, PVF was positively associated with QST-stifle and QST-tail. This demonstrated that an increased centralised pain sensation (translated into low QST thresholds) was leading to a diminished weight bearing of the operated limb, and the contrary was true, a result previously observed with OA induced in rodents [6,17,38]. Likely, "Walk full" was positively associated with QST-stifle. Unexpectedly, "Walk full" was positively associated with the central sensitisation. Similarly, the motor activity recorded by accelerometry was positively associated with both peripheral and central sensitisation. In naturally occurring OA in dogs [9,46] and cats [19], continuous monitoring of daily motor activity revealed the usefulness of increased intensity [19,46] or daily duration [33,34] to detect a treatment

effect. Difference in data acquisition might explain the observed difference between PVF and both "Walk full" and motor activity in the present study. Recording of PVF is dictated by a standardised velocity during locomotion. Thus, PVF might translate joint ability and musculoskeletal adaptation to a limb injury during a forced walk cycle [4,34,57]. In contrast, "Walk full" and motor activity were spontaneous behaviours, recorded in dogs with freedom of movement, and may describe how the dogs with OA desired to walk and manifest restlessness.

Finally, EDA was reliable on central sensitisation only, and spinal SP in our study. A positive correlation between an increased skin conductance response following acute noxious stimulation and central pain activation was observed in human patients [14], particularly at the limbic system of the brain [14,49] that is implicated in the stress response to pain. Therefore, these results argue that EDA measurement is linked to central pain.

4.3. Sensitivity of the behavioural and physiological evaluations to detect a treatment effect

Using QST-stifle and QST-tail as factors highlighted a clear association between different behavioural and physiological methods and sensitisation over time, whereas the responsiveness to treatment allowed the detection of PVF, EDA, and "Walk full" to be related to central pain and differentiate both treatment groups. In a context of concurrent methods validation, these results support the use of PVF [33] and "Walk full" behaviour [15] in assessing OA canine pain symptoms. For EDA, more investigation should be performed, as EDA was not responsive to analgesia in animal pain models [2,48], or was partially responsive [47].

Keeping in mind that the CCLT canine model is a dynamic model [39], levels of inflammation and articular lesions were different from D28 to D56. In this study, only the results of the group comparison allow us to hypothesize an effect of treatment on joint inflammation without taking into account the dynamic process of OA in this model.

Our findings clearly support a major role of a centralised pain occurring in the CCLT dog model. If the spinal neuropeptides SP and TTR look promising, the levels of neuropeptides should be addressed after several months of permanent instability induced in the knee joint, thus conducting to degenerative cartilage and secondary synovitis associated to OA [8]. Further study in larger populations with a variety of validated outcome measures would warrant the inclusion of mechanistic factor(s), such as peripheral and central sensitisation, as it looks encouraging to optimise the responsiveness to treatment and its translational potential to other species and/or painful conditions.

Conflict of interest statement

None of the authors with the exception of Dr Thierry Bertaim have competing interests. Dr Thierry Bertaim is a regular employee of CEVA; he provided the experimental therapeutic agent, participated in the study design, data analysis, and manuscript editing.

Acknowledgements

The authors wish to acknowledge the wonderful technical support provided by the personnel of ArthroLab, Inc., namely Mrs Carolle Sylvestre and Pascale St-Onge.

Dr Pascale Rialland received a doctoral Industrial Innovation Scholarship from the Natural Sciences and Engineering Research Council of Canada (NSERC #406726), the Fonds de recherche du Québec – Nature et Technologies (FRQNT #144812) and ArthroLab, Inc. Maxim Moreau is the recipient of a Doctoral Scholarship from the Canadian Institutes of Health Research – MENTOR Strategic Training Initiative in Health Research Program. This study was supported in part by a grant from CEVA Santé Animale, Libourne, France (Thierry Bertaim), by a grant of the Osteoarthritis Chair of the University of Montreal Hospital Centre, Université de Montréal, QC, Canada; an ongoing New Opportunities Fund (#9483) and a Leader Opportunity Fund (#24601) grants from the Canada Foundation for Innovation (Eric Troncy) for the pain/function equipment; Discovery grants (#327158-2008 and #441651-2013) from the NSERC (Eric Troncy) as operating fund for pain analyses. The funding sources, except CEVA Santé Animale, were not involved in the study design, collection, analysis and interpretation of data, writing of the manuscript, nor in the decision to submit the manuscript for publication.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.pain.2014.07.017.

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