Université de Montréal

Histomorphometric assessment of double-zonal osteons in human cortical bone

Par
Emeline Raguin

Département d'anthropologie
Faculté des arts et des sciences

Thèse présentée à la Faculté des études supérieures
en vue de l'obtention du grade de
Philosophiae Doctor (Ph.D.)
en anthropologie

mai 2018

© Raguin Emeline, 2018

Résumé

Le processus de remodelage osseux produit quatre différents types d'ostéons : type I (les plus communs), type II, « à la dérive » et les ostéons zonaux (DZ). Ces derniers, caractérisés par un anneau hyper minéralisé à l'intérieur des lamelles de l'ostéon, n'ont pas été étudiés de manière extensive dans la littérature anthropologique. Alors qu'ils sont identifiés depuis les années 70, les ostéons DZ ont donné lieu à plus d'hypothèses que de réponses sur les causes possibles de leur formation, probablement dû à leurs mauvaises identifications et au manque d'un protocole d'identification fiable et reproductible. Plus précisément, certains ont proposé que ces ostéons pourraient être le résultat d'un stress physiologique systémique, mais d'autres ont soutenu qu'ils seraient le produit naturel résultant du vieillissement.

Dans ce contexte, le but de ce projet est d'étudier les causes possibles de la formation des ostéons DZ et il a été réalisé en trois parties : 1) l'élaboration d'un protocole d'identification en microscopie à lumière polarisé, testé en comparaison à une méthode de référence; 2) l'évaluation des influences métaboliques liées au sexe, à l'âge ainsi qu'aux pathologies, basés sur une comparaison entre les individus diagnostiqués avec des maladies métaboliques et ceux sans évidence de condition pathologique et 3) l'évaluation des charges mécaniques sur la formation des ostéons zonaux. L'échantillon utilisé dans cette étude est constitué de colons Eurocanadiens du cimetière historique St. Matthew, à Québec (1771-1860).

Les résultats présentés montrent que 1) l'utilisation de la microscopie optique polarisée pour identifier les ostéons DZ est une alternative fiable et précise aux technologies plus coûteuses. De plus, de nouvelles connaissances concernant la formation des ostéons DZ ont été apportées en révélant une altération ou un changement brusque de l'orientation des fibres de collagène au même endroit que l'anneau hyper minéralisé. 2) Aucune différence n'a été observée entre les sexes, les groupes d'âge et les pathologies, suggérant que les ostéons DZ ne sont pas associés à des changements métaboliques. 3) D'après la caractérisation des ostéons DZ par un changement/altération dans l'orientation des fibres de collagène, l'hypothèse selon laquelle des charges biomécaniques plus importantes résulteraient dans la formation des ostéons DZ a pour la première fois été étudié sur des fémurs et humérus appariés. Les résultats ne permettent pas de supporter sans équivoque cette hypothèse : alors que le fémur a une plus

grande densité de DZ que l'humérus, les variations de charges mécaniques interindividuelles sont négativement corrélées avec la fréquence d'ostéons DZ. Par ailleurs, les ostéons DZ sont significativement plus petits que les ostéons de type I, suggérant que la taille de la baie de résorption soit un facteur déterminant de la formation d'ostéons DZ.

Ainsi, l'ensemble des résultats présentés dans cette dissertation suggère que les ostéons DZ ne sont pas une réponse à des instabilités métaboliques ou mécaniques. Plutôt, ils se forment comme les ostéons de type I, mais dans un contexte de baie de résorption à taille réduite, ce qui implique un nombre moindre de cellules responsables de la formation osseuse. Avec ce nombre réduit de cellules formatives, il y a une augmentation des risques d'altération de la synthèse de la matrice de collagène conduisant à une hyper minéralisation subséquente. En conséquence, leur formation serait le résultat de l'activité ostéoclastique et les recherches futures devraient focaliser sur cet aspect.

Mots-clés: Ostéons double-zonaux, Histomorphométrie, Remodelage, Biomécanique, Géométrie de section en coupe, Paléopathologie, Microscopie en lumière Polarisée, Ostéons de type I, Microscopie électronique à balayage.

Abstract

The process of bone remodeling produces four different types of osteons: type I (the most common), type II, drifting and zonal osteons (DZ). The latter, characterized by a hyper-mineralized ring within osteon lamellae, have not been studied extensively in the anthropological literature. While they have been recognized since the 1970s, DZ osteons have given rise to more hypotheses than answers about the possible causes of their formation, probably because of misidentifications and a lack of reproducible protocols. Specifically, it has been proposed that these osteons may be the result of systemic physiological stress, but others have argued that they would be a natural product of aging.

In this context, the purpose of this project is to investigate the possible causes of the formation of DZ osteons and consists of three parts: 1) the development of a protocol in polarized light microscopy that is tested against a gold standard method; 2) the evaluation of metabolic influences linked to sex, age and pathologies, the latter being based on a comparison between individuals diagnosed with metabolic diseases and those without evidence of pathological condition on the formation of DZ osteons and 3) the evaluation of mechanical loads on the formation of DZ osteons. The sample used in this study consists of Eurocanadian settlers from St. Matthew Historical Cemetery, Quebec City (1771-1860).

The results presented show that 1) the use of polarized light microscopy to identify DZ osteons is a reliable and accurate alternative to more expensive technologies. In addition, new insights regarding the formation of DZ osteons has been provided by revealing an abrupt alteration or change in the orientation of collagen fibers at the same location as the hyper-mineralized ring. 2) No difference was observed between the different groups, either by pathological status, age or sex, suggesting that DZ osteons are not associated with metabolic changes. 3) According to the characterization of DZ osteons by a change/alteration in the orientation of collagen fibers, the hypothesis according to which higher biomechanical loads would induce the formation of DZ osteons was explored on paired femurs and humerus. The results do not allow to unequivocally support this hypothesis: whereas the femur has a greater density of DZ than the humerus, the interindividual mechanical loading variation was negatively correlated with DZ osteon frequency. Additionally, DZ osteons were found to be

significantly smaller than type I osteons, suggesting that the size of the resorptive bay is a determining factor in DZ formation.

Thus, the overall results presented in this dissertation suggest that DZ osteons are not the result of metabolic or mechanic instabilities. Instead, they are formed like type I osteons, but in the context of a smaller resorptive bay. Their smaller size implies fewer cells responsible for bone formation, which could increase the risk of impairment in the matrix collagen synthesis, leading to the subsequent hyper-mineralization. Consequently, their formation would be the result of osteoclastic activity and future research should focus on that aspect.

Keywords: Double-zonal osteons, Histomorphometry, Bone remodeling, Biomechanics, Cross-sectional geometry, Paleopathology, Polarized-light Microscopy, Type I osteons, Scanning electron microscopy.

Table of contents

List of tables

List of figures

List of abbreviations and acronyms

AOC: Accumulated osteon creation

BMU: Basic multicellular unit

netBFR: net Bone Remodeling

BSE-SEM: Backscattered scanning electron with scanning electron microscopy

CA or Ct.Ar: Cortical area

%CA: Relative cortical area

CPL: Circular polarized light

DZ: Double-zonal osteons

DZD: Double-zonal osteons density

Es.Ar: Endosteal area

H.Ar: Haversian canal area

$\mathbf{I_{max}}$: Maximum second moment of area

$\mathbf{I_{min}}$**:** Minimum second moment of area

IGF-1: Insulin-like growth factor

J: Polar moment of area

MES: Minimum effective strains

NPV: Negative predictive value

On.Ar: Osteon area

OPD: Osteon population density

OPG: Osteoprotegerin

PLM: Polarized light microscopy

PPV: Positive predicted value

PTH: Parathyroid hormone

RANK: Receptor activator of nuclear factor

RANK-L: Receptor activator of nuclear factor ligand

TA or Tt.Ar: Total subperiosteal area

TGF-B: Transforming growth factor beta

ZH.Ar: Double-zonal Haversian canal area

ZOn. Ar: Double-zonal osteon area

Acknowledgements

First and foremost, I would like to express my gratitude to my advisors: Dr. Michelle Drapeau and Dr. Margaret Streeter without whom this dissertation would not have been possible. I have greatly benefited from their invaluable support and encouragement. I also want to thank them for being readily available whenever help was needed and for always encouraging me throughout this project. Dr. Drapeau provided me with opportunities and guidance throughout my entire career as a graduate student. I am also grateful to Dr. Streeter for introducing me with passion to bone histology. I especially enjoyed our many discussions on bone biology and histology: her excitement and her dedication to the subject were a true source of inspiration.

I would also like to thank the members of my committee: Dr. Isabelle Ribot, for her enthusiasm and the involvement she always had in my projects; Dr. Ariane Burke, thank you very much for our countless discussions; and Dr. Corey Maggiano, for his ongoing advice and encouragement during the conference meetings. I would thank them for their critical comments on this thesis.

I am profoundly grateful to the Anglican community of St. Matthew, particularly Bishop Dennis Drainville from the Anglican Diocese of Quebec and Marie-Sol Gaudreau, for allowing access to the osteological collection and being so open-minded in the realization of this project. I also thank Dr. Réginald Auger (laboratoire d'archéologie de l'université Laval) for giving us access to the collection in his laboratories and to the CT-Scan databank as well as Dr. William Moss (archaeologist, Québec City). Both made it possible to establish and extend the loan agreement for the collection with the Anglican Diocese. The CT scan processing would not have been possible without access to the technologies and resource persons who guided me in the treatment of medical imaging: Dr. Bernard Long, Dr. Geneviève Treyvaud and Louis-Frédérique Daigle at the Laboratoire de scanographie, eau terre environnement (INRS, Québec City). I would like to thank the Archeometry Research Team at Université Laval FRQ-SC (R. Auger) and the Canada France Fund (I. Ribot, anthropologie, Université de Montréal) who supported this project financially. I thank the Laboratoire d'écomorphologie et de paleoanthropologie, and especially Dr. Jihyun Daniel Yi.

I am also thankful to Dr. Antonio Nanci and Dr. Alejandra Rodriguez-Contreras from the Laboratory for the Study of Calcified Tissues and Biomaterials at Université de Montréal for providing access and assistance to the BSE-SEM. This thesis would never have been possible without their kindness. I am sincerely thankful to Dr. Natalie Reznikov for her support and enthusiasm for my research interests. I am grateful for the opportunity she provided to me in order to pursue my academic career.

I would like to thank the undergraduate students, Lara-Maria Breton, Charline Debruyne, William Filion, Tania Bertrand, Malwine Klagba for helping me prepare the innumerable samples from the collection.

Furthermore, there are too many friends that have been part of this process to thank them all. I thank Nancy Grant for her proofreading but even more for making me discover Canada. I am grateful to the many people who encouraged me in one way or another to complete this dissertation, either by helping me directly in this work or by making my life much more enjoyable. I particularly thank my fellow graduate students, Ronan Méhault, Dario Guiducci, Lauriane Bourgeon, Yohan Caillau, Sarah Borgel, Zocha Houle-Wierzbicki, Jean-Baptise Le Moine. A special thanks to Cécile Marcoz-Fellay and, of course, Pakch and Augustine for their support. A special thank you to my friend Sabrina Gloux for being able to calm me down in the most stressful moments!, but mainly for being there. To my friends, Olivier, Karine, Tania, Jimmy, Micka, Ilira, Flori, thank you for the weekend at the cottage and all those evenings spent laughing (a special thanks to Olivier for being my sparring partner in the most difficult moments!). To Erika and now Elise, thank you for being an ongoing source of motivation.

To my Canadian-Argentinean extended family, thank you for all these delicious meals shared in a joyous and relaxed atmosphere, and for a taste of what awaits us at the “quincho”. And especially big thanks to Nico and Sylvie with whom the obstacles seemed easier to cross and for being there at every step, offering lighter moments.

A special thanks to my two doppelgangers Laurence Dumouchel and Luc Doyon, who have kept me focused and who have always been with me despite the distance. I hope that all our dreams will come true.

To my Québécois brother, Vincent Gautier-Doucet, thank you for making my life a lot easier in the past months as well as for always listening, being supportive but mainly for your friendship (and incidentally, correcting my English!). I cannot yet imagine my life without the tacos, the brownies and the apricot beers. I hope that Northern Quebec will become more accessible in the next few years.

Finally, and not least, I owe a special thanks to my family in France for their unconditional support throughout the course of this dissertation project and for always being there for me. They all kept me grounded and continually reminded me of what the important things in life are. My parents have always encouraged me in my decisions, even if those things have taken me further and further from the family nest. My brothers and sisters-in-law have been incredibly supportive and kept me motivated. My niece and nephews have kept my priorities in check and reminded me of the good things in life.

Preface

This dissertation is a thesis by publications. Chapter 1 and Chapter 2 will provide the background needed to establish the theoretical foundation and demonstrate the relevance of the research. One article (Chapter 3) has been accepted and is in press, in collaboration with Dr. Margaret Streeter. This article entitled "*Test of a method to identify double-zonal osteon in polarized light microscopy*" is a brief communication in the peer-reviewed scientific journal *American Journal of Physical Anthropology*. I collected data according to a protocol established jointly with Dr. Streeter. I also did the statistical analysis and was the main editor of the article. The co-author contributed to the revision of the article, both for the quality of the language and in term of its content.

Chapter 4 and Chapter 5 are manuscripts that have not yet been published but are in preparation for submission in summer 2018 in the following expected journals: *International Journal of Paleopathology* and *American Journal of Physical Anthropology* respectively. In both manuscripts, I did the data collection, the statistical analyzes as well as the draft. Dr. Streeter is a co-author in Chapter 4 and Chapter 5 is in collaboration with Dr. Drapeau. All authors provided feedback throughout the process.

In all articles presented in this dissertation I did the sample preparation, from the macroscopic data analysis at the Université Laval (Québec) to the microscopic slide at the *Laboratoire d'écomorphologie et de paléoanthropologie, Département d'anthropologie (Université de Montréal)*. Finally, Chapter 6 will outline the results of this research by linking each back to the specific objectives stated. Conclusive remarks as well as directions for future studies are provided in this section.

1. Introduction and background

1.1 General context of the study

Bone is a dynamic tissue that can grow, repair itself, and respond to mechanical stimuli (Frost, 1985). Skeletal analyses can be performed at several hierarchical levels, ranging from the macroscopic to the molecular level. At the microscopic level, bone histomorphology offers a preferential access to explore bone biology in past and present populations through the process of bone remodeling. Remodeling is a regulated process that ensures bone material integrity and by which existing bone is resorbed and replaced by the deposition of newly formed secondary bone. The result is the creation of secondary osteon, of which four variants are recognized: type I (also called common osteon); drifting osteons; type II (embedded osteon) and zonal osteons, also known as double-zonal (DZ). A brief description of the different variants is provided here, as they will be discussed in detail in section 2.3.2.2.

Type I osteons are characterized by concentrically deposited lamellae surrounding a centrally located Haversian canal, enclosed in a scalloped reversal line (Frost, 1964b). Drifting osteons appear elongated and the Haversian canal is not centered (Epker and Frost, 1965; Robling and Stout, 1999; Sedlin, Villanueva, and Frost, 1963). Both Type I and drifting osteons have been well documented and described in the literature (Epker and Frost, 1965; Frost, 1964; Jaworski, Meunier, and Frost, 1972; Martin, Burr, Sharkey, and Fyhrie, 1998; Streeter, 2010; Takahashi, Epker, and Frost, 1965), particularly for their use in age-at-death estimation (Epker and Frost, 1965; Ericksen, 1991; Kerley and Ubelaker, 1978; Pfeiffer and Zehr, 1996; Stout and Paine, 1992; Streeter et al., 2001; Streeter, 2010; Yoshino, Imaizumi, Miyasaka, and Seta, 1994). The other two morphotypes have been less commonly studied. Type II osteons display two scalloped reversal lines, one within the other. Finally, DZ osteons are characterized by a hyper-mineralized ring within their concentric lamellae (Pankovich, Simmons, and Kulkarini, 1974; Robling and Stout, 2000) and are the least studied secondary osteons. The quantification of bone histology (histomorphometry) provides useful information about the microarchitecture that have been widely employed in anthropological studies related

to age-at-death estimation (Cho, Stout, Madsen, and Streeter, 2002; Ericksen, 1991; Kerley and Ubelaker, 1978; Pfeiffer and Zehr, 1996; Samson and Branigan, 1987; Stout and Paine, 1992; Streeter et al., 2001; Streeter, 2010; Thompson and Galvin, 1983; Yoshino, Imaizumi, Miyasaka, and Seta, 1994), determination of human versus non-human bone (Crescimanno and Stout, 2012; Dominguez and Crowder, 2012; Hillier and Bell, 2007; Mulhern and Ubelaker, 2001; Mulhern and Ubelaker, 2012), mechanical adaptation (Drapeau and Streeter, 2006; Lieberman and Crompton, 1998; Lieberman, Pearson, Polk, Demes, and Crompton, 2003a; Robling and Stout, 2003) and physiological challenges (Cho and Stout, 2003a; Farnum, Shimada, Streeter, and Verano, 2001; Martin and Armelagos, 1985; Stout and Lueck, 1995).

While the first mentions of DZ osteons occur in the early 1960s (Frost, 1964b; Kornblum and Kelly, 1964; Lacroix, 1971; Lacroix and Dhem, 1967; Smith, 1963), the study conducted by Pankovich et al. (1974) initiated a genuine interest in understanding the appearance of this morphotype and was the first to coin the term "double zonal". In this early work, DZ osteons were characterized using microradiographs, which enable the assessment of variations in bone density by the absorption of bone mineral content (hydroxyapatite) through a gray scale (Jowsey et al., 1965). It is from microradiographs that DZ osteons were defined as having one or more hyper-mineralized "halo" visible. Several authors, such as Smith (1963), Kornblum and Kelly (1964), Lacroix and Dhem (1967) and Lacroix (1971), have proposed that the "halo" is a resting or arrest line where growth has stopped long enough to allow the lamellae to become more mineralized. A subsequent resumption in lamellar deposition results in a hyper-mineralized ring separated from the central Haversian canal. While no explanation was suggested at the time to explain the appearance of such features, Pankovich et al. (1974) proposed that DZ osteons result from the aging process and mineral homeostasis and may contribute to increasing porosity in the elderly through intra-osteonal remodeling. However, what Pankovich et al. (1974) describe here is in fact a type II osteon. Results from their research must be put into question for several reasons, in part because of the pathological sample they used, but more importantly, because the authors appear to have confused type II and DZ osteons. Indeed, they failed to distinguish between the smooth arrest line characteristic of DZ osteons and the scalloped, wavy reversal line that defines type II osteon (Lacroix,

1971). The authors suggest that DZ osteons accumulate at a steady rate of 4% per decade in individuals from 20 to 80 years of age but given that they lump DZ and type II osteons together, this rate is questionable.

In the 1980s, it had been suggested that what was previously thought to be a temporary growth arrest seen in DZ could be related to physiological disturbance (Martin, 1983; Martin and Armelagos, 1985; Mays, 1985; Stout and Simmons, 1979). Mays (1985) and Stout and Simmons (1979) argued that these osteons could be akin to the Harris lines formed in subadult long bone epiphysis. These lines are thought to indicate episodes of temporary slowing or cessation of growth at the growth plate due to physiological stress (Harris, 1933). Martin (1983) was the only one to specifically quantify DZ osteon in relationship with health status in her dissertation and in the subsequent article from that work (Martin and Armelagos, 1985). The sample used in her study comes from a prehistoric Nubian population. Bone histomorphometry was assessed according to sex, age as well as the pathological status. The pathological analysis was determined microscopically based on bone loss and each sex was further separated into a normal group and an osteoporotic group. The author found that Nubian females have significantly fewer DZ osteons and more bone loss than males. She proposed that the increased bone loss seen in females compared to males is due to the effect of lactation and pregnancies. While no difference was found in males according to the health status, osteoporotic females were found to have fewer DZ osteons compared to the normal subgroup. The female osteoporotic group was then divided by age where the youngest have more DZ osteons but a better maintenance of their cortex with fewer resorption spaces compared to their older counterparts. Overall, while Martin (1983) assumed that DZ osteons represents past evidence of metabolic disruption and are evidence of a regulation of minerals exchange; individuals able to maintain their cortex will have resumption of remodeling after the arrest, resulting in a DZ. Conversely, individuals who experienced bone loss and are not able to recover from the metabolic stress, never complete the osteon, and thus have a lower frequency of DZ. However, these results do not demonstrate a specific link with metabolic disorders, instead Martin (1983) and Martin and Armelagos (1985) took that relationship for granted. The hypothesis hitherto proposed but never confirmed that DZ osteons resulted from an arrest in osteon formation was questioned in the work of Dhem (1980). When analyzing DZ osteons

in human tibias, Dhem (1980) demonstrated that the cytoplasmic processes of the osteocytes located in the hyper-mineralized ring extend without interruption with the osteocytes found in the lamellae on both sides of the ring, maintaining a functional lacuno-canalicular network. The author argues that if the hyper-mineralized ring was an arrest line, this network should be disrupted between the ring and the subsequent lamellae. Thus, Dhem (1980) rejects the hypothesis that the hyper-mineralized ring is an arrest line analogous to a Harris line and proposes instead that the ring marks an alteration of the bone matrix.

Although the identification of DZ osteons was originally done with microradiography, this technique, principally used in medicine, became progressively obsolete with the improvements of microtomography in the 1980s (Jansen, 2003). This technological change may explain why subsequent studies on DZ are relatively rare. As a result, some researchers have turned to the use of linear Polarized-Light Microscopy (PLM) to assess DZ osteons (Austin and Mulhern, 2015; Bartsiokas and Day, 1993; Pfeiffer and Zehr, 1996). Pfeiffer and Zehr (1996) have used the number of DZ osteons to evaluate the age-at-death of a middle Stone Age humerus from the rate of accumulation proposed by Pankovich et al. (1974) on the ribs. Added to the previously noted drawback of the study of Pankovich et al. (1974) that confused type II and DZ osteons, intraskeletal variability in bone remodeling has since been demonstrated (Cho and Stout, 2011; Stewart, Goliath, Stout, and Hubbe, 2015; Stout, 1982), so it is doubtful that the DZ rate observed in the ribs can be apply to the humerus. Bartsiokas and Day (1993) interpreted the presence of DZ osteons as evidence of lead poisoning of the *Homo heidelbergensis* individual from Broken Hill, Zambia. They based their argument on the observation that Harris lines are caused in some cases by lead poisoning. They assumed that DZ osteons were equivalent to Harris line, but, as stated above, this cause-and-effect relationship has never been demonstrated. Austin and Mulhern (2015) investigated DZ osteons in a medieval Kulbanarti Nubia ribs sample in order to determine whether there were variations related to age and sex. While no difference was observed between sexes, the authors found a negative correlation with age and suggested that DZ osteons may occur during periods of growth arrest when the individuals are young. However, there is no indication that individuals from their sample demonstrate any generalized stress or growth interruption. This negative correlation with age challenges the conclusions of previously mentioned studies that

showed an increase of DZ osteons with age (Pankovich et al., 1974; Simmons, Pritzker, and Grynpas, 1991), or others that did not find a significant correlation (Martin, 1983; Nyssen-Behets et al., 1991; Yoshino et al., 1994). Since no standardized protocol has been developed to identify DZ osteons under PLM, it is difficult to be certain that these studies are indeed all measuring DZ osteons and this lack of standardization raises concerns about their validity and reproducibility.

DZ osteons have been widely acknowledged in the anthropological literature in human bone, but much less in animal studies. Lacroix (1971) has found these osteons in an old dog and proposed that DZ osteons are related to aging. Additionally, Skedros et al. (2007) proposed to evaluate the distribution of secondary osteon variants in order to interpret load history in mammalian bones. The authors did not find any regional cortical variations in the prevalence of osteon variants with habitual loading histories, nor did they report a correlation between osteon variants and the type of load. Results from this study do not clarify DZ formation nor if these osteons are present in nonhuman mammalian bones since the authors grouped DZ osteons in the same category than type II osteons, as a singular phenomenon. According to the Skedros et al. (2007), the choice to quantify these two distinct types of osteons interchangeably reflects their inability to confidently differentiate between the arrest line and the reversal line.

Misidentification, differences in methodology and lack of reproducible protocols are probably the main factors explaining why DZ osteons have not often been the focus of investigations and why a consensus has yet to be reached with regards to the causes of their formation. In this context, this dissertation aims to expand the current knowledge about double-zonal osteons and tries to test previously proposed hypotheses by offering a more global view of the factors that could possibly trigger their development in the human cortical bone.

1.2 Research objectives

The overarching goal of this research is to evaluate the cause behind the formation of DZ osteons, with four specific objectives. The first aim is to establish a standardized protocol to identify DZ osteons under PLM. This step is necessary since no clear methodology is available from previous studies as discussed in the section 1.1 (Austin and Mulhern, 2015; Bartsiokas and Day, 1993; Pfeiffer and Zehr, 1996). A standardized method is particularly needed when using PLM because it cannot detect hyper-mineralization, as discussed in Chapter 3. PLM is a linear light microscope, which allows anisotropic materials, such as bone, to have a double refraction of the beam of light. This property, called birefringence, leads to a variation in brightness when viewed under PLM. Depending on the orientation of the collagen fibers with respect to the direction of light, the image will have an optimal brightness if the fibers are disposed transversely, or the opposite occurrence when the collagen fibers are oriented longitudinally. However, collagen fiber orientation and mineralization are two distinct features. As a result, the hyper-mineralized ring that defines DZ osteons cannot be recognized in PLM, but it is a change in collagen fiber orientation that is actually observed. Therefore, it is necessary to test whether DZ osteons can indeed be identified under PLM, as assumed by all previous researchers that have studies DZ osteons with PLM and if so, to propose a detailed protocol to allow for standardization and reproducibility in future studies.

The second objective is to gain insight into the physiological processes behind DZ formation, which are still in question. It has been hypothesized that DZ osteons could be the result of physiological stress as presented in section 1.1 (Austin and Mulhern, 2015; Martin, 1983; Martin and Armelagos, 1985; Mays, 1985; Stout and Simmons, 1979). A reassessment of this hypothesis is necessary since Martin (1983) was the only one to actually test this hypothesis. Martin (1983) did not find a link between metabolic disorders and the occurrence of DZ osteons. Rather she proposed that individuals experiencing bone loss are not able to resume the growth arrest line of the DZ osteons. Additionally, her study raises several issues: 1) the hyper-mineralized ring of DZ osteons might not to be a growth arrest line as proposed by Dhem (1980); 2) sex-based differences are still poorly understood since her results suggest that males had more past episodes of metabolic stress. Such a conclusion is surprising in light of the fact that females are undergoing more metabolic changes during their lives (Silberberg

and Silberberg, 1972), as it will be discussed in section 2.3.2.3.1. Consequently, the effect of metabolic change on DZ formation needs to be investigated further.

The third objective of this dissertation is to test whether differences in loading history lead to a distinctive DZ osteon remodeling. If zonal osteons are also characterized by a distinctive pattern in the collagen fibers orientation, and collagen fiber orientation affects mechanical properties of bone (Martin et al., 1998; Wang et al., 2000; Wang, Bank, TeKoppele, and Agrawal, 2001), it is reasonable to assume that DZ osteon formation is influenced by variations in load and may reflect past activity levels.

The fourth objective is inherent to the second and third objectives and proposes to investigate the area of DZ osteons. Type I (common) osteon area has been the focus of interest in numerous studies related to age (Britz et al., 2009; Burr et al., 1990; Currey, 1964; Martin, Pickett, and Zinaich, 1980; Takahashi, Epker, and Frost, 1965), sex (Britz et al., 2009; Burr et al., 1990; Borgel, 2017; Denny, 2010; Dominguez and Agnew, 2016; Pfeiffer, 1998; Pfeiffer et al., 2006), and biomechanics (Abbott et al., 1996; Borgel, 2017; Corondan and Haworth, 1986; Denny, 2010; Moyle and Bowden, 1984, Pfeiffer, Crowder, Harrington, and Brown, 2006; Skedros, Keenan, Williams, and Kiser, 2013; Skedros, Mason and Bloebaum, 1994; Skedros, Mendenhall, Kiser, and Winet, 2009; Van Oers et al., 2008, Yeni, 1997). Beyond the fact that a consensus is far from being reached regarding the factors that can influence osteon area as will be detailed in section 2.3.2.3, this variable has never been studied with regard to DZ osteons. If DZ osteons are the result of metabolic changes or if they result from differences in the loading history, their area could be expected to vary accordingly.

1.3 General materials and methods

1.3.1 Overview and selection of the sample

The materials used in this dissertation consists of femur and humerus tissue samples from human skeletal remains that were excavated at the St. Matthew's cemetery in Québec City, Canada. After the British conquest in 1759, the Protestant religion flourished in Québec City, particularly with the arrival of immigrants coming mainly from Western Europe (Hare,

Lafrance, and Ruddel, 1987). In this context of expansion, St. Matthew's cemetery was built in 1771 and served as the first official burial place for the Anglican and Presbyterian populations of the provincial capital of Québec and was subsequently named the "Protestant Burying Ground" (Cloutier, 2000; Simoneau, 2003). Due to sanitary problems, the cemetery was closed in 1860 (Cloutier, 2000; Simoneau, 2003).

Starting in 1982, several archeological interventions unearthed approximately 230 burials (Bélanger, 1993; Cloutier, 2000; Larocque, 1986; Moss, 2010; Simoneau, 2003). This collection has been the subject of numerous bioarchaeological studies on paleodemography (Arpin, 2006), diet (Morland, 2010), activity patterns (Perron, 2006), paleopathology (Houle-Wierzbicki, 2016; Morland, 2010) and on the origin and migration of Québec Protestants (Caron, 2014). The Saint Matthew's Cemetery collection was stored in archeology laboratories at the Université Laval, under the responsibility of archaeologist Dr. Réginald Auger, until it was reburied in November 2015.

This archeological population presents several unique opportunities to investigate bone biology. First, the levels of physical activity and variation in human behavior were much higher among past populations in comparison to modern populations (Parfitt, 1997). Second, the historical context favored the emergence of specific diseases and affected individuals could die in the absence of care unlike current western populations who benefit from health treatment that reduces the mortality rates. Thus, it is possible to study the effect of pathologies while isolating the possible interference of pharmaceutical treatments on the normal physiology of bone and therefore on the modeling and remodeling dynamics (Ott, 2017). Third, the mortality structure of archeological population is more normally distributed in comparison with cadaveric samples where younger individuals are underrepresented, which introduces a bias related to the senescence on bone remodeling dynamics.

The general criteria for the inclusion of St. Matthew's individuals in this study were defined as follows: 1) should be of adult age (greater than 21 years), 2) the periosteal surface should not be eroded, determined upon macroscopic examination, and 3) the histological preservation must be adequate to allow an optimal histomorphometric reading. Thirty individuals satisfy these requirements for inclusion into the sample study of Chapter 3. Moreover, additional criteria were added to meet all the research objectives presented in this

dissertation. In order to test whether double-zonal osteons are associated with metabolic diseases (Chapter 4), a diagnosis of pathology at the macroscopic level has been carried out on the St. Matthew sample (Houle-Wierzbicki, 2016). As a consequence, nine femurs of individuals showing signs of pathology related to metabolic stress were included in the sample and compared to 26 individuals that showed no signs of generalized pathological conditions. Finally, to determine the relationship between double-zonal osteons and biomechanical properties (Chapter 5), only individuals that preserved both the femur and the humerus were included (n=23).

1.3.2 Methods

In order to achieve all the objectives addressed in the section 1.2, distinct and specific methodologies were employed. To avoid redundancy due to the format of this dissertation, only the general methodology employed for the macroscopic data collection and for the production of microscopic slides for the histological analysis will be presented here. The methods specific to each study are detailed in their respective chapter (Chapters 3, 4 and 5).

1.3.2.1 Macroscopic methods

The macroscopic skeletal analyses of the St. Matthew sample were performed at the Université Laval in Québec City and include the determination of the biological profiles of the individuals, the measures of long bones and the recording of paleopathology. This recording of macroscopic data was conducted for a broader project to document the St. Matthew's collection, and several studies investigating different biological aspects have been carried out (B-Hardy, 2017; Caron, 2014; Houle-Wierzbicki, 2016; Toupin, 2016).

The dimension of long bones is particularly useful in estimates of stature and activity pattern. Thus, long bones were systematically measured according to the protocol developed by Buikstra and Ubelaker (1994) and presented in Appendix I. Standard methods were used to assess age-at-death and sex estimation. Age-at-death was assessed from the pubic symphysis metamorphosis (Brooks and Suchey, 1990; Katz and Suchey, 1989) and from the changes to

the auricular surface of the os coxae (Lovejoy, Meindl, Pryzbeck, and Mensforth, 1985; Schmitt, 2005; Schwartz, 1995) (Appendix II). These methods are based on the macroscopic evaluation of the changes of surface features that occur with aging. The mean age range obtained from these methods was calculated for each individual and they were assigned to one of the following categories: 20-29; 30-39; 40-49 and 50+. Despite the relative inaccuracy of such procedure, the age ranges provided are sufficiently broad to minimize biases (Buckberry, 2015; Buikstra and Ubelaker, 1994). Sex was determined from the morphological features of the pelvic bone using the methods proposed by Bruzek (2002) and Buikstra and Ubelaker (1994) (Appendix III). These methods are considered particularly reliable compared to others cited in the literature because of the distinct features adapted to childbearing (Marchal, 2003; Murail, Bruzek, Houët, and Cunha, 2005).

Finally, a thorough paleopathological analysis was conducted on the skeletal remains of St. Matthew's collection and the data of a subsample of the collection was analyzed in a master's thesis (Houle-Wierzbicki, 2016), and a subset was used in Chapter 4 of this dissertation. Paleopathological examination was conducted in accordance with the guideline proposed by Buikstra and Ubelaker (1994) to provide standardized recording techniques and precise descriptions of the lesions found on the skeletons. The skeletal remains were CT scanned using a tomodensitometer Siemens SOMATOM (Definition AS+ 128) at the *Laboratoire de Scanographie – Eau Terre Environnement* (INRS, Quebec City) and came to implement the visual diagnosis. Diagnoses of the pathologies were made according to the differential diagnosis method, commonly used in paleopathological studies (Brickley and Ives, 2008; Ragsdale and Lehmer, 2012). Briefly, the differential diagnosis approach is mainly based on the observation from the macroscopy and from the CT scans, depending on the age and sex of the individual, the geographical origin and the life conditions of the study population. Then a list of exhaustive diseases corresponding to these lesions is established and each are gradually eliminated to identify the most probable etiology. Diagnoses were then divided into the following nonexclusive categories: metabolic; infectious; joint; neoplastic; dysplasia; congenital; developmental; spinal; and finally traumatic lesions (Aufderheide and Rodriguez-Martin, 1998; Ortner, 2003; Roberts and Manchester, 2007). The diseases were scored as 1 (present) or 0 (absent).

1.3.2.2 Microscopic methods

The mid-diaphysis of the long bones was located by measuring the total length of the bone. A three cm block, 1.5 cm on each side of the mid-diaphysis, was collected using a bandsaw. The bone section was then prepared using standard histological preparation protocol (Crowder, Heinrich, and Stout, 2012; Frost, 1958). While the general methodology for producing thin sections is presented here, it differs slightly from the methodology presented in Chapter 3 that needed to meet more specific objectives.

The thick sections were embedded in epoxy resin under a vacuum (EpoThin 2 resin and hardener and Cast N'Vac 1000, Buehler Ltd., Lake Bluff, IL). The vacuum allows evacuating trapped air and ensuring the structural bone integrity for the next procedures. An Isomet precision sectioning saw (Buehler Ltd., Lake Bluff, IL) was used to cut thin sections of around 300 μm after an initial waste cut. The specimens were then ground to a final thickness of approximately 80 μm with a PetroThin thin sectioning system (Buehler Ltd., Lake Bluff, IL). The thin bone sections were finally washed and mounted onto microscopic slides using Permount™ (Fisher Chemicals™) and cover-slipped. Histological examination of the bone slides used in this dissertation was performed using an automated scanning system under linear polarized light with an Olympus BX43 microscope in proper anatomical orientation and at 100X magnification. This technique allows the production of an automatically stitched image of the entire cross-section with the Objective Imaging Surveyor Software. Preparation of microscopic slides and data collection were performed at the *Laboratoire d'écomorphologie et de paléoanthropologie, département d'anthropologie, Université de Montréal.*

Histomorphometric structures were recorded and measured on the entire cross section using ImageJ software (Schneider, Rasband, and Eliceiri, 2012). The histomorphometric variables used and studied in this dissertation are developed in Chapter 3, 4 and 5.

1.4 Questions and Hypotheses

In light of the general context of the study presented in the section 1.1 and the research objectives stated in the section 1.2, the following research questions and hypothesis will be

addressed and tested to fulfill the central aim of this study regarding the possible causes of DZ osteons occurrence:

A) Can DZ osteons be identified under linear polarized-light microscopy following the protocol implemented by an experienced observer?

1. H^A_0: DZ osteons cannot be identified under PLM.
2. H^A_1: DZ osteons can be characterized by a different pattern in collagen orientation when viewed under PLM.

B) Are DZ osteons an indicator of metabolic disturbances or physiological stress?

1. H^B_0: There is no difference in DZ osteon frequency between individuals diagnosed macroscopically with metabolic disease and those without evidence of metabolic disease.
2. H^B_1: Individuals diagnosed macroscopically with metabolic disease have a higher DZ osteon frequency than those without evidence of metabolic disease.

C) Does the density of DZ osteons change with increasing age?

1. H^C_0: There is no difference in DZ osteon frequency between age categories.
2. H^C_1: DZ osteons increased significantly with age.

D) Does the density of DZ osteons vary according to sex?

1. H^D_0: There is no difference between males and females in DZ frequency.
2. H^D_1: There is a significant difference in DZ frequency between males and females, females having greater density than males.

E) Does DZ osteon area changes in individuals experiencing metabolic changes?

1. H^E_0: There is no difference in DZ osteon area with metabolic changes, either induced by bone disease, sex or age.
2. H^E_1: There is a significant difference in DZ osteon area; individuals diagnosed macroscopically with metabolic disease have higher DZ osteon area than those without evidence of metabolic disease, females have higher DZ osteon area than males and there is a decrease in DZ osteon area with age.

F) Does DZ osteons frequency relate to mechanical load history as characterized by bone cross-sectional properties?

1. H^F_0: DZ osteon frequency is not different between humerus and femur and is not related to variation in cross-sectional properties
2. H^F_1: The more loaded femur has a higher density of DZ osteons than the humerus and there is a significant relationship between DZ osteon frequency and cross-sectional properties.

G) Does DZ osteon area relate to mechanical load history as characterized by bone cross-sectional properties?

1. H^G_0: There is no difference in DZ osteon area between humerus and femur and is not related to variation in cross-sectional properties
2. H^G_1: The more loaded femur has smaller DZ osteon area compared to the humerus and there is a significant negative relationship between DZ osteon area and cross-sectional properties.

1.5 Thesis organization

The general context, objectives and hypotheses have already been addressed. Chapter 2 provides a more detailed review of the basic bone anatomy and physiology necessary for the histomorphometric assessment in this dissertation and allows a global view for the interpretation of the results.

Chapters 3, 4 and 5 assess directly the hypotheses previously formulated. Specifically, Chapter 3 tests a method implemented by Dr. Margaret Streeter, an experienced histomophologist, to identify DZ osteon in polarized light microscopy. The aim is to investigate if the PLM protocol used produces accurate results when compared to a gold standard method defined by Backscattered Scanning Electron with Scanning Electron Microscopy (BSE-SEM). The two following chapters, Chapter 4 and 5 ensue from the results and methodology set out in Chapter 3. Chapter 4 proposes to investigate the metabolic effects induced either by age, sex or metabolic disease on the DZ osteons, in relation with others histomorphometric indicators of the general metabolic function. Chapter 5 investigates the relationship between osteon morphotypes, especially type I and DZ osteons, and mechanical loads. Histomorphometric values of each osteon variants are compared in paired humerus and femur of the same individuals and their correlation with cross-sectional properties as an estimate of the mechanical load history of the bone are evaluated.

Lastly, Chapter 6 includes a brief summary and significance of the research established in this dissertation and the hypotheses are revisited in the light of the results obtained previously. This section also includes the inherent limitations of this work and proposes directions for future research.

2. The biology and physiology of bone

2.1 Bone composition and mineralization

Bone is a specialized connective tissue whose main functions are to provide mechanical protection for internal organs and to withstand mechanical stress. Additionally, bone ensures metabolic function to maintain mineral homeostasis as well as to provide housing for the hematopoiesis process (Boskey, 2006).

Bone tissue is composed of organic and mineral components. The organic fraction represents 30% of the component of bone, which largely consists of collagen (90%) and bone cells (10%). The inorganic part constitutes 60% of the bone and is primarily crystalline hydroxyapatite (Feng, 2009). The remaining 10% are water, which serves many functions such as interacting with collagen fibrils and binding to mineral crystals (Boskey, 2013). The organic matrix is largely composed of type I collagen, the most abundant protein found in the human body, particularly in bone, tendons, ligaments and skin (Martin et al., 1998). Bone collagen is responsible for maintaining the structural integrity of the bone matrix and confers mechanical stability, strength and toughness (Feng, 2009; Fratzl, 2008). Type I collagen is composed of fibrils that become organized into larger diameter collagen fibers, presenting gap and overlapping zones (Fratzl, Fratzl-Zelman, and Klaushofer, 1993). This structure offers the scaffold allowing mineral crystals to form within and between the collagen fibers (Wess, 2008). Mineral crystals are hydroxyapatite, a calcium phosphate, which contribute to harden bone tissue when combined with other mineral salts or ions, such as calcium carbonate, magnesium, fluoride, potassium and sulfate (Tortora and Derrickson, 2010). Although the process that precedes the mineral propagation is still unclear, it is thought that hydroxyapatite nucleates from an amorphous calcium phosphate phase in the gap and overlap zones (Mahamid, Sharir, Addadi, and Weiner, 2008). The crystal growth occurs in a second phase, sometimes called secondary mineralization, until the void regions are filled and lead to an extended crystalline aggregate that becomes distinctly packed platelets (Traub, Arad, and Weiner, 1989, 1992). This combination between collagen fibers and minerals crystals confers

to the bone its properties and the resistance to mechanical stress, combining strength and flexibility. Bone tissue is also a reservoir of minerals that can be redistributed to other organs in order to maintain physiological homeostasis (Tortora and Derrickson, 2010).

2.1.1 Bone cells

As a specialized connective tissue, bone includes four major categories of cells that are responsible for bone formation, maintenance and organization of the cellular matrix: osteoblasts, osteocytes, bone lining cells and osteoclasts (Marks and Popoff, 1988). These cells can be grouped into two main categories; the bone-forming cells (osteoblasts, osteocytes and bone lining cells) and the bone resorbing cells (osteoclasts), both types communicate in a close and permanent way by the secretion of soluble factors or by direct contact (Martin et al., 1998).

2.1.1.1 Osteoblasts

Osteoblasts are small mononucleate cuboid cells responsible for bone formation, they originate from pluripotent mesenchymal stem cells (Bassi, Gough, Zakikhani, and Downes, 2011). Mesenchymal stem cells can differentiate themselves into many cell types: myoblasts, fibroblasts, adipocytes, chondrocytes or the osteoprogenitor cells that will become osteoblasts. Through the action of specific transcription factors, mesenchymal stem cells give rise to precursor cells engaged in the osteoblastic pathway, which differentiate into preosteoblasts and then into osteoblasts that will progressively acquire the characteristics of a functional cell (Burr, Bellido, and Kenneth, 2015). The primary function of the mature osteoblasts is to synthetize bone extracellular matrix and the subsequent mineralization process. Osteoblasts secret type I collagen as well as numerous non-collagenic proteins, which form the osteoid. Their production is modulated by a variety of hormones, growth factors and cytokines. While the mechanism underlying the mineralization process is not fully understood, it is generally acknowledged that osteoblasts deposit calcium by mechanisms including phosphate and

calcium transport with alkalinization to absorb acid created by mineral deposition (Blair et al., 2011).

2.1.1.2 Osteocytes

Osteocytes result from the terminal differentiation of osteoblasts, which are embedded in the newly mineralized matrix. They are the most abundant cells in bone; about ten times more numerous than osteoblasts (Manolagas, 2000). Osteocytes are distributed throughout the matrix and maintain a communication network via communicating junctions (Couret, 2004). Morphologically, osteocytes are smaller cells essential for the continuous renewal of the extracellular matrix. They are ovoid in shape with fine and long cytoplasmic extensions. These cells communicate with each other and with bone lining cells forming a syncytium that operates through canaliculi (small channels in the bone tissue) in the bone matrix (Palumbo, Palazzini, Zaffe, and Marotti, 1990). The canaliculi allow the passage of nutrients and oxygen from blood vessels to the osteocytes as well as signaling molecules between the different cells. Thus, bone cells form a functional lacuno-canalicular network that remain connected at all stages of bone formation, from pre-osteoblasts to mature osteocytes (Franz-Odendaal, Hall, and Witten, 2006). Disruption of the network can have negative consequences and has been shown to be associated with microcrack formation (Chen et al., 2015). The location of osteocytes within the matrix confers on them the ability to sense mechanical stimuli placed on the bone, and to respond accordingly: osteocytes can send biomechanical signal to osteoblasts and osteoclasts to regulate their activity through the secretion of various signaling factors (Prideaux, Findlay, and Atkins, 2016). Moreover, osteocytes actively participate in the osteoid mineralization process during bone formation and play an important role in maintaining bone integrity (Atkins and Findlay, 2012).

2.1.1.3 Bone lining cells

After the completion of osteogenesis (bone formation process), some osteoblasts transform into inactive quiescent flat-shaped bone lining cells. They are osteoblasts that,

unlike osteocytes, have escaped burial in the bone matrix and have remained on the surface when bone formation has stopped (Martin et al., 1998). These cells exhibit processes extending into canaliculi, and gap junctions filled with extracellular fluid are also observed between adjacent bone cells and between these cells and osteocytes (Marks and Popoff, 1988; Miller, de Saint-Georges, Bowman, and Jee, 1989). However, unlike osteocytes, bone lining cells can be reactivated into osteoblasts depending on the bone physiological status and initiate bone synthesis (Arnett and Henderson, 1998; Martin et al., 1998). While the function of bone lining cells is not completely understood, they are able to prevent the interaction between osteoclasts and bone matrix when resorption should not occur (Mosley, 2000). Hence, they participate to the maintenance of bone integrity. Additionally, bone lining cells participate in osteoclast differentiation by producing osteoprotegerin (OPG), a factor able to reduce the production of osteoclasts (Florencio-Silva, Sasso, Sasso-Cerri, Simões, and Cerri, 2015).

2.1.1.4 Osteoclasts

Osteoclasts are multi-nucleated giant cells, with four to ten nuclei, that resorb bone. They have a short life span, surviving only a few days (Mentaverri et al., 2000). Stem cells producing osteoclasts are recruited from hematopoietic tissues such as bone marrow and splenic tissues and are transported to the bone by the bloodstream. More specifically, osteoclasts originate from mononuclear precursors (monocytes/macrophages) circulating in hematopoietic tissues under the influence of several factors (Couret, 2004; Frost, 1964a; Udagawa et al., 1990). These factors include a receptor activator of nuclear factor (RANK), present on the surface of osteoclasts. The agonist of this RANK receptor is RANK-L (RANK-ligand), a protein expressed and released by osteoblasts and lymphocytes. RANKL is a crucial factor because when it binds to its receptor RANK in osteoclast precursors, it induces osteoclast formation (Albouy, Rami, and Grimaud, 2006).

Osteoclasts are characterized morphologically by a ruffled border, which has numerous narrow finger-like protrusions penetrating the bone matrix. This morphology gives these cells their unique characteristics for bone resorption: around the ruffled border, the cells bind strongly to the organic matrix via $\alpha_\gamma\beta_3$-type integrin proteins (Suda, Nakamura, and Takahashi,

1997; Väänänen, Zhao, Mulari, and Halleen, 2000). Osteoclasts are sealed to the bone, delimiting a microenvironment poor in organelles but rich in actin filaments necessary for resorption: the clear zone (Fig. 1) (Väänänen and Horton, 1995). The resorption cycle requires cellular activity during which the osteoclast migrates on the resorption site, attaches tightly to the bone surface, dissolves both the organic and inorganic bone matrix, detaches, moves to another resorption site and finally, undergoes apoptosis (Macé, 2008; Väänänen et al., 2000). The release of protons expelled under the ruffled border by the proton pumps decreases the pH of the sub-osteoclastic environment, and thus induces the dissolution of the bone mineral phases (Macé, 2008; Väänänen et al., 2000). Osteoclasts then release lysosomal protease enzyme cathepsin K that degrades the matrix organic elements and forms a resorptive bay called Howship's lacuna (Macé, 2008; Teitelbaum, 2000). The product of this degradation is released to the circulatory system via the cell (Väänänen et al., 2000).

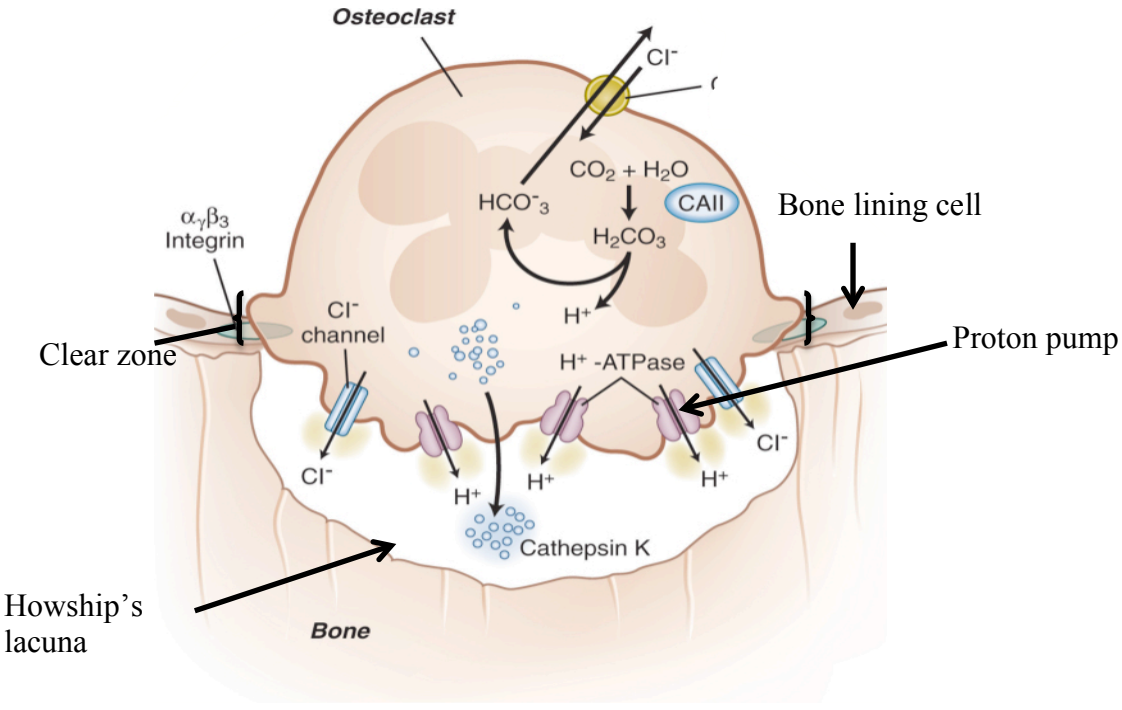


Figure 1. Molecular mechanisms of bone resorption (illustration modified from Tolar, Teitelbaum, and Orchard; 2004)

2.1.1.5 Regulation of bone cells

The functions of osteoblasts and osteoclasts are closely related and involve a complex interaction between the two cell lineages and their precursors (Ducy, Schinke, and Karsenty, 2000). A complex retroactive system, regulated by local factors (cytokines and growth factors) as well as systemic hormonal influences, controls the relative amount of bone formed and resorbed (Teitelbaum, 2002).

Hormonal regulation is dependent on the parathyroid-vitamin D axis, calcitonin, and estrogen. Calcitonin is an inhibitor of bone resorption, which binds to specific osteoclast receptors to gradually slow the cell multiplication. Parathyroid hormone (PTH) and estrogen mediate the differentiation of osteoclasts by acting on osteoblasts. They express on their surface a ligand, RANK-L and the interaction with RANK (RANK-L receptor and transmembrane protein) stimulate both osteoclast precursor differentiation into osteoclasts but also their activities. The differentiation and activity of osteoclasts is modulated by osteoprotegerin (OPG), which binds specifically to RANK-L and inhibits RANK coupling as seen in section 2.1.1.4 (Albouy et al., 2006). PTH inhibits the secretion of OPG and activates the expression of RANK-L. Estrogens stimulate the synthesis of transforming growth factor TGF-β, which in turn allows the synthesis of type I collagen. Hence, estrogen has a direct effect on osteoblasts by blocking the activation of osteoclasts through cytokines. Together, TGF-β and estrogen increase the expression of OPG. Vitamin D stimulates matrix synthesis and alkaline phosphatase (markers of bone formation) among others and acts indirectly by inhibiting the production of cytokines. It also allows better absorption of calcium and phosphorus (Albouy et al., 2006).

Growth hormone acts only moderately on the bone formation by stimulating the synthesis of insulin-like growth factor (IGF-1) by the osteoblast. IGF-1 is synthesized by osteoblasts and increases the number and activity of these cells. Its action, however, is modulated by binding proteins, one of which is called IGFBP-4 that inhibits osteoblastic proliferation. Fibroblast growth factors (FGF) will stimulate the proliferation of preosteoblasts and will secondarily induce an increase in collagen synthesis. At the cellular level, cytokines modulate bone metabolism. Although there are many kinds of cytokines, in bone regulation, they mostly activate osteoclastic differentiation and inhibit bone formation.

2.2 Osteogenesis

Bone usually develops by replacing a pre-existing connective tissue. During skeletal ontogeny, there are two osteogenic pathways, intramembranous ossification and endochondral ossification, but the resulting bone is identical. The distinction between the two processes stems from the fact that endochondral ossification is formed in the presence of cartilaginous frame or anlagen, while there is no anlagen in intramembranous ossification (Karaplis, 2008).

2.2.1 Intramembranous ossification

Membrane bones, such as the flat bones of the skull, develop by intramembranous ossification according to a defined sequence. First, the embryonic mesenchyme turns into a highly vascularized connective tissue (Scheuer and Black, 2000). Second, the mesenchymal cells develop the cuboid shape distinctive of the osteoblasts and begin to secrete the bone matrix. Many ossification centers develop later by the condensation of osteoblasts and merge. Due to the random orientation of the collagen fibers of the newly formed trabeculae, the primary intramembranous bone is called woven bone and forms rapidly during tissue growth or following an injury. New bone is formed by the deposition of calcium phosphate in the bone matrix leading to the inclusion of osteoblasts that become osteocytes and to the partial occlusion of the perivascular channels, which transform the mesenchymal cells into blood precursors (Kierszenbaum, 2006). The woven bone is subsequently replaced by lamellar bone where the newly synthesized collagen fibers align in regular bundles (Fang and Holl, 2013) through the modeling and remodeling process discussed below.

2.2.2 Endochondral ossification

Endochondral ossification is the process by which the embryonic cartilaginous model is replaced by bone. It is through endochondral ossification that long bones grow longitudinally. A primary ossification center is formed during endochondral ossification but, in contrast to intramembranous ossification, this ossification center derives from the

proliferation of chondrocytes responsible for the deposition of an extracellular matrix containing type II collagen, the ground substance of hyaline cartilage (Kierszenbaum, 2006). The chondrocytes undergo hypertrophy secreting growth factors allowing the formation of blood vessels from the perichondrium, which is the connective tissue surrounding the hyaline cartilage. Osteoprogenitor and hematopoietic cells are delivered by the newly formed blood vessels. These events lead to the formation of primary ossification center. Hypertrophic chondrocytes experience apoptosis while calcification of the matrix takes place in the center of the shaft (diaphysis). At the same time, the internal perichondrial cells, derived from osteoblasts, express their osteogenic potential, forming a thin collar at the periosteum. The periosteal collar formed around the medullary area by subperiosteal deposition follows the sequence of intramembranous bone formation and consists of reticular bone (Mackie, Ahmed, Tatarczuch, Chen, and Mirams, 2008). Later stages of endochondral ossification include the invasion of blood vessels into the space previously occupied by the hypertrophic chondrocytes, which will branch out and move to each end of the ossification center. Osteoprogenitor cells and hematopoietic stem cells arrive in the calcified cartilage via perivascular connective tissue surrounding the blood vessels. The osteoprogenitor cells are then differentiated into osteoblasts that accumulate on the surface of the calcified cartilage and begin to deposit bone matrix. Secondary ossification centers then develop in the epiphyses. The growth in length of long bones depends on the interstitial growth of hyaline cartilage at the growth plate while the cartilage center is progressively replaced by bone in equidistant ossification zones (Kierszenbaum, 2006). The cartilage matrix is thus not replaced at the growth plate and on the articular surfaces during growth (Frost, 1964c).

2.2.3 Bone envelopes

Bones can be divided into four distinct envelopes: the periosteal, endosteal, cancellous and intracortical envelopes (Gasser and Kneissel, 2017; Parfitt, 1983). These envelopes are morphologically distinct and play different roles in bone biology. The periosteal envelope is composed of two parts, an outer and an inner layer, which form a dense membrane called the periosteum. The outer fibrous layer contains fibroblasts-like cells, and the inner osteogenic

layer, called cambium, is populated with progenitor cells that develop into osteoblasts and enables bone to increase in diameter (Gasser and Kneissel, 2017). The periosteum is highly vascularized and innervated allowing bone cortical nutrition. The endosteal envelope is a thin layer of connective tissue that surrounds the marrow cavity. The endosteum is very active metabolically and is composed of a discontinuous layer of bone lining cells, associated with capillaries near the bone surface and in the marrow (Burr and Akkus, 2014). Both periosteum and endosteum cooperate during growth and adaptation to maintain bone integrity (Maggiano, 2012). The cancellous or trabecular envelope is similar to the endosteal surface: this surface is covered by a discontinuous layer of bone lining cells that respond to mechanical and biological signals in order to induce bone formation or resorption (Burr and Akkus, 2014; Gasser and Kneissel, 2017). Finally, the intracortical envelope represents the surfaces of the Haversian canals (as discussed in the section 2.3.2.1), which is also covered by a discontinuous layer of resting osteoprogenitor cells (Burr and Akkus, 2014; Gasser and Kneissel, 2017). The Haversian canal contains neurovascular bundle, whose main functions are to regulate nutrient exchange between the vascular system and the extracellular fluid compartments (Burr and Akkus, 2014). This envelope is the site of intracortical remodeling activity as discussed in the section 2.3.2.1.

2.3 Modeling and remodeling

Skeletal development is achieved by growth, modeling and remodeling. Growth and modeling work in concert and are considered together here. Modeling and remodeling refer to the action of osteoblasts and osteoclasts in order to preserve bone properties (Martin et al., 1998).

2.3.1 Bone growth and modeling

While growth increases a bone's length through endochondral ossification and diameter through intramembranous ossification (Enlow, 1963; Macé, 2008), modeling will sculpt the size, shape and curvature of the bone. The aim is to modify this basic architecture

allowed by growth in an optimal way to meet bone's mechanical and metabolic demands (Frost, 1987b). Consequently, when the bone tissue deposited on a bone experiences a change in mechanical loading, modeling selectively adds or removes bone from existing surfaces at the periosteum and endosteum respectively through mechanosensation, resulting in a modeling drift (Frost, 1986). Bone modeling involves osteoclast activation and subsequent resorption or osteoblast activation and subsequent formation on separate locations of cortical periosteal and endosteal surfaces (Frost, 1985; Frost, 1986, 1987b; Martin, 2003a). Thus, the removal of some portions of bone and the creation of other portion allowed by growth and modeling drifts result in a cortex of different boney tissue age. As a result, the mean tissue age of the cortex is less than the chronological age of the individual (Frost, 1987). Bone modeling is the predominant determinant of bone mass and morphology in the adult skeleton (Maggiano, 2012). Once skeletal maturity is reached, modeling reduces to a trivial level but can still occur in some pathological states or when the mechanical loads have been altered radically (Frost, 1986; Robling, 1998).

2.3.1.1 Primary lamellar bone

Modeling accounts for activity at the periosteal and endosteal envelopes and occurs either in rapid deposition of woven bone or in slower forming circumferential primary lamellar bone (Maggiano, 2012). This primary lamellar bone is deposited in parallel laminar sheets to the bone surface (Martin et al., 1998). In human bone, some primary vascular canals can be trapped within the layers of primary lamellae with some distinction between the periosteal and endosteal envelopes. While periosteal vessels are a common feature seen longitudinally, referred either as primary vascular canals or primary osteons (Enlow, 1969), trapped vessels on the endosteum are less common and with a radial progression (Aubry et al., 1990; Maggiano, 2012). Primary vascular canals may be surrounded by few concentric lamellae but do not possess a cement line unlike Haversian system (as seen in the section 2.3.2.1). Additionally, they are not from Haversian origin; they do not result from the coupled actions of resorption and subsequent formation of the remodeling process.

2.3.1.2 Modeling microstructure

Since the primary lamellar formation at the endosteal and periosteal envelopes occurs independently of resorption, the rate of those processes in each envelope differs. The rate of periosteal formation is highest during growth, countered by a rapid endosteal resorption in order to maintain a relatively consistent cortical thickness (Burr and Akkus, 2014). However, it has been shown that during and after growth spurt, endosteal formation occurs and can continue into the fourth or fifth decade of life (Garn, 1972; Maggiano, 2011), producing a distinct meta-feature when viewed in cross-section called the endosteal lamellar pocket (Maggiano, 2011). The persistence of this histomorphometric feature can be explained by the fact that during adulthood, periosteal formation slows and becomes more rapidly remodeled than endosteal envelope (Kerley, 1965).

Modeling formation and resorption occurs in small location leading to different phases, each of them being defined by consecutive and concentric lamellae that are uninterrupted (Maggiano, 2011). When viewed in cross-section, these phases can be identified by a change in lamellar orientation, either with or without disruptions to the lamellar structure (Maggiano, 2011). As a result, when disruption does occur when bone formation ceases and is later reinitiated, a modeling arrest line can easily be identified as a smooth and distinct demarcation (Maggiano, 2011; Morey and Baylink, 1978). While modeling arrest lines have not been extensively studied in human cortical bone, it has received more attention in animal studies, especially in bovids, dinosaurs, birds and reptiles and it is referred as lines of arrested growth (LAG) (Castanet et al., 2004; de Ricqlès. 1983; de Ricqlès et al. 2003, 2008; Sander and Bonn, 2006). LAGs appear brightest under polarized light and are generally lightly hypermineralized. Although the histological features of LAGs seem similar to the modeling arrest line seen in human or to the definition of double-zonal osteons as discussed in sections 1.1 and 2.3.2.2, they correspond to a cyclical growth that correlate with a decrease in metabolic rate during cold season (Castanet et al., 1993; Köhler et al., 2012). LAGs are found at the periosteal envelope and on the whole cross-section before being eventually erased by remodeling (Castanet et al., 2004) unlike human modeling arrest line that shows discontinuity and are found on the endosteal surface. Since no seasonal differences in basal metabolic rate has been observed in human (Anthanont et al., 2017), it is unlikely that LAGs and modeling

arrest line in human share the same origin. Additionally, LAGs are present only in fibro-lamellar bone, a highly vascularized tissue allowing rapid osteogenesis and fast growth (Ray et al., 2004) whereas double-zonal osteons are the product of the coupled action of resorption and formation during the remodeling process (see section 2.3). A better characterization of the collagen as well as the cellular activity at the LAG, modeling arret line and double-zonal osteon could lead to other distinction, but further investigations are needed.

2.3.2 Remodeling

Of particular interest to this study is the action of remodeling on cortical bone. A function of remodeling is the release of nutrients, such as calcium or vitamin D, which are essential components involved in the regulation of the phosphocalcic metabolism and bone homeostasis. Through the remodeling process, portions of primary or secondary bone are replaced with newly formed, secondary bone (Martin et al., 1998). Remodeling is cumulative and it will eventually also replace older secondary bone (Leondes, 2001). During growth, modeling and remodeling act simultaneously in different sites or in some instances, modeling occurs but no remodeling (Macé, 2008). Unlike modeling, which involves either formation or resorption but not at the same location, bone remodeling always follows a well-defined sequence allowing for the replacement of measurable packets of bone (Parfitt, 1979). During intracortical bone remodeling, these replacement packets take the form of the basic multicellular unit (BMU), or osteon, created through the coupled action of bone cells and occurs over an individual lifetime (Frost, 1986). It has been estimated that approximately 5% of the adult compact bone is remodeled each year (Martin et al. 1998).

2.3.2.1 Basic Multicellular Unit and bone remodeling cycle

The cells that remodel bone tissue (osteoblastic and osteoclastic lineages) compose the BMU (Parfitt et al., 1987) and allow the formation of Haversian system, also called osteons (Enlow, 1962). These BMUs contain blood vessels, nerves, and connective tissues that are surrounded by a Haversian canal (Parfitt, 1994a). Lymphatic vessels are not present in Haversian canal but prelymphatic vessels can be found (Burr and Akkus, 2014). In cortical

bone, BMUs form a cylindrical channel, in which they gradually dig into the bone. This is achieved by approximately ten osteoclasts that resorb bone in the direction of the dominant loads (Martin et al., 1998; Petrtýl, Hert, and Fiala, 1996). Osteoblasts are then followed by about a thousand osteoblasts that fill this channel to produce a secondary osteon consisting of the renewed bone (Fig. 2) (Parfitt, 1994a).

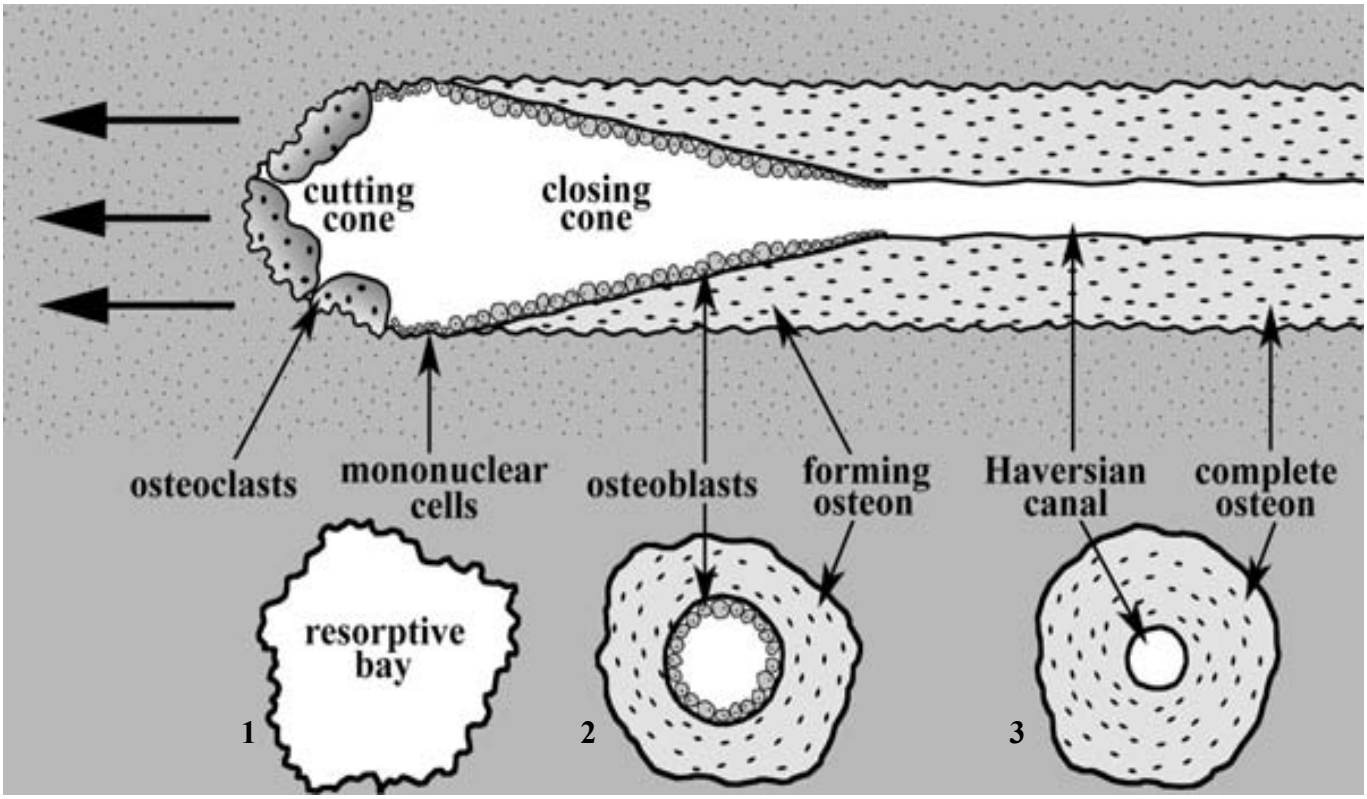


Figure 2. Longitudinal diagram (top) of a basic multicellular unit (BMU) and cross-sectional views (bottom) at different points as the BMU travels through bone tissue space from right to left. The cross-sectional views show the various stages of osteon formation: resorptive bay (1); forming osteon (2); and complete osteon (3). Illustration modified from Robling and Stout (2000).

The bone remodeling sequence is divided into four phases: activation, resorption, inversion and formation (ARIF; Frost, 1985; Frost, 1964b; Parfitt, Mundy, Roodman, Hughes, and Boyce, 1996). The median time of the remodeling cycle in cortical bone is about 120 days, with approximately three weeks for the resorption phase and three month for the formation

phase (Eriksen, 2010). Under some signal that is now considered to emanate from osteocytes (Bonewald, 2007), osteoresorptive factors (hormones and vitamins) are secreted to retract the bone lining cells covering the bone matrix to initiate the activation phase. The mononuclear osteoclasts are then able to anchor to binding sites at the bone surface and merge, thus becoming multinucleated (Macé, 2008). The resorption phase begins, unfolding in two successive stages: the dissolution of the mineral phase by acidification and then the degradation of the organic matrix under the action of enzymes, thus creating the cavity known as a Howship's lacuna. When the osteoclasts have finished digging a lacuna, they die by apoptosis and are first replaced by macrophages, which smooth the bottom of the lacuna, and later by a vascular front of blood vessels: this is the inversion phase. When bone resorption is complete, the osteoprogenitor cells present at the surface of the bone tissue will differentiate into osteoblasts. Their precursors are differentiated at the bottom of the lacuna, called the cement line or reversal line. Finally the bone formation phase involves two stages during which osteoblasts are the main actors: the production of the extracellular matrix and the mineralization of the matrix (Frost, 1964a; Raggatt and Partridge, 2010).

There is growing evidence that bone vascularization is crucial to the remodeling process by maintaining a structural and functional relationships with bone cells in the BMU (Lafage-Proust et al., 2015; Parfitt, 2000). The circular arrangement of cells around the Haversian canal is the most efficient system in order to supply the maximum amount of bone tissue from the minimum number of vessels (Burr and Akkus, 2014). The cells that line the interior surface of the vessels (endothelial cells) contain numerous vesicles that are thought to facilitate the transport of water and nutrients across the capillary wall (Burr and Akkus, 2014). Parfitt (2000) proposed a theory in which vascularization of a progressing BMU could recruit osteoclasts and osteoblasts precursors to sites undergoing active remodeling in order to explain the coupling concept. Specifically, osteoprogenitors from the osteoblastic lineage are recruited from the endothelial cells located in the canopy that isolate the BMU from the bone marrow (Hauge et al., 2001). However, the involvement of vascular cells in the initiation of bone resorption has yet to be empirically demonstrated.

2.3.2.2 Types of osteons

Four morphological variations of Haversian system or osteons have been identified. They occur during intracortical remodeling, reflecting different pattern of formation: type I osteons, drifting osteons, type II osteons and zonal osteons.

Type I osteons

Type I osteons also commonly called secondary osteons, are the most prevalent form of Haversian system in adult humans (Takahashi et al., 1965) (Fig.3A). When viewed in cross section, type I osteons are characterized by a relatively circular shape where concentric lamellar bone is contained within a scalloped cement or reversal line, that delimits the osteoclastic resorption from the subsequent osteoblastic formation (Martin and Burr, 1989). However, three-dimensional studies have shown a more irregular cylindrical structure (Hennig et al., 2015). They are defined by uninterrupted concentrically deposited lamellae surrounding a centrally located Haversian canal (Martin et al., 1998).

Drifting osteons

Drifting osteons appear in cross section to be transversally elongated with a tail of hemicyclic lamellae and an eccentric Haversian canal (Epker and Frost, 1965; Frost, 1964a; Lacroix, 1971; Robling and Stout, 1999; Sedlin et al., 1963; Streeter, 2010) (Fig.3B). Drifting osteons are described as continuous resorption occurring on one side of the structure, evidenced by the scalloped reversal line, and a continuous smooth formation front on the other side (Epker and Frost, 1965). They are the results of a BMU that travels both longitudinally and transversally through the cortex. The stimulus involved in the creation and guidance of this drift remains unclear, but it is thought to be related to a complex strain environment (Robling and Stout, 1999). Several studies have shown that drifting osteons are inversely related to age, and thus, are more prevalent in subadults bones (Burton, Nyssen-Behets, and Dhem, 1989; Coutelier, 1976; Epker and Frost, 1965; Lacroix, 1971; Sedlin et al., 1963; Streeter, 2005; Streeter, 2010). However, they are also observed in adult bone but at lower numbers (Robling and Stout, 1999).

Type II osteons

Type II or embedded osteons result from the intra-osteonal remodeling of a pre-existing Haversian canal (Ericksen, 1991; Lacroix, 1971; Richman et al., 1979) (Fig.3C). Type II osteons are formed by the radial erosion of a previously existing Haversian canal followed by centripetal deposition of new lamellae surrounded by a scalloped reversal line. Therefore, type II osteons have the appearance of an osteon within an osteon in cross section, with two distinct scalloped reversal lines, one within the other (Ericksen, 1980; Ericksen, 1991; Frost, 1964a; Jaworski et al., 1972; Lacroix, 1971; Ortner, 2003; Takahashi et al., 1965). While previously described as being of a limited length of the Haversian canal (Ericksen and Stix, 1991; Jaworski et al., 1972; Ortner, 1975; Richman et al., 1979), recent works on three-dimensional imaging have demonstrated that type II osteons are a longer, continuous structure, reaching even 7.5 mm of length (Arhatari et al., 2011; Maggiano et al., 2016, Maggiano et al., 2017). Their relationship to internal and external factors is not well understood but they are thought to be correlated with non-specific stress (Ericksen, 1991; Frost, 1964a; Ortner, 1975; Richman et al., 1979; Stout and Simmons, 1979; Takahashi and Frost, 1966). Both Ericksen (1991) and Yoshino et al. (1994) have reported an association between type II osteons and increasing age however, Richman and colleagues (1979) found no significant association with age. More specifically, it has been demonstrated that type II osteons are linked to dietary deficiency and a response to the demands of mineral homeostasis (Ericksen, 1980; Ericksen, 1991; Ortner, 1975; Richman, Ortner, and Schulter-Ellis, 1979; Yoshino et al., 1994).

Double-zonal osteons

Double-zonal osteons are the result of a disturbance of normal intracortical osteon production (Martin et al., 1998). Morphologically, they are characterized in a cross section by one or more smooth hyper-mineralized ring inside their lamellae (Frost, 1964b; Kornblum and Kelly, 1964; Lacroix, 1971; Lacroix and Dhem, 1967; Smith, 1963) (Fig.3D). As in the case with type II osteons, some studies have suggested a link between double-zonal osteons and stress. Specifically, it has been hypothesized that DZ osteons could be associated with metabolic diseases and aging (Martin, 1983; Martin and Armelagos, 1985; Mays, 1985; Nyssen-Behets et al., 1991; Pankovich et al., 1974; Stout and Simmons, 1979).

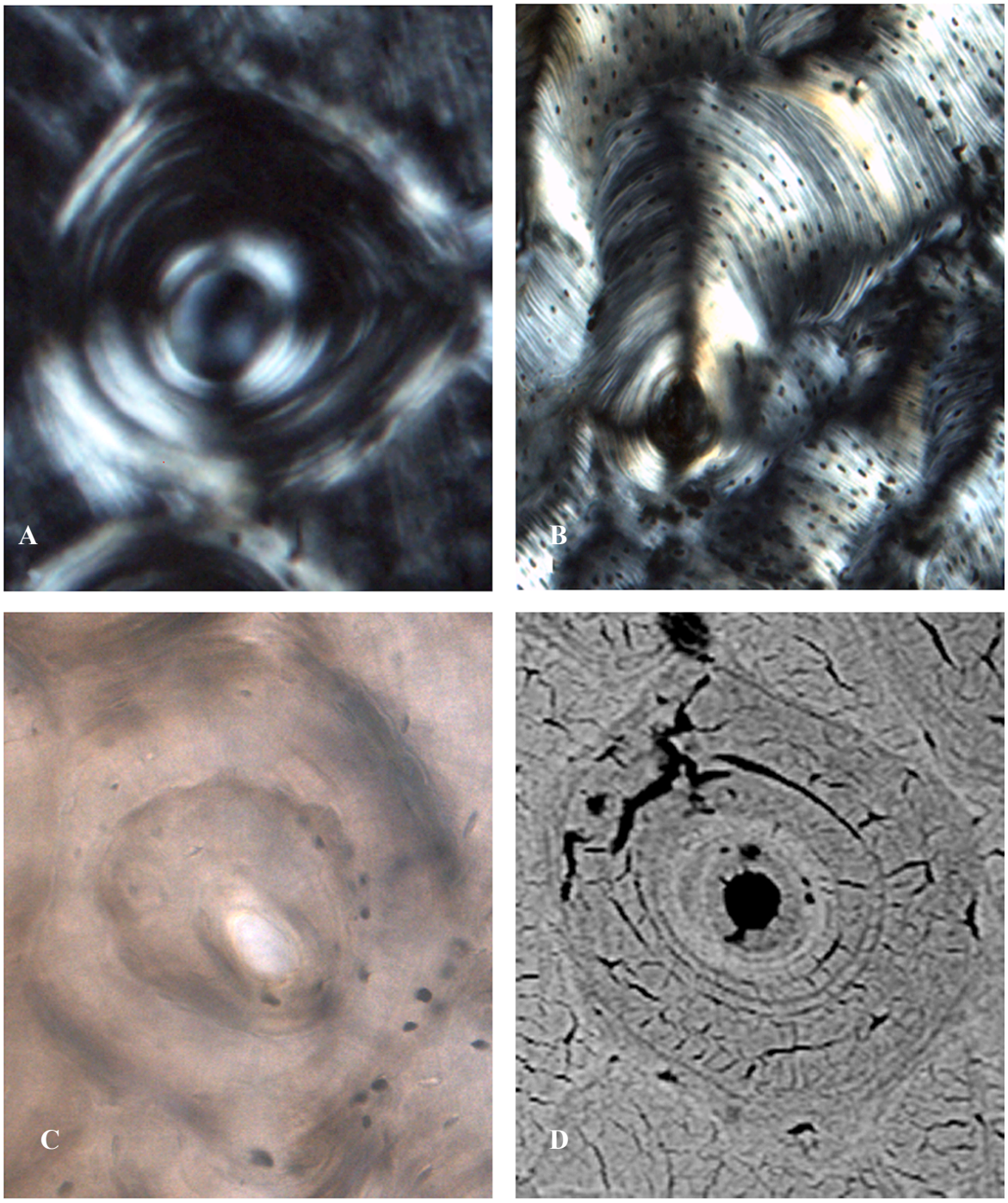


Figure 3. The four morphological types of osteons: A. Type I osteon (polarized light microscopy, 100X magnification); B. Drifting osteon (polarized light microscopy, 100X magnification); C. Type II osteon (bright field microscopy, 200X magnification); D. Double-zonal osteons (Backscattered Electron Microscopy with Scanning Electron Microscope, 70X magnification).

2.3.2.3 General osteon morphology

Studies on three-dimensional perspective of Haversians system have brought new insights into the general osteon morphology and vascular system (Arhatari et al., 2011; Cooper et al., 2011, Hennig et al., 2015; Maggiano et al., 2015, 2016, 2017; Stout et al., 1999). These studies highlight the more complex structures of secondary osteons, either as a single entity or in relation to other osteons. Using a synchrotron radiation-based micro-CT, Maggiano et al (2016) showed that BMU can have different types of branching : lateral branching, dichotomous branching and branching following repathing. Lateral branching results in small unidirectional BMUs that branch off of a previously existing, larger Haversian system. Dichotomous branching are found when Haversian system bifurcates into two osteonal branches of similar size, suggesting that these branches grow simultaneously. They are responsible for the super-osteon or remodeling clusters previously identified in cross-section (Bell et al., 2001). Branching following repathing occurs when previously existing Haversian systems become fragmentary by a repathing system. According to Maggiano and colleagues, a transverse canal created by an angiogenic event supplying the younger Haversian system with its own vessel system, reroutes the canal and potentially the vessel of the fragmented osteons. This last branching system has been identified in Type II osteons, suggesting that osteons fragments may result from a type II osteon (Maggiano et al., 2016).

The general osteon morphology is also defined by the direction of the BMU, which has functional and structural significance. As previously mentioned in the section 2.3.2.1, it is generally accepted that secondary osteons are aligned in the direction of dominant loads experienced by the bone tissue (Martin et al., 1998; Petrtyl et al, 1996). While the continuity of Haversian systems is often represented as being unidirectional, Tappen (1977) found that BMUs can frequently tunnel both in proximal and distal direction, as paired, thus creating two cutting cones. This led Van Oers and colleagues (2008) to confirm these findings using computer simulations. The authors found that paired Haversian systems result from the strain environment around the resorptive bay. According to the authors, this mechanism orients BMUs in the loading direction. Additionally, it has been hypothesized that BMUs have the ability to steer towards microcracks during targeted remodeling (Martin, 2007) (explained in the section 2.3.2.4.2 « physical activity »). This change in the course of an active BMU can be

visualized through three-dimensional imaging : Cooper et al. (2011) have shown the transitions from a roughly cylindrical structure to a drifting form, suggesting that the different type of osteons recognized in 2D cross-section does not necessarily apply at the level of the 3D osteonal network and may account for the osteon size variability.

2.3.2.4 Factors influencing bone remodeling

While bone remodeling is a complex process regulated at the cellular level by local and hormonal factors as discussed in the section 2.1.1.5, biological or environmental influences have the potential to affect bone remodeling. An understanding of such effects is crucial to interpret bone histomorphometry in order to evaluate normal from abnormal remodeling. Such influences include, but are not limited to, local and systemic diseases, nutrition, age, sex, ancestry, and physical activity (Frost, 1987b).

2.3.2.4.1 Biological factors

- **Genetic**

Studies of the incidence of osteoporosis, a severe manifestation of bone loss and a major health issue in modern societies (Cole, Dennison, and Cooper, 2008), have demonstrated unequivocally that genetics plays a major role in bone metabolism and bone mass variation (Heaney et al., 2000; Kelly et al., 1991; Kelly, Morrison, Sambrook, Nguyen, and Eisman, 1995; Matkovic, Fontana, Tominac, Goel, and Chesnut, 1990; Ralston and de Crombrugghe, 2006). However, intracortical remodeling variation has been less systematically explored, mainly because microscopic structures to assess bone remodeling cannot be visualized through in vivo studies of humans (Havill et al., 2013). Nonetheless, Bjørnerem et al. (2015), when analyzing twins remodeling markers in the radius and tibia, found that genetic factors explain 55 to 62% of the variances, consistent with previous findings (Garnero, Arden, Griffiths, Delmas, and Spector, 1996; Wagner, Melhus, Pedersen, and Michaëlsson, 2013). In addition, Havill (2003) have shown in nonhuman primates that osteon size and

Haversian canal size are influenced by genetic variation. In a subsequent study on baboons (Havill et al., 2013), the authors have determined that 48 to 75% of the variance in the osteon area and percentage osteonal bone is due to genetics.

- **Population variation**

Differences in bone remodeling rates have been proposed between populations, although results can be contradictory. Weinstein and Bell (1988) have shown that African Americans have a lower rate of bone turnover in trabecular bone than European Americans. These authors proposed that a lower rate of bone resorption seen in blacks is the result of differences in the amount of bone accumulated during growth and would help to maintain a higher peak bone mass. Similarly, Cho et al. (2002) found that African Americans have lower remodeling rates as well as significantly smaller osteons when compared to European Americans. They used this basis to develop population specific age estimation formulas. On the other hand, larger osteons and larger Haversian canals increase bone porosity, resulting in European Americans being more susceptible to diseases such as osteoporosis (Anderson and Pollitzer, 1994; Cho, Stout, and Bishop, 2006). However, Schnitzler (1993) reached an opposite conclusion when working with bone of both White and Black Africans. He found that African Blacks had higher bone remodeling rates than African Whites. Based on his results, he concludes that the more frequent remodeling cycles ensured better bone quality and indicated adaptation to biomechanical stress. Studies on Inuit samples were also found to show a higher remodeling rates than American whites (Thompson and Gunness-Hey, 1981) and Native American populations (Ericksen, 1973; Richman et al., 1979). Consequently, while evidence of population-level variation has been observed, the lack of control over other influences has led to some uncertainty regarding the degree to which ancestry might influence bone remodeling.

- **Age**

As seen previously, bone remodeling occurs continuously from before birth until death. As a result, the number of osteons in a bone section increases with age (Kerley, 1965). This

principle is the fundamental basis for histological methods of age-at-death estimation (Cho, Stout, Madsen, and Streeter, 2002; Ericksen, 1991; Kerley, 1965; Kerley and Ubelaker, 1978; Stout and Paine, 1992; Streeter et al., 2001; Yoshino et al., 1994). As the density (number per unit area) of osteons increases with age, the cortex becomes crowded with secondary osteons and the creation of each new osteons potentially removes portions of older ones. Thus, osteon fragments are created, which increase in numbers with age (Frost, 1987). In addition, further remodeling will start erasing earlier evidences of remodeling, so that the number of intact and fragmentary osteons per unit area (OPD) stops increasing at a certain age, reaching an asymptote (Frost, 1987a; Robling and Stout, 2007). It has been shown that this asymptote is reached at about 60 years of age in ribs (Frost, 1987), but no estimate exists for other bones of the human skeleton. Studies have also shown that the type of osteon created varies with age. For example, the frequency of drifting osteons has been found to be inversely related to age due to the modeling drift and possibly induced by a complex strain environment incurred by bones and/or by metabolic requirements (Coutelier, 1976; Robling et Stout, 1999; Sedlin et al., 1963) and thus, are more frequently seen in subadults (Burton et al., 1989; Lacroix, 1971; Streeter, 2005; Streeter, 2010).

Age has also been associated with a decrease in the amount of bone deposited in each remodeling cycle, possibly a consequence of the reduction in the number of available cells from the bone-forming lineage (Lee, Fletcher, and Tarantal, 2005; Szulc and Seeman, 2009). This imbalance between bone resorption and bone formation results in an increased bone loss as individuals age. Histomorphological studies have shown that Haversian canal become larger with age, leading to marked porosity (Burr, Ruff, and Thompson, 1990; Cho and Stout, 2003; Jowsey, 1966; Nyssen-Behets, Duchesne, and Dhem, 1997; Pfeiffer, 1998; Thompson, 1980). Severe manifestations of bone loss result in an uncoupling of bone formation and resorption during the remodeling process that can modify bone architecture and have mechanical consequences, leading eventually to osteoporosis (Cho and Stout, 2003).

Finally, numerous studies have shown that osteon area decreases with age (Britz, Thomas, Clement, and Cooper, 2009; Currey, 1964; Dominguez and Agnew, 2016; Martin, Pickett, and Zinaich, 1980; Takahashi et al., 1965; Yoshino et al., 1994), but this relationship still needs to be explained. While Takahashi et al. (1965) argue that this age difference is due

to the deduction that larger osteons have a higher probability than smaller osteons to be overlapped by subsequent remodeling events that increase with age. Martin et al. (1980) propose instead that a decrease in osteoclastic activity with age result in the creation of smaller osteons. There is no evidence, however, to support either of these hypotheses.

- **Sex**

There is considerable evidence of the differences in bone remodeling between males and females. The maturation rate between males and females during growth is different: females form their cortex at an earlier age (Bonjour, Theintz, Buchs, Slosman, and Rizzoli, 1991; Tanner, 1990), and thus the accumulation of osteons is higher than that of males at the same chronological age. An increase of bone remodeling rate is also noted after the onset of menopause where females experienced an increase in remodeling activity compared to males (Parfitt, 1979; Recker, Lappe, Davies, and Heaney, 2004).

Sex steroids (hormones) play a key role in the regulation of bone remodeling and in the maintenance of bone health, as discussed in the section 2.1.1.5 and fluctuation in their production have a direct effect on intracortical remodeling (Compston, 2001; Manolagas, Kousteni, and Jilka, 2002; Samson and Branigan, 1987; Syed and Khosla, 2005). While testosterone production is stable throughout life in males, females experience a diminution in estrogen levels during their menstrual cycle and a major decline after menopause. When estrogen production stops or is significantly reduced, the balance of bone remodeling changes in favor of osteoclastic resorption (Silberberg and Silberberg, 1972), which accounts for the significantly higher remodeling rate usually seen in older females. Studies have also shown that during puberty, females accumulate calcium in bone at a higher and faster rate than do males as a possible adaptation to meet the needs of future reproductive cycles (Bailey, Martin, McKay, Whiting, and Mirwald, 2000; Bowman and Miller, 2001; Wastney et al., 2000; Zanchetta, Plotkin, and Filgueira, 1995). During pregnancy, calcium requirements increase gradually, coinciding with the mineralization of the fetal skeleton (Bowman and Miller, 2001). Bone remodeling also increases during this period and induces an imbalance. While the processes of bone formation and resorption in non-pregnant females is achieved simultaneously, these processes dissociate in pregnant females with a predominance of

resorption over formation (Naylor, Iqbal, Fledelius, Fraser, and Eastell, 2000; Yoon, Lee, Choi, Roh, and Lee, 2000). This significant imbalance between bone resorption and formation has been hypothesized to contribute to the fetal calcium requirement, while maintaining the mother's calcium homeostasis (Black, Topping, Durham, Farquharson, and Fraser, 2000). An increase in bone remodeling with increased bone resorption is also observed during lactation and breastfeeding (Kent et al., 1990; Maryfran Sowers, 1996). After weaning, a period of "recovery" occurs characterized by a dramatic increase of bone formation in association with improved biomechanical properties of bone (Kent et al., 1990; López, González, Reyes, Campino, and Díaz, 1996; Sowers et al., 1993).

In addition to the sex-related variation in bone remodeling rate, differences have also been observed in osteon size. The results of these studies, however, are conflicting. Burr et al. (1990) found that Pecos Pueblos females have larger osteons in the femur than males and Mulhern and Van Gerven (1997) reached the same conclusion in a medieval Nubian population. They both suggest that a sex-based division of labor has led to these results, where males were engaged in more intense activity, resulting in smaller osteon area when compared to their female counterparts. However, the opposite result was found in the metacarpals of an archaeological sample (Denny, 2010) and in the femur of a modern sample (Britz et al., 2009) and some studies did not find significant sex differences (Borgel, 2017; Dominguez and Agnew, 2016; Pfeiffer, 1998). Interestingly, in a modern American sample, Dominguez and Crowder (2015) observed that females have larger osteons than males but the relationship was reversed in the elderly, suggesting that the hormonal status might play a determinant role in osteon area.

2.3.2.4.2 Environmental factors

- **Nutrition**

The development and maintenance of bone mass and remodeling require essential nutrients such as minerals, vitamins, amino acids, fatty acids and water (Myneni and Mezey,

2017; Seibel, 2002). Any imbalance in the dietary intake can have deleterious effects on bone health.

The parathyroid hormone (PTH, section 2.1.1.5) is the principal regulator of the bone remodeling activity by promoting the number of osteoclasts and its secretion is influenced by calcium intake (Heaney, 2001; Parfitt, 1976a). It has been shown that insufficient calcium intake can impair bone development in childhood and accelerates bone loss and may contribute to osteoporosis in adults (Flynn, 2003). Specifically, Heaney et al. (1999) have demonstrated in a longitudinal study that calcium supplementation by the addition of milk to the Western diet resulted in positive bone balance and decreasing levels of PTH. Deficiency in magnesium has a similar effect to insufficient calcium intake by impairing the secretion of PTH, which causes an uncoupling of osteoblasts and osteoclasts and increased bone resorption during remodeling (Castiglioni, Cazzaniga, Albisetti, and Maier, 2013). Phosphorus intake does not seem to play a major role in the regulation of bone metabolism, but excessive intake is associated with low calcium accompanied by an elevation of PTH with bone hyper-resorption.

High protein diet is thought to increase acid production by the dissolution of bone mineral and, thus, to increase renal excretion of acid associated with increased bone loss (Breuil and Euller-Ziegler, 2004). However, recent findings suggest the contrary, that the production of acid due to high-protein intake is counteracted by an increased intestinal calcium uptake rather than a bone loss and has a beneficial effect on bone remodeling (Breuil and Euller-Ziegler, 2004; Cao, Johnson, and Hunt, 2011; Guardia, Roggi, and Cena, 2016). Regardless of the effect on bone quality, Richman et al. (1979) found that the Inuit, who rely mainly on a high-protein diet have more Type II osteons as described in section 2.3.2.2 when compared to the Pueblo and Arikara, who are characterized by low-protein diets. Streeter (2005) suggests instead that the metabolic response to higher levels of acid in the blood stream is a restoration to a more neutral pH by temporary resorption along portions of pre-existing Haversian systems, thus resulting in the formation of type II osteons.

- **Pathological conditions**

Many pathological conditions can affect bone remodeling (Frost, 1985; Frost, 1964a; Ortner, 2003; Schultz, 2001; Stout, 1982; Wu, Schubeck, Frost, and Villanueva, 1970). Pathological conditions can create an imbalance in the normal processes of bone resorption and formation. As a result, bone can react to abnormal conditions by increasing or decreasing the normal processes of formation, resorption, or a combination of both processes (Ortner, 2003).

Metabolic bone diseases can certainly have an impact on bone remodeling by interrupting mineral homeostasis. These diseases describe more generally the disruption in the bone formation process and remodeling processes, and affect the whole skeleton (Albright and Reifenstein, 1948; Brickley and Ives, 2010). For example, osteogenesis imperfecta is associated with an increase in the number of osteons created per year with prolonged modeling through adulthood (Wu et al., 1970). Hyperparathyroidism, Paget's disease and vitamin D deficiency are also associated with increased osteons numbers created per year, but without any effect on bone modeling (Robling and Stout, 2007). In general, these diseases increase the bone remodeling rate. With diabetes mellitus, on the other hand, bone remodeling rates are slowed. Whereas the effects of specific diseases on bone remodeling, such as those named above have been widely documented in clinical samples, the microscopic evaluation of such conditions in past populations is generally based on bone loss and on remodeling rate estimation (Agarwal and Stout, 2003; Brickley and Ives, 2010). Unfortunately, histomorphometric assessment of pathologies regarding osteon size or osteon morphology has not been widely investigated (Martin, 1983).

In contrast to diseases that have systemic effects, bone remodeling rates can also be perturbed locally in the area of a pathology, a factor termed "regional acceleratory phenomenon" (RAP) by Frost (1983a). The effects of RAP are manifested in localized pathologies such as trauma, bone infections, metastases, or local circulatory complications and are not detectable in distant bone sections (Robling and Stout, 2007). Therefore, an area of bone that is suspected to have pathology should be excluded from histomorphological evaluation because of the aberrant remodeling induced by RAP.

- **Physical activity**

Since one of the main functions of remodeling is to maintain bone mechanical properties, variations in strain levels necessarily contribute to changes in bone remodeling. While long bones are generally well adapted to habitual loads incurred in normal activities, repetitive strains produce microdamage, which when accumulated lead eventually to bone failure. It is well established that the remodeling rate increases with physical activity by increasing BMUs activation through the coupled action of osteoblasts and osteoclasts in order to maintain bone integrity through the process of targeted remodeling explained below (Bouvier and Hylander, 1981; Burr, Martin, Schaffler, and Radin, 1985; Lanyon, 1984; Lieberman and Crompton, 1998; Lieberman, Pearson, Polk, Demes, and Crompton, 2003; Robling, 1998; Schaffler and Burr, 1988; Skedros, Mendenhall, Kiser, and Winet, 2009; Yeni, Brown, Wang, and Norman, 1997). Paradoxically, when bones encounter a dramatic decrease in mechanical loading, as seen in astronauts not subject to gravity or in sustained bed rest, the result will also be an increased in remodeling activity (Lau and Guo, 2011; Robling, Castillo, and Turner, 2006), but with a net bone loss because of an imbalance between resorption and formation. Osteoblasts differentiation, lifespan and activity are under regulation of mechanical loading and in the absence of adequate strain, fewer osteoblasts will be recruited in each remodeling unit, leading to a negative bone balance (Hughes and Petit, 2010). Thus, the effects of loading on bone remodeling rate follow a U-shaped curve, where both increase or significantly reduced loading trigger an increased remodeling response, but with very different results in terms of bone mass (Robling, 2006).

Frost (1987b) proposed a mechanical feedback system that regulates bone mass during growth through modeling and remodeling that he named “the mechanostat”. In this model, Frost uses the analogy of the home thermostat to establish a strain threshold called the minimum effective strains (MES). When strains are maintained within the customary strain range, no bone response occurs except normal growth and remodeling. Strains below the threshold of the MES promote bone resorption, which remove bone permanently from the endosteal envelope leading eventually to osteopenia or osteoporosis (Frost, 1997). Conversely, when strains are above the threshold of the MES, bone deposition will be stimulated, eventually leveling the strain incurred by bone and thus, keeping remodeling in a conservation

mode. However, repeated elevated strains in bone eventually lead to microcracks and subsequent bone failure as previously stated (Frost, 2000). To maintain the mechanical properties of the tissue, damaged bone will be replaced through the process of targeted remodeling to prevent microcrack propagation and accumulation (Martin and Burr, 1982; Burr, 2002a; Martin, 2007; Parfitt, 2002). Experimental studies have shown that microcracks in bone can initiate remodeling in order to ultimately stop the cracks (Burr et al., 1985; Bentolila, Boyce, Fyrie, Drumb, Skerry and Schaffler, 1998; Mori and Burr, 1993; Verborgt, Gibson and Schaffler, 2000). Targeted remodeling increases the remodeling rate and thus produces higher osteon counts where loads incurred are higher (Lieberman et al., 2003). Moreover,

The mechanisms responsible for either targeted or non-targeted remodeling are still not fully understood but osteocytes have been identified as the mechanosensory cells that translate the mechanical load to bone cells, which initiate the remodeling response (Burger, Klein-Nulend, Van Der Plas, and Nijweide, 1995; Klein-Nulend et al., 1995; You et al., 2008). Frost (1960) initially proposed that microcracks disrupt the lacuna-canalicular network between osteocytes, providing the stimulus needed to initiate remodeling. Rather, Burr (2002a) suggests that the osteocytes lacuno-canalicular network acts as an inhibitor of the osteoclastic activity. Indeed, microdamage has been associated with reduced fluid flow and osteocytes apoptosis, which are in turn related to new remodeling events (Verborgt, Gibson, and Schaffler, 2000). Both targeted and non-targeted remodeling occurs simultaneously in cortical bone. While the first aims to repair the microdamages, the second occurs randomly in bones as part of normal homeostasis and accounts for the majority of remodeling activities (Martin and Burr, 1982; Burr, 2002a; Martin et al., 1998).

Many studies have attempted to evaluate the impact of biomechanical loads on osteon size (Abbott, Trinkaus, and Burr, 1996, Borgel, 2017; Britz et al., 2009; Corondan and Haworth, 1986; Denny, 2010; Moyle and Bowden, 1984; Pfeiffer, Crowder, Harrington, and Brown, 2006; Skedros, Keenan, Williams, and Kiser, 2013; Skedros et al., 1994; Skedros et al., 2009; van Oers, Ruimerman, van Rietbergen, Hilbers, and Huiskes, 2008; Yeni et al., 1997) and have produced contradictory results. A more detailed review of the studies regarding osteon size and biomechanical properties is provided in Chapter 5. Briefly, while

some authors have demonstrated that larger osteons are associated with increased mechanical demands (Corondan and Haworth, 1986; Borgel, 2017; Moyle and Bowden, 1984; Yeni et al., 1997), others have found a significant correlation between smaller osteons and higher bone strains (Abbott et al. 1996; Britz et al., 2009; Van Oers et al., 2008). Several studies found no significant relationship between osteon area and mechanical loads (Denny, 2010; Dominguez et Agnew, 2016; Pfeiffer et al. 2006; Skedros, Keenan, Williams, and Kiser, 2013). Interestingly, when comparing the osteon size and strain mode in the artiodactyl calcaneus, Skedros et al. (1994) showed that smaller osteons were found in the larger compressive regions of the cortex while the smaller tensile regions housed larger osteons, suggesting that the variation in strain mode could produce both smaller and larger osteon but they acknowledge that this relationship is till poorly understood (Skedros et al., 2013). These studies highlighted the fact that mechanical loads have a possible role in determining osteon size, but also that little is understood regarding the underlying mechanisms.

Bone has the ability to constantly redefine its mass and morphology through modeling and remodeling processes in order to accommodate changes in mechanical and metabolic demands (Sommerfeldt and Rubin, 2001). As a result, environmental or biological factors will affect the ability of the bone to maintain its structural properties.

3. Test of a method to identify double-zonal osteon in polarized light microscopy

Raguin, É. & M.A Streeter

This article is in press and will be published in the *American Journal of Physical Anthropology*.

3.1 Abstract

Objectives: Double-zonal osteons (DZ) have been of interest in paleopathological research because they might be linked to physiological pathology. DZ are thought to be evidence of arrested osteon formation with a brief but abrupt increase in mineralization of lamellae occurring during bone remodeling. Originally identified from microradiographs as hypermineralized rings, recent studies have identified DZ from linear Polarized Light Microscopy (PLM). However, PLM does not guarantee the adequate detection of DZ since PLM captures bone birefringence and not hyper-mineralization. Scanning Electron Microscopy with Backscatter Electrons (BSE-SEM) allows observation of DZ by detecting differences in mineralization. The purpose of this study is to investigate whether DZ, as identified by BSE-SEM, can indeed be identified with PLM.

Materials and Methods: The sample consists of an archaeological collection of adult midshaft femurs (n=30) from St. Matthew cemetery, Quebec City (1771-1860). DZ were identified and counted independently with PLM and BSE-SEM for the same sections. Results from both methods were compared.

Results: Chi-square test shows that there was no significant difference between the two methods (p=0,404). No significant bias was found on Bland-Altman analysis and Cohen's kappa shows a substantial agreement between the two methods (K=0.66). PLM shows a good accuracy (sensitivity 79%, specificity 99.4%) and reliability (Positive Predictive Value: 86.71%; Negative Predictive Value: 99.45%).

Discussion: These findings indicate that the two methods are interchangeable. PLM, using our proposed protocol, is reliable to accurately identify DZ. We discuss how PLM and BSE-SEM that measure different features of the bone tissue can converge on the identification of DZ.

3.2 Introduction

Bone microstructure, specifically osteon morphology, has been widely employed in anthropological studies related to bone adaptation (Drapeau and Streeter, 2006; Schaffler and Burr, 1984), aging (Ericksen, 1991; Kerley and Ubelaker, 1978; Pfeiffer and Zehr, 1996; Stout and Paine, 1992; Streeter et al., 2001; Streeter, 2010; Yoshino et al., 1994), mechanical stress (Burr et al., 1990) and physiological challenge (Farnum, Shimada, Streeter, and Verano, 2001; Martin and Armelagos, 1985). During a process called remodeling, bone can adapt to biomechanical loading and maintain metabolic function through the coupled action of osteoclasts (resulting in bone resorption) and osteoblasts (resulting in bone formation). This action is orchestrated by a complex system involving the activity of proteins, hormones and cytokines located in the extracellular matrix that maintains this equilibrium (Raisz and Rodan, 1998). This cellular activity is collectively called a Basic Multicellular Unit (BMU) and the final product is the creation of a secondary osteon characterized by layers (lamellae) of thin collagen fibers oriented parallel to one another. They provide the essential structure for mineral deposition of calcium phosphate crystals. The plate-shaped crystals are arranged in parallel to the collagen fibers in the extracellular spaces (Landis et al., 1996; Weiner, Arad, and Traub, 1991). The collagen fibers are proteins synthesized by osteoblasts. Their orientation is essential for the toughness of bone while the mineral content confers stiffness to the bone, thus preventing microfracture (Martin, 1991).

Four morphologically distinct types of secondary osteons have been identified: type I (or common osteons), drifting osteons, type II osteons and double-zonal osteons. Type I osteons are the most prevalent form of secondary osteon observed in adult human bone (Enlow, 1962). They consist of a centrally located Haversian canal, surrounded by layers of concentrically deposited lamellae enclosed in a scalloped reversal line (Frost, 1964a). The second kind, the drifting osteon, appears elongated in transverse section. They are defined as an osteon in which there is continuous resorption on one side of the structure and continuous formation on the other side (Epker and Frost, 1965; Frost, 1964; Robling and Stout, 1999; Sedlin, Villanueva, and Frost, 1963). Drifting osteons are the more prevalent type of osteon seen in subadult bone due to the transverse modeling drift (Epker and Frost, 1965; Streeter, 2010). Both type I and drifting osteons have been well documented and described in the

literature (Epker and Frost, 1965; Frost, 1964a; Jaworski, Meunier, and Frost, 1972; Martin, Burr, Sharkey, and Fyhrie, 1998; Streeter, 2010; Takahashi and Frost, 1966). The third kind of osteon is the type II or embedded osteon recognizable in transverse section by the presence of two scalloped reversal lines, one within the other, suggesting that an area of bone along the Haversian canal of a completed osteon has been resorbed and filled in again (Ericksen, 1991; Jaworski et al., 1972; Ortner, 1975; Richman, Ortner, and Schulter-Ellis, 1979).

The fourth kind of osteon is the double-zonal (DZ), characterized by a hyper-calcified ring which could be the result of arrested osteon formation. DZ are hypothesized to be the product of a temporary disruption and then recovery during the formation stage of a type I osteon (Lacroix, 1971; Martin and Armelagos, 1985; Pankovich, Simmons, and Kukarni, 1974; Stout and Simmons, 1979).

While type II osteons are the result of intra-osteonal remodeling of a completed osteon, DZ are created during lamellar apposition, resulting in zones of different mineral density within one osteon (Lacroix, 1971; Pankovich et al., 1974). Type II and DZ osteons have been the focus of interest since the early 1970's (Jaworski et al., 1972; Pankovich et al., 1974) when it was recognized that the variation in osteon morphology had the potential to increase our understanding of the role of bone remodeling as a response to systemic pathology. Ortner (1975) described an increase in type II osteon frequency with aging while Martin (1983) reported that both type II and DZ represent evidence of an episode of physiological stress. Mays (1985) concurred suggesting that the temporary growth arrest seen in DZ could be analogous to Harris lines of the long bones and thus, was possibly related to an episode of stress.

However, the lack of clarity and conformity in the description of the type II and DZ osteons in early studies has led to some uncertainty regarding the identification of these two osteon morphotypes (Lacroix, 1971; Pankovich et al., 1974). Failure to recognize or emphasize the distinction between a cement line and an arrest line is still the potential source of some misidentification. The presence of two, subtle, scalloped or wavy reversal lines, located one within the other, is the defining feature of a type II osteon. Scalloped lines represent resorption by osteoclasts and are fundamentally different from the smooth and microradiographically dense line, as seen in DZ. These lines were previously thought to indicate an interruption and then resumption of lamellar deposition (Fig. 4; Lacroix, 1971).

Type II osteons are readily recognized with the use of bright field microscopy; moving the osteon in and out of focus allows the visualization of the scalloping along the reversal lines. Identification of DZ on the other hand, has presented greater challenges.

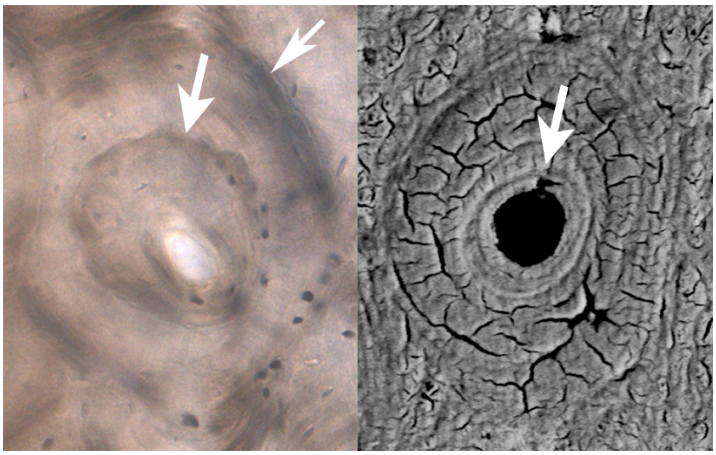


Figure 4. Comparison of type II osteon (left) and double-zonal osteon (right). Note the characteristic two reversal lines of the type II osteon (arrows) (200X, bright field image) while the double-zonal osteon displays a smooth, dense, continuous ring (arrow) (200X, BSE-SEM).

Since DZ are characterized by a ring of hyper-mineralization, they were first identified using microradiographs (Lacroix and Dhem, 1967; Lacroix, 1971; Martin and Armelagos, 1985; Martin, 1983; Pankovich et al., 1974; Simmons, 1985; Stout and Simmons, 1979) but this technology, principally used in medicine, has become obsolete with the development of more advanced imaging methods such as micro-tomography and is no longer available to most researchers (Jansen, 2003). As a result, many have turned to the use of linear Polarized Light Microscopy (PLM) (Austin and Mulhern, 2015; Bartsiokas and Day, 1993; Kim, Jo, Chung, Chung, and Han, 2007; Pfeiffer and Zehr, 1996). PLM uses a light microscope with the sample placed between a polarizer and an analyzer oriented at 90° to the polarizer. Such a configuration is called cross-polarization. In anisotropic materials such as bone, the electromagnetic wave of light has a double refraction. This property, called birefringence, leads to a variation in brightness when viewed by PLM depending on the collagen fibers

orientation. Briefly, the image will have a maximum or minimum brightness if the collagen fibers are disposed transversely or longitudinally to the beam of light (Ascenzi and Bonucci, 1967; Bromage et al., 2003). Marotti (1993) proposed that the brightness of a polarized image is associated with differences in collagen fiber bundle structures rather than their orientation. Giraud-Guille (1988) proposed that the general direction of the collagen fibers varies at an alternating angle from one lamella to another, known as the twisted plywood model, and suggests that this arrangement is responsible for the birefringence of osteons in PLM. It has also been proposed that some collagen fibers are neither longitudinal nor transversal, but rather radially oriented in relation to the Haversian canal and are looser than the dense packing seen in the first two models (Marotti, 1993; Ruth, 1947). Recent findings using a dual beam microscope (FIB-SEM) have shown that collagen fibrils are organized into bundles where thin gaps between the rods and the spaces between adjacent layers are filled with a continuous disordered material (Reznikov, Almany-Magal, Shahar, and Weiner, 2013). This configuration contains collagen fibrils with different orientations as well as a relatively large proportion of interfibrillar ground material due to a looser packing density (Reznikov, Shahar, and Weiner, 2014b). This is of particular interest since the gaps and holes (intramolecular space) provide spaces where the nucleation and growth of mineral crystal can occur (McEwen, Song, and Landis, 1991). However, the intrinsic factors that allow this nucleation of mineral content in association with collagen gaps and holes remains unclear. Plate-shaped mineral crystallites were thought to lie in gap zones inside the collagen fibrils (Katz, Wachtel, Yamauchi, and Mechanic, 1989) but studies on the bone ultrastructure have demonstrated that most of the crystals are actually also located outside the fibrils (McNally, Nan, Botton, and Schwarcz, 2013; Schwarcz, 2015; Schwarcz, McNally, and Botton, 2014). Moreover, when comparing BSE-SEM and PLM images of regions of hyper-mineralization in the proximal femur, Vajda and Bloebaum (1999) found that these regions do not exhibit a birefringent pattern when viewed with PLM. Thus, collagen fiber orientation and degree of mineralization are distinct attributes. As a result, the hyper-mineralized ring that defines DZ cannot be seen in PLM. This implies that the DZ identified by PLM are characterized by a distinct pattern of collagen fiber orientation, possibly due to a looser packing density that allows bigger mineral crystal to form. Previous studies of DZ using PLM have not included a test nor a detailed protocol when attempting to verify whether this pattern of collagen fiber orientation is also characterized by

hyper-mineralization, which is the defining factor of the DZ (Austin and Mulhern, 2015; Bartsiokas and Day, 1993; Kim, Jo, Chung, Chung, and Han, 2007; Pfeiffer and Zehr, 1996). Consequently, this study aims to investigate if the PLM protocol implemented by an experienced observer (M.A.S) to identify DZ is accurate.

Backscattered Scanning Electron with Scanning Electron Microscopy (BSE-SEM) has been used in mineral density studies (Boyde and Jones, 1983; Skedros, Bloebaum, Bachus, Boyce, and Constantz, 1993; Skedros, Holmes, Vajda, and Bloebaum, 2005). Interestingly, BSE-SEM, despite its capacity to identify hyper-mineralized zones in bone, has been underutilized in DZ research. With BSE-SEM, a beam of high-energy electrons interacts with the sample and generates a backscattered electron signal, that when read, results in a contrast image with a gray scale range; highly mineralized zones appear brighter in high-resolution images (Goldman, 2001). A major drawback is that BSE-SEM is more expensive than PLM and the technology is not commonly available in anthropology laboratories. As a result, all recent studies on DZ are performed using PLM without any published test of the accuracy of that method in identifying DZ.

3.3 Materials and Methods

The skeletal sample used in this study consists of a sample of 30 adult femoral midshafts from an historic Euro-Québécois population from the St. Matthew cemetery of Quebec City. The cemetery was active from 1771 to 1860 and is the first official commingled Anglican and Presbyterian cemetery in Quebec City, known as the "Protestant burying ground" (Cloutier, 2000; Moss, 2010; Simoneau, 2003). Diagenetic changes due to bacterial activity can alter mineral density and protein creating the false appearance of a DZ (Fig. 5) (Bell, 2012). Diagenesis can destroy the original architecture of the bone by the dissolution of the original mineral crystallites and the subsequent recrystallization of larger crystals leading to a disruption in the integrity of the collagen depending on the local burial environment (Nielsen-Marsh et al., 2000). In order to avoid misidentification of DZ, only specimens completely free of diagenesis when viewed in PLM and BSE-SEM were included in this study.

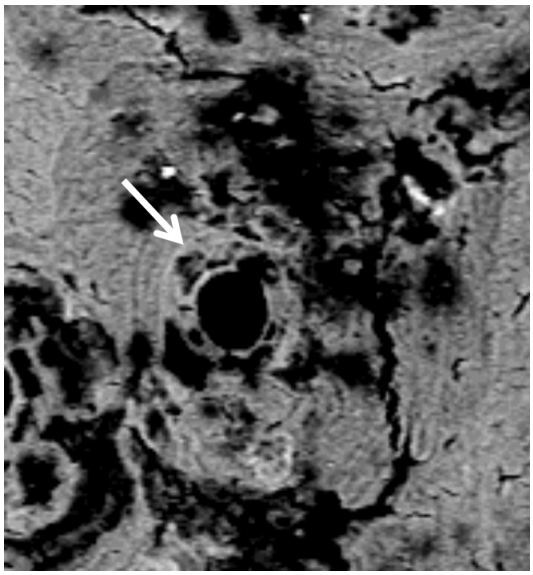


Figure 5. Transverse section of a midshaft femur from the St. Matthew sample (70X) showing bacterial diagenesis. It is not clear if the diagenesis blurs a hyper mineralized ring or if the ring is the result of bacterial activity (arrow). Therefore, this specimen has been removed from this study.

3.3.1 Sample preparation

For each femur, a transverse section of approximately three cm thickness was removed from each midshaft and embedded in a mix of epoxy resin (EpoThin 2 resin and hardener, Buehler Ltd., Lake Bluff, IL) to preserve bone integrity as proposed by Crowder, Heinrich, and Stout (2012). For BSE-SEM analysis, a first cut of the section was made with a Buehler IsoMet saw (Buehler Ltd., Lake Bluff, IL). Since the epoxy resin is nonconductive, a conductive layer of liquid carbon (DAG T-502, Ted Pella) was painted around the freshly cut bone surface of each transverse section. This produced a conductive layer on the section that was then affixed to the BSE-SEM base plate with a double-coated carbon conductive tape (Ted Pella). This protocol was the most suitable in order to preserve the bone surface for the subsequent microscopic slide preparation and was feasible using an environmental BSE-SEM. Environmental BSE-SEM is an advanced SEM technique requiring less sample coating compared to conventional BSE-SEM.This is an important feature since carbon evaporation coating on the entire surface as needed with the conventional BSE-SEM (Jones, 2012), could possibly lead to alteration of histomorphometric characteristics when viewed under PLM.

After scanning with the BSE-SEM, a one mm thick transverse section was cut using a Buehler IsoMet saw. This thin section, which included the bone surface that was scanned with BSE-SEM, was then mounted on a glass slide with the BSE-SEM scanned surface directly glued to the glass slide. The bone section was then ground using a Buehler PetroThin thin sectioning system (Buehler Ltd., Lake Bluff, IL) to obtain a bone wafer with a final thickness of approximately 80 μm. A microscopic slide was then prepared according to the detailed guide by Crowder et al. (2012).

3.3.2 Images processing and data recording

Backscattered (BSE) images of the femurs were collected with a JEOL JSM-6460 LV environmental scanning electron microscope equipped with an energy-dispersive X-ray at the Laboratory for the Study of Calcified Tissues and Biomaterials, Faculty of Dentistry, Université de Montréal. In each anatomical quadrant of bone (anterior, posterior, medial, lateral), a column from the endosteal to the periosteal margin of approximately two mm wide was photographed at 70X and the images were then stitched-together using Photoshop®.

Microscopic slides for each femur were scanned using an Olympus BX43 automated microscope under cross-polarized light at 100X magnification and a stitched-together image of the entire slide was automatically produced with the Objective Imaging's Surveyor™ Software. While circular polarized light microscopy (CPL) has been widely used in collagen orientation studies (Boyde and Riggs, 1990; Bromage et al., 2003), there are several methodological reasons for the choice of linear PLM in this study. First, CPL allows the light from the birefringent structure to be refracted in all 360 degrees with a specific arrangement of filters. Consequently, CPL illuminates all of the fibers almost equally, regardless of their orientation which could have mask the DZ ring (Skedros, Mendenhall, Kiser, and Winet, 2009). Secondly, the protocol established and tested in this study is designed to be used in anthropological studies of histomorphometry and linear PLM is the standard device used in that field. The preparation of the microscopic slides and acquisition of images were performed at the Laboratoire d'écomorphologie et de paléoanthropologie, Département d'anthropologie, Université de Montréal. Using Photoshop®, the images for each quadrant obtained with the

BSE-SEM were superimposed onto the cross-sectional images obtained with PLM to delimit the areas to be compared. These areas of superposition were marked on the PLM images. These identical areas were precisely defined for all quadrants of all the bones (120 zones) on the PLM images to be used as maps to guide the area to read with the PLM. Histological analysis was carried out first on all the 120 zones of the BSE-SEM images and then directly on the histologic slide with the PLM in the areas predefined on the stitched images. The two type of identification were done separately to ensure that the identification of DZ by BSE-SEM did not bias the interpretation of DZ by PLM. For both methods, DZ were counted and marked on the stitched images with the multipoint tool available in ImageJ software (Schneider, Rasband, and Eliceiri, 2012) with the label positioned in the Haversian canal. All sample preparations and data collection were performed by the first author.

3.3.3 Protocols to identify DZ

In the BSE-SEM images, osteons were defined as DZ if they had a clear, continuous bright ring concentric with the other lamellae and it was centered on the Haversian canal (Fig. 6). Older osteons appear more mineralized than newer ones in the BSE-SEM images, which means they were uniformly brighter. However, when adjusting the signal to change the contrast, the continuous ring still showed a much brighter tone than the other lamellae in an older osteon. Similarly, the margins of Haversian canals are often hyper-mineralized (bright) in BSE-SEM images (Fig. 6). This is a common feature that is thought to be associated with the degradation or decomposition of the osteocytes whose lacunae become filled with calcium (Lacroix, 1971; Nyssen-Behets et al., 1997). Because these hyper-mineralized rings result from a process different from that of DZ formation, they are not counted as DZ.

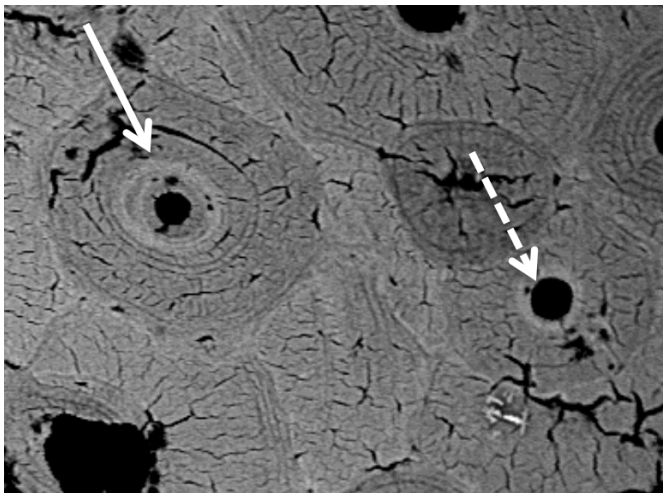


Figure 6. BSE-SEM generated image of a midshaft femur (70X) showing a distinct zonal osteon (solid arrow) and a hyper mineralized Haversian canal (dashed arrow). Some diagenesis is present, but it does not interfere with observation of the hyper-mineralized ring in this image.

With the PLM, DZ were identified directly on the slide following the protocol summarized in Table I and using the predefined maps configuration of the areas to be analyzed. The protocol proposes to examine each osteon in the region of interest at a magnification of 100X. The hyper-mineralization observed in the BSE-SEM image is expected to be seen, in PLM, as a smooth and continuous ring with a change in brightness compared to the adjacent lamellae due to disorganized collagen fibers. Changing the light intensity allows to verify the persistence of the ring and to rule out normal variation in the birefringence of lamellae. It is crucial to focus in and out on the osteon to verify that the ring remains smooth. In order to exclude any misidentification with a type II osteon, it is necessary to examine the osteon under bright field light to confirm that the previously identified ring is still visible as smooth and does not become scalloped. If all the conditions are followed the osteon is labeled on the stitched image as a DZ.

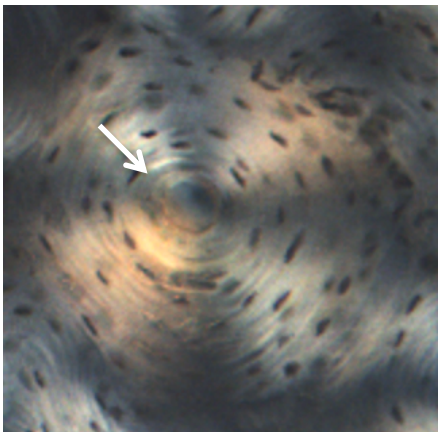
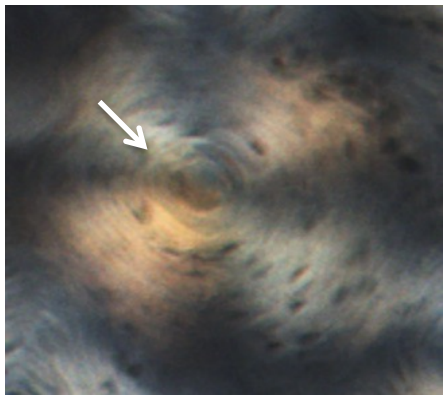
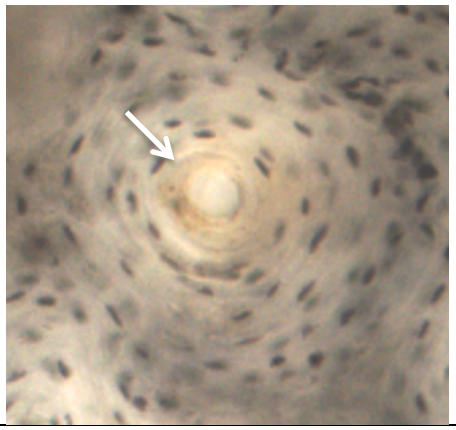
Steps	Illustration
1. In cross-polarized light at 100X, focus on each osteon. When the identification of a smooth, bright and continuous ring is observed (arrow), modify the light intensity and then determine if the ring persists at 200X.	
2. Focus in and out on the osteon at 100X to see if the previously identified ring is still visible, even in the blurred image. The ring must not move and must still be continuous and appear relatively smooth (arrow).	
3. Under bright field light, verify that the ring is still visible as smooth and not scalloped (arrow).	

Table I. Protocol to identify DZ in PLM.

3.3.4 Comparison of Methods

When identification of DZ were completed for all zones with the BSE-SEM images and with the PLM, the corresponding marked images from both methods were superimposed using Photoshop® to identify osteons that were labeled as DZ by both methods, those that were identified as DZ in BSE-SEM only, and those that were identified as DZ in PLM only. The total number of osteons identified as non-zonal by both methods was also recorded for each of the superimposed zones.

Differences between the methods were measured using a chi-square test. A Bland-Altman plot was used to visualize mean bias graphically and the 95% limits of agreement between BSE-SEM and PLM per individual (Bland and Altman, 1986). In our study, BSE-SEM is defined as the gold standard method since the images produced are directly related to mineralization. The Bland-Altman plot represents the reliability of the PLM method: the *x*-axis shows the mean of the osteon counts by the two methods while the *y*-axis represents the difference between the two methods for each individual.

In order to evaluate the accuracy of PLM, Cohen's Kappa corrected for chance was calculated; it quantifies the level of agreement in the identification of DZ between the two methods (Cohen, 1960). The value of Cohen's Kappa ranges from 0 to 1 and is interpreted as follows: less than 0.20 signifies poor agreement; 0.21-0.40 signifies fair agreement; 0.41-0.60 signifies moderate agreement; 0.61-0.80 signifies substantial agreement; and 0.81-0.99 signifies almost perfect agreement (Altman, 1991). However, Cohen's Kappa does not give any information on whether PLM overestimates or underestimates the count of DZ compared to the counts by BSE-SEM. Consequently, sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of PLM were calculated and the 95% confidence intervals of each were determined (Altman and Bland, 1994a, b).

Sensitivity (or the true positive rate) is the capacity of the PLM to correctly identify zonal osteons and is defined as:

$$\text{Sensitivity} = \text{true positives}/(\text{true positives} + \text{false negatives}) \quad (1)$$

where true positives are the number of DZ that were identified as such by both methods and false negatives are the number of DZ that were identified as such with BSE-SEM but not with PLM.

Specificity (or true negative rate) is the capacity to correctly identify non-zonal osteons and is calculated as:

Specificity = true negatives/(true negatives + false positives) (2)

where true negatives are the number of non-zonal osteons identified as such by both methods and false positives are the number of osteons identified as DZ by PLM, but not with BSE-SEM.

PPV and NPV indicate the probability that DZ or non-zonal osteons are correctly identified by PLM, and hence, they assess the reliability of the method. PPV is determined as:

PPV = true positives/total number of positives (3)

where the total number of positives is the number of DZ identified with BSE-SEM (true positive) and the number of DZ identified with PLM but not with BSE-SEM (false positive). NPV is calculated as:

NPV = true negatives/total number of negatives. (4)

where the total number of negatives is the number of non-zonals identified with BSE-SEM (true negative) and the number of non-zonals identified with PLM but not with BSE-SEM (false negative).

3.4 Results

A total of 6735 osteons were analyzed for the 30 individuals included in the sample. Of those, 173 osteons were identified as DZ by BSE-SEM whereas 158 osteons were identified using PLM. The difference between the two methods was determined to be statistically not significant, as shown by the values of the contingency χ^2 test ($p=0.404$; Table II). The Bland-Altman analysis (Fig. 7) showed no significant over- or under-estimation between the two methods of assessment ($p=0.134$), with a mean difference of 0.5 (for an average of about 224 osteons read per individual) and limits of agreement between -2.98 and 3.98.

Method	Zonal Osteons		Total
	+	-	
SEM-BSE	173	6562	6735
PLM	158	6577	6735
χ^2=0.697, d.f.=1, p=0.404			

Table II. χ^2 contingency tables for the number of DZ (+) or non-zonal osteons (-) in the sample for both methods.

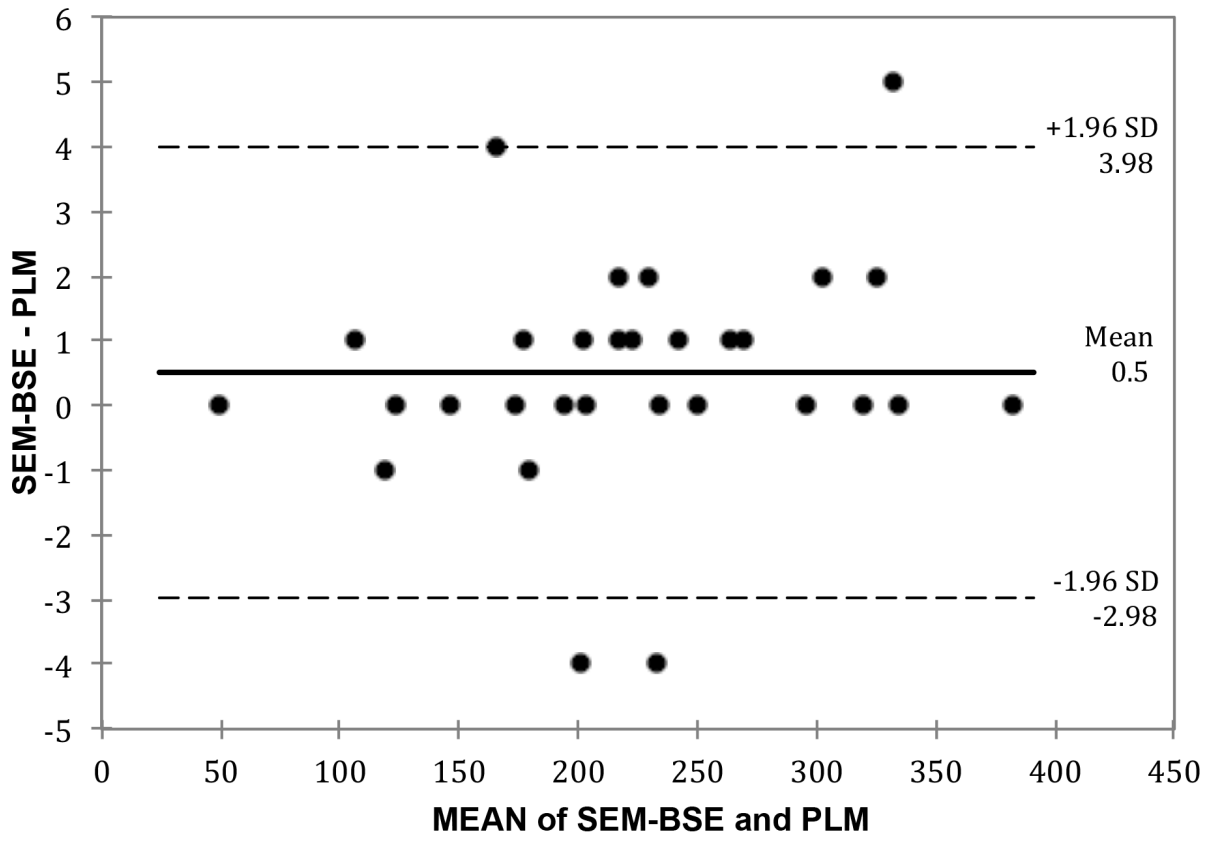


Figure 7. Bland and Altman plot showing the mean differences between the two methods in osteon counts and limits of agreement per individual femur.

Table III shows that the PLM method when compared to BSE-SEM, correctly detected 137 of 173 DZ (sensitivity of 79.2%), whereas the identification as non-zonal osteons was correct in 6520 of 6541 osteons (specificity of 99.7%). Moreover, 21 osteons were assessed as DZ using PLM that were not identified as DZ by the BSE-SEM method, giving a PPV of 86.71%. 6520 osteons have been identified as non-zonal by both methods whereas thirty-six DZ were identified by the BSE-SEM but not diagnosed in PLM, which produces an NPV of 99.45% (Table IV). Cohen's Kappa coefficient for the positive assessment of osteons either as double-zonal or non-zonal using PLM was 0.66, indicating a substantial agreement between the two methods.

		BSE-SEM	
		Double-zonal Osteons	Non-zonal Osteons
PLM	Double-zonal Osteons	137 *true positive*	21 *false positive*
	Non-zonal Osteons	36 *false negative*	6520 *true negative*

Table III. Results of the identification of DZ and non-zonal osteons of the PLM in comparison to the BSE-SEM.

	Sensitivity	Specificity	PPV	NPV
Total number of osteons (n=6735)	79.19 (72.37-84.98)	99.68 (99.51-99.80)	86.71 (80.87-90.96)	99.45 (99.27-99.59)

Values are presented as % with the 95% confidence interval in parentheses; PPV positive predicted value, NPV negative predicted value

Table IV. Sensitivity, Specificity and Predictive Value of double-zonal osteons assessment using PLM relative to BSE-SEM.

3.5 Discussion

In the present study, the ability to correctly identify osteons as DZ or non-zonal with PLM was evaluated, using BSE-SEM as a gold standard. The results presented here reveal that PLM when combined with our protocol is a highly sensitive and specific method for assessing DZ osteons, producing reliable and accurate results. The Bland-Altman plot illustrates the agreement between PLM and BSE-SEM in DZ assessments: the mean is close to zero and data points are equally spread above and below zero. Furthermore, Cohen's Kappa suggests that there is a substantial agreement compared to the gold standard method (BSE-SEM). Hence both methods are equally as good in DZ identification despite some imprecision.

Ideally, a method would flawlessly discriminate between DZ and non-zonal, but in practice, two methods rarely compare perfectly and there will be false positives and negatives (Watson and Petrie, 2010). In our study, PLM assessed correctly 79.2% of the DZ osteons (sensitivity) and 99.7% of the non-zonal (specificity). In other words, PLM is almost perfectly accurate in the determination of non-zonal osteon and still has a high accuracy by detecting four out of five DZ identified by BSE-SEM. In order to evaluate the success of the PLM method, the predictive values are important. Our results show that the probability of DZ osteon being correctly identified as such with PLM was 86.71% (PPV) and the probability of non-zonal osteons being correctly identified is 99.45% (NPV). These highly comparable results indicate that PLM, when used with the protocol established in this study, can be used as an alternative method to BSE-SEM for DZ identification and both methods can be used interchangeably. PLM offers advantages compared to BSE-SEM; it is commonly used in histological studies applied in anthropology and it is inexpensive. Previous researchers have reported results for DZ osteons from PLM (Austin and Mulhern, 2015; Kim et al., 2007; Pfeiffer and Zehr, 1996) but the protocol used in their identification is not detailed and, for that reason it would be difficult to compare and replicate. A beneficial outcome of this study is the description of a clear protocol in the use of DZ identification with PLM that is tested and proven accurate. Future studies will investigate the unidentified and misidentified double-zonal osteons by the PLM compared to the BSE-SEM in order to optimize the protocol and to possibly increase the accuracy of this method.

When comparing BSE-SEM and PLM images of DZ (Fig. 8), the PLM images always appear with a continuous, smooth, brighter ring that corresponds perfectly with the hyper-mineralization ring observed in BSE-SEM. Given that BSE-SEM identifies hyper-mineralization while PLM identifies lamellae with a different orientation of the collagen fibers to the cross section, it is intriguing that the rings are at the same location in both methods. As discussed above, it has been suggested that an alteration of the collagen fibers or an abrupt change in their orientation could produce a bigger gap between the collagen matrix, which would allow a larger amount of mineral crystal to form (Wassen et al., 2000). However, further studies are needed to investigate the relation between collagen fiber orientation and mineral content at the level of the hyper-mineralized ring in DZ in order to understand the changes at the molecular level. Since it is well established that collagen fiber and mineral content contribute significantly to the biomechanical properties of bone and its quality (Burr, 2002b), it would also be interesting to investigate the effect of DZ on those properties.

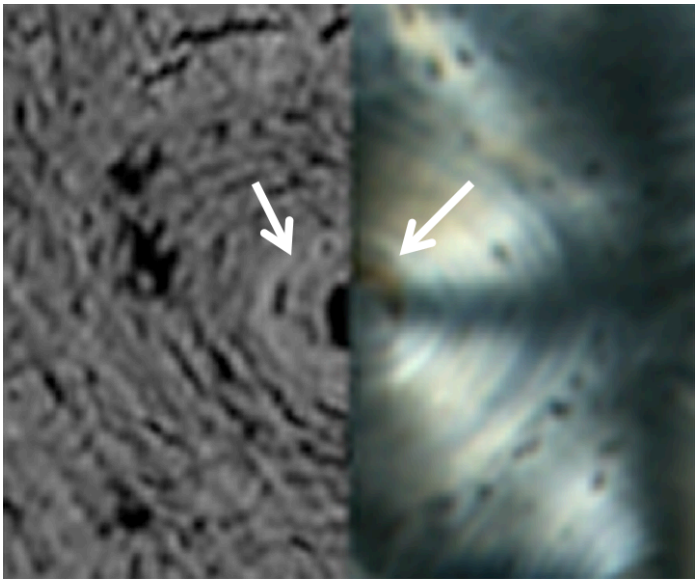


Figure 8. Comparison of a BSE-SEM (left) and PLM (right) image of a zonal osteon (original images taken a 70X with the BSE-SEM and 100X for the PLM and adjusted in size with Photoshop®) showing a ring typically associated with zonal osteon (arrows).

3.6 Conclusion

The results of this study suggest that Polarized Light Microscopy, when following the protocol defined in this study, is a suitable alternative to Backscattered Electron Microscopy with the Scanning Electron Microscope technique for the identification of double zonal osteons. Our results highlight that the hyper-mineralization of DZ osteons corresponds to changes in collagen fiber orientation as seen in Polarized Light Microscopy.

3.7 Acknowledgements

We are grateful to Dr. Antonio Nanci for providing access to the BSE-SEM and to Dr. Alejandra Rodriguez Contreras in her assistance with the device from the Laboratory for the Study of Calcified Tissues and Biomaterials at Université de Montréal. We also thank the following persons for allowing us access to the Quebecois skeletal remains from St-Matthew: Marie-Sol Gaudreau (Anglican Diocese of Quebec), William Moss (Archaeologist, Quebec City), Réginald Auger (Université Laval, Quebec City). Finally, we would also like to extend our gratitude to the two reviewers for their thoughtful comments.

4. An investigation into the nature of double zonal osteons and remodeling activity with the possible effects of age, sex and metabolic diseases

Raguin É. & M.A. Streeter

This article is in preparation for submission in Summer 2018 in the expected following journal: *International Journal of Paleopathology*.

4.1 Abstract

Cortical bone retains evidence of disturbance in normal metabolic processes such as remodeling. Double zonal osteons (DZ), one of the four morphotypes resulting from remodeling, have been of interest to histologists because of their potential to increase our understanding of the health status of past and modern populations. Some researchers have suggested that the appearance of DZ are correlated with systemic physiological stress analogous to the Harris lines of long bones. Other studies hypothesized that they are a naturally occurring product of increasing age. This paper evaluates the effects of metabolic diseases, age and sex on the occurrence of DZ osteons in the femora of adult individuals from an archeological sample (n=35: 10 females, 25 males) of Eurocanadian settlers from the historic St. Matthew cemetery, Quebec City (1771-1860). Comparison was made between individuals assessed to have evidence of metabolic disease based on differential diagnosis from macroscopic and CT scans evaluation, and those with no macroscopic evidence of pathology. In addition to osteon morphology, histomorphometric parameters of remodeling were also determined to ascertain if net bone remodeling rates differ between the pathological and non-pathological groups. Our results show that there is no significant difference in the density of DZ osteons or in the other histomorphometric variables between the pathological and non-pathological groups. In histomorphometric comparison between males and females regardless of the pathological status, no significant differences were found regarding DZ variables. However, females were found to have significantly smaller Type I osteon areas ($p<0.02$) and correspondingly lower net bone remodeling rates (netBFR) ($p<0.03$). Age-

related changes in Type I osteon area only for both sexes are also reported ($p<0.02$) and pairwise analysis reveals a significant increase between the third and fourth decades of life ($p<0.02$) and a significant decrease between the third and fifth decade ($p<0.02$). This study does not support the hypothesis that DZ osteons are associated with metabolic diseases nor did we find significant sex or age-related differences in the occurrence of DZ osteons.

4.2 Introduction

Histological analysis of bone microstructure can provide information on the age, health status, and biomechanical adaptation of past and modern populations. In particular, the quantification of structures observed in bone microarchitecture have been utilized in histomorphological research to develop age-at-death estimation methods (Ericksen, 1991; Kerley and Ubelaker, 1978; Pfeiffer and Zehr, 1996; Stout and Paine, 1992; Streeter et al., 2001; Streeter, 2010; Yoshino, Imaizumi, Miyasaka, and Seta, 1994), and to study bone adaptation and mechanical stress and physiological challenges (Burr, Ruff, and Thompson, 1990; Drapeau and Streeter, 2006; Farnum, Shimada, Streeter, and Verano, 2001; Martin, 1983; Martin and Armelagos, 1985; Martin, Burr, Sharkey, and Fyhrie, 1998; Schaffler and Burr, 1984).

Through the process of bone remodeling, portions of bone are removed and replaced with new bone throughout the life of an individual. Osteoclasts resorb an area of bone followed by a resting or reversal phase, when connective tissue is deposited forming a cement line (also known as a reversal line). Bone formation then proceeds centripetally leaving the centrally located Haversian canal when the process is finished. The result is a secondary osteon. Four distinct types of osteons can develop as the result of bone remodeling, reflecting variation in their pattern of formation: type I, drifting, type II, and double zonal osteons (DZ). While type I and drifting osteons, resulting from normal bone remodeling, have been well documented (Epker and Frost, 1965; Frost, 1964a; Jaworski, Meunier, and Frost, 1972; Martin et al., 1998; Streeter, 2010), the two other variants, type II and double-zonal osteons (DZ) which are thought to differ in morphology and function, remain poorly understood and their relationship to one another and to pathological processes is not clear.

Type II osteons result from intra-osteonal remodeling of a complete Haversian canal. They display two scalloped reversal lines; one within the other (an osteon within an osteon), suggesting that bone has been resorbed and then refilled (Ericksen**,** 1991; Jaworski et al., 1972; Ortner 2003**;** Richman et al., 1979). They are easily recognized with a light microscope using a polarizer. It is generally assumed that type II osteons are associated with metabolic stress incurred during mineral homeostasis (Jaworski, Meunier, and Frost, 1972; Pankovich, Simmons, and Kularkani, 1974).

DZ osteons, on the other hand, are less well understood. They have a smooth hyper-mineralized ring inside their lamellae and are characterized by an abrupt change in the density of lamellae assumed to be resulting from an arrest in osteon formation (Kornblum and Kelly, 1964; Pankovich et al., 1974; Smith, 1963). DZ osteons have not received much attention in histopathological studies because they are challenging to recognize with a light microscope (but see Raguin and Streeter in press), and the exact nature of their function is uncertain.

Research on DZ has also been hampered by a lack of clarity and consistency in their description that leaves this line of inquiry open to question. Pankovich and colleagues (1974) originally reported an increase in the occurrence of double zonal osteons in older individuals. They related that DZ osteons are associated with aging, accumulate at a steady rate of 4% per decade in individuals from 20 to 80 years of age, and are not present in individuals younger than 20 years of age. However, their conclusions are compromised by the inclusion of individuals with metabolic diseases that are known to influence bone remodeling (Frost, 1987b). Several subsequent studies have also found that DZ frequency increases significantly with age (Ortner, 1975; Simmons, Pritzker, and Grynpas, 1991) but others report that DZ density was found to decrease with age (Austin and Mulhern, 2015; Ericksen, 1991; Martin and Armelagos, 1985; Yoshino et al., 1994) while Stout and Simmons (1979) and Nyssen-Behets et al. (1991,1997) detected no significant change associated with age. Lacroix (1971) reported the presence of DZ osteon in the tibial cortex of a 12-year-old individual, contradicting the observations of Pankovich et al. (1974). Moreover, this suggests that age alone is not the only possible cause explaining the appearance of DZ. Finally, Pankovich and colleagues (1974) also failed to make a clear distinction between DZ and type II osteons. They referred to the osteons interchangeably as having undergone focal resorption (as in type II

osteons) but also possessing one or more growth arrest lines (as in DZ) leaving doubt about the morphological features they were actually reporting on.

Attempts to understand the influence of systemic metabolic stress on bone microstructure and the processes it is associated with, require a better understanding of DZ. It has been hypothesized that the hyper-mineralized ring characteristic of DZ osteons is comparable to growth arrest lines found in long bones (Harris Line) and they are more prevalent in individuals experiencing episodes of physiological stress (Mays, 1985; Stout and Simmons, 1979). In this context, Martin and Armelagos (1985) proposed to explore the relationship between bone remodeling rates as an indicator of the overall metabolic function of the skeletal system (Frost, 1964a) and DZ osteons, assuming that they represent direct and immediate response to physiological disruptions. In their study on a large prehistoric Sudanese Nubian sample, they show that females have a lower DZ osteons frequency, lower cortical area and higher remodeling rate compared to males. Moreover, when further investigating the microscopic differences in females from an osteoporotic and a normal group, Martin and Armelagos (1985) found that DZ osteon frequency and cortical area are smaller in the osteoporototic subgroup, but that the remodeling rate was similar to the nonaffected group. The authors conclude that the hyper-mineralized ring is associated with a growth arrest and suggest that the ring has an effect on the regulation of mineral homeostasis in individuals experiencing metabolic challenges. They argue that DZ osteons will be found only in individuals able to recover from the metabolic challenges. Only individuals that recover would be able to continue osteon formation following the growth arrest, thus maintaining bone integrity. However, Martin and Armelagos's hypothesis was contradicted by the findings of Dhem (1980) who has shown that the cytoplasmic processes of the osteocytes located in the hyper-mineralized ring extend without interruption with the osteocytes found in the lamellae on both sides of the ring, maintaining a functional lacuno-canalicular network. Dhem (1980) concludes that DZ osteon cannot represent a period of recovery after an arrest, since this lacuno-canalicular network should be disrupted between the ring and the subsequent lamellae after an arrest in osteon formation.

Raguin and Streeter (in press, chapter 3 of this dissertation) have shown that DZ osteons are also characterized by an alteration of the collagen fibers or an abrupt change in

their lamellae structure at the same location as the hyper-mineralized ring. In normal bone remodeling, collagen is deposited in parallel bundles at their superstructure level and its function is to provide nucleation centers for initiating bone mineralization (Reznikov, Shahar, and Weiner, 2014). Raguin and Streeter (in press, chapter 3 of this dissertation) proposed that the change in collagen orientation could produce bigger gaps in the collagen matrix, allowing a larger amount of mineral crystal to form (Wassen et al., 2000), which is responsible for the hyper-mineralized ring seen in DZ osteons. The collagen fibrillar structure is stabilized by posttranslational modifications that allow the formation of interfibrillar cross-links, responsible for both the structural and mechanical properties of the collagen matrix (Burr and Akkus, 2014; Depalle, Qin, Shefelbine, and Buehler, 2015; Knott and Bailey, 1998). Numerous studies have demonstrated that a change in bone metabolism affects the cross-links, which in turn impair the fibrils normal architecture and lead to randomly arranged collagen fibers (Knott and Bailey, 1998; Teitelbaum and Bullough, 1979).

Age, sex, bone metabolic diseases and nutritional status are known to influence bone metabolism (Ortner, 2003). However, how they might influence DZ osteons formation remains nebulous. Studies have shown that metabolic homeostasis is regulated differently in males and females (Mauvais-Jarvis, 2015) due to the action of sex hormones: while testosterone production is primarily stable throughout life in males, females experience a diminution in estrogen levels during their menstrual cycle and a major decline after menopause, which result in increased bone remodeling (Silberberg and Silberberg, 1972). During puberty, females accumulate calcium in bone at a higher and faster rate than do males as a possible adaptation to meet the needs of future reproductive cycles (Bailey, Martin, McKay, Whiting, and Mirwald, 2000; Bowman and Miller, 2001; Wastney et al., 2000; Zanchetta, Plotkin, and Filgueira, 1995). During pregnancy, calcium requirements increase gradually, coinciding with the mineralization of the fetal skeleton (Bowman and Miller, 2001).

Metabolic bone diseases are related to a disruption in normal bone formation, mineralization and/or maintenance of the bone matrix throughout the skeleton (Mays, 2007). Assessment of metabolic disorders is particularly important in past and present populations because it represents a general indicator of stress and provides insight into health status (Roberts and Manchester, 2007). Age has been shown to influence metabolic homeostasis and

is recognized as a well-known risk factor, eventually leading to metabolic bone diseases with systemic bone loss (Agarwal, 2007; Agarwal and Grynpas, 1996; Agarwal and Stout, 2003; Beauchesne and Agarwal, 2017; Brickley and Ives, 2010). Bone loss occurs at the tissue level of the skeleton and results from an increase in endosteal expansion and higher bone remodeling rates (Cho and Stout, 2003; Martin, 1983; Sedlin, Frost, and Villanueva, 1963; Zebaze, Ghasem-Zadeh, Mbala, and Seeman, 2013). The use of activation frequency as a measure of remodeling rate indicates the frequency at which the bone is being resorbed and reformed and is generally used to assess bone quality and health from archeological skeletal remains at the microscopic level (Abbott, Trinkaus, and Burr, 1996; Agarwal, 2007; Cho, Stout, and Bishop, 2006; Martin and Armelagos, 1985; Mulhern and Van Gerven, 1997; Simmons, 1985; Stout and Simmons, 1979; Stout and Lueck, 1995; Stout and Teitelbaum, 1976; Streeter, Stout, Trinkaus, and Burr, 2010). In other words, it is generally assessed in order to have an indication of the overall bone metabolic function. It has been demonstrated that a higher bone remodeling rate increases intracortical porosity leading to a lower overall bone quality. Higher remodeling rates have been correlated with metabolic diseases (Agarwal, 2007; Beauchesne and Agarwal, 2017; Martin and Armelagos, 1985; Stout and Paine, 1994), nutritional stress (Martin and Armelagos, 1979, 1985; Richman et al., 1979), lactation and pregnancy (Ericksen, 1980; Iwaniec, 1997) and mechanical loading (Burr et al., 1990; Mulhern and Van Gerven, 1997; Robling, 1998; Robling and Stout, 2003).

Some authors have also suggested that osteons and Haversian area could be influenced by metabolic changes: larger Haversian canals relative to osteon size would increase the surface area for calcium exchanges (Brickley and Ives, 2010; Skedros, Clark, Sorenson, Taylor, and Qiu, 2011; Vajda, Kneissel, Muggenburg, and Miller, 1999). As proposed by Dominguez and Crowder (2015), osteon size could be influenced by sexual differences in the endocrine system. These authors have shown that while females had larger osteon areas during the childbearing period compared to males, the relation is reversed in the elderly. An overall age-related decreased in osteon size has been noted in several studies and has been thought to be either related to higher remodeling activity (Takahashi, Epker, and Frost, 1965) or to a decrease in osteoblastic activity (Martin, Pickett, and Zinaich, 1980).

In this context, this study proposes to investigate whether macroscopic evidence of metabolic diseases, sex and age can influence the density of DZ osteons (DZD). Net bone remodeling (netBFR) are also compared to pathological status, age and sex as an indicator of the bone metabolic function. The specific aim is to test whether DZ osteons could reveal biological changes and thus, we predict that individuals with macroscopic evidence of metabolic diseases will have a higher density of DZ osteons, larger osteon area with larger Haversian canal area for both DZ osteons and type I osteons, and a higher net bone remodeling. Such observations may potentially provide valuable information for paleohistopathological studies of archeological samples where a macroscopic diagnosis is not possible. The assessment of the relationship between histomorphometry, age and sex could provide additional information regarding the mechanism responsible for the formation of DZ osteons. Since age and sex are associated with metabolic changes, we also hypothesize that females will have a higher density of DZ, and larger DZ and type I osteons and Haversian canal areas and that there will be differences amongst age categories, with older individuals having a higher density of DZ and larger osteons and Harversian canal areas.

4.3 Materials and Methods

Femoral midshafts were collected from 10 females and 25 males, for a total sample of 35 individuals obtained from a historic Euro-Québécois population from St-Matthew cemetery in Quebec city. This cemetery, active from 1771 to 1860, was known as the “Protestant burying ground” which served as the first official Anglican and Presbyterian cemetery in Quebec city. Individuals buried there were predominantly of western European origins (Cloutier, 2000; Moss, 2010; Simoneau, 2003). This collection has been the subject of several bioarchaeological studies on health and diet based on macroscopic indicators (Caron, 2014; Houle-Wierzbicki, 2016; Morland, 2010; Perron, 2006; Ribot, Morland, Boisjoli, and Leach, 2010). Metabolic disease could affect the entire skeleton and therefore any bone could hold macroscopic evidence of disease and would be useful for study. We chose to investigate the femur because it is an often studied bone in histomorphometry (Abbott, 1996; Kerley, 1978;

Mulhern and Van Gerven, 1997; Robling, 1998), making comparison with other studies possible.

4.3.1 Macroscopic examination

Sex was determined from the morphological features of the pelvic bone (Bruzek, 2002; Buikstra and Ubelaker, 1994; Murail, Bruzek, Houët and Cunha, 2005), while age-at-death was estimated following the methods of the pubic symphysis metamorphosis (Brooks and Suchey, 1990; Katz and Suchey, 1989) and from the changes to the auricular surface of the hip bone (Lovejoy, Meindl, Pryzbeck, and Mensforth, 1985; Schmitt, 2005; Schwartz, 1995). Paleopathological examinations were conducted according to the protocol developed by Buikstra and Ubelaker (1994) in order to have standardized recording techniques and precise descriptions of the lesions found on the skeletons. Gross pathological examinations were supplemented by CT scans, performed using a tomodensitometer Siemens SOMATOM (Definition AS+ 128) (*Laboratoire de Scanographie – Eau Terre Environnement*, INRS, Quebec City), to further enhance the pathological diagnosis and rule out post-mortem processes. For each individual of the sample, metabolic disorder was assessed by differential diagnosis and was scored as either present (1) or absent (0). The diagnostic criteria were based on macroscopic evidence using a combination of the following documented morphological features (Aufderheide and Rodriguez-Martin, 1998; Brickley and Ives, 2010; Eleazer, 2013; Ortner, 2003; Roberts and Manchester, 2007): 1) osteoperiostitis (Fig.9A); 2) porosity on the external surface of the skull (orbital and vault lesions) (Fig.9B and C); and 3) diffuse osteopenia.

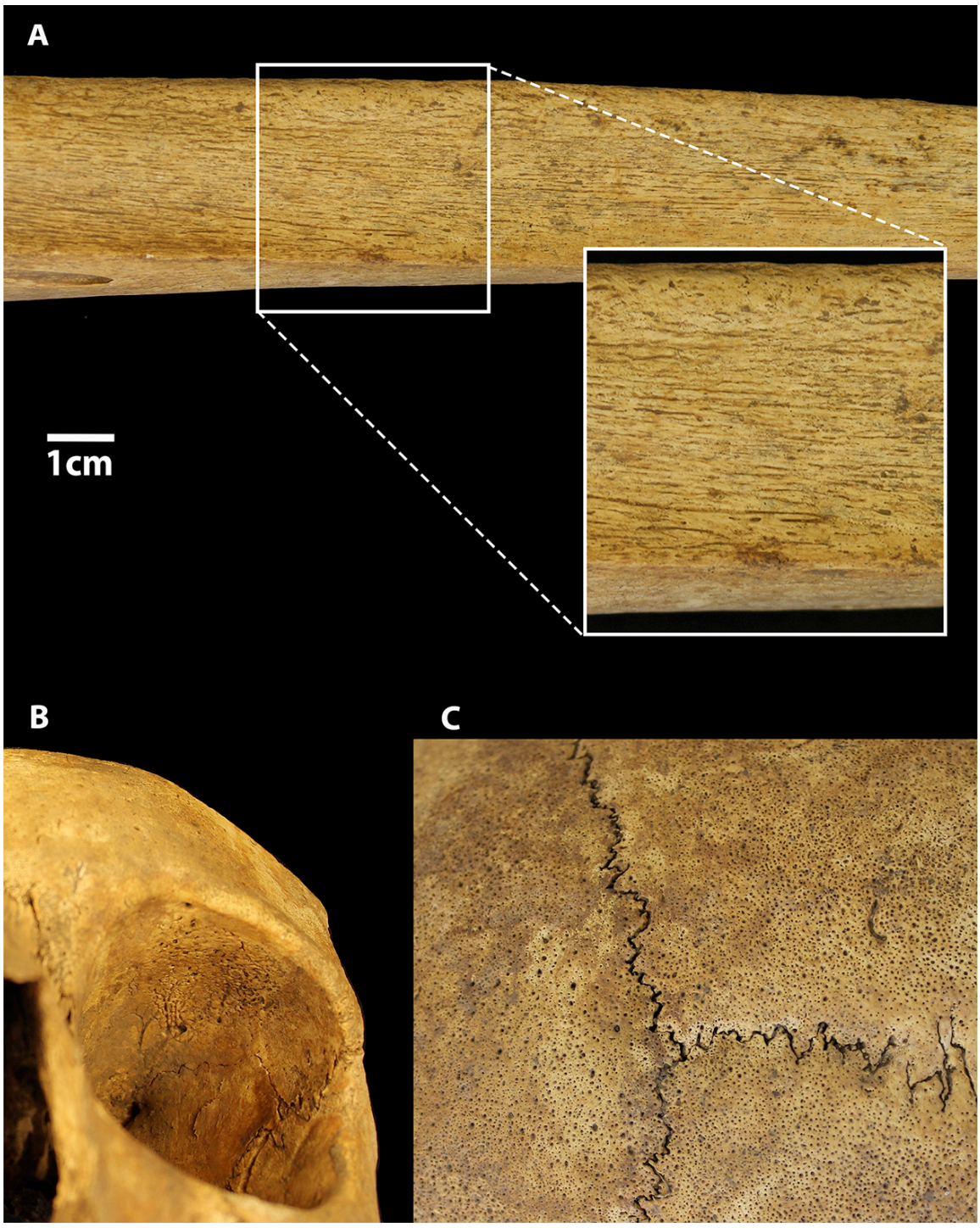


Figure 9. Example of the diagnostic criteria observed on skeletons from St. Matthew: A. Osteoperiostitis on the shaft of a tibia of 12A2gr5ind1; B. Porotic hyperostosis on roof of orbits (cribra orbitalia) of 8F1gr3; C. Porotic hyperostosis on the frontal and parietal bones of 8F1gr3.

1) *Osteoperiostitis* is new bone formation with periosteal reaction, in relation to physiological stressors or trauma (Goodman and Martin, 2002; Roberts and Manchester, 2007). These lesions have been linked to malnutrition and metabolic disorder, such as rickets and scurvy (Mensforth, Lovejoy, Lallo, and Armelagos, 1978; Ortner, 2003). However, while osteoperiostitis due to malnutrition or metabolic disorder can be found on the entire skeleton, such lesions can appear locally on long bones, with a predilection for the tibia. These localized periosteal reactions are probably due to the proximity of the skin, which increase the vulnerability to minor and repeated injuries. Consequently, they are more likely the results of a trauma or infection of the surrounding tissue when they are localized and thus, these localized lesions were not considered evidence of metabolic disorder and were not recorded in our study (Eleazer, 2013; Ortner, 2003).

2) *Porosity on the external surface of the skull: orbital and vault lesions.* Porotic hyperostosis lesions and cribra orbitalia have been shown to likely share the same etiology and are considered a powerful indicator of metabolic stress (Stuart-Macadam, 1989, 1992; Walker, Bathurst, Richman, Gjerdrum, and Andrushko, 2009). They consist of macroporosity of various size and distribution penetrating the outer compact bone (Ribot and Roberts, 1996; Stuart-Macadam, 1989). These lesions have been associated with iron-deficiency anemia, malnutrition, scurvy, rickets and infectious disease (Ortner, 2003; Walker et al., 2009).

3) *Diffuse osteopenia* is a general descriptive term that identifies bone loss. Osteopenia can be assessed using computed tomography by trabecular architectural disorganization and thinning of the cortical wall of long bones. In addition to age, it is associated with several metabolic disorders including osteoporosis, osteomalacia, rickets and hyperparathyroidism (Curate, Lopes, and Cunha, 2010; Ortner, 2003).

Individuals that present one or more of the skeletal lesions defined above were included in the metabolic pathological group, while those without visible lesions of metabolic etiology, both macroscopic and radiographic, were classified in the non-pathological group for metabolic disorder. The age, sex and pathological distributions of the sample are illustrated in table V.

Age	Females		Males		Combined
	Pathological	Non-pathological	Pathological	Non-pathological	
20-29	2	3	3	7	15
30-39	-	3	1	6	10
40-49	-	1	3	3	7
50+	-	1	-	2	3
Total	2	8	7	18	35

[1]Pathological assessment based on macroscopic evaluation for metabolic diseases; non-pathological individuals are those without macroscopic evidence of metabolic diseases

Table V. Sex, age and pathological[1] distribution of the St. Matthew sample.

4.3.2 Microscopic analysis

For each individual, three-centimeter blocks were removed from the midshaft of the femur using a band saw. Undecalcified sections were prepared according to the proposed guide by Crowder, Heinrich, and Stout (2012), following the method developed by Frost (1958). Accordingly, bone thick sections were embedded in epoxy resin (EpoThin 2 resin and hardener, Buehler Ltd., Lake Bluff, IL), thin sectioned using an Isomet saw (Buehler Ltd., Lake Bluff, IL), and then ground to a thickness of approximately 80 μm with a PetroThin thin sectioning system (Buehler Ltd., Lake Bluff, IL). Thin sections were then mounted with Permount™ (Fisher Chemicals™) and cover-slipped on glass slides.

The entire femoral slides were scanned using an automated Olympus BX43 microscope in proper anatomical orientation at 100X magnification under polarized light and a stitched together image was automatically produced with the Objective Imaging Surveyor Software. The preparation of the microscopic slides and acquisition of images were performed at *Laboratoire d'écomorphologie et de paléoanthropologie, département d'anthropologie, Université de Montréal*. ImageJ software (Schneider, Rasband, and Eliceiri, 2012) was used to count and measure the different variables recorded on the entire cross-section. DZ osteons were identified using the protocol developed in Raguin and Streeter (in press, chapter 3 of this dissertation), which produces a reliable and accurate assessment of DZ osteons under polarized light microscopy. Briefly, DZ osteons can be identified under cross-polarized light microscope by the presence of a smooth, bright and continuous ring when observed at a

magnification of 100X. This ring must persist after increasing the magnification to 200X, as well as changing the light to bright field. DZ osteons were recorded using the point count tool available in Photoshop® to avoid confusion with other types of osteons during the measurements on the digitalized images (Fig.10).

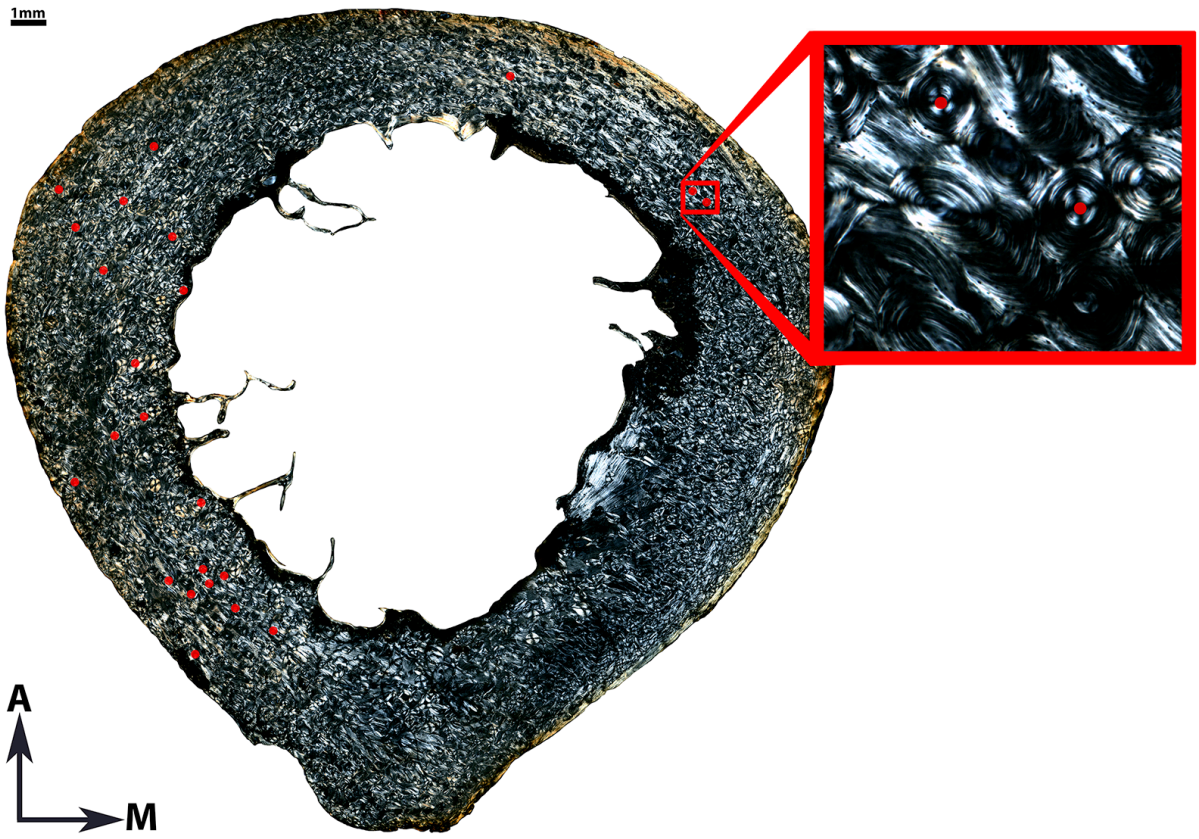


Figure 10. Stitched together cross-section of a complete femur under polarized light at 100X magnification from St. Matthew showing double-zonal osteons recorded with the point count tool in Photoshop®.

The following variables were counted, measured or calculated on the entire section of each femur:

1. *Total area* (Tt.Ar) = the total cross-section area, including the marrow cavity (mm^2).
2. *Medullary area* (Es.Ar) = the area of the marrow cavity (mm^2).
3. *Cortical area* (Ct.Ar) = the total bone area excluding the marrow cavity (mm^2):

$$Ct.Ar = Tt.Ar – Es.Ar \quad \text{(Eq. 1)}$$

4. *Percent cortical area* (%Ct.Ar) = the percentage of cortical area relative to total area:

$$\%Ct.Ar = (Ct.Ar/Tt.Ar) \times 100 \quad \text{(Eq. 2)}$$

5. *Number of double-zonal osteon* (DZ) = the number of double-zonal osteons; counted on the entire cross section.
6. *Number of intact secondary osteon* (N.On) = the number of all types of osteons that have at least 90% of their Haversian canal perimeter complete; counted on the entire cross section.
7. *Number of fragmentary osteon* (N.On.Fg) = the number of partially remodeled osteons that have at least 10% of the Haversian canals missing due to remodeling; counted on the entire cross section.
8. *Osteon Population Density* (OPD) = the total number of intact and fragmentary osteons per unit area (mm^2):

$$OPD=(N.On + N.On.Fg)/Ct.Ar \quad \text{(Eq. 3)}$$

9. *Double-Zonal Osteon Density* (DZD) = the number of double-zonal osteon per unit area (mm^2):

$$DZD=\#DZ/Ct.Ar \quad \text{(Eq.4)}$$

10. *Mean Osteon Area* (On.Ar) = the average area of bone (mm^2), including the Haversian canal, contained within the cement line of intact type I osteons; calculated from the measurement of 200 osteons per cross section (mm^2).
11. *Mean Haversian canal Area* (H.Ar) = the average area of a Haversian canals (mm^2); calculated from the measurement of 200 canals per cross section (mm^2).
12. *Mean Double-Zonal Osteon Area* (ZOn.Ar) = the average area of bone (mm^2), including the Haversian canal, contained within the cement lines of double-zonal

osteons; calculated from the measurement of all DZ osteon recorded per cross section (mm^2).

13. *Mean Double-zonal Haversian canal Area* (ZH.Ar) = the average area of double-zonal Haversian canals (mm^2); calculated from the measurement of all DZ canals recorded per cross section (mm^2).

OPD and On.Ar are parameters that served to calculate the net bone remodeling using the method proposed by Frost (1987a) and Wu et al. (1970). Stout and Teitelbaum (1976) and Stout and Paine (1994) have acknowledged the validity of this algorithm, which reasonably estimates the remodeling rate when applied to archeological bone.

14. *Accumulated Osteon Creations* (AOC) = the total number of intact, fragmentary and missing osteons for a given OPD, calculated using the formula:

$$\text{AOC} = \beta(\text{OPD}) \qquad \text{(Eq.5)}$$

As remodeling activity eventually reaches an asymptote of OPD, newly created osteons will obliterate all evidence of older osteons creation. Frost (1987a) developed the algorithm with the scaling operator $\beta = (1\text{-}\alpha^x)^{-1}$, which when multiplied by the OPD, gives the estimation of AOC. The exponent x is equal to 3.5 according to Frost (1987a) and α is the observed OPD normalized to its asymptotic value $\alpha = \text{OPD}(\text{OPD asymptote})^{-1}$. The OPD asymptote is calculated using the formula:

$$\text{OPD asymptote} = k\left[\left(2\times\sqrt{\frac{On.Ar}{\pi}}\right)^2\right]^{-1} \qquad \text{(Eq.6)}$$

k is a fragment packing operator accounting for the overlap of intact osteon and osteon fragments distribution in a unit area of bone and is specific for each bone. The k value for the femur was determined to be 2 as reported by Robling (1998) based on a modern autopsy sample. Abbott et al. (1996) have provided a value of 1.38 but as pointed out by Robling (1998), several methodological issues make this value inaccurate: they used the original erroneous data from Kerley (1965) instead of the corrected data (Kerley and Ubelaker, 1978). In addition, Kerley (1965) presented distinct graph of intact and fragmentary osteons, making impossible to determine the maximum OPD.

15. *Net bone remodeling* (netBFR) = the total amount of remodeling that occurred over the lifetime of an individual (mm^2/mm^2) and calculated by the formula:

$$netBFR = AOC(On.Ar) \quad \text{(Eq.7)}$$

In a sample of unknown age, it is only possible to calculate the net bone remodeling because the activation frequency and bone remodeling rate (which uses activation frequency) require a specific chronological age (Abbott et al., 1996; Stout and Paine, 1994).

4.3.3 Statistical analyses

The SPSS 24 statistical package was used to compute the variables and to perform statistical analyses. The Mann-Whitney U Test was used to test the differences between the variables assessed and the pathological status as well as between these variables and sex. The difference between age categories and the histomorphometric features have been tested using the Kruskal-Wallis test. The Monte Carlo method was applied for age comparisons because of the small sample size in the category of 50 and over. This method relies on repeated random sampling, which is necessary to compare statistics for small samples (Creedon and Hayes, 2015). Unfortunately, the health status could not be addressed for the combined effect of age and sex due to small subsample size.

4.4 Results

Table VI presents the descriptive statistics for the St. Matthew sample and the different variables measured or calculated. The DZD, ZOn.Ar, ZH.Ar and histomorphometric variables used to calculate the net bone remodeling were compared between pathological and non-pathological individuals. Descriptive statistics and results from the Mann-Whitney U test are presented in Table VII. There are no significant differences between individuals diagnosed with and those without evidence of metabolic conditions.

	Mean	SD
DZD (#/mm^2)	0,0834	0.0535
ZOn.Ar (mm^2)	0.0344	0.0080
ZH.Ar (mm^2)	0.00190	0.00064
OPD (#/mm^2)	10.95	1.90
On.Ar (mm^2)	0.0528	0.0108
H.Ar (mm^2)	0.00256	0.00054
DZD/OPD	0.00781	0.00485
%Ct.Ar	72,23	6.64
Tt.Ar (mm^2)	539.83	110.45
Es.Ar (mm^2)	151.77	55.41
Ct.Ar (mm^2)	388.06	76.41
AOC (nbr)	11.35	2.08
netBFR (mm^2/mm^2)	0.591	0.129

[1] DZD, Double-zonal Osteon Density; ZOn.Ar, Double-zonal osteon area; ZH.Ar, Double-zonal Haversian canal area; OPD, Osteon Population Density; On.Ar, Osteon area; H.Ar, Haversian Area; DZD/OPD, Double-zonal osteon density controlled by the total osteon population density; %Ct.Ar, relative cortical Area; Tt.Ar, Total Area; Es.Ar, Endosteal Area; Ct.Ar, Cortical Area; AOC, Accumulated Osteon Creation; netBFR, net Bone Remodeling.

Table VI. Descriptive statistics for the St. Matthew sample for all the variables measured or calculated[1].

	Pathological (n=9)		Non-pathological (n=26)		Mann-Whitney	
	Mean	SD	Mean	SD	Z	*p-value*
DZD (#/mm^2)	0.1102	0.0824	0.0741	0.0363	-1.057	0.291
ZOn.Ar (mm^2)	0.0366	0.0043	0.0337	0.0088	-1.396	0.163
ZH.Ar (mm^2)	0.00182	0.00029	0.00193	0.00073	-0.340	0.734
OPD (#/mm^2)	11.58	1.54	10.74	1.99	-1.170	0.242
On.Ar (mm^2)	0.0518	0.0087	0.0531	0.0115	-0.189	0.850
H.Ar (mm^2)	0.00258	0.00071	0.00255	0.00047	-0.642	0.521
DZD/OPD	0.0096	0.0071	0.0072	0.0038	-0.528	0.597
netBFR (mm^2/mm^2)	0.633	0.169	0.575	0.113	-0.604	0.546

[1] See abbreviations of the variables in Table VI.

Table VII. Descriptive statistics of histological variables[1] and Mann-Whitney U-tests comparing pathological and non-pathological groups.

Table VIII summarizes the males and females values as well as results from the Mann-Whitney U-test comparing the sexes. Osteon Area (On.Ar) differs significantly between males

and females ($p<0.02$). Females have a smaller osteon area of 0.046 mm^2 on average than men who have 0.055 mm^2 (Fig. 11A). As a result, net bone remodeling (netBFR) is significantly different ($p<0.03$) for the mean value of females (0.52 mm^2/mm^2) compared to that of males (0.62 mm^2/mm^2) (Fig. 11B). No statistically significant difference was found between sexes regarding the other variables tested.

	Females (n=10)		Males (n=25)		Mann-Whitney	
	Mean	SD	Mean	SD	Z	*p-value*
DZD (#/mm²)	0.0588	0.0358	0.0932	0.0557	-1.789	0.074
ZOn.Ar (mm²)	0.0327	0.0069	0.0351	0.0082	-0.803	0.422
ZH.Ar (mm²)	0.00199	0.00090	0.00187	0.00052	-0.256	0.798
OPD (#/mm²)	11.19	2.20	10.86	1.77	-0.475	0.635
On.Ar (mm²)	0.0462	0.0088	0.0554	0.0103	-2.300	0.021*
H.Ar (mm²)	0.00238	0.00050	0.00263	0.00053	-0.803	0.422
DZD/OPD	0.0056	0.0037	0.0087	0.0050	-1.826	0.068
netBFR (mm²/mm²)	0.520	0.100	0.619	0.130	-2.191	0.028*

*Significant at $p<0.05$

[1]See abbreviations of the variables in table VI.

Table VIII. Descriptive statistics of histological variables[1] and Mann-Whitney U-tests comparing males and females.

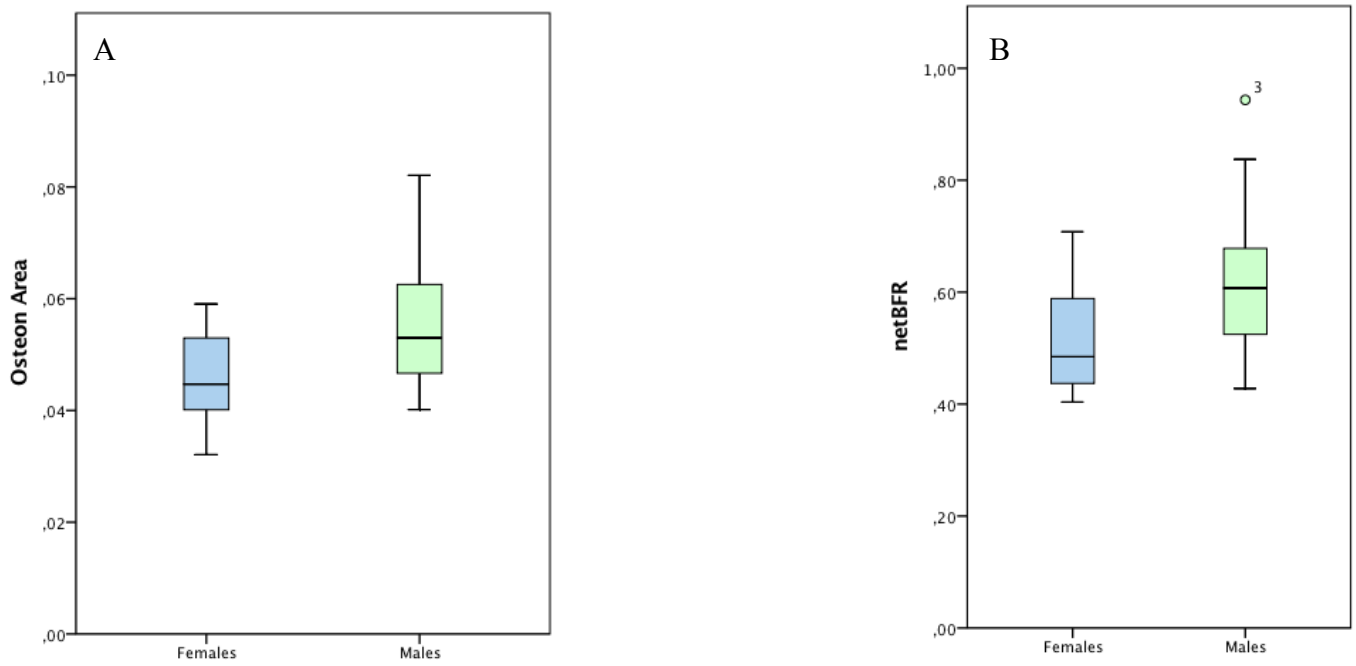


Figure 11. Boxplot of the osteon area (type I osteons)(A) and net bone remodeling (B) between females (n=10) and males (n=25). These females-males differences are statistically significant.

The comparison amongst age categories of DZD and netBFR, regardless of sex and pathological status, did not reveal significant differences. The means for all variables by age categories are shown in table IX. However, the Kruskal-Wallis rank analysis shows that osteon area (On.Ar) differs significantly with age ($p<0.02$), with an increase from 0.048 mm^2 to 0.056 mm^2 in the second and third decades and 0.061 mm^2 in the fourth decade, and then a decrease to 0.047 mm^2 in the fifth decade (Fig. 12). A pairwise analysis comparing the age categories with each other yielded significant differences between the third and fourth decades ($p<0.02$) and the third and fifth decade ($p<0.02$). Interestingly, no differences in netBFR were found most likely because OPD shows an opposit trend to the On.Ar. For all other variables, no significant differences were found between age categories.

	20-29 (yr)		30-39 (yr)		40-49 (yr)		50 + (yr)		Kruskal-Wallis	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	χ^2	*p-value*
DZD (#/mm²)	0.0760	0.0467	0.0771	0.0394	0.1073	0.0857	0.0852	0.0422	0.749	0.876
ZOn.Ar (mm²)	0.0319	0.0056	0.0343	0.0088	0.0409	0.0098	0.0321	0.0047	6.344	0.089
ZH.Ar (mm²)	0.00174	0.00030	0.00170	0.00051	0.00221	0.00069	0.00266	0.00150	4.060	0.265
OPD (#/mm²)	11.41	1.57	10.44	1.22	10.70	3.01	10.98	2.67	1.780	0.641
On.Ar (mm²)	0.0479	0.008	0.0562	0.0044	0.0609	0.0173	0.0468	0.0019	9.946	0.012*
H.Ar (mm²)	0.00238	0.00048	0.00265	0.00027	0.00284	0.00086	0.00248	0.00032	3.309	0.365
DZD/OPD	0.0069	0.0048	0.0074	0.0035	0.0104	0.0071	0.0075	0.0019	2.030	0.584
netBFR (mm²/mm²)	0.567	0.145	0.610	0.100	0.642	0.134	0.526	0.129	3.771	0.304

*Significant at $p<0.02$

[1]See abbreviations of the variables in Table VI.

Table IX. Descriptive statistics of histological variables[1] and Kruskal-Wallis rank test comparing the age groups.

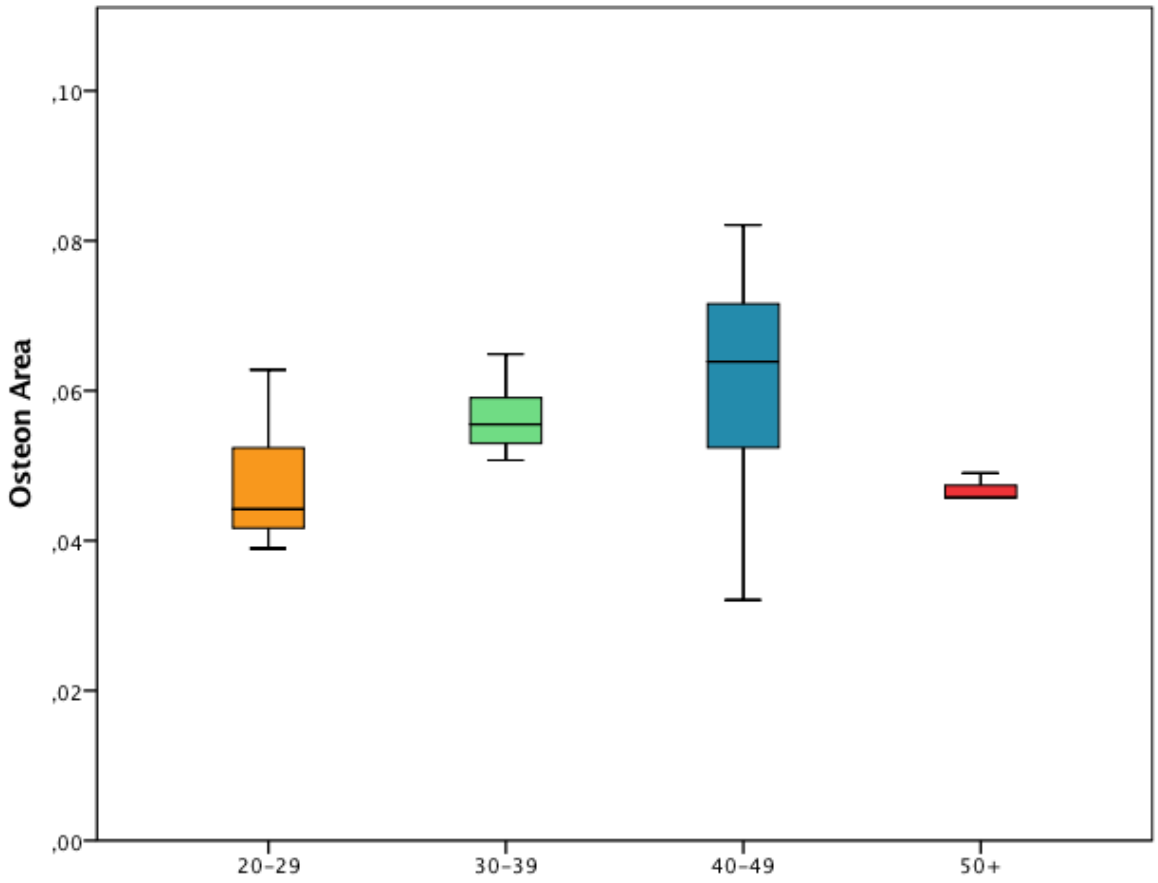


Figure 12. Boxplot of the osteon area (type I osteons) between the four age categories. Significant differences were found only between the third and fourth decade ($p<0.02$), and the third and fifth decade ($p<0.02$).

4.5 Discussion

This study proposed to investigate how metabolic diseases, age and sex influence DZ osteons formation as well as netBFR in the femurs of a historic sample. While DZ osteons have often been reported to result from non-specific stress induced by metabolic change (Austin and Mulhern, 2015; Bartsiokas and Day, 1993; Martin and Armelagos, 1985; Mays, 1985; Stout and Simmons, 1979), our study suggests that DZD is not related to age, sex or pathological status. Specifically, no statistical increases were found in DZD between the different age categories, in agreement with some studies (Martin and Armelagos, 1985; Nyssen-Behets et al., 1991; Nyssen-Behets, Duchesne, and Dhem, 1997; Yoshino et al., 1994) but in contrast to others (Pankovich et al., 1974; Simmons et al., 1991) that have reported an increase in DZD with age. Pankovich's study cannot be compared directly to ours since they did not distinguish between DZ and type II osteons. On the other hand, Simmons et al. (1991) detected age changes on a very small sample of 15 femurs. They report eight individuals older than 60 years and the rest of the sample was consisted of three individuals between 40 and 45 years old and four individuals between 20 and 25 years old, with no representation in the 25-40 years of age ranges. The difference in composition of our sample, particularly our small sample size in the age category of 50 and above may have impeded the detection of any age-related changes that might be more obvious in a later age group. Given the size of our sample and the idiosyncrasies of Simmons and colleagues' sample, definitive conclusions on the effect of age on DZ formation remains inconclusive.

Our results did not reveal further significant sex-related differences in absolute or relative DZD nor in ZOn.Ar or ZH.Ar. Martin and Armelagos (1985) found that Nubian females have significantly fewer DZ osteons than males after the age of 30 with significant results in the fourth and sixth decades. They proposed that females experienced more bone loss from past event of childbearing and breastfeeding, and thus, show less evidence of DZ osteons since they are more likely to have been removed by subsequent remodeling events. However, more recent studies have demonstrated no evidence of significant bone loss during pregnancy (Black et al., 2000; Kent et al., 1993; Naylor et al., 2000). Lactation has been shown to increase bone loss in cancellous bone only, and the bones of the individuals recover completely a few months after weaning (López et al. 1996). Martin and Armelagos (1985)

sampled at a different location on the femur, below the lesser trochanter, which may have contributed to some differences relative to our study. Hence, further investigations are needed to explore the potential differences between sexes regarding the formation of DZ osteons.

When females were further analyzed according to their health status, Martin and Armelagos (1985) showed that osteoporotic individuals have fewer DZ osteons and a decrease in relative cortical area but with a similar remodeling rate compared to the non-osteoporotic females. When they divided the female osteoporotic group by age, they observed that the youngest individuals had more DZ osteons, a similar relative cortical area and remodeling rate, but with less resorption cavities. This led the authors to conclude that youngest osteoporotic individuals are able to maintain mineral homeostasis with an increase in DZ frequency and experienced less bone loss compared to the oldest. They demonstrated a significant relationship between the relative cortical area and DZ frequency: as relative cortical increases, the number of DZ osteons increases for both males and females. They interpreted the hyper-mineralized ring as evidence of mineral regulation. Figure 13 shows the relationship between DZD and relative cortical area for our sample, which does not show the positive correlation that Martin and Armelagos (1985) observed. Our results, instead, suggest that DZ osteons are not related to the maintenance of bone integrity as hypothesized by Martin and Armelagos (1985). However, these authors based their argumentation on the assumption that the hyper-mineralized ring of DZ osteons is a growth arrest line, which has been refuted by Dhem (1980). Given that we found no differences between pathological and non-pathological individuals in DZD, it appears likely that DZ osteons are not related to a function of maintaining homeostasis in individuals experiencing episodes of physiological stress.

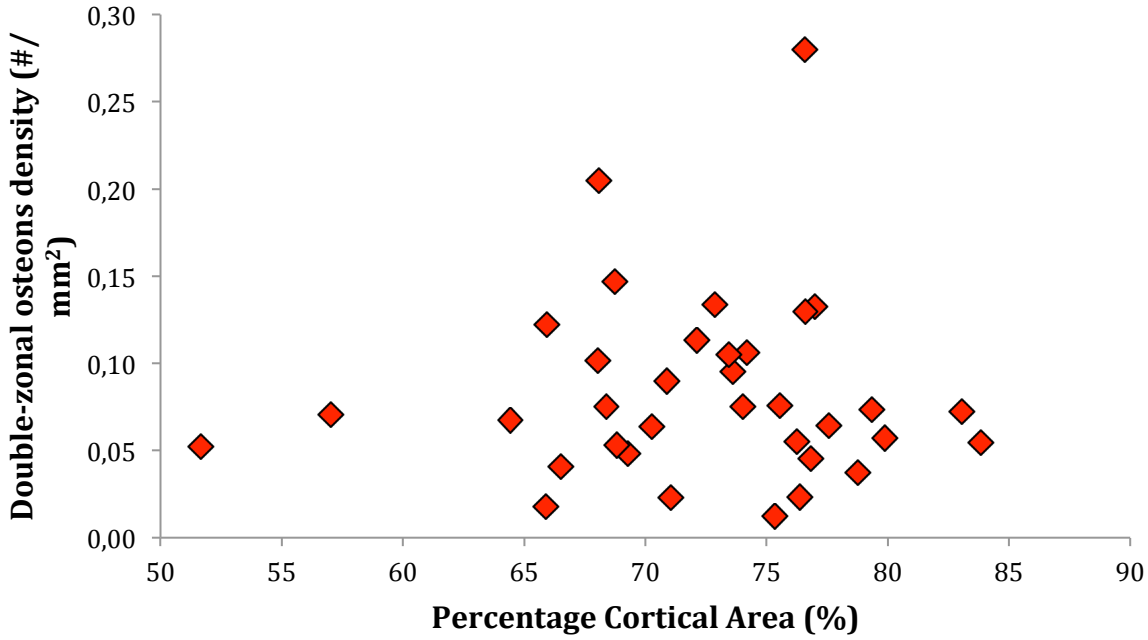


Figure 13. Scatterplot showing the relationship between DZD and the relative cortical area. The correlation (r=0.037) is not significant.

The overall results of our study reveal that DZ osteons are not a general indicator of metabolic disorder contrary to what was previously hypothesized (Mays, 1985; Stout and Simmons, 1979). No statistical differences were observed between the normal and pathological groups regarding DZD, ZOn.Ar or ZH.Ar. Methodological issues arising from the limitations of making a diagnosis in paleopathology, known as the osteological paradox (Wood et al., 1992), may have influenced to our results. The inferences of pathological status depend largely on the formation of the lesions, but individuals can die quickly from a stressful event before the initiation of a macroscopic skeletal response (Wood et al., 1992). Thus, the distinction between pathological and non-pathological groups may not reflect an accurate representation of the health status of deceased individuals, a limitation inherent in any paleopathological studies. Moreover, the lesions left by metabolic diseases are sometimes very localized with a great intraskeletal variability, and will not necessarily be reflected on the whole skeleton (Brickley and Ives, 2008), adding an additional challenge in the distinction between pathological and non-pathological groups. Alternatively, since it has been

demonstrated that the hyper-mineralized ring is not an arrest line, it is conceivable that DZ osteons are not at all related to metabolic changes and further studies are needed to investigate other possible causes.

The parameters used to calculate netBFR, such as osteon area and Haversian canal area for type I osteons, reveal interesting underlying dynamics of bone remodeling. First, our results show changes in the size of type I osteons across age categories: while the osteon area increases significantly until the fourth decade, there is a decrease in osteon size in individuals older than 50. However, this trend is based only on three individuals. The overall trend of reducing osteon area with older age while maintaining the same mean Haversian canal area confirms the results of numerous researchers that report a decrease in osteon area with age (Dominguez and Agnew, 2016; Grynpas, 1993; Jowsey, 1966; Martin, Pickett, and Zinaich, 1980; Takahashi, Epker, and Frost, 1965; Thompson and Galvin, 1983; Yoshino et al., 1994). Few explanations have been proposed to explain the relationship between age and osteon area. Takahashi, Epker, and Frost (1965) argue that larger osteons have a higher probability of getting overlapped by subsequent remodeling events and are, therefore, more often erased than the smaller ones, inducing a size bias as individuals age. However, in our study, no significant difference is observed in OPD among the different age categories. It is possible that given the low mean age of our sample and the underrepresentation of older individuals, the increase of OPD with age that is normally observed is blurred. Others have proposed that a decrease in osteon size with age results from an age-related decline in osteoclastic activity, which defines the size of the osteon and results in smaller resorbed areas and less refilling (Martin, Pickett, and Zinaich, 1980; Qiu, Fyhrie, Palnitkar, and Rao, 2003). Unfortunately, test of that hypothesis is beyond the scope of this study.

Second, our study has shown differences in type I osteons area between males and females, but contrary to our hypothesis, females have a smaller average osteons area (0.046 mm^2) compared to males (0.055 mm^2). Similarly, Britz et al. (2009) found that females had smaller osteons than males in the femurs of a forensic sample, but without offering any explanation for the difference. In contrast, other studies found no difference between sexes (Borgel, 2017; Domingez and Agnew, 2016; Pfeiffer, 1998, 2006; Streeter, 2010). Burr et al. (1990), in a sample of Pecos Pueblos, and Mulhern and Van Gerven (1997), in a sample of

Kulubnarti Nubians from the Middle Ages, found larger osteons in the femur of females compared to males. Dominguez and Crowder (2015) have demonstrated in a modern American sample that females have larger osteons during the reproductive age compared to males but this trend was reversed in the elderly, suggesting that a hormonal component is at play, and possibly explains some of the contradictory results of previous studies. Both studies of Burr et al. (1990) and Mulhern and Van Gerven (1997) proposed that the differences observed in their sample reflect sexual division of labor where males, engaged in more strenuous activities, have smaller osteon. However, the influence of mechanical loads on osteon area, if any, is still unresolved. While few studies have suggested that osteon area decreases with higher strain levels (Abbott et al., 1996; Britz, Thomas, Clement, and Cooper, 2009; van Oers, Ruimerman, van Rietbergen, Hilbers, and Huiskes, 2008), others suggested the opposite trend (Borgel, 2017; Corondan and Haworth, 1986) and some found no relationship with physical activity (Denny, 2010; Pfeiffer, 1998; Pfeiffer, Crowder, Harrington, and Brown, 2006). The relationship between osteon area, biomechanics, sex and age is still poorly understood and needs further investigation, particularly since a strong genetic component has recently been established (Havill et al., 2013). Hence, the differences observed in our sample could be attributed to differences in activity patterns and hormonal influences. Additionally, while no difference in double-zonal osteon size between the sexes or among the age groups has been found, on average DZ osteons are smaller than the type I osteons, suggesting variability among osteon types.

NetBFR was found to be significantly lower in females (0.52 mm^2/mm^2) compared to males (0.62 mm^2/mm^2). Martin and Armelagos (1985), instead, reported higher bone remodeling rate in females compared to males in a prehistoric Nubian sample. The authors attribute this to the physiological and metabolic disruption occurring in females that increases bone remodeling rates. Since they did not report the values of osteon area, we cannot tell whether their results are similar to ours. Mulhern and Van Gerven (1997), in the Nubian sample, also found higher netBFR in females (0.452 mm^2/mm^2) than in males (0.426 mm^2/mm^2), which they attributed to sexual division of labor. They use the femoral k value of 1.38 for netBFR as proposed by Abbott et al. (1996) but this value has been shown to be population specific so caution is recommended when applying values used on other

pouplations such as Neandertals (Robling, 1998). For the comparison, we recalculated their results with the k value of 2 as in our study. The mean netBFR would thus be 0.436 mm^2/mm^2 for females and 0.423 mm^2/mm^2 for males. Beyond indicating an opposite trend in remodeling dynamics between the sexes, our study also found higher values (0.520 mm^2/mm^2 for females and 0.619 mm^2/mm^2 for males) compared to Mulhern and Van Gerven (1997). It is possible that the St Matthew sample reached skeletal maturity earlier than the populations studied by Mulhern and Van Gerven (1997), thus having a shorter period of rapid bone modeling, resulting in higher OPD that accumulate for a longer period and therefore, higher netBFR (Stout and Lueck, 1995). To our knowledge, no data showing differences in skeletal maturation among past and present populations is currently available to corroborate this hypothesis.

Bone remodeling occurs at different rates in the various bones of the skeleton, and hence, direct comparisons with other studies are limited. Stout and Lueck (1995) have compared the bone remodeling rate between four distinct populations representing different mode of subsistence in North America: two foraging populations from the Early Archaic and the Middle Woodland, a Late Woodland population, that practiced agriculture and a modern autopsy sample. They suggest that the higher remodeling rate seen in the agricultural sample may reflect episodes of malnutrition compared to the two foraging groups. Historical records documenting the population of St. Matthew have related that there were periods of famine between 1769 and 1832 in Canada (Hare et al., 1987; Ruddel, 1991), making it conceivable that the higher remodeling rate found in our study reflects this stress. However, comparison between individuals diagnosed with metabolic disease and those without evidence of macroscopic pathology did not reveal differences in the bone remodeling dynamics. While numerous studies have shown the influence of metabolic diseases on remodeling rates (Eriksen, Steiniche, Mosekilde, and Melsen, 1989; Eriksen, 1986; Frost, 1964a; Parfitt, 1976a, 1976b), the absence of significant differences between males and females in our study might be due to a number of factors including, as mentioned above, the limitations in identifying individuals as pathological in archeological collections. Nonetheless, our results are consistent with Eleazer (2013), which found no differences in remodeling pattern between individuals

diagnosed with metabolic versus those diagnosed with non-metabolic diseases in an archeological juvenile sample.

While Canadian pioneering settlers such as the St Matthew cemetery's individuals offer a unique opportunity to explore the relationship between health and skeletal biology, the inherent limitations of paleopathological assessments in archeological samples may have influenced these results. Thus, further study should investigate DZD and netBFR in a modern, documented sample to confidently support or refute the relationship between histological parameters and pathological status, age, and sex.

4.6 Conclusion

Bone remodeling dynamics, more specifically double-zonal density and net bone remodeling, were evaluated in femoral cortical bone from the St Matthew's historic sample, Quebec City, Canada, in order to investigate if pathology, sex or age could be factors influencing DZ formation. The results of this study revealed that double-zonal osteons are linked to neither pathological status, sex, nor age differences. This suggests to us that DZ osteons are not related to mineral maintenance as previously suggested. However, these questions need to be investigated further on a larger sample for which health status and age would be confidently assessed. Our study was limited by the difficulty and uncertainty of paleopathological diagnosis of metabolic disease in archeological collections. Nonetheless, we did find sex-related differences in Type I osteon area and bone remodeling rates: females have smaller type I osteons and, thus, lower remodeling rates when compared to their male counterparts. Our results are particularly challenging since no consensus has been reached regarding the potential causes affecting osteon area, but the wide variation in observed male-female differences suggest that other factors, such as loading or activity, might be more important in determining osteon size. Given our relatively small sample size and the fairly low density of DZ osteons, the fact that we found no difference in double-zonal osteon area between age, sex and pathological conditions cannot be considered a definitive conclusion. Further studies might elucidate the role of other factors, such as mechanical loading that is likely to influence the formation of different types of osteon and their size.

4.7 Acknowledgments

This research is supported by the Archeometry Research Team at Université Laval FRQ-SC (R. Auger) and the Canada France Fund (I. Ribot, anthropologie, Université de Montréal). We are grateful to Dr. Bernard Long, Dr. Geneviève Treyvaud and Louis-Frédérique Daigle for providing access and assistance with the CT-Scan at the Laboratoire de scanographie, eau terre environnement (INRS, Québec City). We thank the following persons for allowing us access to the skeletal remains from St-Matthew: Marie-Sol Gaudreau (Anglican Diocese of Quebec), William Moss (Archaeologist, Quebec City), Réginald Auger (Université Laval, Quebec City).

5. Load history as a determinant of osteon frequency and morphology

Raguin, É. & M.S.M Drapeau

This article is in preparation for submission in Summer 2018 in the expected following journal: *American Journal of Physical Anthropology.*

5.1 Abstract

Double-zonal osteons (DZ), a product of the remodeling process, have generally been hypothesized to be the result of physiological stress, but recent work found no support for that hypothesis. While DZ are characterized by a hyper-mineralized ring inside their lamellae, recent findings suggest that this ring is also defined by a change in the collagen fibers orientation. Collagen and minerals are essential components to the maintenance of adequate bone strength and their alteration can modify the mechanical properties of the bone tissue. Consequently, the aim of this study is to explore the effect of past loads on the formation of DZ osteons compared to type I (common) osteons and cross-sectional geometric properties, which reflect bone strength and rigidity. The sample consists of paired humerus and femur midshaft sections (n=23) of Eurocanadian settlers from the historical St. Matthew cemetery, Quebec City (1771-1860). Histomorphometric variables included in this study are osteon density for DZ and type I osteons (DZD; OPD), osteon area (ZOn.Ar; On.Ar), and Haversian canal area (ZH.Ar; H.Ar). Loading history is estimated from cross-sectional properties and the variables included in the study are cortical and total area (CA, TA), maximum and minimum second moment of area (I_{max}, I_{min}) and polar moment of area (J). When the humerus and femur are compared, the femur has a higher OPD, DZD, and relative DZD (DZD/OPD). Compared to type I osteons, DZ osteons have a smaller osteon area and Haversian canal area. Correlations between the residual scores of the regression of histomorphometric variables and cross-sectional properties of the humerus on the femur were not significant. This lack of correlation between variations of loads and of remodeling, combined with the significant differences between humerus and femur suggests that the creation of DZ or type I osteons in the bone tissue is not simply regulated by biomechanical loads, but instead, that there is a bone specific response that needs to be further investigated.

5.2 Introduction

Bone is a complex tissue whose primary functions are to provide structural support for the body and to maintain mineral homeostasis (Martin, Burr, Sharkey, and Fyhrie, 1998). To maintain equilibrium in strain levels within the tissue, bone structure adapts to the incurred loads at both the macroscopic and microscopic level through modeling and remodeling (Carter, 1987; Frost, 1987; Lanyon, 1984). Modeling sculpts the size, shape and curvature of the bone in response to mechanical demands during growth and usually stops once maturity is reached (Frost, 1973), while remodeling, in addition to maintaining mineral homeostasis in the blood stream, provides maintenance and repair to preserve bone tissue integrity throughout the lifetime of an individual (Martin, 2003b). Thus, bone remodeling can be either stochastic as a normal part of mineral homeostasis (Martin and Burr, 1982; Burr, 2002; Frost, 1990b) or targeted as a mechanical adaptation to loading to replace damaged bone tissue (Burr, 2002; Frost, 2003; Parfitt, 2002). Remodeling removes and replaces portions of bone by a complex arrangement of cells, mainly osteoblasts and osteoclasts that respectively form and resorb bone. The cells are collectively called a basic multicellular unit (BMU) and their activity results in the creation of secondary osteons (Parfitt, 2003).

The effect of mechanical loading on bone modeling and remodeling has been hypothesized by Frost (1983b) in his mechanostat theory, which proposes that they are modulated by a negative feedback system based on the magnitude of the strain and/or the duration of loading. Under high strain levels, cortical bone will be added through modeling and remodeling will be inhibited while under disuse strain levels, remodeling will be activated and modeling inhibited. As pointed out by Martin (2000b), this theory is limited to the effects of strain on modeling and remodeling and does not include the removal of damaged tissue resulting from high strains. As mentioned above, bone remodels also to repair micro-damage that normally occurs when bone incurs loads (Martin, 2000b; Parfitt, 2002). Consequently, Frost (2003) revised his theory to include remodeling that repairs the accumulated fatigue damage caused by repeated strains. Bone response to loads by modeling and remodeling and the repair process allows cortical bone to maintain its mechanical integrity.

The mechanisms that trigger remodeling to replace damaged bone are still not entirely understood but numerous histomorphometric studies have found an increase in bone

remodeling in more loaded bones (Bentolila et al., 1998; Burr, 2002; Burr, Ruff, and Thompson, 1990; Drapeau and Streeter, 2006; Lanyon, 1984; Lieberman and Crompton, 1998; Lieberman, Pearson, Polk, Demes, and Crompton, 2003; Martin, 2007; Rubin, McLeod, and Bain, 1990) and found that remodeling appears to be targeted to areas of greater microdamage (Burr, 2002; Parfitt, 2002). Additionally, it has been shown in experimental studies that microcracks in bone can initiate remodeling in order to ultimately remove the cracks to maintain bone integrity (Burr et al., 1985; Bentolila, Boyce, Fyrie, Drumb, Skerry and Schaffler, 1998; Mori and Burr, 1993; Verborgt, Gibson and Schaffler, 2000).

Four distinct types of secondary osteons are recognized in human cortical bone; type I, the drifting osteon, the type II and the double-zonal osteon (DZ). Type I osteons are the most common osteons and are characterized by uninterrupted and concentrically deposited lamellae surrounding a central Haversian canal (Martin and Burr, 1989; Takahashi and Frost, 1966). Drifting osteons are defined by an elongated "tail" and a non-centrally located Haversian canal resulting from a continuous resorption on one side and continuous formation on the other (Frost, 1964; Robling and Stout, 1999) and are preponderant in subadult bones (Epker and Frost, 1965; Streeter, 2010). Both type I and drifting osteons are the result of normal bone remodeling and their formation as well as their morphology have been well described in previous studies (Enlow, 1962; Epker and Frost, 1965; Frost, 1963, 1964; Lacroix, 1971; Robling and Stout, 1999; Sedlin, Villanueva, and Frost, 1963). Type II or embedded osteons are a smaller version of type I osteons that form within the cement line of a preexisting secondary osteons by radial erosion of a preexisting Haversian canal (Jaworski, Meunier, and Frost, 1972; Lacroix, 1971; Ortner, 1975). Type II are thought to be associated with aging, possibly occurring to maintain mineral homeostasis (Ericksen, 1991; Ortner, 1975; Yoshino, Imaizumi, Miyasaka, and Seta, 1994), while other studies have shown a possible link with dietary variations (Ericksen, 1980; Richman, Ortner, and Schulter-Ellis, 1979). Finally, DZ osteons are similar to type I osteons except that they can be distinguished by a hyper-mineralized ring within the lamellae of the osteon. They have been hypothesized to be the result of a temporary arrest in bone deposition during the formation of a type I osteon (Lacroix, 1971; Pankovich, Simmons, and Kulkarni, 1974) resulting in a greater mineralization of the tissue and a change in collagen fibers orientation (Raguin et Streeter, In

press, Chapter 3 of this dissertation). However, as seen in Chapter 4, their formation does not appear to be related to metabolic pathologies, nor to variation in age.

This change in the ultrastructure of the osteon is relevant to bone structural integrity since it can affect the mechanical properties of the bone tissue (Evans and Bang, 1967; Portigliatti Barbos, Bianco, Ascenzi, and Boyde, 1984). Collagen fibers are proteins synthesized by osteoblasts providing the essential structures for mineral deposition formed by calcium phosphate crystal plate-shaped in a parallel arrangement to these fibers in extracellular spaces, within the collagen fibrils and at their surface (Landis et al., 1996; Weiner, Arad, and Traub, 1991). Ascenzi and Bonucci (1964, 1967, 1968, 1972) have presented a classification of several types of osteons according to the predominant orientation of their collagen fibers: dark, bright and alternating when viewed under linear polarized light (PLM). They found that osteons with collagen fibers oriented parallel to the long axis of the bone (dark) have the greatest tensile strength but are the weakest in shear; osteons with alternating collagen fiber orientation (alternating) are more able to withstand loading by bending; and osteons with circumferentially oriented collagen fibers (bright) are stronger in compression. Alternatively, Marotti (1993) proposes another model of bone lamellation using electron microscopy, where collagen fibers are organized according to two different patterns: looser and denser packed lamellae. The looser packed lamellae consisted of thick collagen fibers, sparsely distributed, and not oriented in a predominant direction while the dense lamellae are constituted of thin, but dense collagen fiber bundles that are oriented in a predominant direction. Marotti (1993) showed that the calcium and phosphorous concentration in loose lamellae were 10 to 15% higher than in the dense lamellae. The author postulated that these differences in size, density and orientation result in the tone variations when viewed under PLM, the loose lamellae appearing darker while the dense lamellae are brighter. In a subsequent study, Marotti et al. (1994) suggested that the dense, collagen-rich and low-mineralized lamellae are able to resist tensile strains alternating with loose, collagen-poor and highly mineralized lamellae, more suitable to withstand compression. However, the works of Ascenzi and Bonucci (1964, 1967, 1968, 1972) and Marotti (1993) on their interpretation of the birefringence pattern in PLM regarding collagen orientation were questioned by Martin et al. (1996a; 1996b). The authors proposed that since osteons are not perfectly aligned along bone axis, the birefringence pattern would depend on the local orientation of the osteon as well

as the fiber organization relative to this axis. Moreover, they argue that birefringence in PLM is also a function of the orientation of the section with respect to the polarization plane, which superimposes artifactual extinction patterns, known as the "Maltese cross" (Bromage et al., 2003). Martin et al. (1996a; 1996b) suggest that while a change in collagen fiber orientation can be determined under PLM, the precise orientation linked to biomechanical load can be characterized only in circular polarized light (CPL), which eliminate this artifact. Their results have highlighted the greater variability of the collagen fiber birefringence in PLM when compared to CPL. Consequently, the most accepted model regarding the arrangement of the collagen fibers is the twisted plywood structure proposed by Giraud-Guille (1988) which states that collagen fibers lay in parallel to each other in each lamella of an osteon at an angle from the long axis of the bone with an alternating orientation of fibrils from one lamella to another. Finally, recent studies unite several of these models with the description of two different types of materials in collagen fibrils organization in demineralized human femoral section: the ordered material characterized by aligned bundles of collagen arranged in twisted an oscillating plywood patterns; and the disordered material, which contains randomly distributed collagen fibrils and a larger proportion of ground substance due to a looser packing density (Reznikov, Almany-Magal, Shahar, and Weiner, 2013; Reznikov, Shahar, and Weiner, 2014a, 2014b). The bigger gaps between the collagen matrix allow a larger amount of mineral crystal to form (Wassen et al., 2000).

Regardless of the debate about collagen fiber orientations and fiber density, the hierarchical organization of collagen fibrils and mineralization is believed to be influenced by cells from the osteoblastic lineage that are able to tune the extracellular matrix in response to the mechanical load and thus, mineral and collagen are good indicator of bone strength (Currey, 2003; Martin, Lau, Mathews, Gibson, and Stover, 1996; Rohrbach et al., 2012; Wagner and Weiner, 1992). In addition, it is widely accepted that collagen is responsible for the toughness of the bone whereas mineral content is involved in bone strength and stiffness (Martin, 1991; Wang, 20000, 2001). While the distribution of osteons according to their collagen fiber orientation has been correlated with the distribution of strain mode (Portigliatti Barbos, Bianco, and Ascenzi, 1983; Portigliatti Barbos et al., 1984; Riggs, Lanyon, and Boyde, 1993), no studies, to our knowledge, have focused on the relationship between the frequency of DZ, and the mechanical properties of bones. A reason that could explain this lack

of comparative data is that DZ osteons have always been hypothesized as being the result of metabolic disorder due to the presence of the hyper-mineralized ring, thought to be the consequence of an arrested growth formation analogous to Harris lines (Austin and Mulhern, 2015; Lacroix, 1971; Lacroix and Dhem, 1967; Martin and Armelagos, 1985; Pankovich et al., 1974). However, Dhem (1980) demonstrated that the hyper-mineralized ring was not an arrest line since the cytoplasmic processes of the osteocytes located in the hyper-mineralized ring extended without interruption with the osteocytes found in the lamellae on both sides of the ring, maintaining a functional lacuno-canalicular network. The author argues that after an arrest in osteon formation, this lacuno-canalicular network should be disrupted. Additionally, Raguin and Streeter (chapter 4) found no differences in DZ osteon density between individuals with and without metabolic diseases. Since DZ osteons are also defined by a change in collagen fiber orientation (Raguin and Streeter, in press, Chapter 3 of this dissertation) we hypothesize that their formation could be regulated by differences in mechanical loads incurred by the bone.

Osteon size also appears to affect mechanical properties of cortical bone, although numerous studies have produced conflicting results (Borgel, 2017; Denny, 2010; Pfeiffer, Crowder, Harrington, and Brown, 2006; Skedros, Keenan, Williams, and Kiser, 2013; Skedros, Mendenhall, Kiser, and Winet, 2009). Corondan and Haworth (1986) found that humans cortical bone tends to break where osteons are smaller. They proposed that an increase in osteon size would increase the resistance to crack propagation. Moyle and Bowden (1984) showed a complex and U-shaped relation between osteon size and toughness, where greater energy absorption in area of a developing crack can be achieved when osteon are smaller and as osteon size increases, energy absorption declines. However, they also found that much larger osteons (with a diameter greater than 200 μm) also allow greater energy absorption. Yeni et al. (1997) demonstrated that crack initiation and fracture decrease with increasing osteon area and proposed that larger osteons allow a better diffusion of microcracks and increase the resistance of the bone. In a study of adult human femurs, Britz et al. (2009) have shown that osteon size in inversely related to body weight. Thus, if larger body weights result in higher bone strains, higher strains can be inferred to result in smaller osteons. Similarly, Van Oers et al. (2008) have shown a decrease in the perimeter of the osteons when bones incur higher mechanical loads. Abbott et al. (1996) also interpreted smaller osteons in Pleistocene

populations in comparison to modern populations as the product of higher mechanical loads in the prehistoric sample. However, when comparing ribs and femurs of the same individuals from samples of Late Stone Age foragers from South Africa and historic Canadians settlers, Pfeiffer et al. (2006) found that rib osteon size was smaller than that of the femur while variation in size was similar in the two bones. Since the rib most likely incur much lower loads than the femur, they conclude that osteon size was not a good indicator of physical activity in past populations. However, Dominguez and Agnew (2016), while acknowledging that many elements are at play such as the strain mode, proposed that a larger cortex would allow bigger osteons to form, which could explain the observed differences observed by Pfeiffer et al. (2006) between the femur and the much smaller rib. Osteon size, thus, would be a function of chronological age and bone porosity. Finally, when comparing the osteon size and strain mode in the artiodactyl calcaneus, Skedros et al. (1994) showed that smaller osteons were found in the larger compressive regions of the cortex while the smaller tensile regions housed larger osteons. The authors suggest that the variation in loading is more determinant than the size of the cortex.

Besides biomechanical loads, other factors such as age and sex have been proposed to influence osteon area. Most studies agree on the inverse relationship between age and osteon area (Britz et al., 2009; Burr et al, 1990; Currey, 1964; Martin, Pickett, and Zinaich, 1980; Takahashi, Epker, and Frost, 1965). Burr et al. (1990) hypothesized that reduction in osteon size is an adaptive response to maintain bone properties to compensate for bone loss associated with the accumulation of remodeling events as an individual's age. However, studies of sex-related differences do not yield consistent results. Burr et al. (1990) and Mulhern and Van Gerven (1997) found, on archeological samples, that females have larger osteons than males. Both attribute these results to sex-based division of labor, where males engaged in more strenuous activity and had smaller osteons. These results are supported by the aforementioned work by Van Oers et al. (2008), Abbott et al. (1996), and Britz et al. (2009) that proposed that osteons were smaller in more loaded bones. However, Britz and colleagues (2009), in the same study on a modern sample, demonstrated that females had smaller osteons compared to males, without offering an explanation for this observed difference. Similarly, Denny (2010) found smaller osteons in females compared to males in the second metacarpals of a pioneer population from Canada, contemporary to our sample. She proposed that the

larger cortex in males would allow bigger osteons to form, as suggested by Dominguez and Agnew (2016). Other studies found no sex-related differences (Borgel, 2017; Dominguez and Agnew, 2016; Pfeiffer, 1998; Pfeiffer et al., 2006) Finally, Havill et al. (2013) have shown that genetics is an important component in the determination of osteon size.

Results from all these studies underscore how little is understood of the factors that determine osteon formation and size, particularly for DZ osteons. In this context, the purpose of this study is to investigate the relationship between DZ osteons morphotype and mechanical loads and how it differs from the formation of 'normal' type I osteons. We will also compare histomorphometric values in paired femur and humerus of the same individuals to investigate how these osteons, their numbers and size, correlate with cross-sectional properties as an estimate of the mechanical load history of the bone. Because some remodeling is targeted to damaged bone and because DZ osteons are characterized by collagen lamellae disturbance that we believe occurs in the context of large loads, we hypothesize that bones that have incurred greater loads will have more type I and proportionally more DZ osteons than bones that incur smaller loads. Also, because we believe that large loads favor the formation of smaller osteons, we hypothesize that osteons, particularly DZ, will be smaller and will have smaller Haversian canals in more loaded bones.

5.3 Materials and Methods

The study sample comprised paired femurs and humerus (n=23) from 18 and 19th century Eurocanadians settlers from St-Matthew cemetery in Quebec City, Quebec. The cemetery was active between 1771 and 1860 (Cloutier, 2000; Simoneau, 2003). Age was estimated and only adults between 20 to 50 years old were included in this analysis. The bone from the right side was selected, but if it was missing or significantly damaged, the left side was used.

The undecalcified bone sections were prepared using standard histological preparation protocol (Crowder, Heinrich, and Stout, 2012; Frost, 1958). After cutting a section of approximately three cm thick with a bandsaw at the midshaft of each bone, the sections were then embedded in epoxy resin under a vacuum (EpoThin 2 resin and hardener and Cast N'Vac

1000, Buehler Ltd., Lake Bluff, IL). The vacuum forces the evacuation of trapped air and the penetration of the resin deep into the bone to insure that bone structural integrity is preserved. An Isomet precision sectioning saw (Buehler Ltd., Lake Bluff, IL) was used to cut thin sections of around 300 µm and then ground to a final thickness of approximately 80 µm with a PetroThin thin sectioning system (Buehler Ltd., Lake Bluff, IL). The thin bone sections were finally mounted onto microscopic slides using Permount™ (Fisher Chemicals™) and cover-slipped. Histological digital images of each femur and humerus at midshaft were acquired with automated scanning under linear polarized light with an Olympus BX43 microscope in proper anatomical orientation at 100X magnification with the Objective Imaging Surveyor Software. This technique allows the production of an automatically stitched image of the entire cross section. Preparation of microscopic slides and data collection were performed at *Laboratoire d'écomorphologie et the paléoanthropologie, département d'anthropologie, Université de Montréal* by one of us (ER). Histomorphometrical structures were recorded and measured on the entire cross section using ImageJ software (Schneider, Rasband, and Eliceiri, 2012). We recorded Osteon Population Density (the number of intact and fragmentary osteons per unit of area; OPD), Zonal Osteon Density (number of intact DZ osteons per unit of area, DZD), as well as osteon area and Haversian canal area for both type I (On.Ar, H.Ar) and DZ (ZOn.Ar, ZH.Ar). Osteon area and Haversian canal area are defined, respectively, as the average area of bone including the Haversian canal contained within the cement lines and the average area of the Haversian canal. The average values are based on approximately 200 type I osteons for the femur and 100 for the humerus sampled throughout the cross sections. Because of their much smaller numbers, all DZ osteons were used to calculate the same parameters.

DZ osteons were identified following the protocol developed by Raguin and Streeter (in press, Chapter 3 of this dissertation). Osteons that showed a smooth and continuous ring with a difference in brightness compared to adjacent lamellae that persists by changing the intensity of light and by focusing in and out were positively identified as DZ. Each of these osteons was then examined under transmitted light to ensure that the ring did not become scalloped to avoid misidentification with type II osteons. This method proved to be reliable and accurate alternative to Backscattered Electron Microscopy with the Scanning Electron Microscope technique for the identification of DZ (Raguin and Streeter, In press).

The concept that bone is adapted to its mechanical environment during life has been widely applied in anthropological studies in order to understand the variations of past and present populations regarding subsistence strategy and mobility (Ruff and Hayes, 1983a; Ruff and Larsen, 1990; Ruff, Larsen, and Hayes, 1984; Sládek, Berner, and Sailer, 2006; Trinkaus and Ruff, 1999), sexual dimorphism (Berner, Sládek, Holt, Niskanen and Ruff, 2017) and adaptation to the environment (Ruff, 2005; Stock and Pfeiffer, 2001). Bone adapts to mechanical loads by subtly modifying its mass and architecture to match the strains incurred by the skeleton (Frost, 1990a; Sommerfeldt and Rubin, 2001; Turner, 1998). It has been shown that long bones, particularly at midshaft, are loaded predominantly in bending (Rubin et al., 1990) and, as a consequence, measures of bending rigidity provide a means to infer the history of mechanical loading (Robling and Stout, 2003). Considering that area of the cross section and the distribution of this area about the neutral axis of bending, it is possible to evaluate the bending rigidity of the bone's diaphysis known as the second moment of area (SMA). Thus, beams with greater SMAs have a greater resistance in bending and the opposite is observed when SMAs are smaller (Van der Meulen, Jepsen, and Mikić, 2001).

The stitched digitized cross sections were imported into ImageJ using the BoneJ plugin (Doube et al., 2010) for the calculation of cross-sectional properties. The following macrostructural variables were thus quantified for each bone sections: cortical area (CA); total subperiosteal area (TA), maximum second moment of area (I_{max}), minimum second moment of area (I_{min}), and polar moment of area (J). Cortical area and subperiostal area provide measures of the quantity and relative distribution of bone in the section (Ruff, 2007). Second moment of areas (I) measure the quantity and distribution in order to quantify bending rigidity or, in other words, the capacity of the bone to resist bending in a given axis. I_{max} and I_{min} represent the maximum and minimum rigidities of the section when flexed about its minor and major axes. Polar moment of area (J) measures the capacity of the bone to resist torsional loads, it is also used as a measure of average bending rigidity since it is calculated at the sum of any two perpendicular second moment of areas of a section (Ruff and Hayes, 1983a, 1983b).

Cross-sectional geometric properties of long bones are functions of body mass and bone length (Ruff et al.; 1993). However, in this study we use the paired humerus and femur of the same individuals, so it not necessary to control for body mass since it is a constant. To

control for different bone length between humerus and femur, SMAs and polar moment of inertia were standardized by bone length (I/L and J/L).

To test whether type I and DZ osteons had different morphologies, we test whether DZ are smaller than type I osteons using a non-parametric Wilcoxon tests in SPSS 24 statistical package. TO explore the effects of loading on type I and DZ osteons, we did two types of analyses: direct comparison of paired humerus and femur and correlation analyses of the variation of macroscopic and microscopic variables.

Since the femur is used in locomotion while the humerus is not, we assumed that the femur incurs greater loads than the humerus, an assumption that is supported by the observation that the cross-sectional size of the femur at the midshaft is always greater than that of the humerus in non-pathological individuals (Macintosh, Davies, Ryan, Shaw, and Stock, 2013; Ruff, 2003b; Sumner and Andriacchi, 1996; Table X). In a first step, we compared histomorphometric parameters of the humerus directly to the femurs using a Wilcoxon test for paired values in SPSS 24 statistical package.

To explore more subtle loading differences, we examined the correlation between macroscopic and microscopic variables, using a method slightly modified from Robling (1998). Robling used the histological values of the rib to control for the systemic remodeling of the femur and 'isolate' the remodeling variation due to variation in loading. For the current study, since we did not have the rib, we modified the method by regressing the values of the humerus on the femur. We choose to regress the humerus on the femur for two reasons. First, humans only use only their lower limbs in locomotion, leaving the upper limb for manual activities that are generally fairly variables in human societies. Second, because of the fragmentary nature of archaeological collections, we could not systematically use the humerus or femur of the same side in our study. Since humans are characterized by greater asymmetry of the upper limb relative to the lower limb (Rhodes and Knüsel, 2005; Trinkaus and Churchill, 1999; Trinkaus, Churchill, and Ruff, 1994; Trinkaus and Ruff, 1999), right and left femurs were less likely to be different and either side reflected the general activity and mobility level for each individual. The residuals of a regression of the values of the humerus on the femur reflect the variation in the humerus that is not correlated to (or explained by) the variation in the femur (which includes interindividual variation in general activity level as well as metabolic variations). In other words, the residuals quantify the deviations of the humeral

values from those expected from the femur. Individuals that loaded their upper limbs (i.e., the right or left humerus) more than what would be expected from the femur will have positive values while individuals that loaded the upper limbs less would have negative values. It is expected that individuals that have greater deviation from the 'average' population macroscopic values will also have greater deviations of the microscopic values, so we expected positive correlations between macroscopic and microscopic deviations.

In order to explore the correlation of macroscopic and microscopic variables, the cross-sectional properties (I_{max}, I_{min}, J, TA, CA) and histomorphometric variables (OPD, DZD, On.Ar, ZOn.Ar, H.Ar and ZH.Ar) for the humerus as well as for the femur were log-transformed (ln-transformed). Each of the logged variables from the humerus were regressed onto the same variables from the femur using reduced major axis regression (RMA) to determine the underlying biological relationship between the two bones (Aiello, 1992; Hofman, 1988; Smith, 2009). RMA is the most appropriate line-fitting technique when error variances of the two variables are unknown (Rayner, 1985). From these regressions, residual scores (^{R}) were calculated for each individual and, as explained above, they reflect the degree of deviation of the humeral value based on the expected value from the femur. In other words, residual scores represent the histomorphometric and cross-sectional properties in the humerus that are not accounted for by the same variables in the femur. To estimate relationships between the residual scores of the microscopic and cross-sectional properties variables, we used Pearson's correlation coefficients when the residuals were normally distributed, but Spearman's correlation (non-parametric) was used when the residuals were not normally distributed. These analyses were performed using the PAST statistical package (Hammer, Harper, and Ryan, 2001).

5.4 Results

Table X presents the descriptive histomorphometric values and cross-sectional properties for the femur and the humerus. When bones are analyzed separately, osteon and Haversian canal areas are significantly smaller in DZ osteons than in type I, for both the humerus and femur (Table XI and Fig. 14). As predicted, osteon population density and double-zonal osteon density is significantly greater in the femur than in the humerus (Table

XII and Fig. 15A and B). When controlling for the frequency of the DZ by the frequency of the OPD (DZD/OPD), DZ are also proportionally more common in the femur than in the humerus (Table XII and Fig. 15C). However, no difference was found between the humerus and femur in size of the osteons, whether they are type I or DZ osteons, failing to support our hypothesis of smaller osteons in more loaded bones.

	CA (mm^2)	TA (mm^2)	I_{max}/L (mm^3)	I_{min}/L (mm^3)	J/L (mm^3)	OPD (#/mm^2)	DZD (#/mm^2)	On.Ar (mm^2)	ZOn.Ar (mm^2)	H.Ar (mm^2)	ZH.Ar (mm^2)
Humerus											
Mean	221.24	335.47	30.20	20.87	51.07	9.91	0.043	0.0528	0.0334	0.00279	0.00170
SD	61.68	91.59	9.62	6.46	15.49	1.93	0.032	0.0090	0.0134	0.00053	0.00076
Femur											
Mean	405.07	559.69	60.57	47.65	108.22	10.84	0.085	0.0538	0.0348	0.00261	0.00176
SD	66.87	102.10	20.46	14.61	34.37	1.58	0.055	0.0099	0.0077	0.00047	0.00038

[1]CA, cortical area; TA, total subperiosteal area; I_{max}/L, maximum second moment of area divided by bone length; I_{min}/L, minimum second moment of area divided by bone length; J/L, polar moment of area divided by bone length; OPD, osteon population density; DZD, double-zonal osteon density; On.Ar, osteon area; ZOn.Ar; double-zonal osteon area; H.Ar, Haversian canal area; ZH.Ar, double-zonal Haversian canal area.

Table X. Mean and standard deviation for cross-sectional areas and size-standardized second moment of area and polar moment of area and histomorphometric variables[1]

	Humerus		Femur	
	Z	*p-value*	*Z*	*p-value*
Osteon area	-5.508[1]	0.000*	-4.197	0.000*
Haversian canal area	-5.660	0.000*	-4.015	0.000*

*Significant at p=0.000

[1]Negative values indicate that type I osteon area and Haversian canal area are larger than those of DZ osteons

Table XI. Results of the Wilcoxon tests comparing osteon area and Haversian canal area of type I and zonal osteons in the humerus and in the femur.

	OPD	DZD	DZD/OPD[2]	On.Ar	ZOn.Ar	H.Ar	ZH.Ar
Z[3]	-2.342	-3.893	-3.425	-0.912	-0.156	-1.247	-0.434
p-value	0.019*	0.000*	0.001*	0.362	0.876	0.212	0.664

*Significant at $p<0.05$
[1]See abbreviations of the variables in Table X.
[2]Double-zonal osteon density controlled for the total osteon population density
[3]Negative values indicate greater average in the femur than in the humerus

Table XII. Results of the Wilcoxon tests comparing histomorphometric variables of the humerus to the femur[1].

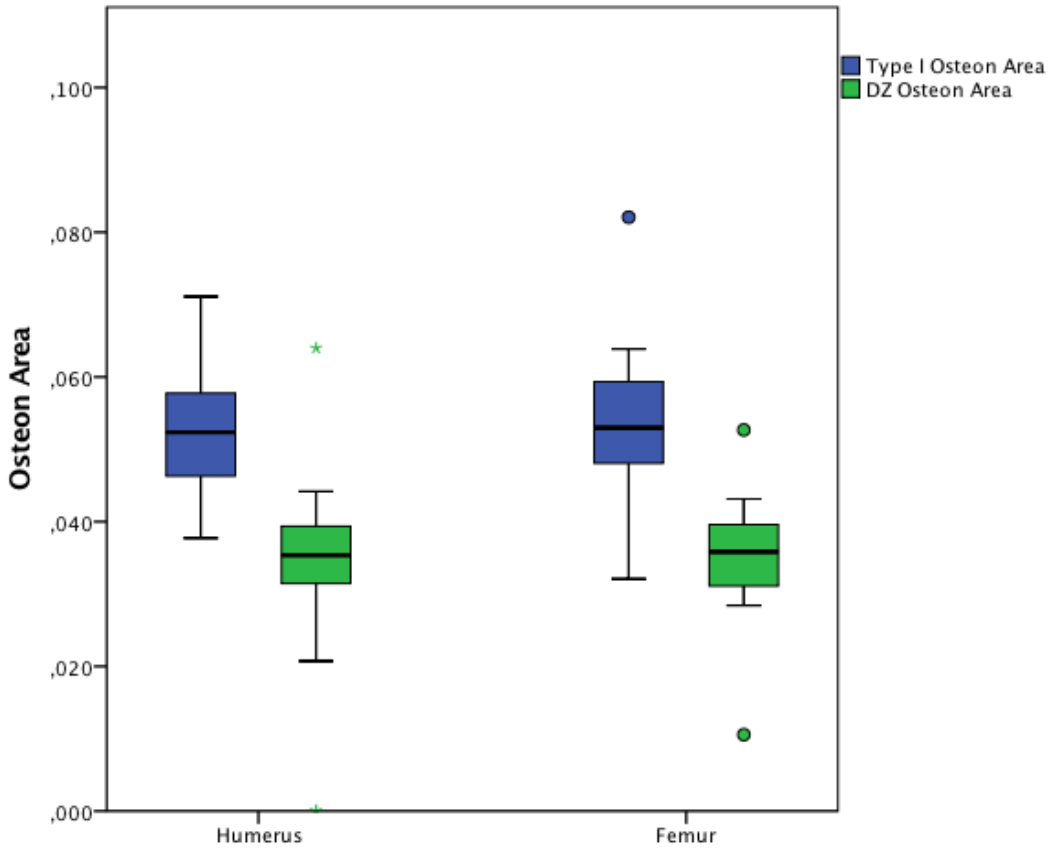


Figure 14. Boxplot of the difference of osteon area between Type I osteon and DZ osteon in the humerus and in the femur.

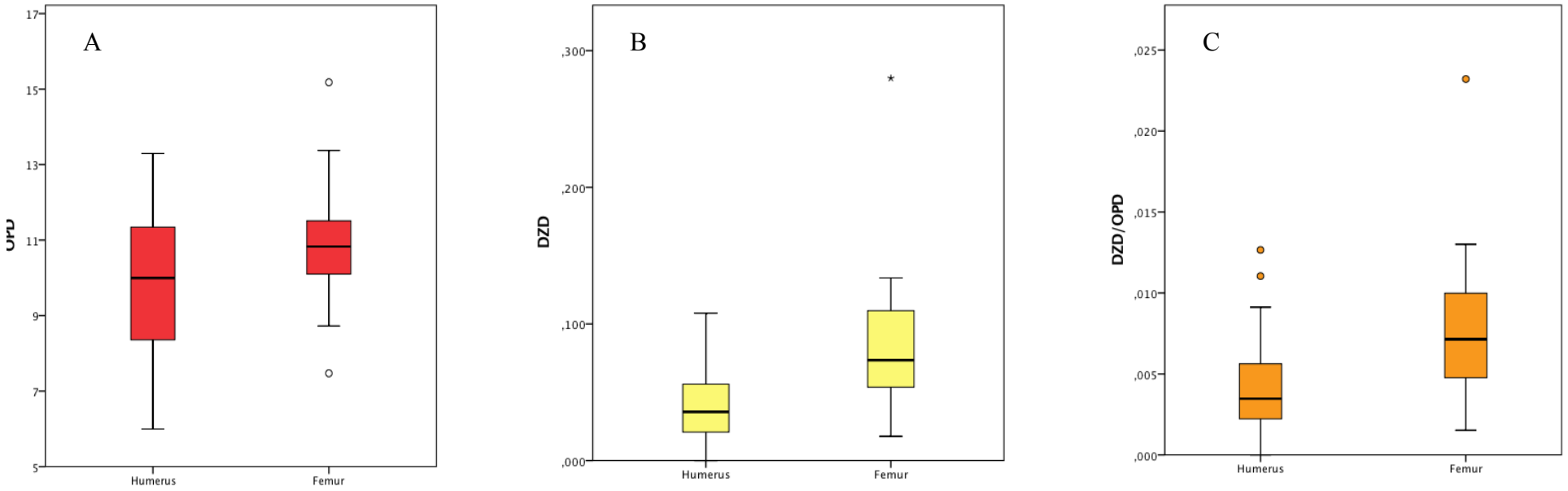


Figure 15. Boxplot of the osteon population density (A), double-zonal density (B) and relative double-zonal density (C) in the humerus compared to the femur. All humerus-femur differences are statistically significant.

Correlations of the residuals from the RMA regressions of the different variables (Table XIII) revealed that ^{R}DZD is significantly and negatively correlated with the total subperiosteal area, $I_{max,}$ I_{min} and J. Thus, it appears that a significant portion of the residual variation in the DZD of the humerus is explained by the mechanical loading environment, but the trend is opposite of what was expected. As cross-sectional properties of the humerus are larger than what would be expected from the femur, there are fewer DZ osteons than when the cross-sectional properties are smaller. There is no such trend with Type I OPD (Fig.16).

	^{R}CA		^{R}TA		$^{R}I_{max}$		$^{R}I_{min}$		^{R}J	
	cc	*p-value*	cc	*p-value*	cc	*p-value*	cc	*p-value*	cc	*p-value*
^{R}OPD	-0.235	0.280	-0.288	0.183	-0.245	0.260	-0.269	0.214	-0.275	0.204
^{R}DZD	-0.430	0.052	-0.592	0.005*	-0.533	0.013*	-0.535	0.012*	-0.589	0.005*
^{R}On.Ar	-0.166	0.448	-0.126	0.567	-0.245	0.260	-0.071	0.747	-0.207	0.344
^{R}ZOn.Ar	0.056[2]	0.810	0.201[2]	0.382	0.052[2]	0.823	0.152[2]	0.511	0.053[2]	0.819
^{R}H.Ar	-0.328	0.127	-0.089	0.686	-0.327	0.127	-0.077	0.725	-0.266	0.219
^{R}ZH.Ar	0.071	0.761	0.121	0.600	0.031	0.893	-0.015	0.947	0.016	0.944

*Significant at $p<0.05$ prior to Bonferroni correction, but not significant after the correction
[1]See abbreviations in Table X. Pearson's correlations are presented unless otherwise noted.
[2]Spearman correlation coefficient

Table XIII. Correlation coefficients (cc) and associated p-values between the residuals scores of the histomorphometric variables and those of the cross-sectional properties[1].

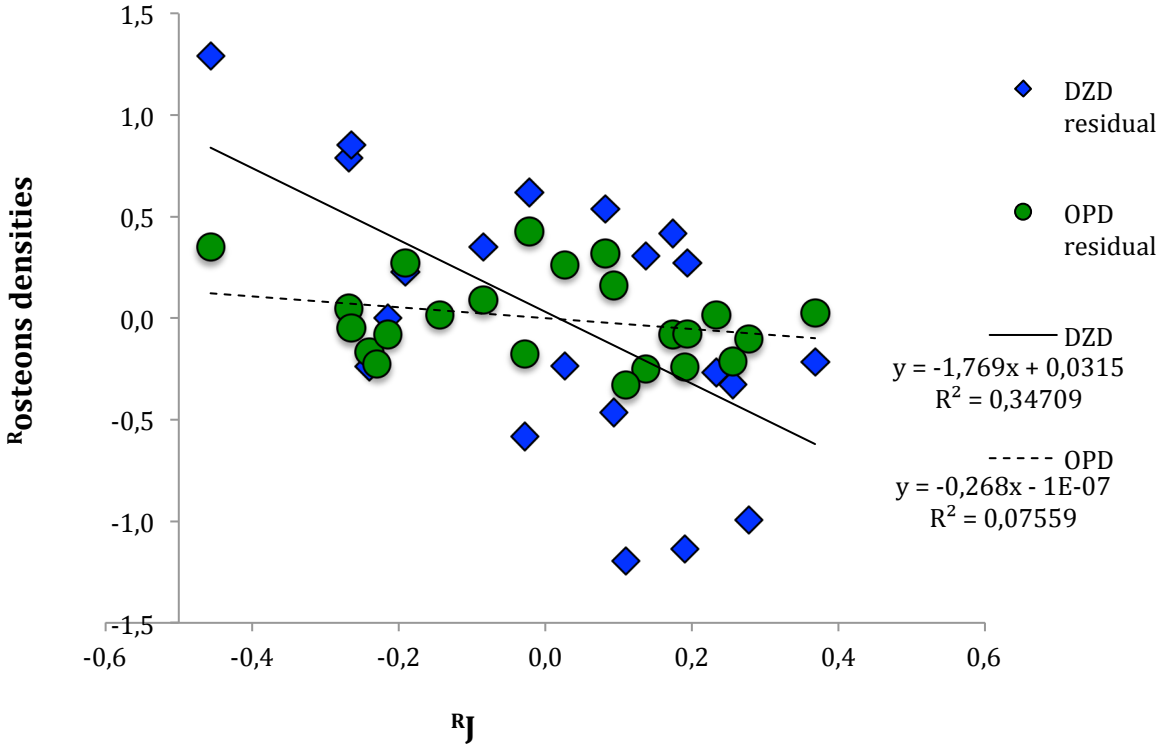


Figure 16. Relation between the residual osteon densities of type I (^{R}OPD) and DZ (^{R}DZD) and the residual polar moment of inertia (^{R}J).

5.5 Discussion

The present study shows that the lower limb, when compared to the upper limb, has more remodeling of type I and DZ osteons. These differences suggest that greater loading, as experienced by the femur relative to the humerus, results in more remodeling events. In addition, the proportion of DZ osteons relative to type I (DZD/OPD) is also greater in the lower limb, indicating an accentuated response of DZ osteons relative to type I. The greater remodeling in the most loaded bone supports the findings of previous studies (Bouvier and Hylander, 1981; Burr, Martin, Schaffler, and Radin, 1985; Lanyon, 1984; Lieberman and Crompton, 1998; Lieberman et al., 2003; Robling, 1998; Schaffler and Burr, 1988; Skedros et al., 2013, 2009; Yeni et al., 1997) that suggested that loading results in more remodeling events. In addition, the proportionally greater remodeling of DZ osteons supports our hypothesis that these osteons occur in context of greater strains in the bony tissue.

However, this postulate is questioned by the negative correlation between the residuals of DZD and cross-sectional properties. Indeed, there were negative correlations between the residual DZD (i.e, DZD of the humerus not accounted for by the DZD of the femur) and residuals of the total subperiosteal area (TA), maximum bending rigidity (I_{max}), minimum bending rigidity (I_{min}) and polar moment of area (J). In other words, individuals that have a humeral cross section that is bigger relative to the femur have lower relative DZD. There is a similar trend with type I OPD, although the correlations are not significant. These findings are not consistent with previous studies on the rib and femur (Robling, 1998; Robling and Stout, 2003; Walker, Lovejoy, and Meindl, 1994) that show a positive correlation between remodeling events and cross-sectional properties. Our results are also not consistent with predictions of the mechanostat theory (Frost, 1987, 2003), which states that when subject to high mechanical loads, bone increase its modeling rate resulting in a larger cross section but also its remodeling activity in order to repair the micro-damage to maintain bone strength. However, many authors have pointed out the inconsistencies in the mechanostat theory with regards to experimental observations (Martin, 2000; Skedros et al., 2001; Turner, 1999). This led Turner (1999) to propose the principle of cellular accommodation, in which the cellular responses to mechanical loading result in an adaptation of the cellular mechanical behavior (Vahdati et al., 2008). Moreover, bone cells retain a cellular memory of their previous loading

environment (Turner et al., 2002) and thus, are only stimulated when the new strain stimulus is higher than the past one (Schriefer et al., 2005). As a result, it is possible that greater loads incurred by the humerus during growth have favored a greater modeling activity, but a change in loading later in life does not increase remodeling activity because the bone is already adapted.

Since we focused on DZ osteons, our results suggest that this type of osteons may not occur predominantly in a context of high loads. However, the greater remodeling of all types of osteons that we observed in the femur relative to the humerus suggests that there might be bone-specific rates of remodeling. Intraskeletal variability between weight-bearing and non weight-bearing bones has previously been demonstrated regarding bone mass (Peck and Stout, 2007; Ruff, 2000; Stewart, Goliath, Stout, and Hubbe, 2015) but much less is known at the microscopic level. To our knowledge, only comparisons between the femur and the ribs have been investigated (Cho and Stout, 2011; Mulhern, 2000; Mulhern and Van Gerven, 1997; Robling, 1998; Robling and Stout, 2003). Although several studies have found higher remodeling rates in the rib compared to the femur (Cho and Stout, 2011; Mulhern, 2000; Mulhern and Van Gerven, 1997), others have shown opposite results (Robling, 1998; Robling and Stout, 2003). Robling and Stout (2003) argue that the dynamic loading incurred by the femur induces higher remodeling rates compared to the rib. Conversely, Cho and Stout (2011) hypothesized that the higher remodeling rates in the rib compared to the femur is due to the accumulation of micro-damage induced by the respiration loading cycle while Mulhern (2000) proposes that the femur takes a longer time to mature than the rib, leading to fewer secondary osteons accumulation at any chronological age and therefore differences between the two bones may not reflect differences in loading. Our results of a higher OPD in the more loaded femur support Robling and Stout's hypothesis (2003) but are more difficult to conciliate with Mulhern's hypothesis (2000) of maturation differences. Although it is not inconceivable that the humerus matures later than the femur, they are two long bones with similar growth patterns that undergo epiphyseal fusion at broadly similar ages (Buikstra and Ubelaker, 1994). However, when comparing the amount of modeling drift in paired femur and humerus at three sampling locations (proximal, midshaft and distal) along the diaphysis of each bones, Maggiano et al. (2015) showed that while the femur has a consistent drift pattern, dominantly lateral with a slight anterior tendency, the humerus has a general posterio-medial drift and the

entire bone is drifting rather than a precise location to intiate curvature or positionning. According to the authors, this pattern observed in the humerus may be correlated with the development of humeral torsion. Previous researches have suggested that the ontogenic change in the humeral torsion ceases between 16 and 20 years of age (Krahl, 1945; Edelson, 2000). Additionnally, when evaluating the growth-related changes in an archeological population, Sumner and Andriacchi (1996) have shown that the relative cortical thickness, cortical area and moment of inertia increased at a greater rate in the femur than in the humerus prior to the age of ten, even when accounting for the rate of increase in bone length. The authors suggest that this increase in cross-sectional geometry of the femur compared to the humerus is linked to differences in muscle strength and also to the type of loading. These results are corroborated by Ruff (2003a, 2003b) in a longitudinal study of modern individuals, which found that the muscle cross-sectional area of the thigh increases more rapidly than that of the forearm until 12 years of age. This pattern was reversed through the rest of adolescence, as forearm muscles cross-sectional area continues to increase while thigh muscle area stabilizes. The author also found that between 1 and 2 years, the femoral strength increases almost three times as fast as humeral strength, continue to rise rapidly between 2 and 3 years, and then settle into a much slower increase until mid-adolescence when, according to the author, adult proportions are reached. Thus, the femur would attain a near-adult size at an earlier age than the humerus. Moreover, Sumner and Andriacchi (1996) reported that the diametric growth continued until the third decade in the humerus and the femur, but that there is a basal adaptive lag in the humerus, which increases in magnitude as the mechanical demands on the bone increase. Thus, it is possible in our sample that the humerus cross-section consists of younger bone since it goes through cortical drift and modeling at a later age than the femur. With less drift, the femur bone compacta would be older and would, hence, have more remodeling events, not necessarily because of the greater loads, but because of the older age of the bone tissue that had more time to accumulate remodeling events. In addition, if we assume greater cortical drift in the more loaded humerus, it might explain the actual smaller number of DZ osteons (and possibly smaller OPD) in the larger humerus since they would have drifted more and, hence, consist of younger bone than in the smaller, less drifted humerus. If the humerus drifts more than the femur, the smaller number of osteons (type I and DZ) in the humerus relative to the femur could be explained by compacta age differences

instead of loading differences. In order to be confident that the observed differences between humerus and femur are indeed due to different ages of adult compacta would require the determination of these ages in the femur and in the humerus. Unfortunately, the effective age for the birth of adult compacta has only been determined for the middle third of the rib and is estimated at 12.5 years (Wu, Schubeck, Frost, and Villanueva, 1970), a value often used as a default age for adult compact of other bones (Abbott et al., 1996; Martin and Armelagos, 1985; Robling, 1998), but without having ever been verified.

We further predicted that the most loaded femurs would have smaller osteon area and Haversian canal area than the humerus. However, our results demonstrate no differences of osteon and Haversian canal areas in type I and DZ between paired femur and humerus. Those results suggest that osteon size does not appear to be directly dependent of the size of the cortical area as proposed by Dominguez and Agnew (2016), at least not in long bone cortices that are relatively thick (unlike that of the rib). Moreover, our results do not support the hypothesis proposed by some that larger osteons are an adaptation to increase bone toughness by absorbing more energy during micro-crack propagation and accumulating a greater number of micro-cracks (Bernhard et al., 2013; Corondan and Haworth, 1986; Moyle and Bowden, 1984; Yeni et al., 1997), nor do they support the hypothesis that loading induces osteons to be smaller (Abbott et al., 1996; Britz et al., 2009; van Oers et al., 2008). Given that we did not differentiate tensile and compressive quadrants, we are unable to address Skedros and colleagues' (1994, 2013) hypothesis that compressive loads result in smaller osteons and tensile loads result in bigger osteons.

Although there is no difference in osteon size between the humerus and femur, we did find size differences between type I and DZ osteons and their Harversian canals, with DZ being significantly smaller. In addition, DZ osteons are more prevalent in the femur than in the humerus, even after controlling for general remodeling (OPD) difference between the two bones (Table XII). Even though it is not currently possible to confidently identify causation, we might hypothesize that the smaller DZ osteon and Haversian canal size compared to type I could result from variation in osteoclastic activity. Factors influencing the coupling activities of bone resorption and formation within the BMU remains to be determined, but an essential requirement implies that a similar amount of bone removed by resorption be replaced to maintain balanced remodeling (Sims and Martin, 2014). Hence, osteoclasts determine the size

of the resorptive pit to be filled and thus, control osteoblastic formation (Sims and Gooi, 2008). During this process of osteon refilling, some osteoblasts are embedded in the bone matrix to become osteocytes, connected to each other through a lacuno-canalicular network, while many of them died by apoptosis leading to a decline in intraosteonal formation rate (Bonewald, 2011). As a result, the number of osteocytes increases with bone apposition and it is thought that osteocytes control the infilling of osteons through an inhibitory effect on osteoblasts to reduce the appositional rate (Martin, 2000a, 2000b; Metz, Martin, and Turner, 2003). Nonetheless, even with a reduced number of active forming cells, osteon refilling occurs until an optimum Haversian canal size for a given osteon is reached (Mishra and Knothe Tate, 2003; Qiu, Fyhrie, Palnitkar, and Rao, 2003). Qiu et al. (2003) estimated this ideal Haversian canal size to be about 4-5% of the osteon area. In our study, average DZ Haversian canal area represents 5,09% of the osteon area in the femur and 5,08% in the humerus while the average type I Haversian canal area correspond respectively to 5,28% and 4,85% of the osteon area. These similar numbers suggest that both types of osteon result from balanced remodeling processes and thus, maintain bone integrity. It has been shown that the volume of the matrix built by the osteoblasts before they become osteocytes is proportional to the number of available cells within the space of the BMU (Buenzli, Pivonka, and Smith, 2014; Pazzaglia et al., 2013). Thus, smaller osteons, such as DZ, had less osteoblasts when they are forming. Since the amount and orientation of collagen deposited is influenced by bone-forming cell density (Chen and Raghunath, 2009), it can be assumed that smaller osteons have a reduced number of fibrils deposited. This reduced number could lead more readily to temporary disturbance of collagen fibril arrangement, which then would allow bigger mineral crystals to form in the interfibrillar spaces (Wassen et al., 2000). As a consequence, smaller osteons would be more likely to have hypermineralized rings. This implies that smaller resorptive bays are more likely to become DZ than larger ones, resulting in DZ being smaller, in average, than type I osteons. Some studies found that DZ frequency increases with age (Ericksen, 1991; Simmons, Pritzker, and Grynpas, 1991). If this increase with age is indeed real, it does not contradict our hypothesis since aging is associated with a decline in bone cellular activities (Martin et al., 1980; Saito and Marumo, 2010; Sethe, Scutt, and Stolzing, 2006; Stenderup, Justesen, Clausen, and Kassem, 2003) and smaller osteon areas (Britz et al., 2009; Currey, 1964; Martin, Pickett, and Zinaich, 1980; Takahashi, Epker, and Frost, 1965;

but see Chapter 4). Thus, DZ appearance would result from a decrease in cellular activity and smaller resorptive bay size. Consequently, one explanation for the formation of DZ osteons could be that they form as a normal, but smaller type I osteon and their particular morphology would be a consequence of a reduced number of available osteoblasts leading to collagen synthesis impairment. This is a hypothesis, and more research on the ultrastructure of osteons, particularly on the relationship between osteoblasts numbers and collagen fibril morphology and disposition, is needed to better understand the formation and morphological variation of osteons.

Their relatively larger number of DZ osteons in the femur could suggest that resorptive bay size is modulated, in part, by loads with smaller bays in more loaded bones. However, since we found a negative correlation between variation in DZ osteon densities and cross-sectional properties, we believe that more work is needed to confidently link load intensity to the formation of DZ osteons.

A few limitations need to be addressed for future work. First, this analysis is based on a relatively small sample, and some non-significant trends observed deserve to be explored further with larger samples. Second, it would be ideal to investigate the frequency of DZ osteons in a sample for which age and occupation are better documented. And finally, intraskeletal variability regarding the formation of different osteon type, the remodeling rates as well as the determination of the effective birth of the adult compacta are important issues that will need to be addressed for a clear understanding of the formation and morphological variation of osteons, including DZ osteons.

5.6 Conclusion

This study explored the influence of load on DZ osteon formation. Our results do not unequivocally support the hypothesis that DZ osteons are related to the mechanical load history. Although DZD is higher in the femur than in the humerus, even when controlled for the higher type I remodeling rate of the femur, there are no correlations between variation in loads (as measured from cross-sectional dimensions) and variation in type I remodeling. Instead, we found a negative correlation with DZ remodeling. These results, that appear to

conflict, could be explained by intraskeletal variability in the remodeling processes, at least between the humerus and femur, possibly due to differential ages of adult bone compacta throughout the skeleton. While we did not find any differences between osteon area and Haversian canal area between femur and humerus, DZ osteons are significantly smaller compared to the more common type I osteon in both bones. We propose that DZ osteon forms as a normal type I osteon, but due to its smaller size and thus, a lower number of available bone forming cells, impairment in the matrix collagen synthesis would be more prevalent and result in a hypermineralized ring. Further investigations, however, are needed to fully clarify the cause of the appearance of DZ osteons and to test this hypothesis.

5.7 Acknowledgments

We thank the following persons for allowing us access to the skeletal remains from St-Matthew: Marie-Sol Gaudreau (Anglican Diocese of Quebec), William Moss (Archaeologist, Quebec City), Réginald Auger (Université Laval, Quebec City).

6. Overview and Concluding Remarks

6.1 Hypotheses revised

In the light of the foregoing analyses and conclusions, the hypotheses defined in section 1.4 can be addressed directly, as presented in table XIV:

Table XIV. Results and interpretation of the tested hypotheses.

Research questions	Hypotheses	Accept/reject H_0	Interpretation
A	H^A_0: DZ osteons cannot be identified under PLM. H^A_1: DZ osteons can be characterized by a different pattern in collagen orientation when viewed under PLM.	REJECT	When following the protocol defined in this study, DZ osteons can indeed be identified under polarized-light microscopy.
B	H^B_0: There is no difference in DZ osteon frequency between individuals diagnosed macroscopically with metabolic disease and those without evidence of metabolic disease. H^B_1: Individuals diagnosed macroscopically with metabolic disease have a higher DZ osteon frequency than those without evidence of metabolic disease.	ACCEPT	There is no difference in DZ osteon frequency relative to the health status. DZ osteons are not an indicator of metabolic disturbances.
C	H^C_0: There is no difference in DZ osteon frequency between age categories. H^C_1: DZ osteons increased significantly with age.	ACCEPT	Since there is no difference, DZ osteons are not the result of the metabolic change due to age.
D	H^D_0: There is no difference between males and females in DZ frequency. H^D_1: There is a significant difference in DZ frequency between males and females, females having greater density than males.	ACCEPT	Since there is no difference between males and females, DZ osteons do not appear to be induced by hormonal changes.
E	H^E_0: There is no difference in DZ osteon area with metabolic changes, either induced by bone disease, sex or age. H^E_1: There is a significant difference in DZ osteon area; individuals diagnosed macroscopically with metabolic disease have higher DZ osteon area than those without evidence of metabolic disease, females have higher DZ osteon area than males and there is a decrease in DZ osteon area with age.	ACCEPT	Since there is no difference relative to the health status, sex or age, DZ osteon area do not appear to vary with metabolic changes.

F	H^F_0: DZ osteon frequency is not different between humerus and femur and is not related to variation in cross-sectional properties H^F_1: The more loaded femur has a higher density of DZ osteons than the humerus and there is a significant relationship between DZ osteon frequency and cross-sectional properties.	Partially REJECTED	The more loaded femur has a higher density of DZ osteons than the humerus. However, a negative correlation between DZ osteon frequency and cross-sectional properties was found, suggesting that intraskeletal variability is the most determining factor in remodeling processes.
G	H^G_0: There is no difference in DZ osteon area between humerus and femur and is not related to variation in cross-sectional properties H^G_1: The more loaded femur has bigger DZ osteon area compared to the humerus and there is a significant relationship between DZ osteon area and cross-sectional properties.	ACCEPT	Since there is no difference between femur and humerus or with cross-sectional properties,DZ osteon area do not appear to vary with mechanical loads.

6.2 Summary and significance

The main objective of this dissertation was to evaluate the potential causes behind the formation of DZ osteons. Specifically, the aims were, first, to establish a methodology to identify DZ osteons; second, to evaluate the potential effect of metabolic disturbances induced either by age, sex or diseases on the appearance of DZ osteons and finally, to investigate if differences in loading history can lead to the distinctive DZ osteons remodeling. Several contributions to skeletal biology have resulted from this research.

First, the data presented in this dissertation demonstrate that the use of polarized light microscopy to identify DZ osteons is an accurate and a reliable alternative to more costly technologies such as BSE-SEM. In addition, this study has implemented a protocol for the identification of DZ osteons in PLM, something that was lacking in the literature and impeded reliable comparisons of past studies. This study also provided interesting insights into the formation of DZ osteons by revealing an alteration or an abrupt change in collagen fibers orientation at the exact same location as the hyper-mineralized ring.

Second, this thesis provided a test of previous hypotheses that DZ osteons, and more specifically the hyper-mineralized ring, represented a growth arrest that occurred in the context of the regulation of mineral homeostasis. These osteons were thought to represent evidence of metabolic disruption (Martin, 1983; Martin and Armelagos, 1985; Mays, 1985; Stout and Simmons, 1979). Since it has been shown that the hyper-mineralization ring is not an arrest line (Dhem, 1980), it was necessary to reevaluate if metabolic disturbances induced by aging, hormonal changes, or metabolic bone disease did indeed result in the formation of DZ. A valuable contribution of this research was to show that metabolic disturbances are not responsible for the appearance of the DZ osteons. Thus, DZ osteons are not related to mineral maintenance as previously suggested.

Third, based on the observation that DZ osteons are characterized by changes/alterations of collagen fibers orientation that are assumed to be regulated by mechanical strains, this thesis explored the influence of load on DZ osteon formation, a relationship that was never explored. As predicted, the more loaded femur exhibits a higher DZ osteons density than the humerus. However, interindividual mechanical loading variation

was negatively correlated with DZ remodeling, failing to unequivocally support the hypothesis that greater loads result in more DZ osteons formation. Instead, the results suggest intraskeletal variability in the remodeling processes, with some bones remodeling more than others either due to the different type of loadings (e.g., habitual, high strain) or to genetically determined differences of the bones. Precisely, although further studies are needed to corroborate this hypothesis, the intraskeletal variability could be due to different ages in adult bone tissue: it is possible that the femur attains a near-adult size at an earlier age than the humerus, leading to less drift. As a result, the femur bone tissue age would be older and would have more time to accumulate remodeling events compared to the humerus that will continue to drift and thus, could erase some of the previous remodeling events. Alternatively, Maggiano and colleagues (2015), who compared the amount of modeling drift in the femur and in the humerus in the same individuals have shown that while the femur as a consistent drift pattern, the humerus drift rotates because of a relationship between drift and humeral torsion. Thus, if not because of the bone tissue age, the differences observed in this work could be due to the way bones develop, supporting the idea of intra-skeletal variability.

The fourth contribution of this analysis is the recognition that DZ osteons are significantly smaller compared to the more common type I osteon. While metabolic changes or biomechanical loads do not seem to directly modulate the formation of DZ osteons, their formation may instead be a consequence of the small size of the resorptive bay. The overall results of this dissertation suggest that DZ osteons form as a normal type I osteons, but when the resorptive bay is smaller, there is a smaller number of available osteoblasts that increases the chances of impairment in the matrix collagen synthesis. DZ formation would be an accidental by-product of smaller resorptive bays.

The conclusions of this work lead us to rethink previous hypotheses and studies on DZ osteons, particularly in regards to interpretations of pathological conditions since DZ osteons do not appear form in the context of metabolic disturbances. Given the negative results obtained in this dissertation, it must be concluded that DZ osteons cannot provide a method to identify pathological states at the microscopic level and should not be used as an indicator of physiological status in past populations.

Additionally, this study may lead us to revisit the way we classify osteons, since DZ do not appear to be fundamentally different from type I osteons. Osteon area, instead, might be a more interesting variable to investigate further since DZ osteons are smaller than type I osteons. The main factors responsible for the variation observed in osteon area (i.e. biomechanics, age, sex, genetics) remain poorly understood and a comprehensive study of type I, type II and DZ osteons areas might be a very promising line of investigation, possibly allowing us to better understand past populations' health, activity level, etc. from their skeleton.

The results of this dissertation also demonstrate that DZ osteons are not good indicators of activity levels and do not enable interpretation of the activities of past populations. Nonetheless, the significant intra-skeletal variability in remodeling events, especially in osteons density and area, underscore the need to 1) better document that variability through the entire skeleton, and 2) to avoid comparisons between studies that have worked on different bones. Many apparent inconsistencies in the anthropological literature might in fact be due to fundamentally different remodeling processes among bones. Results of this dissertation have shown differences between femur and humerus regarding rates of remodeling for both type I and DZ osteons, but they do not appear to be solely related to loading differences. If two relatively similar bones such as the humerus and femur are found to be different, it can be assumed that remodeling in other bones, such as ribs, clavicles, or metacarpals, that are all extremely different in shape, growth, or mechanical loading regime, will be dramatically different and that direct comparisons can only lead to spurious conclusions. This thesis underscores that observations and conclusions are bone-specific, thus, the systematic exploration of intra-skeletal variability will allow for a better understanding of skeletal biology, which in turn would provide new insights into the cultural and biological aspects, including health, mobility and aging, of past and present societies.

6.3 Limitations of the study

The sample size used in the different studies of this dissertation is relatively small, especially when metabolic changes are assessed. Thus, conclusions reached in this work

should be corroborating on a larger sample. Additionally, since the pathological assessment is particularly challenging in archeological remains, and sex and age are estimated, it would be ideal to evaluate the effect of metabolic bone disease from a medical or well-documented sample in order to draw more definitive pattern of the relationship between DZ osteons and metabolic changes.

6.4 Expansion and future research

It is becoming increasingly clear that bone remodeling is largely due to genetic factors. It could be interesting to evaluate the occurrence of DZ osteon and to study osteon size in distinct population to shed light on the genetic influences on skeletal physiology. Similarly, it would be interesting to study the incidence of these osteons in a ontogenic sequence to determine if they can be found before maturity.

Additionally, the protocol tested in this dissertation was implemented by Dr. Margaret Streeter, an experienced observer in bone histology. Thus, now that this protocol has been proved to be reliable and accurate, efforts should be made to refine it by trying to characterize DZ osteons that were not identified in PLM but which was seen in BSE-SEM and by reassessing the false positive, identified in PLM but not in BSE-SEM. It is possible that the protocol could be enhanced to provide an even more accurate methodology to identify DZ osteon in PLM.

Several areas of investigation have merged as a result of the conclusions presented in this dissertation. First, a more systematic exploration of intraskeletal differences is needed in order to provide a better understanding of the remodeling dynamics of type I and DZ osteons. Second, although this goes well beyond habitual concerns of anthropological studies, it seems necessary to have a better understanding of the bone ultrastructure and its formation. In particular, a greater comprehension of the exchange between collagen deposition and mineralization would help understand more fully how and why DZ osteons are formed. New technologies such as Fourier Tranform Infrared Spectroscopic Microscopy or Raman Microscopy could bring additional informations of the exact nature of the optical features seen

in double-zonal ring. Another perspective can be draw with the Focused Ion Beam with Scanning Electron Microscope (FIB-SEM) in order to quantify the change of collagen orientation at the hyper-mineralized ring. A more systematic exploration of frequency different osteon variants, especially type I, DZ and type II, could also help to understand how and why these osteons are formed.

Literature cited

Abbott, S., Trinkaus, E., and Burr, D. B. (1996). Dynamic bone remodeling in later Pleistocene fossil hominids. *American Journal of Physical Anthropology*, 99, 585-601.

Agarwal, S. C. (2007). Light and Broken Bones: Examining and Interpreting Bone Loss and Osteoporosis in Past Populations. In A. Katzenberg and S.R. Saunders (Eds). *Biological anthropology of the human skeleton (Second edition),* (pp.387–410). New York, NY: Wiley-Liss.

Agarwal, S. C., and Grynpas, M. D. (1996). Bone quantity and quality in past populations. *The Anatomical Record*, 246, 423–432.

Agarwal, S. C., and Stout, S. D. (2003). *Bone loss and osteoporosis: an anthropological perspective*. New York, NY: Springer.

Aiello, L. C. (1992). Allometry and the analysis of size and shape in human evolution. *Journal of Human Evolution*, 22, 127-147.

Albouy S., Rami L., and Grimaud D. (2006). Métabolisme phosphocalcique. In: Martin C, Riou B, and Vallet B, editors. *Physiologie humaine appliquée*, (pp 471-484). Paris, FR: Arnett Blackwell.

Albright, F., and Reifenstein, E. C. (1948). *The Parathyroid Glands and Metabolic Bone Disease: Selected Studies*. Baltimore, MD : The Williams and Wilkins Compagny.

Altman, D. G. (1991). *Practical statistics for medical research*. London: Chapman and Hall.

Altman, D. G., and Bland, J. M. (1994a). Diagnostic tests. 1: Sensitivity and specificity. *British Medical Journal*, 308, 1552.

Altman, D. G., and Bland, J. M. (1994b). Statistics Notes: Diagnostic tests 2: predictive values. *British Medical Journal*, 309, 102.

Anderson, J. J. B., and Pollitzer, W. S. (1994). Ethnic and Genetic Differences in Susceptibility to Osteoporotic Fractures. In H.H. Draper (Ed.). *Nutrition and Osteoporosis,* (pp. 129-149). Boston, MA : Springer.

Anthanont, P., Levine, J.A., McCrady-Spitzer, S.K., and Jensen, M.D. (2017). Lack of Seasonal Differences in Basal Metabolic Rate in Humans: A Cross-Sectional Study. *Hormone and Metabolic Research*. 49, 30-35.

Arhatari, B.D., Cooper, D.M.L., Thomas, C.D.L, Clement, J.G., and Peele, A.G. (2011).

Imaging the 3D structure of secondary osteons in human cortical bone using phase-retrieval tomography. *Physics in Medicine and Biology*. 56, 5265.

Arnett, T., and Henderson, B. (1998). *Methods in Bone Biology*. London, UK : Chapman and Hall.

Arpin, C. (2006). *Sépultures du cimetière St.Matthew. Étude sur les critères paléodémographiques et la représentativité d'une collection d'ossements témoignant de la présence protestante à Québec entre 1771 et 1860*. Mémoire de maîtrise, Université de Laval, Québec.

Ascenzi, A., and Bonucci, E. (1964). The ultimate tensile strength of single osteons. *Cells Tissues Organs*, 58, 160-183.

Ascenzi, A., and Bonucci, E. (1967). The tensile properties of single osteons. *The Anatomical Record*, 158, 375–386.

Ascenzi, A., and Bonucci, E. (1968). The compressive properties of single osteons. *The Anatomical Record*, 161, 377-391.

Ascenzi, A., and Bonucci, E. (1972). The shearing properties of single osteons. *The Anatomical Record*, 172, 499-510.

Atkins, G. J., and Findlay, D. M. (2012). Osteocyte regulation of bone mineral: a little give and take. *Osteoporosis International*, 23, 2067-2079.

Aubry P., Mialhe C., Morel M., Amicabile C. (1990). Experimental Pseudarthrosis in Dogs: Vascular Pathogenesis and Cellular Evolution. In: J. Arlet, B. Mazières (Eds). *Bone Circulation and Bone Necrosis*. Berlin, Heidelberg : Springer.

Aufderheide, A. C., and Rodriguez-Martin, C. (1998). *The Cambridge encyclopedia of human paleopathology.* Cambridge, UK : Cambridge University Press.

Austin, R. M., and Mulhern, D. (2015). The sum of their parts: Assessing double-zonal osteons within medieval Kulubnarti, Nubia. *American Journal ofPphysical Anthropology*, 156, 73-74.

Bailey, D. A., Martin, A. D., McKay, H. A., Whiting, S., and Mirwald, R. (2000). Calcium Accretion in Girls and Boys During Puberty: A Longitudinal Analysis. *Journal of Bone and Mineral Research*, 15, 2245-2250.

Bartsiokas, A., and Day, M. H. (1993). Lead poisoning and dental caries in the Broken Hill hominid. *Journal of HumanEevolution*, 24, 243–249.

Bassi, A., Gough, J., Zakikhani, M., and Downes, S. (2011). 5 - Bone tissue regeneration. In

L. Bosworth and S. Downes (Eds.), *Electrospinning for Tissue Regeneration,* (pp. 93-110). Cambridge, UK: Woodhead Publishing.

Beauchesne, P., and Agarwal, S. C. (2017). A multi-method assessment of bone maintenance and loss in an Imperial Roman population: Implications for future studies of age-related bone loss in the past. *American Journal of Physical Anthropology*, 164, 41-61.

Bélanger, R. (1993). *Rue Saint-Jean, Québec*. Ministère de la Culture et des Communications du Québec (Unpublished Archaeological Report).

Bell, K. L., Loveridge, N., Reeve, J., Thomas, C. D., Feik, S. A., and Clement, J. G. (2001). Super-osteons (remodeling clusters) in the cortex of the femoral shaft: influence of age and gender. *The Anatomical Record*, 264, 378-386.

Bell, L. S. (2012). Identifying postmortem microstructural change to skeletal and dental tissues using Backscattered Electron Imaging. In L.S. Bell (Ed.)*, Forensic Microscopy for Skeletal Tissues: Methods and Protocols* (pp. 173-190). Springer, New York: Humana Press.

Bentolila, V., Boyce, T. M., Fyhrie, D. P., Drumb, R., Skerry, T. M., and Schaffler, M. B. (1998). Intracortical remodeling in adult rat long bones after fatigue loading. *Bone*, 23, 275–281.

Berner M., Sládek V., Holt B., Niskanen M., and Ruff C. B. (2017). In C. B. Ruff (Ed.). Sexual Dimorphism. *Skeletal Variation and Adaptation in Europeans*, (pp.133-161). Hoboken, NJ : Wiley.

Bernhard, A., Milovanovic, P., Zimmermann, E. A., Hahn, M., Djonic, D., Krause, M., … Busse, B. (2013). Micro-morphological properties of osteons reveal changes in cortical bone stability during aging, osteoporosis, and bisphosphonate treatment in women. *Osteoporosis International*, 24, 2671-2680.

B-Hardy, M.-H. (2017). *Étude de la diversité des populations historiques de Montréal et de Québec par l'analyse de la morphologie dentaire : le cimetière catholique de la première église Notre-Dame (1691-1796) et le cimetière protestant Saint-Matthew de Québec (1771-1860)*. Mémoire de maîtrise, Université de Montréal, Montréal, Québec.

Bjørnerem, A., Bui, M., Wang, X., Ghasem-Zadeh, A., Hopper, J. L., Zebaze, R., and Seeman, E. (2015). Genetic and environmental variances of bone microarchitecture and bone remodeling markers: a twin study. *Journal of Bone and Mineral Research*, 30, 519–527.

Black, A. J., Topping, J., Durham, B., Farquharson, R. G., and Fraser, W. D. (2000). A detailed assessment of alterations in bone turnover, calcium homeostasis, and bone density in normal pregnancy. *Journal of Bone and Mineral Research*, 15, 557–563.

Blair, H. C., Robinson, L. J., Huang, C. L.-H., Sun, L., Friedman, P. A., Schlesinger, P. H., and Zaidi, M. (2011). Calcium and bone disease. *BioFactors (Oxford, England)*, 37, 159-167.

Bland, J. M., and Altman, D. (1986). Statistical methods for assessing agreement between two methods of clinical measurement. *The Lancet*, 327, 307–310.

Bonewald, L. F. (2007). Osteocytes as Dynamic Multifunctional Cells. *Annals of the New York Academy of Sciences*, 1116, 281-290.

Bonewald, L. F. (2011). The amazing osteocyte. *Journal of Bone and Mineral Research: The Official Journal of the American Society for Bone and Mineral Research*, 26, 229-238.

Bonjour, J.-P., Theintz, G., Buchs, B., Slosman, D., and Rizzoli, R. (1991). Critical Years and Stages of Puberty for Spinal and Femoral Bone Mass Accumulation during Adolescence. *The Journal of Clinical Endocrinology and Metabolism*, 73, 555-563.

Borgel, S. (2017). *Manifestations microscopiques des charges biomécaniques sur le second métacarpe humain*. (Mémoire de maîtrise). Université de Montréal, Montréal.

Boskey, A. L. (2006). Mineralization, structure and function of bone. *Dynamics of Bone and Cartilage Metabolism: Principles and Clinical Applications, 2,* 201–209.

Boskey, A. L. (2013). Bone composition: relationship to bone fragility and antiosteoporotic drug effects. *BoneKEy Reports*, 2, 447.

Bouvier, M., and Hylander, W. L. (1981). Effect of bone strain on cortical bone structure in macaques (Macaca mulatta). *Journal of Morphology*, 167, 1-12.

Bowman, B. M., and Miller, S. C. (2001). Skeletal adaptations during mammalian reproduction. *Journal of Musculoskeletal and Neuronal Interactions*, 1, 347–55.

Boyde, A., and Jones, S. J. (1983). Back-scattered electron imaging of skeletal tissues. *Metabolic Bone Disease and Related Research*, 5, 145–150.

Boyde, A., and Riggs, C. M. (1990). The quantitative study of the orientation of collagen in compact bone slices. *Bone*, 11, 35-39.

Breuil, V., and Euller-Ziegler, L. (2004). Nutrition et vieillissement osseux: L'ostéoporose. *Nutrition Clinique et Métabolisme*, 18, 212-218.

Brickley, M., and Ives, R. (2008). Chapter 1 - Introduction. In *The Bioarchaeology of Metabolic Bone Disease* (pp. 1-5). San Diego, CA: Academic Press.

Brickley, M., and Ives, R. (2010). *The Bioarchaeology of Metabolic Bone Disease*. Elsevier, San Diego, CA: Academic Press.

Britz, H. M., Thomas, C. D. L., Clement, J. G., and Cooper, D. M. L. (2009). The relation of femoral osteon geometry to age, sex, height and weight. *Bone*, 45, 77-83.

Bromage, T. G., Goldman, H. M., McFarlin, S. C., Warshaw, J., Boyde, A., and Riggs, C. M. (2003). Circularly polarized light standards for investigations of collagen fiber orientation in bone. *The Anatomical Record*, 274, 157–168.

Brooks, S., and Suchey, J. M. (1990). Skeletal age determination based on the os pubis: A comparison of the Acsádi-Nemeskéri and Suchey-Brooks methods. *Human Evolution*, 5, 227-238.

Bruzek, J. (2002). A method for visual determination of sex, using the human hip bone. *American Journal of Physical Anthropology*, 117, 157-168.

Buckberry, J. (2015). The (mis)use of adult age estimates in osteology. *Annals of Human Biology*, 42, 323-331.

Buenzli, P. R., Pivonka, P., and Smith, D. W. (2014). Bone refilling in cortical basic multicellular units: insights into tetracycline double labelling from a computational model. *Biomechanics and modeling in mechanobiology*, 13, 185–203.

Buikstra, J. E., and Ubelaker, D. H. (1994). Standards for data collection from human skeletal remains: Proceedings of a seminar at the Field Museum of Natural History. Arkansas Archaeological Survey Research Series, 44, Fayetteville, AR.

Burger, E. H., Klein-Nulend, J., Van Der Plas, A., and Nijweide, P. J. (1995). Function of osteocytes in bone—their role in mechanotransduction. *The Journal of nutrition*, 125, 2020S–2023S.

Burr, D. B., Martin, R. B., Schaffler, M. B., and Radin, E. L. (1985). Bone remodeling in response to in vivo fatigue microdamage. *Journal of biomechanics*, 18, 189–200.

Burr, D. B., Ruff, C. B., and Thompson, D. D. (1990). Patterns of skeletal histologic change through time: comparison of an archaic native American population with modern populations. *The Anatomical Record*, 226, 307-313.

Burr, D. B. (2002a). Targeted and nontargeted remodeling. *Bone*, 30, 2-4.

Burr, D. B. (2002b). The contribution of the organic matrix to bone's material properties. *Bone*, 31, 8–11.

Burr, D. B., and Akkus, O. (2014). Bone Morphology and Organization. In D. B. Burr and M. Allen, *Basic and Applied Bone Biology,* (pp. 3-25). San Diego, CA: Academic Press.

Burr, D. B, Bellido, T. M., and Kenneth, E. W. (2015). Bone structure and function. In M.C. Hochberg, A. J. Silman, J.S. Smolen, M.E.Weinblatt and M. H. Weisman (Eds) *Rheumatology: Sixth Edition* (p. 42-55). Philadelphia, PA: Elsevier.

Burton, P., Nyssen-Behets, C., and Dhem, A. (1989). Haversian Bone Remodelling in Human Fetus. *Cells Tissues Organs*, 135, 171-175.

Cao, J. J., Johnson, L. K., and Hunt, J. R. (2011). A diet high in meat protein and potential renal acid load increases fractional calcium absorption and urinary calcium excretion without affecting markers of bone resorption or formation in postmenopausal women. *The Journal of Nutrition*, 141, 391-397.

Caron, D. (2014). *Essai de détection de processus migratoires à travers les isotopes de strontium et d'oxygène : étude des restes humains du cimetière Saint-Matthew (Québec, 1771-1860).* (Mémoire de maîtrise). Université de Montréal, Montréal.

Carter, D. R. (1987). Mechanical loading history and skeletal biology. *Journal of Biomechanics*, 20, 1095-1109.

Castanet, J., Francillon-Vieillot, H., Meunier, F.J. and de Ricqlès, A.J. (1993). Bone and individual aging. In B.K. Hall (Ed), *Bone, Volume 7*. (pp. 245-283). Boca Raton, FL : CRC Press.

Castanet J., Croci, S., Aujard, F., Perret, M, Cubo, J., de Margerie E. (2004). Lines of arrested growth in bone and age estimation in a small primate: *Microcebus murinus. Journal of Zoology.* 263, 31-39.

Castiglioni, S., Cazzaniga, A., Albisetti, W., and Maier, J. A. M. (2013). Magnesium and Osteoporosis: Current State of Knowledge and Future Research Directions. *Nutrients*, 5, 3022-3033.

Chen, C. Z., and Raghunath, M. (2009). Focus on collagen: in vitro systems to study fibrogenesis and antifibrosis, state of the art. *Fibrogenesis and Tissue Repair*, 2, 7.

Chen, H., Senda, T. and Kubo, K.Y. (2015). The osteocyte plays multiple roles in bone remodeling and mienral homeostasis. *Medical Molecular Morphology*. 48, 61-68.

Cho, H., Stout, S. D., Madsen, R. W., and Streeter, M. A. (2002). Population-specific histological age-estimating method: a model for known African-American and European-American skeletal remains. *Journal of Forensic Science*, 47, 12–18.

Cho, H., and Stout, S. D. (2003). Bone remodeling and age-associated bone loss in the past: a

histomorphometric analysis of the Imperial Roman skeletal population of Isola Sacra. In S.C. Agarwal and S.D. Stout (Eds.), *Bone Loss and Osteoporosis* (pp. 207–228). New York, NY: Springer.

Cho, H., Stout, S. D., and Bishop, T. A. (2006). Cortical bone remodeling rates in a sample of African American and European American descent groups from the American Midwest: comparisons of age and sex in ribs. *American journal of physical anthropology*, 130, 214–226.

Cho, H., and Stout, S. D. (2011). Age-associated bone loss and intraskeletal variability in the Imperial Romans. *Journal of Anthropological Science*, 89, 109–125.

Cloutier, C. (2000). Tombeaux, cercueils, caveaux et linceuls: les témoins archéologiques au dernier repos. Cimetière St. Matthew. Ministère de la Culture et des Communications du Québec (Unpublished Archaeological Report).

Cohen, J. (1960). A coefficient of agreement for nominal scales. *Educational and Psychological Measurement*, 20, 37–46.

Cole, Z. A., Dennison, E. M., and Cooper, C. (2008). Osteoporosis epidemiology update. *Current Rheumatology Reports*, 10, 92-96.

Compston, J. E. (2001). Sex steroids and bone. *Physiological reviews*, 81, 419–447.

Cooper, D.M.L., Erickson, B., Peele, A.G., Hannah, K., Thomas, C.D.L. and Clement, J.G. (2011). Visualization of 3D osteon morphology by synchrotron radiation micro-CT. *Journal of anatomy.* 219, 481-489.

Corondan, G., and Haworth, W. L. (1986). A fractographic study of human long bone. *Journal of Biomechanics*, 19, 207–218.

Couret, I. (2004). Biologie du remodelage osseux. *Médecine Nucléaire*, 28, 57-65.

Coutelier, L. (1976). Le remaniement de l'os compact chez l'enfant. *Bulletin de l'Association des Anatomistes (Nancy)*, 60, 95–110.

Creedon, P.J., and A.F. Hayes. (2015). Small sample mediation analysis: how far can we push the bootstrap. *Annual conference of the Association for Psychological Science*. New York, NY.

Crescimanno, A., and Stout, S. D. (2012). Differentiating fragmented human and nonhuman long bone using osteon circularity. *Journal of Forensic Sciences*, 57, 287–294.

Crowder, C., Heinrich, J., and Stout, S. D. (2012). Rib histomorphometry for adult age estimation. In L.S. Bell (Ed.), *Forensic Microscopy for Skeletal Tissues: Methods and*

Protocols, (pp.109–127). New York, NY: Humana Press.

Curate, F., Lopes, C., and Cunha, E. (2010). A 14th–17th century osteoporotic hip fracture from the Santa Clara-a-Velha Convent in Coimbra (Portugal). *International Journal of Osteoarchaeology*, 20, 591–596.

Currey, J. D. (1964). Some effects of ageing in human Haversian systems. *Journal of Anatomy*, 98, 69.

Currey, J. D. (2003). Role of collagen and other organics in the mechanical properties of bone. *Osteoporosis International*, 14, 29–36.

Dhem, A . (1980). Étude histologique et microradiographique des manifestations biologiques propres au tissu osseux compact. Bulletin et Mémoire de l'Académie Royale de Médecine de Belgique, 135, 368-381.

Denny, B. (2010). *Does Mean Osteon Size Change with Age, Sex or Handedness? Analysis of the Second Metacarpal in a 19th Century Sample from Belleville, Ontario, Canada*. (Master thesis). Boise State University, Boise.

Depalle, B., Qin, Z., Shefelbine, S. J., and Buehler, M. J. (2015). Influence of cross-link structure, density and mechanical properties in the mesoscale deformation mechanisms of collagen fibrils. *Journal of the Mechanical Behavior of Biomedical Materials*, 52, 1-13.

Dominguez, V. M., and Agnew, A. M. (2016). Examination of factors potentially influencing osteon size in the human rib. *The Anatomical Record*, 299, 313–324.

Dominguez, V. M., and Crowder, C. (2015). Sex differences in measures of bone remodeling. *Proceedings of the 67th Annual Scientific Meeting of the American Academy of Forensic Sciences* (pp. 185-186). Orlando, FL.

Dominguez, V. M., and Crowder, C. M. (2012). The utility of osteon shape and circularity for differentiating human and non-human Haversian bone. *American journal of physical anthropology*, 149, 84–91.

Doube, M., Kłosowski, M. M., Arganda-Carreras, I., Cordelières, F. P., Dougherty, R. P., Jackson, J. S., … Shefelbine, S. J. (2010). BoneJ: Free and extensible bone image analysis in ImageJ. *Bone*, 47, 1076-1079.

Drapeau, M. S., and Streeter, M. A. (2006). Modeling and remodeling responses to normal loading in the human lower limb. *American journal of physical anthropology*, 129, 403–409.

Ducy, P., Schinke, T., and Karsenty, G. (2000). The Osteoblast: A Sophisticated Fibroblast under Central Surveillance. *Science*, 289, 1501-1504.

Edelson, G. (2000). The development of humeral head torsion. *Journal of Shoulder and Elbow Surgery*. 9, 316-318.

Eleazer, C. (2013). *The interaction of mechanical loading and metabolic stress on human cortical bone: testing anthropological ssumptions using cross-sectional geometry and histomorphology*. (PhD dissertation). The University of Tennessee, Knoxville.

Enlow, D. H. (1962). Functions of the Haversian system. *The American Journal of Anatomy*, 110, 269-305.

Enlow, D. H. (1963). *Principles of Bone Remodeling: An Account of Post-natal Growth and Remodeling Processes in Long Bones and the Mandible*. Springfield, IL: Charles C. Thomas.

Epker, B. N., and Frost, H. M. (1965). The Direction of Transverse Drift of Actively Forming Osteons in Human Rib Cortex. *Journal of Bone and Joint Surgery*, 47, 1211–1215.

Eriksen, E. F., Steiniche, T., Mosekilde, L., and Melsen, F. (1989). Histomorphometric analysis of bone in metabolic bone disease. *Endocrinology and Metabolism Clinics of North America*, 18, 919-954.

Eriksen, E. F. (1986). Normal and pathological remodeling of human trabecular bone: three dimensional reconstruction of the remodeling sequence in normals and in metabolic bone disease. *Endocrine reviews*, 7, 379–408.

Ericksen, M. F. (1973). *Age-related bone remodeling in three aboriginal American populations.* (PhD dissertation). George Washington University, Washington DC.

Ericksen, M. F. (1980). Patterns of microscopic bone remodeling in three aboriginal American populations. In D.L. Bowman (Ed.), *Early Native Americans: prehistoric demography, economy, and technology.* The Hague: Mouton, 239–270.

Ericksen, M. F. (1991). Histologic estimation of age at death using the anterior cortex of the femur. *American Journal of Physical Anthropology*, 84, 171-179.

Evans, F. G., and Bang, S. (1967). Differences and relationships between the physical properties and the microscopic structure of human femoral, tibial and fibular cortical bone. *American Journal of Anatomy*, *120*(1), 79-88.

Fang, M., and Holl, M. M. B. (2013). Variation in type I collagen fibril nanomorphology: the significance and origin. *BoneKEy reports*, 2, 1-7.

Farnum, J., Shimada, I., Streeter, M. A., and Verano, J. (2001). Recent advances in the bioarchaeology of the Prehispanic Sican on the North Coast of Peru. *American Journal of Physical Anthropology*, S32, 64.

Feng, X. (2009). Chemical and Biochemical Basis of Cell-Bone Matrix Interaction in Health and Disease. *Current Chemical Biology*, 3, 189-196.

Florencio-Silva, R., Sasso, G. R. da S., Sasso-Cerri, E., Simões, M. J., and Cerri, P. S. (2015). Biology of Bone Tissue: Structure, Function, and Factors that Influence Bone Cells. *BioMed Research International*, 2015, 1-17.

Flynn, A. (2003). The role of dietary calcium in bone health. *Proceedings of the Nutrition Society*, 62, 851-858.

Franz-Odendaal, T. A., Hall, B. K., and Witten, P. E. (2006). Buried alive: how osteoblasts become osteocytes. *Developmental Dynamics: An Official Publication of the American Association of Anatomists*, 235, 176-190.

Fratzl, P. (2008). Collagen: Structure and Mechanics, an Introduction. In *Collagen* (pp. 1-13). New York, NY: Springer.

Fratzl, P., Fratzl-Zelman, N., and Klaushofer, K. (1993). Collagen packing and mineralization. An x-ray scattering investigation of turkey leg tendon. *Biophysical Journal*, 64, 260-266.

Frost, H. M. (1958). Preparation of thin undecalcified bone sections by rapid manual method. *Stain technology*, 33, 273–277.

Frost, H. M. (1960). Presence of microscopic cracks in vivo in bone. *Henry Ford Hospital Medical Journal*, 8, 25–35.

Frost, H. M. (1963). Measurement of human bone formation by means of tetracycline labelling. *Canadian Journal of Biochemistry and Physiology*, 41, 31–42.

Frost, H. M. (1964a). *Bone remodeling dynamics.* Springfield, IL: Charles C. Thomas.

Frost, H. M. (1964b). Dynamics of bone remodeling. In *Bone biodynamics* (pp. 315-333). Springfield, IL: Charles C. Thomas.

Frost, H. M. (1964c). *The laws of bone structure*. Springfield, IL: Charles C. Thomas.

Frost, H. M. (1969). Tetracycline-based histological analysis of bone remodeling. *Calcified Tissue Research*, 3, 211-237.

Frost, H. M. (1973). *Bone modeling and skeletal modeling errors*. Springfield, IL: Charles C.

Thomas.

Frost, H. M. (1983a). The regional acceleratory phenomenon: a review. *Henry Ford Hospital Medical Journal*, 31, 3.

Frost, H. M. (1983b). A determinant of bone architecture: the minimum effective strain. *Clinical orthopaedics and related research*, *175*, 286–292.

Frost, H. M. (1985). The "new bone": Some anthropological potentials. *American Journal of Physical Anthropology*, 28, 211-226.

Frost, H. M. (1987a). Secondary osteon populations: an algorithm for determining mean bone tissue age. *American Journal of Physical Anthropology*, 30, 221–238.

Frost, H. M. (1987b). Bone "mass" and the "mechanostat": a proposal. *The anatomical record*, 219, 1–9.

Frost, H. M. (1990a). Skeletal structural adaptations to mechanical usage (SATMU): 1. Redefining Wolff's law: the bone modeling problem. *The Anatomical Record*, 226, 403–413.

Frost, H. M. (1990b). Skeletal structural adaptations to mechanical usage (SATMU): 2. Redefining Wolff's law: the remodeling problem. *The Anatomical Record*, 226, 414–422.

Frost, H. M. (1997). Why do marathon runners have less bone than weight lifters? A vital-biomechanical view and explanation. *Bone*, 20, 183-189.

Frost, H. M. (2000). Does bone design intend to minimize fatigue failures? A case for the affirmative. *Journal of Bone and Mineral Metabolism*, 18, 278-282.

Frost, H. M. (2003). Bone's mechanostat: a 2003 update. *The anatomical record*, 275, 1081–1101.

Garnero, P., Arden, N. K., Griffiths, G., Delmas, P. D., and Spector, T. D. (1996). Genetic influence on bone turnover in postmenopausal twins. *The Journal of Clinical Endocrinology and Metabolism*, 81, 140-146.

Garn, S. (1972). The course of bone gain and the phases of bone loss. *Orthopedic Clinics of North America*. 3, 503-509.

Gasser, J.A., Kneissel, M. (2017). Bone physiology and Biology. In S.Y. Smith, A. Varela and R. Samadfam (Eds.) *Bone Toxicology*, (pp. 27-94). Cham, Switzerland: Springer.

Giraud-Guille, M. M. (1988). Twisted plywood architecture of collagen fibrils in human compact bone osteons. *Calcified Tissue International*, 42, 167–180.

Goldman, H. M. (2001). *Histocomposition and geometry at the human mid-shaft femur.* (PhD dissertation). City University of New York, New York, NY.

Goodman, A. H., and Martin, D. L. (2002). Reconstructing Health Profiles from Skeletal Remains. In R. Steckel and J. Rose (Eds.). *The Backbone of History: Health and Nutrition in the Western Hemisphere,* (pp. 11-60). Cambridge, UK : Cambridge University Press.

Grynpas, M. (1993). Age and disease-related changes in the mineral of bone. *Calcified Tissue International*, 53, S57–S64.

Guardia, L. D., Roggi, C., and Cena, H. (2016). Diet-induced acidosis and alkali supplementation. *International Journal of Food Sciences and Nutrition*, 67, 754-761.

Hammer, Ø., Harper, D. A. ., and Ryan, P. . (2001). PAST: Paleontological statistics software package for education and data analysis. *Palaeontologia Electronica*, 4, 1-9.

Hare, J., Lafrance, M., and Ruddel, D.-T. (1987). *Histoire de la ville de Québec, 1608-1871.* (Editions Boréales et Musée canadien des civilisations). Montréal, Québec.

Harris, H. A. (1933). *Bone Growth in Health and Disease: The Biological Principles Underlying the Clinical, Radiological, and Histological Diagnosis of Perversions of Growth and Disease in the Skeleton*. London, UK: Oxford University Press.

Hauge, E.M., Qvesel, D., Eriksen, E.F., Mosekilde, L., Melsen, F. (2001). Cancellous bone remodeling occurs in specialized compartments lined by cells expressing osteoblastic markers. *Journal of Bone and Mineral Research*. 16, 1575–1582.

Havill, L. M., Allen, M. R., Harris, J. A. K., Levine, S. M., Coan, H. B., Mahaney, M. C., and Nicolella, D. P. (2013). Intracortical bone remodeling variation shows strong genetic effects. *Calcified tissue international*, 93, 472–480.

Havill, Lorena M. (2003). Osteon remodeling dynamics in the Cayo Santiago Macaca mulatta: the effect of matriline. *American journal of physical anthropology*, 121, 354–360.

Heaney, R. P. (2001). The bone remodeling transient: interpreting interventions involving bone-related nutrients. *Nutrition Reviews*, 59, 327-334.

Heaney, R. P., Abrams, S., Dawson-Hughes, B., Looker, A., Marcus, R., Matkovic, V., and Weaver, C. (2000). Peak bone mass. *Osteoporosis international*, 11, 985–1009.

Heaney, R. P., McCarron, D. A., Dawson-hughes, B., Oparil, S., Berga, S. L., Stern, J. S., Barr, S.I., Rosen, C. J. (1999). Dietary Changes Favorably Affect Bone Remodeling

in Older Adults. *Journal of the American Dietetic Association*, 99, 1228-1233.

Hennig, C., Thomas, C.D.L, Clement, J.G., and Cooper, D.M.L. (2015). Does 3D orientation account for variation in osteon morphology assessed by 2D histology? *Journal of Anatomy*. 227, 497-505.

Hillier, M. L., and Bell, L. S. (2007). Differentiating human bone from animal bone: a review of histological methods. *Journal of forensic sciences*, 52, 249–263.

Hofman, M. A. (1988). Allometric scaling in palaeontology: A critical survey. *Human Evolution*, 3, 177-188.

Houle-Wierzbicki, Z. (2016). Étude paléopathologique préliminaire à travers l'analyse macroscopique et scanographique : exemple du cimetière St. Matthew, ville de Québec (1771-1860). (Mémoire de maîtrise). Université de Montréal, Montréal.

Hughes, J.M. and Petit, M.A. (2010). Biological undepinnings of Frost's mechanostat thresholds: The important role of osteocytes. *Journal of Musculoskeletal and Neuronal Interaction.* 10, 128-135.

Iwaniec, U. T. (1997). *Effects of dietary acidity on cortical bone remodeling: a histomorphometric assessment*. (PhD Dissertation) University of Wisconsin, Madison.

Jansen, J. A. (2003). Histological Analysis of Bone-Implant Interface. In Y. H. An and K. L. Martin (Eds), *Handbook of Histology Methods for Bone and Cartilage* (pp. 353-360). Totowa, NJ: Humana Press.

Jaworski, Z. F., Meunier, P., and Frost, H. M. (1972). Observations on two types of resorption cavities in human lamellar cortical bone. *Clinical Orthopaedics and Related Research*, 83, 279-285.

Jones, C. G. (2012). Scanning Electron Microscopy: Preparation and Imaging for SEM. In L.S. Bell (Ed.), *Forensic Microscopy for Skeletal Tissues: Methods and Protocols*, (p. 1-20). Springer, New York: Humana Press.

Jowsey, J., Kelly, P. J., Riggs, B. L., Bianco Jr, A. J., Scholz, D. A., and Gershon-Cohen, J. (1965). Quantitative microradiographic studies of normal and osteoporotic bone. *Journal of Bone and Joint Sugery*, 47, 785–872.

Jowsey, J. (1966). Studies of Haversian systems in man and some animals. *Journal of Anatomy*, 100, 857.

Karaplis, A. C. (2008). Chapter 3 - Embryonic Development of Bone and Regulation of

Intramembranous and Endochondral Bone Formation. In J. P. Bilezikian, L. G. Raisz, and T. J. Martin (Eds.), *Principles of Bone Biology (Third Edition)* (pp. 53-84). San Diego, CA: Academic Press.

Katz, D. et J. M. Suchey. (1989). Race Differences in Pubic Symphyseal Aging Patterns in the Male. *American Journal of Physical Anthropology*, 80, 167-172.

Katz, E. P., Wachtel, E., Yamauchi, M., and Mechanic, G. L. (1989). The Structure of Mineralized Collagen Fibrils. *Connective Tissue Research*, 21, 149-158.

Kelly, P. J., Hopper, J. L., Macaskill, G. T., Pocock, N. A., Sambrook, P. N., and Eisman, J. A. (1991). Genetic Factors in Bone Turnover. *The Journal of Clinical Endocrinology and Metabolism*, 72, 808-813.

Kelly, P. J., Morrison, N. A., Sambrook, P. N., Nguyen, T. V., and Eisman, J. A. (1995). Genetic influences on bone turnover, bone density and fracture. *European Journal of Endocrinology*, 133, 265-271.

Kent, G. N., Price, R. I., Gutteridge, D. H., Allen, J. R., Barnes, M. P., Hickling, C. J., Retallack, R.W, …Smith, M. (1990). Human lactation: forearm trabecular bone loss, increased bone turnover, and renal conservation of calcium and inorganic phosphate with recovery of bone mass following weaning. *Journal of Bone and Mineral Research*, 5, 361–369.

Kerley, E. R. (1965). The microscopic determination of age in human bone. *American journal of physical anthropology*, 23, 149–163.

Kerley, E. R., and Ubelaker, D. H. (1978). Revisions in the microscopic method of estimating age at death in human cortical bone. *American Journal of Physical Anthropology*, 49, 545-546.

Kierszenbaum, A. L. (2006). *Histologie et biologie cellulaire: Une introduction à l'anatomie pathologique*. Bruxelles, BE: De Boeck Supérieur.

Kim, Y. S., Jo, S. M., Chung, I. J., Chung, N. E., and Han, S. H. (2007). Preparation of Bone Slide for Histomorphometry in Forensic Anthropology. *Korean Journal of Legal Medicine*, 31, 151–156.

Klein-Nulend, J., Van der Plas, A., Semeins, C. M., Ajubi, N. E., Frangos, J. A., Nijweide, P. J., and Burger, E. H. (1995). Sensitivity of osteocytes to biomechanical stress in vitro. *The FASEB Journal*, 9, 441–445.

Knott, L., and Bailey, A. J. (1998). Collagen cross-links in mineralizing tissues: A review of their chemistry, function, and clinical relevance. *Bone*, 22, 181-187.

Köhler, M., Marin-Moratalla, N., Jordana, X. and Aanes R. (2012). Seasonal bone growth and physiology in endotherms shed light on dinosaur physiology. *Nature*. 487, 358-361.

Kornblum, S. S., and Kelly, P. J. (1964). The lacunae and Haversian canals in tibial cortical bone from ischemic and non-ischemic limbs: a comparative microradiographic study. *Journal of Bone and Joint Surgery*, 46, 797–810.

Krahl, V.E., Evans, F.G. (1945). Humeral torsion in man. *American Journal of Physical Anthropology*. 3, 229-253.

Lacroix, P., and Dhem, A. (1967). Le vieillissement des os. Etude microradiographique de l'os compact. *Acta Orthopaedica Belgica*, 33, 745–760.

Lacroix, P. (1971). The internal remodeling of bones. In G.H. Bourne (Ed), *The Biochemistry and Physiology of Bone (Second Edition)* (pp. 119–144). New York, NY: Academic Press.

Lafage-Proust, H., Roche, B., Langer, M., CLeret, D., Vanden Bossche, A., Olivier, T., and Vico, L. (2015). Assessment of bone vascularization and its role in bone remodeling. *BoneKEy reports*. 4, 1-8.

Landis, W. J., Hodgens, K. J., Song, M. J., Arena, J., Kiyonaga, S., Marko, M., Owen, C., and McEwen, B. F. (1996). Mineralization of collagen may occur on fibril surfaces: evidence from conventional and high-voltage electron microscopy and three-dimensional imaging. *Journal of Structural Biology*, 117, 24–35.

Lanyon, L. E. (1984). Functional strain as a determinant for bone remodeling. *Calcified Tissue International*, 36, S56-S61.

Larocque, R. (1986). *Intervention archéologique au cimetière St. Matthew.* Ministère de la Culture et des Communications du Québec (Unpublished Archaeological Report).

Lau, R. Y., and Guo, X. (2011). A review on current osteoporosis research: with special focus on disuse bone loss. *Journal of osteoporosis*, 2011:293808.

.

Lee, C. C. I., Fletcher, M. D., and Tarantal, A. F. (2005). Effect of age on the frequency, cell cycle, and lineage maturation of rhesus monkey (Macaca mulatta) CD34+ and hematopoietic progenitor cells. *Pediatric Research*, 58, 315-322.

Leondes, C. T. (2001). *Biomechanical Systems: Techniques and Applications, Volume III: Musculoskeletal Models and Techniques* (Vol. 3). Boca Raton, Florida: CRC Press.

Lieberman, D. E., and Crompton, A. W. (1998). Responses of bone to stress. In. E. Wiebel, C. Taylor and L. Bolis (eds.). *Principles of Biological Design: The Optimization and Symmorphosis Debate*, (pp.78–86). Cambridge, UK: Cambridge University Press.

Lieberman, D. E., Pearson, O. M., Polk, J. D., Demes, B., and Crompton, A. W. (2003). Optimization of bone growth and remodeling in response to loading in tapered mammalian limbs. *Journal of Experimental Biology*, 206, 3125-3138.

López, J. M., González, G., Reyes, V., Campino, C., and Díaz, S. (1996). Bone turnover and density in healthy women during breastfeeding and after weaning. *Osteoporosis International*, 6, 153-159.

Lovejoy, C. O., Meindl, R. S., Pryzbeck, T. R., and Mensforth, R. P. (1985). Chronological metamorphosis of the auricular surface of the ilium: A new method for the determination of adult skeletal age at death. *American Journal of Physical Anthropology*, 68, 15-28.

Macé, B. (2008). *Histologie : bases fondamentales* (Broché). Paris: Sophia-Antipolis: Omniscience.

Macintosh, A. A., Davies, T. G., Ryan, T. M., Shaw, C. N., and Stock, J. T. (2013). Periosteal versus true cross-sectional geometry: a comparison along humeral, femoral, and tibial diaphyses. *American Journal of Physical Anthropology*, 150, 442-452.

Mackie, E. J., Ahmed, Y. A., Tatarczuch, L., Chen, K.-S., and Mirams, M. (2008). Endochondral ossification: How cartilage is converted into bone in the developing skeleton. *The International Journal of Biochemistry and Cell Biology*, 40, 46-62.

Maggiano, C. M. (2012). Making the mold: a microstructural perspective on bone modeling during growth and mechanical adaptation. In S. Stout and C. Crowder (Eds). *Bone Histology-An anthropological perspective,* (pp. 45-90). Boca Raton, FL: CRC Press.

Maggiano, C.M., Maggiano, I.S., and Cooper D. (2015). Osteonal repathing: A broader context for the interpretation of type II osteons and bone remodeling vasculature across development. . *American Journal of Physical Anthropology*, 156, 210.

Maggiano, I.S., Maggiano C.M., Tiesler V.G., Kierdorf, H., Stout, S.D. and Schultz, M. (2011). A distinct region of microarchitectursl variation in femoral compact bone: histomorphology of the endosteal lamellar pocket. *International Journal of Osteoarchaeology*. 21, 743-750.

Maggiano, I.S., Maggiano C.M., Tiesler V.G., Chi-Keb J.R. and Stout, S.D. (2015). Drifting diaphysis: asymmetry in diametric growth and adaptation along the humeral and femoral length. *The Anatomical Record*, 298, 1689-1699.

Maggiano, I.S., Clement, J.G., Thomas, D., Carter, Y., and Cooper, D.M.L. (2015). Synchrotronic three-dimensional reconstruction of cortical bone for analysis of osteonal branching and interconnectivity across age. *American Journal of Physical Anthropology*, 156, 210-211.

Maggiano, I.S., Maggiano, C.M., Clement, J.G., David, C., Thomas, L., Carter, Y. and Cooper, D.M. (2016). Three-dimensional reconstruction of Haversian systems in human cortical bone using synchrotron radiation-based micro-CT: morphology and quantification of branching and transverse connections across age. *Journal of Anatomy*. 228, 719-732.

Maggiano, I.S., Maggiano, C.M., Clement, J.G., Thomas, D., and Cooper, D.M. (2017). Longitudinal variation of osteon circularity in three-dimensional reconstructions of Haversian networks. *American Journal of Physical Anthropology*, 162, 273.

Mahamid, J., Sharir, A., Addadi, L., and Weiner, S. (2008). Amorphous calcium phosphate is a major component of the forming fin bones of zebrafish: Indications for an amorphous precursor phase. *Proceedings of the National Academy of Sciences*, 105, 12748-12753.

Manolagas, S. C., Kousteni, S., and Jilka, R. L. (2002). Sex steroids and bone. *Recent progress in hormone research*, 57, 385–410.

Manolagas, S. C. (2000). Birth and Death of Bone Cells: Basic Regulatory Mechanisms and Implications for the Pathogenesis and Treatment of Osteoporosis. *Endocrine Reviews*, 21, 115-137.

Marchal, F. (2003). Le dimorphisme sexuel de conformation de l'os coxal humain. Bases biologiques et nouvelles applications possibles. *Bulletins et mémoires de la Société d'Anthropologie de Paris*, 15, 7-24.

Marks, S. C., and Popoff, S. N. (1988). Bone cell biology: the regulation of development, structure, and function in the skeleton. *Developmental Dynamics*, 1–44.

Marotti, G. (1993). A new theory of bone lamellation. *Calcified Tissue International*, 53, S47–S56.

Marotti, G., Muglia, M. A., Palumbo, C., and Zaffe, D. (1994). The microscopic determinants of bone mechanical properties. *Italian Journal of Mineral and Electrolyte Metabolism*, 8, 167–175.

Martin, D. L., and Armelagos, G. J. (1979). Morphometrics of compact bone: An example from Sudanese Nubia. *American Journal of Physical Anthropology*, 51, 571-577.

Martin, D. L. (1983). *Paleophysiological aspects of bone remodeling in the Meroitic, X-Group and Christian populations from Sudanese Nubia*. (PhD Dissertation). University of Massachusetts, Amherst, MA.

Martin, D. L., and Armelagos, G. J. (1985). Skeletal remodeling and mineralization as

indicators of health: an example from prehistoric Sudanese Nubia. *Journal of Human Evolution*, 14, 527–537.

Martin, R. B., Pickett, J. C., and Zinaich, S. (1980). Studies of skeletal remodeling in aging men. *Clinical Orthopaedics and Related Research*, 149, 268-282.

Martin, R.B., and Burr, D. B. (1982). A hypothetical mechanism for the stimulation of osteonal remodelling by fatigue damage. *Journal of Biomechanics*, 15, 137-139.

Martin, R. B., and Burr, D. B. (1989). *Structure, function, and adaptation of compact bone*. Raven Press, New York, NY.

Martin, R. B. (1991). Determinants of the mechanical properties of bones. *Journal of Biomechanics*, 24, 79–88.

Martin, R. B., Lau, S. T., Mathews, P. V., Gibson, V. A., and Stover, S. M. (1996). Collagen fiber organization is related to mechanical properties and remodeling in equine bone. A comparsion of two methods. *Journal of biomechanics*, 29, 1515–1521.

Martin, R. B., Gibson, V. A., Stover, S. M., Gibeling, J. C., and Griffins, L. V. (1996). Osteonal structure in the equine third metacarpus. *Bone*, 19, 165–171.

Martin, R. B., Burr, D. B., Sharkey, N. A., and Fyhrie, D. P. (1998). *Skeletal tissue mechanics*. Springer, New York, NY.

Martin, R. B. (2000a). Does osteocyte formation cause the nonlinear refilling of osteons? *Bone*, 26, 71-78.

Martin, R. B. (2000b). Toward a unifying theory of bone remodeling. *Bone*, 26, 1–6.

Martin, R. B. (2007). Targeted bone remodeling involves BMU steering as well as activation. *Bone*, 40, 1574–1580.

Martin, R. B. (2003a). Functional Adaptation and Fragility of the Skeleton. In S.C. Agarwal and S.D. Stout (Eds.), *Bone Loss and Osteoporosis* (pp. 121-138). New York, NY: Springer.

Martin, R. B. (2003b). Fatigue Damage, Remodeling, and the Minimization of Skeletal Weight. *Journal of Theoretical Biology*, 220, 271-276.

Mashiba, T., Hirano, T., Turner, C. H., Forwood, M. R., Johnston, C. C., and Burr, D. B. (2000). Suppressed bone turnover by bisphosphonates increases microdamage accumulation and reduces some biomechanical properties in dog rib. *Journal of Bone and Mineral Research*, 15, 613–620.

Matkovic, V., Fontana, D., Tominac, C., Goel, P., and Chesnut, C. H. (1990). Factors that influence peak bone mass formation: a study of calcium balance and the inheritance of bone mass in adolescent females. *The American Journal of Clinical Nutrition*, 52, 878-888.

Mays, S. A. (1985). The relationship between harris line formation and bone growth and development. *Journal of Archaeological Science*, 12, 207-220.

Mays S. A. (2007). Metabolic Bone Disease. In R. Pinhasi and S. Mays (Eds.). *Advances in Human Palaeopathology,* (pp.215-251). New York, NY: Wiley.

McEwen, B. F., Song, M. J., and Landis, W. J. (1991). Quantitative determination of the mineral distribution in different collagen zones of calcifying tendon using high voltage electron microscopic tomography. *Journal of Computer-Assisted Microscopy*, 3, 201-210.

McNally, E., Nan, F., Botton, G. A., and Schwarcz, H. P. (2013). Scanning transmission electron microscopic tomography of cortical bone using Z-contrast imaging. *Micron*, 49, 46-53.

Mensforth, R. P., Lovejoy, C. O., Lallo, J. W., and Armelagos, G. J. (1978). Part Two: The role of constitutional factors, diet, and infectious disease in the etiology of porotic hyperostosis and periosteal reactions in prehistoric infants and children. *Medical Anthropology*, 2, 1-59.

Mentaverri, R., Lorget, F., Wattel, A., Maamer, M., Kamel, S., and Brazier, M. (2000). Régulation ostéoblastique de la survie des ostéoclastes : effets du calcitriol. *Comptes Rendus de l'Académie des Sciences - Series III - Sciences de la Vie*, 323, 951-957.

Metz, L. N., Martin, R. B., and Turner, A. S. (2003). Histomorphometric analysis of the effects of osteocyte density on osteonal morphology and remodeling. *Bone*, 33, 753-759.

Meulen, M. C. H. van der, Jepsen, K. J., and Mikić, B. (2001). Understanding bone strength: size isn't everything. *Bone*, 29, 101-104.

Miller, S. C., de Saint-Georges, L., Bowman, B. M., and Jee, W. S. (1989). Bone lining cells: structure and function. *Scanning Microscopy*, 3, 953-960.

Mishra, S., and Knothe Tate, M. L. (2003). Effect of lacunocanalicular architecture on hydraulic conductance in bone tissue: implications for bone health and evolution. *The Anatomical Record. Part A, Discoveries in Molecular, Cellular, and Evolutionary Biology*, 273, 752-762.

Morey, E.R. and Baylink, D.J. (1978) Inhibition of bone formation during space flight.

Science. 201, 1138-1141.

Mori S., Burr D.B. (1993). Increased intracortical remodeling following fatigue damage. *Bone* 14, 103–109

Morland, F. (2010). *Nutrition et état de santé : études paléochimique et paléopathologique de la population exhumée du cimetière protestant Saint-Matthew, ville de Québec, Canada (1771-1860).* (Mémoire de maîtrise). Université de Montréal, Montréal.

Mosley, J. R. (2000). Osteoporosis and bone functional adaptation: Mechanobiological regulation of bone architecture in growing and adult bone, a review. *Journal of Rehabilitation Research and Development*, 37, 189-199.

Moss, W. (2010). *Fouilles et surveillance archéologiques dans le cadre du réaménagement et de la restauration du Parc-cimetière St-Matthew (CeEt-41).* Ministère de la Culture et des Communications du Québec (Unpublished Archaeological Report).

Moyle, D. D., and Bowden, R. W. (1984). Fracture of human femoral bone. *Journal of Biomechanics*, 17, 203–213.

Mulhern, D. M., and Van Gerven, D. P. (1997). Patterns of femoral bone remodeling dynamics in a medieval Nubian population. *American Journal of Physical Anthropology*, 104, 133-146.

Mulhern, D. M. (2000). Rib remodeling dynamics in a skeletal population from Kulubnarti, Nubia. *American Journal of Physical Anthropology*, 111, 519–530.

Mulhern, D. M., and Ubelaker, D. H. (2001). Differences in osteon banding between human and nonhuman bone. *Journal of Forensic Science*, 46, 220–222.

Mulhern, D. M., and Ubelaker, D. H. (2012). Differentiating human from nonhuman bone microstructure. In S. Stout and C. Crowder (Eds). *Bone Histology-An anthropological perspective,* (pp. 109-134). Boca Raton, FL: CRC Press.

Murail, P., Bruzek, J., Houët, F., and Cunha, E. (2005). DSP: A tool for probabilistic sex diagnosis using worldwide variability in hip-bone measurements. *Bulletins et Mémoires de la Société d'Anthropologie de Paris*, 17, 167-176.

Myneni, V. D., and Mezey, E. (2017). Regulation of bone remodeling by vitamin K2. *Oral Diseases*, 23, 1021-1028.

Naylor, K. E., Iqbal, P., Fledelius, C., Fraser, R. B., and Eastell, R. (2000). The effect of pregnancy on bone density and bone turnover. *Journal of Bone and Mineral Research: The Official Journal of the American Society for Bone and Mineral Research*, 15, 129-137.

Nielsen-Marsh, C.M., Gernaey, A., Turner-Walker, G., Hedges, R.E.M., Pike, A.W.G., Collins, M.J. (2000). The chemical degradation of bone. In: Cox, M., Mays, S. (Eds.), *Human Osteology: In Archaeology and Forensic Science*, (pp. 439-454). London: Greenwich Medical Media.

Nyssen-Behets, C., Arnould, V., Benoit, C., and Dhem, A. (1991). Histomorphometry and photodensitometry of osteons with hypercalcified ring of human compact bone. *Bulletin de l'Association des anatomistes*, 75, 127–129.

Nyssen-Behets, C., Duchesne, P.-Y., and Dhem, A. (1997). Structural changes with aging in cortical bone of the human tibia. *Gerontology*, 43, 316–325.

Ortner, D. J. (1975). Aging effects on osteon remodeling. *Calcified Tissue Research*, 18, 27-36.

Ortner, D. J. (2003). *Identification of pathological conditions in human skeletal remains*. London, UK: Academic Press.

Ott, S. M. (2017). Chapter 2 – Bone biology and effects of pharmaceutical intervention on bone quality. In S. Bose and A. Bandyopadhyay (Eds). *Materials and devices for bone disorders,* pp 29-82. Cambridge, MA: Academic Press.

Palumbo, C., Palazzini, S., Zaffe, D., and Marotti, G. (1990). Osteocyte differentiation in the tibia of newborn rabbit: an ultrastructural study of the formation of cytoplasmic processes. *Acta Anatomica*, 137, 350-358.

Pankovich, A. M., Simmons, D. J., and Kulakrini, V. V. (1974). Zonal Osteons in Cortical Bone. *Clinical Orthopaedics and Related Research*, 100, 356-363.

Parfitt, A. M. (1976a). The actions of parathyroid hormone on bone: relation to bone remodeling and turnover, calcium homeostasis, and metabolic bone disease. Part III of IV parts; PTH and osteoblasts, the relationship between bone turnover and bone loss, and the state of the bones in primary hyperparathyroidism. *Metabolism: Clinical and Experimental*, 25, 1033-1069.

Parfitt, A. M. (1976b). The actions of parathyroid hormone on bone: Relation to bone remodeling and turnover, calcium homeostasis, and metabolic bone disease: Part IV of IV parts: The state of the bones in uremic hyperparathyroidism—The mechanisms of skeletal resistance to PTH in renal failure and pseudohypoparathyroidism and the role of PTH in osteoporosis, osteopetrosis, and osteofluorosis. *Metabolism-Clinical and Experimental*, 25, 1157–1188.

Parfitt, A. M. (1979). Quantum concept of bone remodeling and turnover: implications for the pathogenesis of osteoporosis. *Calcified Tissue International,* 28, 1-5.

Parfitt, A. M., Drezner, M. K., Glorieux, F. H., Kanis, J. A., Malluche, H., Meunier, P. J., … Recker, R. R. (1987). Bone histomorphometry: Standardization of nomenclature, symbols, and units: Report of the asbmr histomorphometry nomenclature committee. *Journal of Bone and Mineral Research*, 2, 595-610.

Parfitt, A. M. (1994a). Osteonal and hemi-osteonal remodeling: The spatial and temporal framework for signal traffic in adult human bone. *Journal of Cellular Biochemistry*, 55, 273-286.

Parfitt, A. M. (1994b). The two faces of growth: Benefits and risks to bone integrity. *Osteoporosis International*, 4, 382-398.

Parfitt, A. M., Mundy, G. R., Roodman, G. D., Hughes, D. E., and Boyce, B. F. (1996). A new model for the regulation of bone resorption, with particular reference to the effects of bisphosphonates. *Journal of Bone and Mineral Research*, 11, 150-159.

Parfitt, A. M. (1997). Bone density and fragility, age related changes. In R. Dulbecco (Ed.). *Encyclopedia of Human Biology* (Vol. 2), (pp. 95-107). New York, NY: Academic Press.

Parfitt, A. M. (2000). The mechanism of coupling: a role for the vasculature. *Bone*, 26, 319-323.

Parfitt, A. M. (2002). Targeted and nontargeted bone remodeling: relationship to basic multicellular unit origination and progression. *Bone*, 30, 5-7.

Parfitt, A. M. (2003). New Concepts of Bone Remodeling: A Unified Spatial and Temporal Model with Physiologic and Pathophysiologic Implications. In S.C. Agarwal and S.D. Stout (Eds.), *Bone Loss and Osteoporosis* (pp. 3-17). New York, NY: Springer.

Pazzaglia, U. E., Congiu, T., Pienazza, A., Zakaria, M., Gnecchi, M., and Dell'Orbo, C. (2013). Morphometric analysis of osteonal architecture in bones from healthy young human male subjects using scanning electron microscopy. *Journal of Anatomy*, 223, 242-254.

Peck, J. J., and Stout, S. D. (2007). Intraskeletal variability in bone mass. *American Journal of Physical Anthropology*, 132, 89-97.

Perron, J. S. (2006). *Les marqueurs osseux d'activit!e physique: une étude des restes humains du cimetère St-Matthew à Québec (XVIII ème et XIX ème siècles).* (Mémoire de maîtrise). Université de Laval, Québec.

Petrtýl, M., Hert, J., and Fiala, P. (1996). Spatial organization of the haversian bone in man. *Journal of Biomechanics*, 29, 161-169.

Pfeiffer, S. (1998). Variability in osteon size in recent human populations. *American Journal of Physical Anthropology*, 106, 219-227.

Pfeiffer, S., Crowder, C., Harrington, L., and Brown, M. (2006). Secondary osteon and Haversian canal dimensions as behavioral indicators. *American Journal of Physical Anthropology*, 131, 460–468.

Pfeiffer, S., and Zehr, M. K. (1996). A morphological and histological study of the human humerus from Border Cave. *Journal of Human Evolution*, 31, 49–59.

Portigliatti Barbos, M., Bianco, P., and Ascenzi, A. (1983). Distribution of osteonic and interstitial components in the human femoral shaft with reference to structure, calcification and mechanical properties. *Acta Anatomica*, 115, 178-186.

Portigliatti Barbos, M., Bianco, P., Ascenzi, A., and Boyde, A. (1984). Collagen orientation in compact bone: II. Distribution of lamellae in the whole of the human femoral shaft with reference to its mechanical properties. *Metabolic Bone Disease and Related Research*, 5, 309-315.

Prideaux, M., Findlay, D. M., and Atkins, G. J. (2016). Osteocytes: The master cells in bone remodelling. *Current Opinion in Pharmacology*, 28, 24-30.

Qiu, S., Fyhrie, D. P., Palnitkar, S., and Rao, D. S. (2003). Histomorphometric assessment of Haversian canal and osteocyte lacunae in different-sized osteons in human rib. *The Anatomical Record. Part A, Discoveries in Molecular, Cellular, and Evolutionary Biology*, 272, 520-525.

Raggatt, L. J., and Partridge, N. C. (2010). Cellular and molecular mechanisms of bone remodeling. *Journal of Biological Chemistry*, 285, 25103–25108.

Ragsdale, B. D., and Lehmer, L. M. (2012). A knowledge of bone at the cellular (histological) level is essential to paleopathology. In A.L. Grauer (ed). *A companion to paleopathology,* (pp. 225–249). Chichester, UK: Blackwell Publishing Ltd.

Ralston, S. H., and de Crombrugghe, B. (2006). Genetic regulation of bone mass and susceptibility to osteoporosis. *Genes and development*, 20, 2492–2506.

Raisz, L. G., and Rodan, G. A. (1998). Chapter 1 - Embryology and Cellular Biology of Bone. In L. V. Avioli and S. M. Krane (Eds.), *Metabolic Bone Disease and Clinically Related Disorders (Third Edition)* (pp. 1–22). San Diego: Academic Press.

Ray, S., Botha, J., and Chinsamy, A. (2004). Bone histology and growth patterns of some nonmammalian therapsids. *Journal of Vertebrate Paleontology*. 24, 634, 648.

Rayner, J. M. V. (1985). Linear relations in biomechanics: the statistics of scaling functions.

Journal of Zoology, 206, 415-439.

Recker, R., Lappe, J., Davies, K. M., and Heaney, R. (2004). Bone remodeling increases substantially in the years after menopause and remains increased in older osteoporosis patients. *Journal of Bone and Mineral Research*, 19, 1628–1633.

Reznikov, N., Almany-Magal, R., Shahar, R., and Weiner, S. (2013). Three-dimensional imaging of collagen fibril organization in rat circumferential lamellar bone using a dual beam electron microscope reveals ordered and disordered sub-lamellar structures. *Bone*, 52, 676–683.

Reznikov, N., Shahar, R., and Weiner, S. (2014a). Bone hierarchical structure in three dimensions. *Acta Biomaterialia*, 10, 3815–3826.

Reznikov, N., Shahar, R., and Weiner, S. (2014b). Three-dimensional structure of human lamellar bone: The presence of two different materials and new insights into the hierarchical organization. *Bone*, 59, 93-104.

Ribot, I., and C. Roberts. (1996). A Study of Non-specific Stress Indicators and Skeletal Growth in Two Mediaeval Subadult Populations. *Journal of Archaeological Science,* 23, 67-79.

Ribot, I., Morland, F., Boisjoli, M.-È., and Leach, P. (2010). La bioarchéologie humaine, à la frontière entre le «social» et le «biologique» Démographie, archéologie et état de santé de populations historiques euroquébécoises. *Collection Paléo-Québec*, 34, 27–54.

De Ricqlès, A. (1983). Cyclical growth in the long limb bones of a sauropod dinosaur. *Acta Paleontologica*. 28, 225–232.

De Ricqlès, A.J., Padian, J. R., Horner , E.-T., Lamm and Myhrvold, N. (2003). Osteohistology of Confuciusornis sanctus (Theropoda: Aves). *Journal of Vertebrate Paleontology*. 23, 373-386.

De Ricqlès A.J., Padian, K., Knoll, F., Horner, J.R. (2008). On the origin of high growth rates in archosaurs and their ancient relatives: Complementary histological studies on Triassic archosauriforms and the problem of a "phylogenetic signal" in bone histology. *Annales de Paléontologie*. 94, 57–76.

Rhodes, J. A., and Knüsel, C. J. (2005). Activity-related skeletal change in medieval humeri: Cross-sectional and architectural alterations. *American Journal of Physical Anthropology*, 128, 536–546.

Richman, E. A., Ortner, D. J., and Schulter-Ellis, F. P. (1979). Differences in intracortical bone remodeling in three aboriginal American populations: possible dietary factors. *Calcified Tissue International*, 28, 209–214.

Riggs, C. M., Lanyon, L. E., and Boyde, A. (1993). Functional associations between collagen fibre orientation and locomotor strain direction in cortical bone of the equine radius. *Anatomy and Embryology*, 187, 231–238.

Roberts, C. A., and Manchester, K. (2007). *The archaeology of disease (Third Edition)*. Ithaca, NY: Cornell University Press.

Robling, A. G. (1998). *Histomorphometric Assessment of Mechanical Loading History from Human Skeletal Remains: The Relation Between Micromorphology and Macromorphology at the Femoral Midshaft.* (PhD dissertation). University of Missouri, Columbia.

Robling, A. G., and Stout, S. D. (1999). Morphology of the drifting osteon. *Cells Tissues Organs*, 164, 192–204.

Robling, A. G., and Stout, S. D. (2000). Methods of determining age at death using bone microstructure. In A. Katzenberg and S.R. Saunders (Eds). *Biological anthropology of the human skeleton (First edition),* (pp.187–205). New York, NY: Wiley-Liss.

Robling, A. G., and Stout, S. D. (2003). Histomorphology, geometry, and mechanical loading in past populations. In S.C. Agarwal and S.D. Stout (Eds.), *Bone Loss and Osteoporosis* (pp. 189-205). New York, NY: Springer.

Robling, A. G., Castillo, A. B., and Turner, C. H. (2006). Biomechanical and molecular regulation of bone remodeling. *Annual Review of Biomedical Engineering*, 8, 455–498.

Robling, A. G., and Stout, S. D. (2007). Histomorphometry of human cortical bone: applications to age estimation. In A. Katzenberg and S.R. Saunders (Eds). *Biological anthropology of the human skeleton (Second edition),* (pp.149–182). New York, NY: Wiley-Liss.

Rohrbach, D., Lakshmanan, S., Peyrin, F., Langer, M., Gerisch, A., Grimal, Q., … Raum, K. (2012). Spatial distribution of tissue level properties in a human femoral cortical bone. *Journal of Biomechanics*, *45*(13), 2264-2270.

Rubin, C. T., McLeod, K. J., and Bain, S. D. (1990). Functional strains and cortical bone adaptation: Epigenetic assurance of skeletal integrity. *Journal of Biomechanics*, 23, 43-54.

Ruddel, D.T. (1991). *Québec, 1765-1832: l'Évolution d'une Ville Coloniale.* Québec, Hull: Musée Canadien des Civilisations.

Ruff, C. B., and Hayes, W. C. (1983a). Cross-sectional geometry of Pecos Pueblo femora and

tibiae—A biomechanical investigation: I. Method and general patterns of variation. *American journal of physical anthropology*, 60, 359–381.

Ruff, C. B., and Hayes, W. C. (1983b). Cross-sectional geometry of Pecos Pueblo femora and tibiae—A biomechanical investigation: II. Sex, age, and side differences. *American journal of physical anthropology*, 60, 383–400.

Ruff, C. B., and Larsen, C. S. (1990). Postcranial biomechanical adaptations to subsistence strategy changes on the Georgia coast. *Anthropolical Papers of the American Museum of Natural Hist*ory, 68, 94–120.

Ruff, C. B., Larsen, C. S., and Hayes, W. C. (1984). Structural changes in the femur with the transition to agriculture on the Georgia coast. *American Journal of Physical Anthropology*, 64, 125–136.

Ruff, C. B., Trinkaus, E., Walker, A., and Larsen, C. S. (1993). Postcranial robusticity in Homo. I: Temporal trends and mechanical interpretation. *American journal of physical anthropology*, 91, 21–53.

Ruff, C. B. (2000). Body size, body shape, and long bone strength in modern humans. *Journal of Human Evolution*, 38, 269-290.

Ruff, C. B. (2003a). Growth in bone strength, body size, and muscle size in a juvenile longitudinal sample. *Bone*, 33, 317-329.

Ruff, C. B. (2003b). Ontogenetic adaptation to bipedalism: age changes in femoral to humeral length and strength proportions in humans, with a comparison to baboons. *Journal of Human Evolution*, 45, 317-349.

Ruff C. B. (2007). Biomechanical Analyses of Archaeological Human Skeletons. In A. Katzenberg and S.R. Saunders (Eds). *Biological anthropology of the human skeleton (Second edition),* (pp.183–206). New York, NY: Wiley-Liss.

Ruth, E. B. (1947). Bone studies. I. Fibrillar structure of adult human bone. *Developmental Dynamics*, 80, 35–53.

Saito, M., and Marumo, K. (2010). Collagen cross-links as a determinant of bone quality: a possible explanation for bone fragility in aging, osteoporosis, and diabetes mellitus. *Osteoporosis international*, 21, 195–214.

Samson, C., and Branigan, K. (1987). A new method of estimating age at death from fragmentary and weathered bone. In A. Boddington, A.N. Garland and R.C. Janaway (Eds.). *Death, decay and reconstruction: approaches to archaeology and forensic science*, (pp.101–108). Manchester, UK: Manchester University Press.

Sander, P.M. and Andrassy, P. Lines of arrested growth and long bone histology in

Pleistocene large mammals from Germany: What do they tell us about dinosaur physiology? *Palaeontographica Abteilung*. 277, 143-159.

Schaffler, M. B., and Burr, D. B. (1984). Primate cortical bone microstructure: Relationship to locomotion. *American Journal of Physical Anthropology*, 65, 191–197.

Schaffler, M. B., and Burr, D. B. (1988). Stiffness of compact bone: Effects of porosity and density. *Journal of Biomechanics*, 21, 13-16.

Scheuer, L., and Black, S. (2000). Bone Development. In *Developmental Juvenile Osteology* (pp. 18-31). London, UK: Academic Press.

Schmitt, A. (2005). Une nouvelle méthode pour estimer l'âge au décès des adultes à partir de la surface sacro-pelvienne iliaque. *Bulletins et mémoires de la Société d'anthropologie de Paris*, 17, 89-101.

Schneider, C. A., Rasband, W. S., and Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nature Methods*, *9*, 671-675.

Schnitzler, C. M. (1993). Bone quality: a determinant for certain risk factors for bone fragility. *Calcified Tissue International*, *53*, S27-31.

Schriefer, J.L., Warden, S.J., Saxon, L.K., Robling, A.G., Turner, C.H. (2005). Cellular accommodation and the response of bone to mechanical loading. *Journal of Biomechanics*. 38, 1838-1845.

Schultz, M. (2001). Paleohistopathology of bone: A new approach to the study of ancient diseases. *American Journal of Physical Anthropology*, 116, 106–147.

Schwarcz, H. P. (2015). The ultrastructure of bone as revealed in electron microscopy of ion-milled sections. *Seminars in Cell and Developmental Biology*, 46, 44-50.

Schwarcz, H. P., McNally, E. A., and Botton, G. A. (2014). Dark-field transmission electron microscopy of cortical bone reveals details of extrafibrillar crystals. *Journal of Structural Biology*, 188, 240-248.

Schwartz, J. (1995). *Skeleton Keys: An Introduction to Human Skeletal Morphology, Development, and Analysis*. New York, NY : Oxford University Press.

Sedlin, E. D., Villanueva, A. R., and Frost, H. M. (1963). Age variations in the specific surface of howship's lacunae as an index of human bone resorption. *The Anatomical Record*, 146, 201-207.

Seibel, M. J. (2002). Nutrition and molecular markers of bone remodelling. *Current Opinion in Clinical Nutrition and Metabolic Care*, 5, 525-531.

Sethe, S., Scutt, A., and Stolzing, A. (2006). Aging of mesenchymal stem cells. *Ageing research reviews*, 5, 91–116.

Silberberg, M., and Silberberg, R. (1972). Steroid hormones and bone. In G. H. Bourne (Ed.), *The Biochemistry and Physiology of Bone. Second edition* (p. 401-484). London, UK: Academic Press.

Simmons, D. J. (1985). Options for bone aging with the microscope. *American Journal of Physical Anthropology*, 28, 249–263.

Simmons, E. D., Pritzker, K. P. H., and Grynpas, M. D. (1991). Age-related changes in the human femoral cortex. *Journal of Orthopaedic Research*, 9, 155–167.

Simoneau, D. (2003). Église et cimetière Saint-Matthew, rapport de surveillance archéologique. Ministère de la Culture et des Communications du Québec, Division design et patrimoine, Centre de développement économique et urbain, Ville de Québec (Unpublished Archaeological Report).

Sims, N. A., and Gooi, J. H. (2008). Bone remodeling: Multiple cellular interactions required for coupling of bone formation and resorption. *Seminars in Cell and DevelopmentalBbiology,* 19, 444–451.

Sims, N. A., and Martin, T. J. (2014). Coupling the activities of bone formation and resorption: a multitude of signals within the basic multicellular unit. *BoneKEy Reports*, *3*.

Seeman, E. (2013). Age- and Menopause-Related Bone Loss Compromise Cortical and Trabecular Microstructure. *The Journals of Gerontology: Series A*, 68, 1218-1225

Skedros, J. G., Bloebaum, R. D., Bachus, K. N., Boyce, T. M., and Constantz, B. (1993). Influence of mineral content and composition on graylevels in backscattered electron images of bone. *Journal of Biomedical Materials Research*, 27, 57–64.

Skedros, J. G., Bloebaum, R. D., Mason, M. W., and Bramble, D. M. (1994). Analysis of a tension/compression skeletal system: Possible strain-specific differences in the hierarchical organization of bone. *The Anatomical Record*, 239, 396-404.

Skedros, J. G., Mason, M. W., and Bloebaum, R. D. (1994). Differences in osteonal micromorphology between tensile and compressive cortices of a bending skeletal system: Indications of potential strain-specific differences in bone microstructure. *The Anatomical Record*, 239, 405-413.

Skedros, J.G., Mason, M.W., amd Bloebaum, R.D. (2001). Modeling and remodeling in a developing artiodactyl calcaneus: A model for evaluating Frost's Mechanostat

hypothesis and its corollaries. The Anatomical Record. 263, 167-185.

Skedros, J. G., Holmes, J. L., Vajda, E. G., and Bloebaum, R. D. (2005). Cement lines of secondary osteons in human bone are not mineral-deficient: New data in a historical perspective. *The Anatomical Record*, 286, 781–803.

Skedros, J. G., Sorenson, S. M., and Jenson, N. H. (2007). Are distributions of secondary osteon variants useful for interpreting load history in mammalian bones? *Cells, Tissues, Organs*, 185, 285-307.

Skedros, J. G., Mendenhall, S. D., Kiser, C. J., and Winet, H. (2009). Interpreting cortical bone adaptation and load history by quantifying osteon morphotypes in circularly polarized light images. *Bone*, 44, 392-403.

Skedros, J. G., Clark, G. C., Sorenson, S. M., Taylor, K. W., and Qiu, S. (2011). Analysis of the Effect of Osteon Diameter on the Potential Relationship of Osteocyte Lacuna Density and Osteon Wall Thickness. *The Anatomical Record*, 294, 1472-1485.

Skedros, J. G., Keenan, K. E., Williams, T. J., and Kiser, C. J. (2013). Secondary osteon size and collagen/lamellar organization ("osteon morphotypes") are not coupled, but potentially adapt independently for local strain mode or magnitude. *Journal of Structural Biology*, 181, 95-107.

Sládek, V., Berner, M., and Sailer, R. (2006). Mobility in Central European Late Eneolithic and Early Bronze Age: Femoral cross-sectional geometry. *American Journal of Physical Anthropology*, 130, 320–332.

Smith, J. W. (1963). Age changes in the organic fraction of bone. *The Journal of Bone and Joint Surgery. British volume*, 45, 761-769.

Smith, R. J. (2009). Use and misuse of the reduced major axis for line-fitting. *American Journal of Physical Anthropology*, 140, 476-486.

Sommerfeldt, D., and Rubin, C. (2001). Biology of bone and how it orchestrates the form and function of the skeleton. *European Spine Journal*, 10, S86-S95.

Sowers, M., Corton, G., Shapiro, B., Jannausch, M. L., Crutchfield, M., Smith, M. L., … Hollis, B. (1993). Changes in bone density with lactation. *Journal of the American Medical Association*, 269, 3130–3135.

Sowers, M. (1996). Pregnancy and lactation as risk factors for subsequent bone loss and osteoporosis. *Journal of Bone and Mineral Research*, 11, 1052–1060.

Stenderup, K., Justesen, J., Clausen, C., and Kassem, M. (2003). Aging is associated with decreased maximal life span and accelerated senescence of bone marrow stromal

cells. *Bone*, 33, 919–926.

Stewart, M. C., Goliath, J. R., Stout, S. D., and Hubbe, M. (2015). Intraskeletal Variability of Relative Cortical Area in Humans. *The Anatomical Record*, 298, 1635-1643.

Stout, S. D., and Teitelbaum, S. L. (1976). Histological analysis of undecalcified thin sections of archeological bone. *American Journal of Physical Anthropology*, 44, 263–269.

Stout, S. D., and Simmons, D. J. (1979). Use of Histology in Ancient Bone Research. *Yearbook of Physical Anthropology*, 22, 229-249.

Stout, S. D. (1982). The effects of long-term immobilization on the histomorphology of human cortical bone. *Calcified Tissue International*, 34, 337–342.

Stout, S. D., and Paine, R. R. (1992). Histological age estimation using rib and clavicle. *American Journal of Physical Anthropology*, 87, 111-115.

Stout, S. D., and Paine, R. R. (1994). Bone remodeling rates: a test of an algorithm for estimating missing osteons. *American journal of physical anthropology*, 93, 123–129.

Stout, S. D., and Lueck, R. (1995). Bone remodeling rates and skeletal maturation in three archaeoloqical skeletal populations. *American Journal of Physical Anthropology*, 98, 161–171.

Stout, S.D., Brunsden, B.S., Hidelbolt, C.F., COmmean, P.K., Smith, K.E., Tappen, N.C. (1999). Computer-Assisted 3D reconstruction of serial sections of cortical bone to determine the 3D structure of osteons. *Calcified Tissue International*. 65, 280-284.

Stout, S. D., and Crowder, C. (2012). Bone remodeling, histomorphology, and histomorphometry. In S. Stout and C. Crowder (Eds). *Bone Histology-An anthropological perspective,* (pp. 1-22). Boca Raton, FL: CRC Press.

Streeter, M., Stout, S. D., Trinkaus, E., Stringer, C. B., Roberts, M. B., and Parfitt, S. A. (2001). Histomorphometric age assessment of the Boxgrove 1 tibial diaphysis. *Journal of Human Evolution*, 40, 331-338.

Streeter, M. A. (2005). *Histomorphometric Characteristics of the Subadult Rib Cortex: Normal Patterns of Dynamic Bone Modeling and Remodeling During Growth and Development*. (PhD disseration). University of Missouri, Columbia.

Streeter, M. A. (2010). A Four-Stage Method of Age at Death Estimation for Use in the Subadult Rib Cortex*,†. *Journal of Forensic Sciences*, 55, 1019-1024.

Streeter, M. A., Stout, S., Trinkaus, E., and Burr, D. (2010). Brief communication: Bone remodeling rates in Pleistocene humans are not slower than the rates observed in

modern populations: A reexamination of Abbott et al. (1996). *American Journal of Physical Anthropology*, 141, 315-318.

Stuart-Macadam, P. (1989). Porotic hyperostosis: relationship between orbital and vault lesions. *American Journal of Physical Anthropology*, 80, 187-193.

Stuart-Macadam, P. (1992). Porotic hyperostosis: a new perspective. *American Journal of Physical Anthropology*, 87, 39-47.

Suda, T., Nakamura, I., and Takahashi, N. (1997). Regulation of Osteoclast Function. *Journal of Bone and Mineral Research*, 12, 869-879.

Sumner, D. R., and Andriacchi, T. P. (1996). Adaptation to differential loading: Comparison of growth-related changes in cross-sectional properties of the human femur and humerus. *Bone*, 19, 121-126.

Syed, F., and Khosla, S. (2005). Mechanisms of sex steroid effects on bone. *Biochemical and Biophysical Research Communications*, 328, 688-696.

Szulc, P., and Seeman, E. (2009). Thinking inside and outside the envelopes of bone: dedicated to PDD. *Osteoporosis International*, 20, 1281-1288.

Takahashi, H., Epker, B., and Frost, H. M. (1965). Relation between age and size of osteons in man. *Henry Ford Hospital Medical Journal*, 13, 25–31.

Takahashi, H., and Frost, H. M. (1966). Age and sex related changes in the amount of cortex of normal human ribs. *Acta Orthopaedica Scandinavica*, 37, 122Orthop

Tanner, J. M. (1990). *Foetus into man: Physical growth from conception to maturity*. Cambridge, MA: Harvard University Press.

Tappen, N.C. (1977). Three-dimensional studies on resorption spaces and developing osteons. *American Journal of Anatomy*. 149, 301–317.

Teitelbaum, S. L., and Bullough, P. G. (1979). The pathophysiology of bone and joint disease. *The American journal of pathology*, 96, 282.

Teitelbaum, S. L. (2000). Bone Resorption by Osteoclasts. *Science*, 289, 1504-1508.

Thompson, D. D. (1980). Age changes in bone mineralization, cortical thickness, and haversian canal area. *Calcified Tissue International*, 31, 5–11.

Thompson, D. D., and Galvin, C. A. (1983). Estimation of age at death by tibial osteon remodeling in an autopsy series. *Forensic Science International*, 22, 203-211.

Thompson, D. D., and Gunness-Hey, M. (1981). Bone mineral-osteon analysis of Yupik-

inupiaq skeletons. *American Journal of Physical Anthropology*, 55, 1–7.

Tolar, J., Teitelbaum, S. L., and Orchard, P. J. (2004). Osteopetrosis. *New England Journal of Medicine*, 351, 2839-2849.

Tortora, G. J., and Derrickson, B. H. (2010). *Principles of Anatomy and Physiology, 13th Edition*. Hoboken, NJ: Wiley.

Toupin, R. (2016). *Évolution des pratiques alimentaires en cours de vie : une étude isotopique multi-matérielle de la population du cimetière Saint-Matthew (Québec, 1771-1860)*. (Mémoire de maîtrise). Université de Montréal, Montréal, Québec.

Traub, W., Arad, T., and Weiner, S. (1989). Three-dimensional ordered distribution of crystals in turkey tendon collagen fibers. *Proceedings of the National Academy of Sciences*, 86, 9822–9826.

Traub, W., Arad, T., and Weiner, S. (1992). Origin of Mineral Crystal Growth in Collagen Fibrils. *Matrix*, 12, 251-255.

Trinkaus, E., Churchill, S. E., and Ruff, C. B. (1994). Postcranial robusticity in Homo. II: Humeral bilateral asymmetry and bone plasticity. *American Journal of Physical Anthropology*, 93, 1–34.

Trinkaus, E., and Churchill, S. E. (1999). Diaphyseal cross-sectional geometry of Near Eastern Middle Palaeolithic humans: the humerus. *Journal of Archaeological Science*, 26, 173–184.

Trinkaus, E., and Ruff, C. B. (1999). Diaphyseal cross-sectional geometry of Near Eastern Middle Palaeolithic humans: the femur. *Journal of Archaeological Science*, 26, 409–424.

Turner, C. H. (1998). Three rules for bone adaptation to mechanical stimuli. *Bone*, 23, 399-407.

Turner, C.H. (1999). Toward a mathematical description of bone biology: The principle of cellular accommodation. *Calcified Tissue International*. 65 ,466–481.

Turner, C.H., Robling A.G., Duncan, R.L., Burr, D.B. (2002). Do bone cells behave like a neuronal network? *Calcified Tissue International*. 70, 435–442.

Udagawa, N., Takahashi, N., Akatsu, T., Tanaka, H., Sasaki, T., Nishihara, T., … Suda, T. (1990). Origin of osteoclasts: mature monocytes and macrophages are capable of differentiating into osteoclasts under a suitable microenvironment prepared by bone marrow-derived stromal cells. *Proceedings of the National Academy of Sciences*, 87, 7260-7264.

Väänänen, H. K., Zhao, H., Mulari, M., and Halleen, J. M. (2000). The cell biology of osteoclast function. *Journal of Cell Science*, 113, 377-381.

Väänänen, H. Kalervo, and Horton, M. (1995). The osteoclast clear zone is a specialized cell-extracellular matrix adhesion structure. *Journal of Cell Science*, 108, 2729-2732.

Vahdati, A., Rouhi, G., Ghalichi, F., Tahani, M. (2008). Mechanically Induced Trabecular Bone Remodeling Including Cellular Accommodation Effect: a Computer Simulation. Transactions of the Canadian Society for Mechanical Engineering. 32, 371-381.

van Oers, R. F. M., Ruimerman, R., van Rietbergen, B., Hilbers, P. A. J., and Huiskes, R. (2008). Relating osteon diameter to strain. *Bone*, 43, 476-482.

Vajda, E. G., and Bloebaum, R. D. (1999). Age-related hypermineralization in the female proximal human femur. *The Anatomical Record*, 255, 202-211.

Vajda, E. G., Kneissel, M., Muggenburg, B., and Miller, S. C. (1999). Increased Intracortical Bone Remodeling During Lactation in Beagle Dogs. *Biology of Reproduction*, 61, 1439-1444.

Verborgt, O., Gibson, G. J., and Schaffler, M. B. (2000). Loss of osteocyte integrity in association with microdamage and bone remodeling after fatigue in vivo. *Journal of Bone and Mineral Research*, 15, 60–67.

Wagner, H. D., and Weiner, S. (1992). On the relationship between the microstructure of bone and its mechanical stiffness. *Journal of Biomechanics*, 25, 1311-1320.

Wagner, H., Melhus, H., Pedersen, N. L., and Michaëlsson, K. (2013). Genetic influence on bone phenotypes and body composition: a Swedish twin study. *Journal of Bone and Mineral Metabolism*, 31, 681-689.

Walker, R. A., Lovejoy, C. O., and Meindl, R. S. (1994). Histomorphological and geometric properties of human femoral cortex in individuals over 50: Implications for histomorphological determination of age-at-death. *American Journal of Human Biology*, 6, 659-667.

Walker, P. L., Bathurst, R. R., Richman, R., Gjerdrum, T., and Andrushko, V. A. (2009). The causes of porotic hyperostosis and cribra orbitalia: a reappraisal of the iron-deficiency-anemia hypothesis. *American Journal of Physical Anthropology*, 139, 109-125.

Wang, X., Bank, R. A., TeKoppele, J. M., Hubbard, G. B., Althanasiou, K. A., and Agrawal, C. M. (2000). Effect of collagen denaturation on the toughness of bone. *Clinical*

Orthopaedics and Related Research, 371, 228–239.

Wang, X., Bank, R. A., TeKoppele, J. M., and Agrawal, C. M. (2001). The role of collagen in determining bone mechanical properties. *Journal of Orthopaedic Research*, 19, 1021–1026.

Wassen, M. H. M., Lammens, J., Tekoppele, J. M., Sakkers, R. J. B., Liu, Z., Verbout, A. J., and Bank, R. A. (2000). Collagen Structure Regulates Fibril Mineralization in Osteogenesis as Revealed by Cross-Link Patterns in Calcifying Callus. *Journal of Bone and Mineral Research*, 15, 1776–1785.

Wastney, M. E., Martin, B. R., Peacock, M., Smith, D., Jiang, X.-Y., Jackman, L. A., and Weaver, C. M. (2000). Changes in calcium kinetics in adolescent girls induced by high calcium intake. *The Journal of Clinical Endocrinology and Metabolism*, 85, 4470–4475.

Watson, P. F., and Petrie, A. (2010). Method agreement analysis: a review of correct methodology. *Theriogenology*, 73, 1167–1179.

Weiner, S., Arad, T., and Traub, W. (1991). Crystal organization in rat bone lamellae. *Febs Letters*, 285, 49–54.

Weinstein, R. S., and Bell, N. H. (1988). Diminished rates of bone formation in normal black adults. *The New England Journal of Medicine*, 319, 1698-1701.

Wess, T. J. (2008). Collagen Fibrillar Structure and Hierarchies. In P. Fratzl (Ed.). *Collagen,* (pp. 49-80). New York, NY: Springer.

Wood, J. W., Milner, G. R., Harpending, H. C., Weiss, K. M., Cohen, M. N., Eisenberg, L. E., … Wilkinson, R. G. (1992). The Osteological Paradox: Problems of Inferring Prehistoric Health from Skeletal Samples [and Comments and Reply]. *Current Anthropology*, 33, 343-370.

Wu, K., Schubeck, K. E., Frost, H. M., and Villanueva, A. (1970). Haversian bone formation rates determined by a new method in a mastodon, and in human diabetes mellitus and osteoporosis. *Calcified Tissue Research*, 6, 204–219.

Yeni, Y. N., Brown, C. U., Wang, Z., and Norman, T. L. (1997). The influence of bone morphology on fracture toughness of the human femur and tibia. *Bone*, 21, 453-459.

Yoon, B. K., Lee, J. W., Choi, D. S., Roh, C. R., and Lee, J. H. (2000). Changes in biochemical bone markers during pregnancy and puerperium. *Journal of Korean Medical Science*, 15, 189–93.

Yoshino, M., Imaizumi, K., Miyasaka, S., and Seta, S. (1994). Histological estimation of age

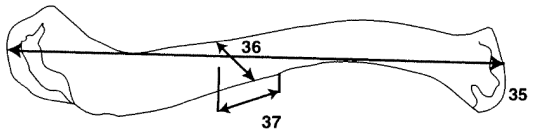
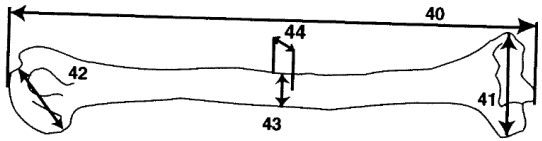
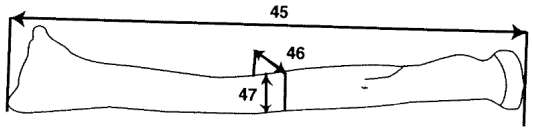
at death using microradiographs of humeral compact bone. *Forensic Science International*, 64, 191-198.

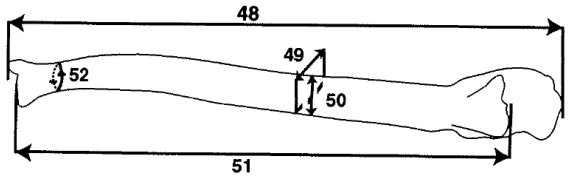
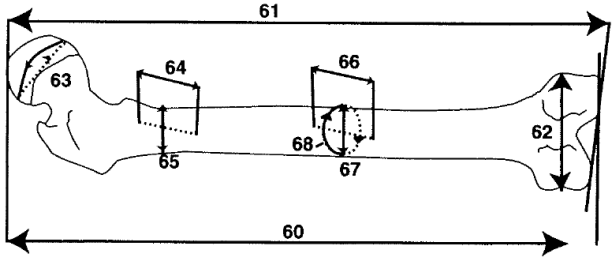
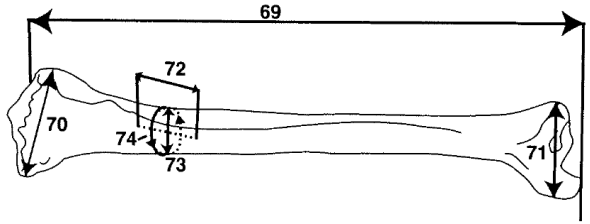
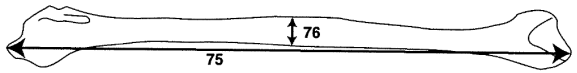
You, L., Temiyasathit, S., Lee, P., Kim, C. H., Tummala, P., Yao, W., … Jacobs, C. R. (2008). Osteocytes as mechanosensors in the inhibition of bone resorption due to mechanical loading. *Bone*, 42, 172–179.

Zanchetta, J. R., Plotkin, H., and Filgueira, M. A. (1995). Bone mass in children: normative values for the 2–20-year-old population. *Bone*, 16, S393–S399.

Zebaze, R., Ghasem-Zadeh, A., Mbala, A., and Seeman, E. (2013). A new method of segmentation of compact-appearing, transitional and trabecular compartments and quantification of cortical porosity from high resolution peripheral quantitative computed tomographic images. *Bone*, 54, 8–20.

Appendix I: Long bones measurements methods

Bone	Measurements (Buikstra and Ubelaker, 1994)	Definition
Clavicle		35: Maximum length 36: Antero-posterieur diameter at midshaft 37: Supero-inferior diameter at midshaft
Humerus		40: Maximum length 41: Epicondylar breadth 42: Vertical diameter of head 43: Maximum diameter at midshaft 44: Minimum diameter at midshaft
Radius		45: Maximum length 46: Antero-posterior diameter at midshaft 47: Medio-lateral diameter at midshaft

Ulna		48: Maximum length 49: Antero-posterior diameter 50: Medio-lateral dimater 51: Physiological length 52: Minimum circumference
Femur		60: Maximum length 61: Bicondylar length 62: Epicondylar breadth 63: Maximum head diameter 64: Antero-posterior subtrochantric diameter 65: Medio-lateral subtrochanteric diameter 66: Antero-posterior midshaft diameter 67: Medio-lateral diameter 68: Midshaft circumference
Tibia		69: Length 70: Maximum proximal epiphyseal breadth 71: Maximum distal epiphyseal breadth 72: Maximum diameter at the nutrient foramen 73: Medio-lateral diameter at the nutrient foramen 74: Circumference at the nutrient foramen
Fibula		75: Maximum length 76: Maximum diameter at midshaft

Appendix II: Age estimation methods

Anatomical element	Description	Range (year)	Reference populations	Date	N	Authors
Auricular surface of the ilium	- Evaluation of the following aspects with scoring: • Undulations or striations on part (or all) of the articular surface • Granulations and porosity • Morphological changes of the apex • Aspects of the posterior region	10 years	• Europeans: -Portugal -England • North America: -Cleveland	19th-20th 18th-19th Early 20th	126 163 174	Schmitt, 2005
	- Comparison of the auricular surface morphology based on 8 predefined phases	5 years until 60 years old.	• Natives • North America: -Cleveland	Late Woodland Early 20th	250 500	Schwartz, 1995. (Reassessment of Lovejoy et al., 1985)
	- Comparison of the auricular surface morphology based on 8 predefined phases according to the sex of the individuals	5 years until 60 years old.	• Natives • North America: -Cleveland	Late Woodland Early 20th	250 500	Lovejoy et al., 1985
Pubic symphysis	- Comparison of the pubic morphology based on 6 predefined phases according to the sex of the individuals	Phase I: 5 years Phase II: 10 years Phase III: 9 years	• North America: - Los Angeles	Late 20th (autopsy sample)	1225	Brooks and Suchey, 1990

		Phase IV: 19 years Phase V: 30 years Phase VI: 22 years				
	- Comparison of the pubic morphology based on 6 predefined phases for males only and depending on the ancestry. (The ranges provided are those for Europeans Americans, used in this study)	Phase I: 2 years Phase II: 4.3 years Phase III: 4.1 years Phase IV: 9.4 years Phase V: 13.7 years Phase VI: 1.1 years	• North America: -Europeans Americans -African Americans -Mexicans	Late 20th (autopsy sample)	486 140 78	Katz and Suchey, 1989

Appendix III: Sex estimation methods

Bone	Type	Description of the method	Reference population	N (♂/♀)	Authors
Os coxae	Metric	-Measurements of the following variables: • Acetabulo-symphyseal pubic length • Cotylo-pubic width • Innominate or coxal length • Greater sciatic notch height • Ischium post-acetabular length • Iliac or coxal breadth • Spino-sciatic length • Spino-auricular length • Cotylo-sciatic breadth • Vertical acetabular diameter - Application of a discriminant function using at least four of these measures - Possible sex determination if the combination of measures provides reliability of 95% or greater	• Europeans: -Portugal -France -England -Lithuania • Africans: -South Africa • North America: -Cleveland -Washington DC • Asia: -Thailand	♂=102; ♀=130 ♂=98; ♀=62 ♂=31; ♀=31 ♂=108; ♀=112 ♂=261; ♀=267 ♂=112; ♀=113 ♂=203; ♀=212 ♂=96; ♀=102	Murail et al., 2005
Os coxae	Visual determination	- Evaluation of the following aspects: • Aspects of the preauricular surface • Aspect of the greater sciatic notch • Form of the composite arch • Morphology of the inferior pelvis • Ischiopubic proportion aspects	• Europeans: -Portugal -France	♂=106; ♀=134 ♂=98; ♀=64	Bruzek, 2002

Os coxae and sacrum	Visual determination	- Evaluation of the following aspects: • Attributes of the subpubic region (ventral arc; subpubic concavity; ischiopubic ramus ridge) • Aspect of the greater sciatic notch • Preauricular sulcus	Unspecified	Unspecified	Buikstra and Ubelaker, 1994