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Effect of exercise training on preeclampsia superimposed on chronic hypertension in a mouse model

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Résumé

La prééclampsie est l'une des causes primaires de mortalité et morbidité périnatales, touchant 2-7% des grossesses. Sa prévalence augmente à 10-25% chez les femmes hypertendues. Jusqu'à maintenant, aucun traitement, mis à part l'accouchement précoce, n'est connu. Néanmoins, plusieurs études épidémiologiques suggèrent une diminution de l'incidence de la prééclampsie chez les femmes entraînées quoique, ces études sont considérées insuffisantes. Ainsi, le but de cette étude est de déterminer si l'entraînement avant et pendant la grossesse prévient la maladie dans un modèle animal de prééclampsie superposée à de l'hypertension chronique (SPE).

Nous avons utilisé des souris double transgéniques, surexpirmant la rénine et l'angiotensinogène humaines (R^+A^+), puisqu'elles sont hypertensives à la base, et développent plusieurs symptômes de la prééclampsie. Pour l'entraînement, les souris ont été mises dans des cages d'exercice 4 semaines avant leur grossesse et y sont restées jusqu'au sacrifice.

L'entraînement physique a prévenu la hausse de pression artérielle en fin de gestation présente chez les souris R^+A^+ sédentaires, possiblement via l'axe de l'angiotensine-(1-7). Le rapport entre l'albumine: créatinine a également été réduit avec l'entraînement. Les altérations placentaires ont été prévenues chez les souris entraînées, améliorant le développement placentaire et fœtal. Ceci était accompagné d'une normalisation de sFlt-1 circulant et placentaire. De plus, l'augmentation du récepteur à l'angiotensine II de type 1 et la diminution du récepteur Mas dans le placenta étaient renversées.

L'entraînement semble prévenir plusieurs symptômes de la SPE dans un modèle animal suggérant qu'il pourrait être d'une grande utilité dans la prévention de la maladie chez la femme.

Mots-clés : Prééclampsie superposée à de l'hypertension chronique, entraînement physique, modèle transgénique, système rénine-angiotensine, placenta.

Abstract

Preeclampsia is among the leading causes of perinatal mortality and morbidity, affecting 2-7% of pregnancies. Its incidence increases to 10-25% in already hypertensive women. To date, no treatment, aside from delivery, is known. Interestingly, several studies have reported that exercise training (ExT) can reduce preeclampsia prevalence although the available studies are considered insufficient. Therefore, the aim of this study is to determine the impact of ExT when practiced before and during gestation on pregnancy outcome in a mouse model of preeclampsia superimposed on chronic hypertension (SPE).

To do so, mice overexpressing both human angiotensinogen and renin (R^+A^+) were used because they are hypertensive at baseline and they develop many hallmark features of SPE. Mice were trained by placing them in a cage with access to a running wheel 4 weeks before and during gestation.

ExT in this study prevented the rise in blood pressure at term observed in the sedentary transgenic mothers. This may be realized through an increased activity of the angiotensin-(1-7) axis in the aorta. In addition, ExT prevented the increase in albumin/creatinine ratio. Moreover, placental alterations were prevented with training in transgenic mice, leading to improvements in placental and fetal development. Placental mRNA and circulating levels of sFlt-1 were normalized with training. Additionally, the increase in angiotensin II type I receptor and the decrease in Mas receptor protein were reversed with training.

ExT appears to prevent many SPE-like features that develop in this animal model and may be of use in the prevention of preeclampsia in women.

Keywords: Superimposed preeclampsia on chronic hypertension, exercise training, transgenic model, renin-angiotensin system, placenta.

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(P)RR: Pro-renin receptor

ACE: Angiotensin-converting enzyme

ACE2: Angiotensin-converting enzyme 2

ACEI: Angiotensin-converting enzyme inhibitors

ACOG: American College of Obstetricians and Gynecologists

ACR: Albumin/creatinine ratio

ADH: antidiuretic hormone

AGT: Angiotensinogen

AMPA: Aminopeptidase A

AMPM: Aminopeptidase M

AngI: Angiotensin I

Ang-(1-7): Angiotensin-(1-7)

AngII: Angiotensin II

AngIV: Angiotensin IV

AP: Arterial pressure

ARB: AT_1 receptor blockers

AT1R: Angiotensin II type 1 receptor

AT2R: Angiotensin II type 2 receptor

AT1-AA: AT1R auto-antibodies

BNP: Brain natriuretic peptide

$CaCl_2$: Calcium chloride

cDNA: complementary DNA

cGMP: cyclic guanosine monophosphate

CO: Cardiac output

CVD: Cardiovascular diseases

DNA: Deoxyribonucleic acid

E-cadherin: Epithelial cadherin

EDTA: Ethylenediaminotetraacetic acid

EF: Ejection fraction

ELISA: Enzyme-linked immunosorbent assay

eNOS: endothelial nitric oxide synthase

ExT: Exercise training

FS: Fractional shortening

GAPDH: Glyceraldehyde 3-phosphate dehydrogenase

GFR: Glomerular filtration rate

GPx: Glutathione peroxidase

hANG: Human angiotensinogen

HR: Heart rate

HELLP: Hemolysis elevated liver enzymes low platelet

HIF-1α: Hypoxia-inducible factor-1α

HLA: Human leukocyte antigen

HPS: Hematoxylin phloxine saffron

hREN: human renin

HSP: Heat shock protein

ICAM-1: Intercellular adhesion molecule-1

IL: interleukin

IFN-γ: Interferon-γ

iNOS: inducible nitric oxide synthase

IRAP: Insulin-regulated aminopeptidase – Angiotensin IV receptor

IUGR: intra-uterine growth restriction

IVS: Interventricular septum

KIR: Killer-cell immunoglobulin-like receptors

LV: Left ventricle

LVD: Left ventricular diameter

LVEDD: Left ventricular end-diastolic diameter

LVEDV: Left ventricular end-diastolic volume

LVESD: Left ventricular end-systolic diameter

LVESV: Left ventricular end-systolic volume

LVID: Left ventricular internal diameter

LVPW: Left ventricular posterior wall thickness

MAP: Mean arterial pressure

MasR: Angiotensin-(1-7) Mas oncogene receptor

$MgCl_2$: Magnesium chloride

M-MLV: Moloney murine leukemia virus reverse transcriptase

MTHFR: Methyltetrahydrofolate reductase

Na_3VO_4: Sodium ortovanadate

Nab1: NGFI-A binding protein 1

NaCl: Sodium chloride

NEP: Neutral endopeptidase

NF-κβ: nuclear factor kappa activated by B cells

NK: Natural killer

nNOS: Neuronal nitric oxide synthase

NO: Nitric oxide

OT: Oxytocin

PAI-1: Plasminogen activator inhibitor-1

PCR: Polymerase chain reaction

PE: preeclampsia

PGI2: Prostacyclin

PlGF: Placental growth factor

PMSF: Phenylmethanesulphonylfluoride

PO_2: Oxygen partial pressure

RAS: Renin angiotensin system

RBF: Renal blood flow

RNS: Reactive nitrogen species

ROS: reactive oxygen species

RT: Reverse transcription

S16: 40S ribosomal protein S16

SDS: Sodium dodecyl sulfate

sEng: Soluble endoglin

sFlt-1: Soluble Fms-like tyrosine kinase-1

SHR: Spontaneously hypertensive rat

SOD: Superoxide dismutase

SPE: Preeclampsia superimposed on chronic hypertension

STBM: Syncytiotrophoblastic microfragments

SV: Stroke volume

TGFβ1: Transforming growth factor β1

TGFβ3: Transforming growth factor β3

TLR: Toll-like receptor

TNFα: Tumor necrosis factor α

TPR: Total peripheral resistance

TBS: Tris buffered saline

VE-cadherin: Vascular endothelial cadherin

VEGF: Vascular endothelial growth factor

VEGFR: Vascular endothelial growth factor receptor

À tous ceux qui ont cru en moi

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Chapter 1 - Introduction

Preeclampsia (PE) is a pathology that develops during pregnancy. Although the disease has been known for centuries, PE remains a disease of theories. Several components have been found to be implicated in disease progression, but are not seen consistently among all women who develop PE. Importantly, underlying medical conditions, like chronic hypertension and diabetes, are known to increase a woman's risk of developing PE.

Although research concerning PE has blossomed in the last 2 decades, treatment options are still lacking. The risk of developing this gestational disease will continue to rise, given the increase in prevalence of contributing factors (i.e. obesity, chronic hypertension and diabetes)[1]. As such, PE prevention appears to be the best option for minimizing the prevalence of the disease worldwide.

The purpose of this memoire is thus to investigate the therapeutic effect of exercise training in a mouse model of preeclampsia superimposed on chronic hypertension (SPE), as well as to investigate potential mechanisms implicated.

Chapter 2 - Pregnancy

2.1 The placenta

2.1.1 Placentation

Pregnancy is characterized by the development of a new organ: the placenta. The fetal side of the placenta develops from cells originating from the fertilized egg, while the maternal side develops from the mother's uterine tissue. This feto-maternal organ plays a primordial role throughout pregnancy by providing the nutrients and oxygen needed for fetal growth, and eliminating waste products. Human pregnancy, much like that of rodents, is characterized by a hemochorial placentation, in which the maternal blood comes in direct contact with the fetal chorion[2].

The effectiveness of this placental system is a result of its unique anatomical structure. The intricate network of vessels scattered throughout the organ effectively perfuses the placenta. In addition, the extensive villous tree structures enhance the area available for gas, nutrient and waste exchange for the growing fetus. As such, placental vasculogenesis, angiogenesis and pseudovasculogenesis are critical processes for a successful pregnancy[3]. It is important to understand that the placenta contains both maternal and fetal vessels. On the maternal side, remodeling of the vasculature is indispensable to ensure an adequate delivery of blood for placental development and fetal growth. The fetus is however

responsible for the formation of an intricate vascular tree, which will exponentially enhance the surface area available for exchange of substances[4].

The placental villous tree development begins after implantation[4]. The initial step involves the formation of primary villi, which consist of columns of cytotrophoblasts, located on the fetal side. These primary villi become secondary villi following invasion of the mesenchyme. Once fetal capillaries can be seen within the villi structure, they are termed tertiary mesenchymal villi. These three steps are repeated throughout pregnancy and contribute to the villous tree structure[3]. Some placental villi become anchored to the basement membrane of the uterus, while others remain bathed in maternal blood, both of which are derived from cytotrophoblasts. Floating villi develop as a result of fusion of cytotrophoblasts to form multinucleated syncytiotrophoblasts. Distal cytotrophoblasts will attach themselves to the maternal tissue, creating bridges between mother and fetus. Additionally, the cytotrophoblast penetrate deeply into the uterine and maternal vasculature, via interstitial and endovascular invasion, respectively. Endovascular invasion leads to the acquisition of an endothelial adhesion molecular phenotype by cytotrophoblasts, a process known as pseudovasculogenesis, and the removal of the endothelial and muscular linings of uterine arterioles[2, 5]. Endovascular invasion occurs directly via the lumen and within the vessel itself, below the endothelium[6]. Vascular remodeling reaches its full extent by mid pregnancy, having modified the spiral arteries of the

endometrium and superficial region of the myometrium[6]. The remodeled segments are lined exclusively with cytotrophoblasts; endothelial and smooth muscle cells are no longer detectable. The disappearance of the muscular tunica media converts these normally high resistance vessels into high capacitance (low resistance) vessels[7]. Indeed, the diameter of the uterine artery increases two-fold by 21 weeks of pregnancy, thereby contributing to an increase in placental perfusion and, thus fetal growth[8]. In fact, uterine blood flow increases 20-fold during pregnancy as a result of this remodeling[9]. These vessels also become unresponsive to vasoactive substances as they lack an endothelium, thereby maintaining an adequate and consistent blood flow to the growing fetus.

2.1.2 Role of oxygen tension

The first trimester is of critical importance for the success of a pregnancy. During this period, changes in oxygen tension modulate cytotrophoblastic proliferation and differentiation[10, 11]. Blood flow to the placenta is initially minimal, creating a relatively hypoxic environment. Indeed, oxygen tension is suspected to be around 20 and 40 mmHg at 8-10 weeks in the intervillous space of the endometrium and decidua, respectively, in comparison to 60 and 45 mmHg, respectively, when evaluated at 12-13 weeks, after trophoblastic invasion has begun[12]. Very little endovascular invasion takes place at low oxygen tensions, further contributing to low placental perfusion. This stage of pregnancy is thus characterized by a rapid placental growth and contributes to the future growth and

development of the fetus by ensuring the placenta's ability to promote gas, nutrient and waste exchange.

Under low oxygen tension, cytotrophoblasts are highly proliferative[10]. Indeed, *in vitro* studies have demonstrated that cyclin B, a protein required for entering the mitosis phase of the cell cycle, is significantly increased under low oxygen tension (oxygen partial pressure (pO_2) of 2%), compared to standard conditions (pO_2 of 20%)[10]. Additionally, this group also observed a decrease in $p21^{WF1/CIP1}$, a protein involved in cell cycle arrest under hypoxic conditions, demonstrating a reduction in mitotic inhibition thereby favouring proliferation. This reduction in p21 is likely mediated in part by the transforming growth factor β (TGFβ) 3, in line with the premise that TGFβ inhibits trophoblastic invasion[13]. This highly proliferative state is accompanied by a concomitant decrease in the cell's ability to differentiate, as demonstrated by specific antigen expression, such as a reduction in α1 integrin required for invasiveness[10]. As proliferation continues to take place, peripheral cytotrophoblasts gain access to maternal arterial blood, rich in oxygen. Cytotrophoblastic cells go from being proliferative to invasive around week 10[10, 14, 15].This change in oxygen tension consequently causes a decrease in the cells' ability to proliferate[5]. Instead, the endovascular cytotrophoblasts begin to differentiate and acquire an endothelial-like expression pattern of cell adhesion molecules, which enhances their motility and invasiveness[5]. Indeed, *in vitro* studies have demonstrated that an increase in oxygen modifies αV integrin family members expression spatially, notably increasing α1β1 and α3β3 integrins

which are specific to endothelial cells, and decreasing α6β4 and α6β3 integrins, which are characteristic of epithelial cells[5, 16]. Invasive trophoblasts also have a reduced E-cadherin (epithelial cadherin) and increased VE-cadherin (endothelial cadherin) expression, providing further evidence that cytotrophoblasts undergo differentiation by mimicking endothelial cell antigen expression[5]. Relatively high oxygen tension thus promotes differentiation of cytotrophoblasts and endovascular invasion, and may explain why invasion of the arterial rather than the venous side of the uterine circulation is favoured.

Oxygen tension is known to modulate hypoxia-inducible factor-1α expression (HIF-1α)[17]. Under hypoxic conditions, much like during early pregnancy, HIF-1α levels are increased, as is TGF-β3[14]. HIF-1α stimulates the expression of the vascular endothelial growth factor (VEGF), a potent mediator of angiogenesis and vasodilation. This family of angiogenic factors, along with angiopoietins, are critical for pregnancy. The VEGF family is involved in endothelial cell proliferation, angiogenesis, vascular permeability and inflammation while angiopoietins are involved in endothelial survival, capillary sprouting and vascular stabilization[18, 19]. Angiogenesis and vasculogenesis dominate during the first trimester of pregnancy, when HIF-1α is elevated. By 10 weeks of pregnancy, the expression of HIF-1α and TGF-β3 are diminished, which is in line with the increase in oxygen tension that occurs following trophoblastic invasion[14]. Subsequently, a decrease in VEGF occurs as pregnancy progresses[20].

Importantly, modulation in VEGF levels has been postulated to also contribute to trophoblastic invasiveness since VEGF and VEGFR mRNA expression are decreased in biopsies of preeclamptic placentas[21].

2.2 Cardiovascular and hemodynamic adaptations

Pregnancy is a physiological process that necessitates cardiovascular and hemodynamic adaptations to ensure the survival of both mother and fetus. The systemic renin-angiotensin system (RAS) is a key player in many of these changes as it is critical for arterial pressure (AP) control as well as fluid and salt homeostasis in the non-pregnant state (see Figure 1 for detailed depiction of the RAS). Normal pregnancy is characterized by an increase in the circulating levels of prorenin, renin and angiotensinogen[22]. Via this cascade, circulating levels of angiotensin II (AngII) are increased, although this is coupled with a diminished endothelial sensitivity to AngII[23]. AngII is the dominant physiologically active compound produced by the RAS and has several downstream effects, mediated by the AngII type 1 receptor (AT1R). Notably, AngII stimulates vasoconstriction, and the release of aldosterone and antidiuretic hormone (ADH). The increase in circulating levels of aldosterone and ADH (also known as arginine vasopressin (AVP)) during pregnancy contributes to the enhanced renal sodium and water reabsorption, respectively, observed in this condition. Although the systemic RAS plays a pivotal role, the implication of the local RASs cannot be ignored. Their roles have become more apparent as a result of their investigation during diseased-states. For instance, in patients with cerebral aneurysms, hypertension and renal

disease present with inappropriate activation of the RAS thereby contributing to the disease development[24, 25]. Moreover, a functional uteroplacental RAS also exists as all of its components are expressed at the level of the uterus, and the placental and fetal membranes[26]. The fetal RAS is hypothesized to be implicated in fetoplacental blood flow regulation, while that on the maternal side is thought to be involved in the vascular remodelling required for normal pregnancy[22].

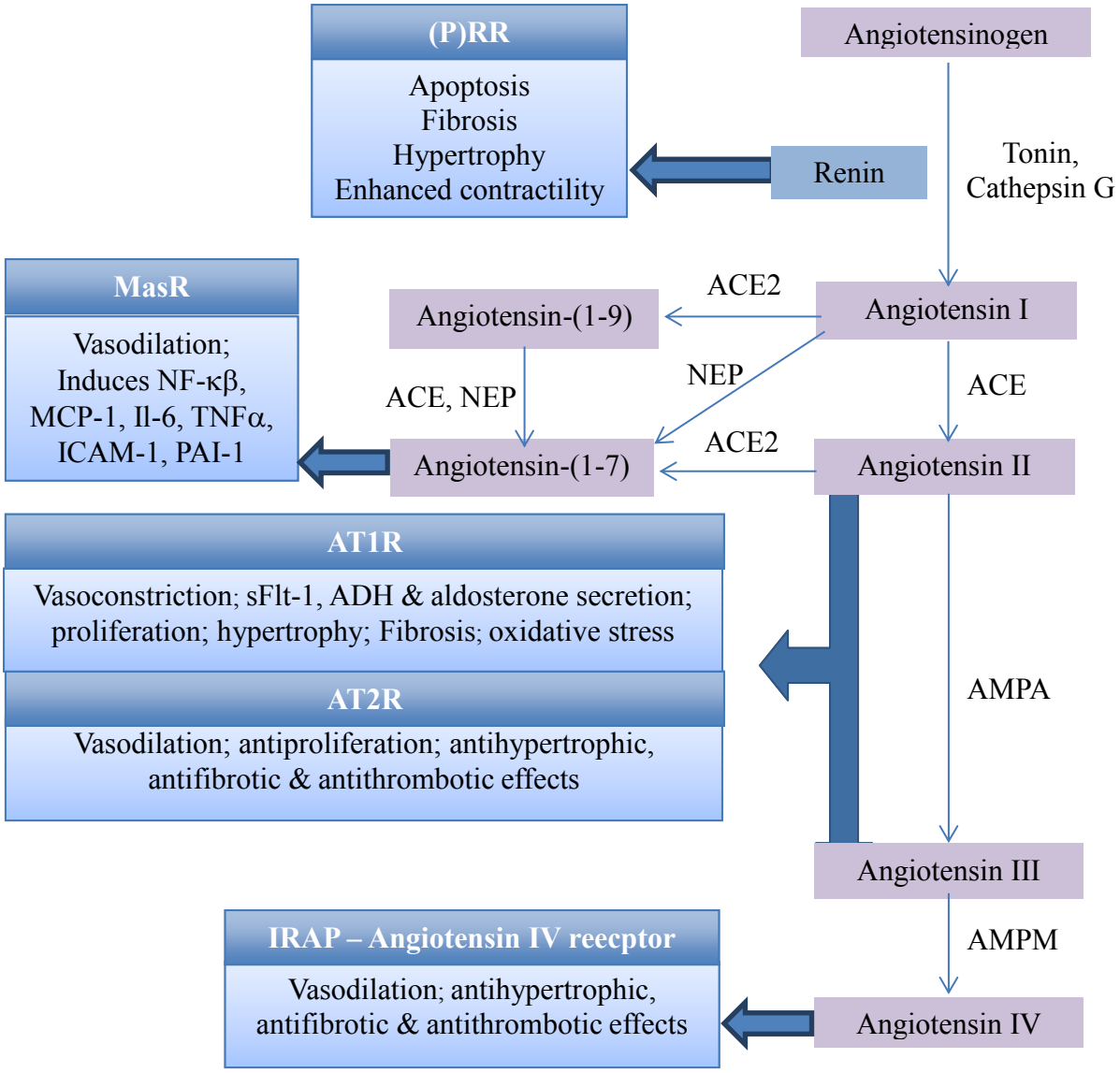

Figure 1: The RAS and the effects of the different components via their respective receptors

Thin arrows describe the conversion reactions that make up the RAS pathway. Thick arrows demonstrate the receptor(s) by which these RAS peptides mediate their effects. ACE, angiotensin-converting enzyme; ADH, antidiuretic hormone; AMPA, Aminopeptidase A; AT1R, angiotensin II type 1 receptor; ICAM-1, intercellular adhesion molecule-1; IL-6; interleukin-6; IRAP, insulin regulated aminopeptidase; MasR, angiotensin-(1-7) Mas oncogene receptor; MCP-1, monocyte chemoattractant protein-1; NEP, neutral endopeptidase; NF-κβ, nuclear factor kappa activated by B cells; PAI-1, plasminogen activator inhibitor-1; (P)RR,

pro-renin receptor; TNFα, tumor necrosis factor α[39].

The pregnancy-induced volume expansion is characterized by a 45-55% increase in extracellular volume, including a 20-30% elevation in plasma volume[27]. An adequate increase in plasma volume will help maintain an adequate blood flow, and thus nutrient and oxygen delivery, to the growing fetus. Moreover, the increase in circulating blood volume gives rise to a proportionate increase in stroke volume (SV) and thus cardiac output (CO), which will be maintained throughout pregnancy[28]. As a result of the pregnancy-induced hypervolemia, heart rate (HR) also increases to maintain organ perfusion[29]. Paradoxically, AP does not increase during normal pregnancy. In fact, AP decreases during the first and second trimester[30]. During the third trimester, AP rises, returning to pre-pregnancy baseline values, and in some cases somewhat higher, by the end of pregnancy[31].

The vasodilatory state of pregnancy is primarily mediated by a pregnancy-induced reduced response to vasoconstrictors, notably AngII, vasopressin and norepinephrine, by smooth muscle cells and the endothelium[22, 32, 33]. This pressor response is not mediated by changes in receptor affinity or receptor number, but rather results from an increased activity and expression of angiotensin-converting enzyme 2 (ACE2) during pregnancy[34, 35]. Enhanced ACE2 expression was observed in the kidney, the placenta and to a lesser extent,

the uterus[34, 36]. ACE2, a homologue of the angiotensin-converting enzyme (ACE), converts AngII into the vasodilatory angiotensin-(1-7) (Ang-(1-7)) (see Figure 2)[37, 38]. Indeed, plasma Ang-(1-7) is increased during pregnancy in women[39]. Additionally, animal studies have demonstrated enhanced Ang-(1-7) expression in the uteroplacental unit and kidneys[34, 40]. Further supporting the role of ACE2 on blood pressure control are studies demonstrating a reduction in ACE2 expression in several hypertensive animal models[41, 42]. Furthermore, *Gurley et al* have observed an increase in baseline blood pressure in certain genetic strains of ACE2-deficient mice[43]. Degradation of AngII and production of Ang-(1-7) by ACE2 therefore counterbalances the AngII mediated vasopressor effect. Ang-(1-7) mediates its effects by binding to the Ang-(1-7) Mas oncogene receptor (MasR). MasR is a G-protein coupled receptors that ultimately leads to the nitric oxide (NO) production by endothelial nitric oxide synthase (eNOS) and prostaglandin production[44]. MasR may also be implicated in the pregnancy-induced pressor response as Yamaleyeva and colleagues have noted an increase in MasR mRNA expression in the utero-placental unit of an animal model during early gestation[45].

Another important component that contributes to reducing the vasoconstrictive tone of pregnancy involves the generation of vasodilatory mediators like NO. NO is a biologically active signalling molecule that binds soluble guanylate cyclase in smooth muscle cells, and in doing so, produces cGMP (cyclic guanosine monophosphate). cGMP activates protein kinase G which ultimately causes

smooth muscle relaxation. NO is generated by NO synthase (NOS) from L-arginine, O_2 and NADPH. There exists three subtypes of this enzyme: endothelial, inducible and neuronal NOS (eNOS, iNOS and nNOS, respectively)[46]. Inhibition of NOS prevents the pregnancy-induced pressure drop in rats, implicating this system in AP control[47]. Several possible mechanisms exist for eNOS activation during pregnancy. Enhanced flow-induced shear stress resulting from pregnancy-induced hypervolemia contributes to NO production[48]. As previously mentioned, the binding of Ang-(1-7) to MasR leads to eNOS activation and NO production, through the activation of Akt-dependent pathways[44]. *In vitro* studies have demonstrated the induction of NO production by estrogen through the effects of eNOS[1, 49]. Elevated estrogen levels throughout pregnancy contribute to the observed vasodilatory state and maintained endothelial function. Abnormal estrogen levels during pregnancy may be involved in gestational pathologies; similarly to the increased cardiovascular risk observed in post-menopausal women[50-52].

Systemic and renal NO production are enhanced during pregnancy, which accounts for the increase in renal blood flow (RBF) and glomerular filtration rate (GFR), along with a decrease in total peripheral resistance (TPR)[23, 28, 53].

Another possible mediator involved in the vasopressor response of pregnancy includes the insulin-regulated aminopeptidase (IRAP), also known as the AngIV receptor. It is expressed by several tissues, notably the placenta and the heart. In the circulation, this enzyme not only degrades oxytocin (OT), but also

vasoconstrictors such as angiotensin III (AngIII), and promoters of cardiac hypertrophy, such as AVP[54, 55]. Circulating levels and activity of IRAP have been reported to be increased during pregnancy[56] and as such, may play a role in maintaining homeostasis during pregnancy[57]. See figure 2 for a summary of the mediators implicated in AP control during pregnancy.

The homeostatic adaptations during pregnancy promote the development of a physiological cardiac hypertrophy primarily due to an increase in left ventricular (LV) thickness[23]. LV function is also improved because of a higher preload, a lower afterload and improved intrinsic contractile properties of the myocardium[23]. Pregnancy, although physiological, is temporary, and as a result, cardiac properties and function return to pre-pregnancy values following delivery, as does AP, SV and HR.

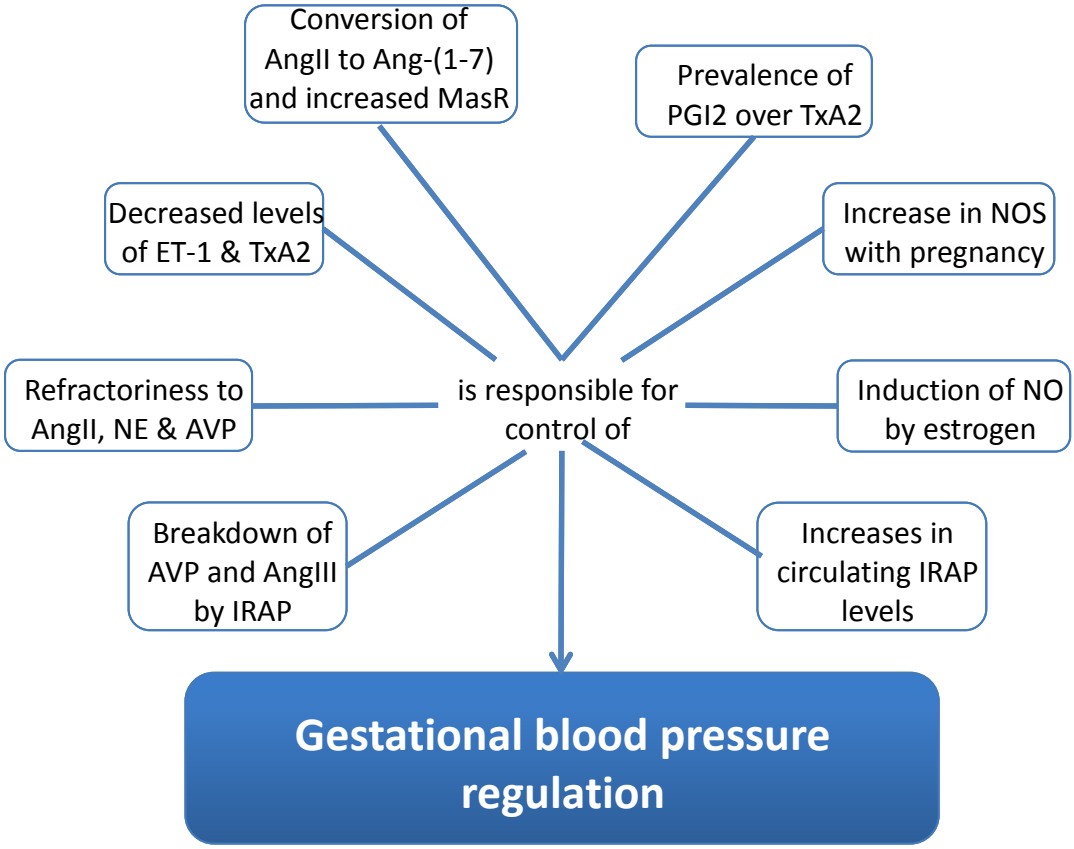

Figure 2 : Regulators of gestational blood pressure control

White boxes depict the mechanisms implicated in blood pressure control during pregnancy. Ang-(1-7) angiotensin-(1-7); AngII, angiotensin II; AngIII, angiotensin III; AVP, arginine vasopressin; ET-1, endothelin-1; IRAP, insulin-regulated aminopeptidase; NE, Norepinephrine; NO, nitric oxide; NOS, nitric oxide synthase; PGI2, prostacyclin; TxA2, thromboxane A2.

Chapter 3 - Preeclampsia

3.1 Symptoms

Mild preeclampsia (PE) is diagnosed by an increase in AP above 140/90 mmHg and *de novo* proteinuria above 300mg/24h after 20 weeks of gestation[58] while severe PE is defined by a diastolic and systolic pressure above 110 and 160mmHg, respectively with the new appearance of proteinuria[59].. As one would expect, the risk of perinatal mortality and morbidity is heightened in cases of severe PE [60].

SPE is characterized by a significant increase in AP (above their already hypertensive values) and *de novo* proteinuria after 20 weeks of pregnancy[61]. SPE is difficult to diagnose in patients with undiagnosed pre-pregnancy hypertension because, these women can present with normal AP during their first and second trimester as a result of the pregnancy-induced AP decrease. The diagnosis is often made several months after delivery, when AP has still not returned to its normal range.

It is important to understand that PE is a systemic disease that affects virtually every organ, either overtly or subclinically, depending on the cause and severity of the disease. As such, the clinical spectrum of PE varies enormously. This heterogeneity is likely a result of the existence of multiple etiologies[62]. Consequently, several treatment options will likely have to be identified/developed according to these respective subtypes.

PE is normally associated with reversible renal and cardiovascular anatomical and pathophysiological adaptations. The cardiovascular adaptations normally ensue as a result of the increase in afterload[63]. Indeed, high AP is known to cause concentric cardiac hypertrophy in men and non-pregnant women[64]. Similar cardiac findings are observed among preeclamptic patients[65]. Paradoxically, PE is normally associated with a hypovolemic state. Interestingly, preeclamptic women are more susceptible to cardiovascular diseases (CVD) later in life[66, 67]. This may be a result of the development of a pathological cardiac hypertrophy or may be a manifestation of a pre-existing medical condition, which merely progresses with time to CVD, irrespective of whether the patient developed PE, rather than the actual cause of CVD post-PE.

GFR and RBF are normally increased in normal pregnancy as a result of the greater blood volume[28, 30]. However, in PE, both of these components are diminished compared to normal pregnancy[68], resulting in hyperuricemia[69]. Hyperuricemia, if severe, can cause renal failure[70], thereby creating a vicious circle, in addition to being a risk factor for hypertension and CVD[71]. Systemic endothelial dysfunction, commonly observed during PE, can also cause a reduction in both GFR and RBF[72]. Worsening proteinuria or sudden oliguria are signs of severe PE. In very severe cases, kidney failure can arise, necessitating dialysis for the remainder of a woman's life.

Overt hepatic damage is also usually indicative of severe PE. Generally, 10-15% of PE patients develop HELLP syndrome, which is characterized by hemolysis, elevated liver enzymes and low platelet count[73], and normally requires induction of fetal delivery. A correlation has been reported between low platelet count and perinatal mortality and morbidity[74], as well as between high serum hepatic transaminases levels, a marker of liver damage, and PE severity[75]. HELLP syndrome normally develops in response to over-activation of the coagulation cascade as is observed during PE; thereby promoting the development of microthrombi and giving rise to ischemia and necrosis[35].

Less than 1% of PE cases progress to eclampsia, a disease characterized by convulsions[76]. Those PE patients that do however develop eclampsia will sometimes present with neurological impairment, including headaches, visual symptoms, changes in mental status and lethargy, as a result of cerebral hyperperfusion-induced edema[77]. These patients require close monitoring as an increase in cerebral AP may lead to stroke. Although severe, the prevalence of eclampsia has decreased significantly in the last 20 years because of the use of magnesium sulfate to prevent the progression of PE to eclampsia[78].

Fetal prematurity, resulting from the necessity to induce premature delivery to protect the mother from PE-induced maternal complications is responsible for elevated rates of fetal mortality and morbidity[34]. Fetal symptoms will thus vary

depending on the severity of PE. Since no other treatment exists aside from inducing fetal delivery, fetal consequences are common. These premature neonates may suffer from intra-uterine growth restriction (IUGR). Suboptimal perfusion resulting from PE-induced placental alterations is likely responsible for IUGR [79]. Prematurity and IUGR may have developmental repercussions later in life[34].

Although PE symptoms generally disappear following delivery, these women remain at risk of CVD as previously mentioned earlier in the text. Indeed, several studies have observed an increased risk of CVD in women previously diagnosed with PE[66, 67] and research is now focusing on this issue to better protect these women from prospective diseases. PE will have long-term implications on women's health and their diagnosis should be used as a marker for underlying diseased state. Similarly, although the apparent fetal consequences of PE are those associated with preterm delivery and IUGR, fetal programming is likely intricately implicated[80-82]. Alterations in placental development, fetal substrate availability and, of importance, the hormonal environment will arise as a result of PE-associated placental hypoxia and oxidative stress. Exposure to high levels of glucocorticoids and nutrient deprivation will alter the expression of transcription factors, receptors, and cell mediators, and in doing so, will increase the fetus' risk of disorders of adulthood, like CVD, neuroendocrine disorders and psychiatric disorders, among others[83].

3.2 Risk factors of PE

3.2.1 Epidemiology

PE is observed in about 2-7% of healthy nulliparous pregnancies[59, 84]. MacGillivray noted in a well characterized Scottish population that 5.6% of women who experienced a preeclamptic birth were primiparous, while 0.3% of cases occurred during a second pregnancy[85]. It is commonly found in women of a young age, during their first pregnancy[62]. Although an association appears to exist between young age and PE, nulliparity may be the source for this apparent link. It is hypothesized that desensitization or tolerance to paternal antigens is responsible for protecting the mother during future pregnancies with the same mate[86]. Therefore, all factors that modify maternal recognition of paternal antigens tend to have an effect. Indeed, women who have had an abortion[87-89] have a reduced risk of PE while the frequency of PE is superior in women using donor sperm insemination[90] and among those using barrier method contraceptives[91]. Importantly, a pregnancy with a new mate eliminates the protection that was once conferred, lending support to the implication of an immunogenic response as a possible mechanism for PE development[92]. Further supporting this hypothesis is the observation that pregnant women are more likely to develop PE if their partner has previously given rise to a preeclamptic pregnancy[93, 94]. The so-called “dangerous father” therefore demonstrates the existence of paternal factors that contribute to PE risk.

PE prevalence is heightened in women with CVD, including chronic hypertension, metabolic syndrome, obesity, pre-gestational diabetes mellitus, dyslipidemia, and pre-existing thrombophilias[62]. Many of these diseases share common characteristics, like systemic inflammation and endothelial dysfunction. Unfortunately, the rising incidence of CVD will contribute to a further increase in PE prevalence. There is a higher occurrence of PE among women of African descent, which may be due to the higher incidence of hypertension among this group of women[95].

It is estimated that 10-20% of women of childbearing age suffer from hypertension worldwide[83]. This CVD is characterized by an increase in AP, above 140/90mmHg[96]. If left uncontrolled, high AP can lead to stroke and heart attack, among other CVD[97]. Studies have shown a direct link between lowering AP and a decreased risk of CVD and death[98]. Underlying hypertension is an important risk factor for PE, as the prevalence of SPE increases to 10-25% in previously hypertensive women[99, 100]. During pregnancy however, these women also experience a physiological decrease in AP as great as 15-20 mmHg, rendering it difficult to diagnose SPE in previously undiagnosed hypertensive mothers[101].

An increase in AP alone poses a significant risk on fetal outcome[99]. Indeed, perinatal mortality is higher among pregnancies associated with hypertension, with a relative risk of 2.3 compared to normotensive, uncomplicated pregnancies[99]. In an attempt to control AP during pregnancy, care must be taken when choosing a

treatment option, as exposure to anti-hypertensive medications can pose major risks to the fetus. Methyldopa is presently the preferred drug prescribed to hypertensive women during pregnancy, as many others are contraindicated[95, 102]. Inhibitors of the RAS, like AT_1 receptor blockers (ARB) or angiotensin-converting enzyme inhibitors (ACEI), have been linked with fetal abnormalities and neonatal death[35]. Interestingly, a recent study by *Diav-Citrin et al.* fails to observe ARB- and ACEI-mediated teratogenic effects when used during the first trimester[103]. The use of diuretics is also discouraged because of its effect on plasma volume, particularly as PE is associated with a hypovolemic state. Importantly, controlling AP by antihypertensive medication does not treat the disease; it minimizes the risk of developing severe PE. When antihypertensive therapies are not effective at controlling AP, fetal delivery is required, in an attempt to limit negative pregnancy outcomes.

For a more complete list of known PE risk factors, see table 1 below.

Table 1: PE risk factors

Young age (less than 20 years)	Family history (CVD)
Old age (above 45 years)	Adiposity
Primiparity	Prepregnancy obesity
New Paternity	Prepregnancy diabetes mellitus
Prolonged interval between pregnancies	Prepregnancy hypertension (chronic hypertension
Excessive placental size	Weight gain during pregnancy
Hydrops fetalis*	Thrombophilia
Multifetal pregnancies	Sedentary lifestyle
Lack of previous abortion	Lack of smoking
Barrier contraception	Dietary factors
Donor oocyte or insemination	Hyperglycemia
Intracytoplasmic sperm injection	Insulin resistance
Polycystic ovarian syndrome	African American ethnicity
Low calcium intake	Triploidy
Short-term oral or semen exposure (shorter cohabitation period)	Hydatiform mole**

* Hydrops fetalis: Fetal disorder characterized by an abnormal accumulation of fluide in a minimum of two (2) fetal compartments[104].

* Hyaditiform mole: Abnormal pregnancy in which the embryo, amniotic membrane and cord are lacking[105].

3.2.2 Genetic factors

PE is commonly found within members of a same family, providing evidence for the existence of genetics risk factors. For example, a woman born from a preeclamptic birth runs a greater risk (20-40%) of developing PE [106, 107]. Among sisters, the risk can climb to 11-37%[108]. The daughter of a man who was born from a preeclamptic birth is also at a greater risk of suffering from PE[109]. Paternal genes may play a different kind of role in disease development, as depicted by the “dangerous father”, triggering an abnormal immune response against seminal antigens[92, 110]. The link between genetics and PE is far from

simple however. There are various genetic components in play, in addition to their interaction with the environment, which can predispose an individual to this gestational disease[34]. Alone, genetics are unlikely to be responsible for inducing the disease, but they are likely to be implicated in altering an individual's susceptibility[111].

Association studies have helped elucidate common alleles that are found in women who develop PE. Those extensively studied include methylenetetrahydrofolate reductase (MTHFR), factor V Leiden, prothrombin (factor II), angiotensinogen (AGT), ACE, endothelial NOS (also referred to as NOS3) and human leukocyte antigen (HLA)[112]. MTHFR, factor II and V are involved in inherited thrombophilias, which is a known risk factor for PE. AGT and ACE are critical players in the classical RAS, which has been postulated to be implicated in PE because circulating levels of several RAS components are abnormal in this condition, as mentioned previously. For instance, the AGT T235 polymorphism predisposes women to essential hypertension and, thus, PE, by enhancing plasma and tissue AGT expression, ultimately leading to an increased production of AngII[113, 114]. As previously mentioned, PE is characterized by systemic endothelial dysfunction, rendering the endothelium more responsive to vasoconstrictors and less so to vasodilators. As such, NOS3 is involved in the maintenance of endothelial function. The NOS3 variant Glu298Asp, linked with PE minimize the availability of NO, thereby reducing the potential extent of vasodilation and steering it more towards a vasoconstrictive state[115, 116].

Lastly, HLAs are involved in maternal immune response during pregnancy. The human leukocyte type 2 DR antigens (HLA-DR) are responsible for paternally-derived fetal antigen recognition by the mother. An increase in HLA-DR homozygosity in both parents is associated with the generation of a preeclamptic birth, suggesting that the absence of genetic compatibility between mother and father may play a role in PE initiation[117]. Polymorphisms in other candidate genes have also been identified, including glutathione S-transferase[118].

It is important to understand that maternal and fetal genotypes are not identical. Additionally, the placenta and fetus originate from maternal and paternal genes and thus represents an allograph. An abnormal immune response by the mother could be at the heart of the disease. Interestingly, diseases related to T-cell immunodeficiency, like HIV, have a lower risk of developing PE[119, 120].

3.2.3 Dietary supplementation, stress, smoking and exercise training

To date, the only cure for PE is delivery of the fetus. There are however consequences to this option, as fetal growth and development may be jeopardized in the process if early delivery is chosen. As a result, research has focused on identifying possible pharmaceutical therapies and lifestyle modifications to prevent and control progression of the disease.

Unfortunately, most of the studies investigating the role of dietary supplements in PE have shown no beneficial role. Although oxidative stress has been implicated

in PE development, antioxidant supplementation has been investigated without much success[121]. Interestingly, many of the studies investigating the effects of antioxidants on PE prevention begin supplementation after 14-16 weeks of gestation. At this stage of pregnancy, the processes involved in PE development have already been initiated. Antioxidant supplementation may not be sufficient at this stage to counter the systemic oxidative stress and its damages. Calcium supplementation was shown to be effective only among women with a reduced calcium intake[122]. There is recent evidence indicating that vitamin D supplementation prior to and/or during pregnancy can prevent PE in women[123], although further investigation is required as the exact mechanisms by which vitamin D positively impacts pregnancy remain unclear.

Chronic stress is an environmental factor that can negatively affect CVD, certain cancers, respiratory diseases, etc[124]. Mothers living or working in a stress-ridden environment during pregnancy have been shown to be at a greater risk of delivering prematurely[125]. Additionally, stress at work has been associated with an increased risk of PE[126]. For instance, female resident physicians were more likely to become preeclamptic compared to the wives of their male classmates[127].

Also, although there are many adverse effects of smoking during pregnancy, including fetal growth restriction and placental abruption, it is interesting to note that smokers have a reduced risk of developing PE[128]. The protective effect of smoking is thought to be mediated by carbon monoxide in the placenta[129]. It is

reported that carbon monoxide reduces the production of anti-angiogenic mediators which can cause endothelial dysfunction and thus lead to hypertension and proteinuria[130].

The role of exercise training in modulating PE risk will be discussed in detail in chapter 3.

3.3 Etiologies of PE

PE is a disease of theories; however our understanding of the possible etiologies has increased drastically in the last two decades. There are several mechanisms that have been implicated in the development of PE including abnormal placentation, endothelial dysfunction, oxidative stress, inflammation, immunity and the RAS[62].

3.3.1 Abnormal placentation

The role of the placenta has long been suspected, particularly given that the only treatment for PE is induction of fetal delivery and as such, removal of the placenta. Moreover, the disease may occur during molar and is more frequent during multiple pregnancies, further lending support to a placental role[131, 132]. Placental abnormalities is among the most supported hypotheses for PE development. Indeed, preeclamptic placentas are characterized by the presence of

sclerotic villi, a loss of intermediate villi, a growth in intervillous space and an abundance of syncytial knots[133]. The vasculature is characterized by the presence of foam cells, fibrinoid necrosis and perivascular lymphocyte infiltration, possibly a result of an immunological response[134]. Vascular and thrombotic lesions are detected in vessels on both the fetal and maternal side of the uteroplacental unit and may contribute to the diminished placental perfusion[135] and tissue hypoxia. It is estimated that this reduction in placental blood flow is of the order of 50-70%[136]. Importantly, reduced uterine perfusion pressure has been identified as an animal model of PE[137, 138]. Further aggravating this situation is the abundance of vasoconstrictors, like thromboxane A2 and endothelin-1, in addition to an increased sensitivity to AngII without any changes in that of vasodilators, like prostacyclin (PGI2), NO and Ang-(1-7)[139-141]. The overpowering vasoconstrictive effect, which diminishes placental perfusion, results from a superficial invasion of the spiral arteries by cytotrophoblasts and defective pseudovasculogenesis. Furthermore, the failure of trophoblasts to acquire an endothelial-like adhesion molecule phenotype ensures that the vessels retain their elastic and smooth muscle-like properties, and continue responding to circulating vasoconstrictive substances, further decreasing placental perfusion[135, 142]. Together, adequate placental blood flow is jeopardized, as is fetal growth and development.

The soluble Fms-like tyrosine kinase-1 (sFlt-1) and soluble endoglin (sEng) are anti-angiogenic mediators that have been found to be elevated during PE[143, 144]. Hypoxia causes the placenta to synthesize and release these mediators into the circulation, which is thought to further impact placental development and aggravate hypoxia. Placental sFlt-1 production is also induced by pro-inflammatory cytokines, like TNF-α, and its secretion is stimulated by AT1R[145, 146]. In addition to their local effects in the placenta, these mediators enter the circulation and promote systemic endothelial dysfunction, inflammatory responses and oxidative stress.

sFlt1 is a splice variant of the vascular endothelial growth factor receptor (VEGFR or Flt-1), which lacks the transmembrane domain. It therefore circulates in the blood and binds circulating VEGF and placental growth factor (PlGF) which reduces their capacity to attach to their membrane receptors, VEGFR1 (also named Flt-1). As such, the effects on angiogenesis and vascular permeability are blunted[147]. Decreased VEGF availability may also prevent pseudovasculogenesis, since conversion of hematopoietic stem cells into endothelial cells is induced by VEGF[148]. In addition, under hypoxic conditions, a decrease in PlGF gene expression along with an increase in VEGF gene expression in cytotrophoblasts and syncytiotrophoblasts is observed *in vitro*[149, 150]. Although counterintuitive, the increase in VEGF expression may merely be a compensatory mechanism to counteract the increase in circulating sFlt-1. Finally, *in vitro* studies have demonstrated that sFlt-1 inhibits cytotrophoblastic invasion

and differentiation[151] by blocking ligand binding. Indeed, the expression of integrin α1, which is normally expressed by endovascular cytotrophoblasts, was significantly reduced in the presence of sFlt-1[151]. Moreover, Zhou et al. also observed an increase in TUNEL staining, thereby demonstrating higher levels of apoptosis[151]. Interestingly however, Flt-1 deletion in an animal model does not affect placentation, suggesting that sFlt-1 during PE may not be implicated in placental alterations. This however cannot be concluded with certainty since sFlt-1 modulates Flt-1, Flk-1 and neuropilin signalling[48, 152]. Moreover, administration of sFlt-1 to pregnant and non-pregnant rats causes hypertension and proteinuria, similarly to the symptoms observed during PE[153]. Similarly, overexpressing sFlt-1 in mice led to the development of PE-like manifestations in pregnant mice, however Lu et al did not observe similar symptoms when transfecting non-pregnant mice[154]. Thus far, sFlt-1 overexpression has been depicted as a deleterious anti-angiogenic state, however lower levels of sFlt-1 during the first trimester have been observed in women who go on to miscarry compared to women who deliver healthy babies[39]. It is important to note that some women with elevated sFlt1 levels do not develop PE, and vice versa, supporting the existence of various etiologies for this disease[143, 155]. Elevated plasma sFlt-1 levels are therefore not specific to preeclampsia.

Endoglin, a coreceptor for TGF β1 and β3, is released by the placenta into the maternal circulation in cases of PE[144]. Antagonization of TGFβ1 and TGFβ3 by sEng inhibits TGFβ signalling pathways, which include eNOS phosphorylation

thereby hindering systemic endothelial function[156-158]. Animal models have shown that sEng alone cannot give rise to all PE features, but does cause an increase in mean arterial pressure (MAP) in pregnant rats[144]. The effects of sEng adrenoviral expression in non-pregnant mice was not however investigated in this study. Animal models in which both sFlt1 and sEng were overexpressed caused severe PE-like features, including a more important increase in MAP compared to sEng alone, proteinuria, HELLP syndrome and fetal growth restriction in pregnant rats and severe vascular injury among their non-pregnant counterparts[144]. These antiangiogenic components thus seem to work together to mediate systemic endothelial dysfunction, inflammation and oxidative stress[159].

Abnormal placentation will negatively impact fetal growth and development, possibly resulting in fetal growth restriction. Fetal growth restriction does not however occur in all women affected by PE, suggesting that the extent of trophoblastic invasion relates to disease severity and that compensatory mechanisms may arise to minimize the effects on the fetus.

3.3.2 Endothelial dysfunction

The endothelium consists of a layer of cells separating vascular smooth muscle cells from circulating blood components. It is responsible for modulating vascular tone and permeability, and plays a critical role in targeting immune cells to the site of injury and controlling the coagulation cascade[160]. Systemic endothelial cell

injury, as is the case in PE, will consequently affect the endothelium's ability to adequately respond to vasoactive substances, to control vascular permeability and to maintain hemostasis[161]. Systemic endothelial dysfunction is observed biochemically by the synthesis and secretion of markers of endothelial cell injury, like endothelin-1, fibronectin and selectins[162, 163]. Indeed, circulating endothelin-1 levels are greater among PE patients[164]. Additionally, PE patients often present with activation of the coagulation pathway, resulting in microthrombi, and thus contributing to an impaired organ perfusion and endothelial dysfunction[165]. Additionally, the endothelium will acquire new properties, including the ability to produce vasoconstrictors and pro-coagulants like endothelin-1 and thromboxane A2, respectively, further aggravating maternal symptoms[141, 164]. Endothelial dysfunction is present in many vascular beds, and consequently, it has the ability to mediate diffused organ damage, much like what is observed during PE.

Inadequate trophoblastic invasion is believed to be critical for the initiation of PE, and as such, the placental vasculature is likely one of the first tissues affected. Indeed, the endothelial lining of the spiral arteries fails to undergo denudation or remodelling in PE, thus causing the superficial invasion of the maternal vasculature[9, 166-168]. This ultimately leads to placental hypoxia, as the vessels continue to respond to vasorestrictive substances, thereby diminishing placental blood flow. As mentioned previously, anti-angiogenic mediators are released form

the placenta into the circulation and cause systemic endothelial dysfunction by antagonizing VEGF and PlGF. Indeed, decreased levels of VEGF are reported with PE as a result of the increase in circulating sFlt-1[169, 170], although data are inconsistent as some have reported increased levels of VEGF[171, 172]. This discrepancy likely results from the existence of two distinct and publishable VEGF measures[173, 174]. In PE, because of the increase in sFlt-1, a decrease in free circulating VEGF concentrations is often detected. Groups measuring total VEGF concentrations may observe an increase in circulating VEGF, as a result of a compensatory mechanism to counter the higher levels of sFlt-1 normally observed during PE[171, 172]. Elevated levels of circulating VEGF have been linked to transient hypotension while inhibitors of VEGF have been shown to induce proteinuria and hypertension[175]. Similarly, sFlt-1 antagonizes VEGF, and thus reduces the free form of VEGF in the circulation. It is interesting to note the existence of placental derived syncytiotrophoblast microfragments (STBM) in the maternal circulation[176], that appear as a result of necrosis, that may be involved in mediating endothelial cell injury by stimulating an exaggerated maternal inflammatory response.

Circulating components are implicated in promoting endothelial cell activation and thus dysfunction in PE. A whole slew of mediators have been identified, some of which have been mentioned previously, including angiogenic mediators.

Inflammatory mediators and plasma factors, like TNFα and AngII, are commonly increased in PE[176-178]. As mentioned previously, there is an absence of

refractoriness to AngII in PE patients compared to normal pregnant controls[179]. Downstream signalling of AngII includes NADPH oxidase activation (which induces oxidative damage), TNFα production and sFlt-1 secretion, all of which contribute to endothelial dysfunction[145, 180]. As a results of the increase placental hypoxia and ischemia, concentrations of inflammatory cytokines, like TNFα, have been reported to be greater in PE compared to normal pregnancy[181]. Interestingly, in the circulation, TNFα impairs endothelium relaxation[182] via activation of NADPH oxidase[183] and negatively impacts NO signalling and may stimulate the production of endothelin-1[184], further demonstrating the ability of TNFα to promote endothelial dysfunction by several means.

Alternately, diminished response to vasodilatory substances, like prostaglandins, NO, acetylcholine and bradykinin has been reported[185-188]. Diminished PGI2 production has also been observed in preeclamptic women[189]. An abnormal vasodilatory response can even be observed prior to the onset of clinical symptoms[36, 187, 190, 191]. Lower flow-mediated dilation and asymmetric dimethylarginine (endogenous inhibitor of eNOS) were observed in women that went on to develop PE, when compared to those with normal pregnancy[36, 191].

For a summary of the circulating factors present in pregnancy and preeclampsia, see table 2.

Table 2 - Circulating factors in pregnancy and preeclampsia

Circulating factor	Pregnancy	PE
AngII	↑ with ↓ sensitivity	↑ with ↑ sensitivity
Endothelin-1	↑ with ↓ sensitivity	↑ with ↑ sensitivity
NO	↑	↑, ↓ or = ↓ response
PGI_2	↑	↓ with ↓ response
sFlt-1/sEng	Low/absent	↑
STBM	Low/absent	↑
Thromboxane A2	↓	↑
TNFα	↓	↑
VEGF (free ligand)	↑	↓
VEGF (total ligand)	↑	↑

3.3.3 Oxidative stress

Oxidative stress is implicated in a number of pathologies, including hypertension and obesity. It is the result of an imbalance between reactive oxygen species (ROS) production and the body's ability to ward them off, via antioxidant defenses such as superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx). ROS are critical for many biological processes, including normal pregnancy. For example, maternal vascular remodeling within the placenta is mediated by ROS, as is fetal growth and development[192]. Conversely, overactivation of NADPH oxidase is observed in PE patients, which contributes to the production of endothelial dysfunction and an exaggerated state of oxidative stress[193]. Indeed, circulating ROS, like superoxide anion, hydrogen peroxide and hydroxyl free

radicals directly and indirectly play a role in the induction of endothelial dysfunction[194]. For instance, they induce lipid peroxidation of the membrane bilayer of endothelial cells and promote oxidation of lipoproteins. Compromising the integrity of the bilayer and the functioning of signaling proteins at the membrane jeopardizes the proper functioning of the cell. Very high levels of lipid peroxidation, originating from the placenta, are observed in the circulation of women who go on to suffer from PE, thereby revealing the placenta as a point of origin for the production of oxidative damage[195, 196]. Indirectly, ROS scavenge NO, thereby minimizing NO stores, and blunting vasodilatory response[197, 198]. In addition, studies have reported that antioxidant defenses are compromised in PE, as seen by a decrease in SOD, GPx and catalase in erythrocytes hemolysates and at the mRNA level in the placenta[45, 199-201]. Moreover, placental ROS levels were found to be elevated in an animal model of spontaneous PE (BPH/5 mice), implicating this system in the production of placental alterations[202]. This increase was associated with a decrease in placental murine SOD expression. In PE, the placental vasculature is also laden with nitrotyrosine, an oxidative bi-product[203]. Oxidative damage observed in PE is proposed to originate as a result of the inadequate placental perfusion and development[204].

3.3.4 Renin-angiotensin system

The RAS is an important mediator in the control of AP and sodium and water handling. Normal pregnancy is associated with cardiovascular and hemodynamic

changes that are mediated by different RAS components. As described above, abnormalities in the RAS are associated with PE (see diagram of the intricate RAS in Figure 2).

Normal pregnancy is associated with an increase in circulating levels of AngII, along with a diminished sensitivity of the AT1R for its ligand[23]. Paradoxically, AngII levels are diminished while maternal sensitivity to this vasoactive substance is increased during PE[205]. A proposed mechanism for this effect is the 4-5 times increase in bradykinin 2 receptors density on platelets and placental omental vessels, compared to healthy pregnancy[206]. Indeed, these can form a heterodimer with the AT1R, which produces an increased sensitivity to AngII[206]. In addition, AT1 auto-antibodies (AT1-AA) have recently been found in the circulation of preeclamptic women[207]. These have a high affinity for the AT1 receptor. As such, AT1-AA and AngII both can stimulate the downstream signalling pathways of the AT1R, which include oxidative stress, endothelial dysfunction[208], sFlt-1 secretion and vasoconstriction[209]. Furthermore, the effects of AngII and AT1-AA appear to be additive[210].

As previously mentioned, ACE2 and Ang-(1-7) are involved in AP regulation during pregnancy. ACE2 converts AngII, a vasoconstrictor, into the Ang-(-1-7), a vasodilator. Modulation of these RAS components may contribute to PE. Indeed, circulating Ang-(1-7) are decreased in preeclamptic women, compared to women with uncomplicated pregnancy[211]. The same group later observed a reduction in

ACE2 mRNA expression and Ang-(1-7) in the uterus of an animal model of PE. Lower levels of Ang-(1-7) likely contribute to the hypertensive state of PE[212].

A diminished level of circulating IRAP has also been observed during PE, compared to normal pregnancy[56]. This is potentially as a result of the necrosis of placental syncytiotrophoblasts, which normally synthesize IRAP during pregnancy[56]. IRAP is critical for glucose uptake and its decrease may be implicated in mediating hyperglycemia and insulin intolerance in PE[213, 214]. In addition, as IRAP is responsible for the degradation of OT, AngIII and arginine vasopressin[54, 55], its decreased circulating levels during PE may thus contribute to the associated vasoconstrictive state and may be involved in inducing pathological cardiac hypertrophy.

3.3.5 Inflammation and immunity

The fetus is analogous to an allograft in the woman's womb. In order to prevent rejection, the mother's immune system must adapt. As such, there is a shift away from cell mediated immunity (Th1) towards a humoral one (Th2), in addition to a diminished adaptive immune response[7]. Towards the end of pregnancy, a shift back towards cell-mediated immunity occurs in order to induce the timely delivery of the fetus[7]. Unfortunately, in the case of PE, cell-mediated immunity dominates throughout the pregnancy, resulting in an altered cytokine expression pattern[7], where pro-inflammatory cytokines, such as the tumor necrosis factor alpha (TNFα), interferon gamma (IFN-γ) and several interleukins (IL) show

enhanced expression[215]. This can have deleterious effects on endothelial function, the coagulation pathway and can modulate the production of ROS[216].

Many cells are involved in the PE mediated inflammatory response, among them, are endothelial cells. Not only do they respond to inflammatory mediators, but they themselves can secrete cytokines, ROS and vasoconstrictors, and up-regulate adhesion molecules to in turn recruit leukocytes. Because of their abundance throughout the body, local inflammatory responses can be propagated to distant locations. These inflammatory responses prompt endothelial dysfunction by i) promoting oxidative damage, ii) activating the coagulation pathway and iii) inducing the production of vasoconstrictors (AngII and endothelin-1) and surface adhesion molecules, and thus contribute to the pathophysiology of PE[119, 120, 217].

Natural killer (NK) cells and monocytes may also play a role in PE initiation and progression. Indeed, NK cells are involved in immune recognition. They have the ability to recognize invasive cytotrophoblasts via killer-cell immunoglobulin-like receptors (KIR) and in turn mediate spiral artery remodeling. Insufficient NK cell activation may thus prevent adequate arterial remodeling and as a result, contribute to placental alterations[218-220]. PE patients often express inhibitory KIR, and as such, their NK and monocytes responses are often abnormal. This abnormal immune response is postulated to be a disease-promoting entity, which leads to inflammation via the production of type 1 cytokines[215].

Placental hypoxia, often observed in PE, also leads to, induction of HIF-1α[17]. Among the downstream mediators of HIF-1α is the activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), which is intimately involved in many inflammatory responses[215].

In addition, an increase in circulating levels of syncytiotrophoblastic microfragments, or STBM, is often observed during PE[176]. These fragments are phagocytosed by macrophages and dendritic cells. Exposure of endothelial cells *in vitro* to STBM originating from preeclamptic women interfered with endothelial cell proliferation suggesting that these microfragments may be involved in mediating PE-associated endothelial damage[221]. These microparticles also cause the release of pro-inflammatory mediators by the endothelium, causing further damage[221, 222]. Neutrophils are induced by STBM to produce superoxide radicals in preeclamptic women, which also contribute to endothelial dysfunction and inflammation[223].

Although there appears to be several processes involved in the disease, there is likely considerable overlap, and identifying which is the initiator will prove to be difficult. Nevertheless, understanding the mechanisms implicated in its development are indispensable to identify treatment opportunities and reducing the significant risk of future cardiovascular and cardiometabolic diseases in women who have suffered from PE.

Chapter 4 - Exercise training

4.1 Exercise training benefits for non-pregnant individuals

Exercise has long been considered the ultimate means for preventing and treating CVD as it improves cardiac function and lowers AP[224]. Moreover, it improves an individual's lipid profile and insulin sensitivity as well as preserves muscle mass[225]. In addition, it has been shown to promote mental, psychological and emotional health by diminishing anxiety and improving mood state and self-esteem[226]. The wide range of beneficial effects produced by exercise is a result of its ability to affect most bodily systems, in addition to targeting the endothelium. For example, the shear stress resulting from exercise training minimizes plaque buildup and thus reduces the risk of developing atherosclerosis[227]. Moreover, exercise induces a physiological cardiac hypertrophy and promotes peripheral muscle development and strength; the heart and skeletal muscle both being tissues that necessitate vascular adaptations to ensure an adequate delivery of oxygen and nutrients[228]. Exercise promotes angiogenesis by mobilizing progenitor endothelial cells[228]. It also improves the vasculature's vasodilatory capabilities by increasing eNOS expression and by enhancing the endothelium's sensitivity to vasodilators[229, 230]. This increase in eNOS thus enhances the production of the vasodilatory NO[231]. Additionally, sensitivity to vasoconstrictors, like endothelin-1, is diminished, likely via a post-receptor mechanism[228]. Through these mechanisms, an individual's risk of

becoming hypertensive and developing associated-diseases are also greatly diminished[232].

Better vasodilation and perfusion of the heart tissue following a myocardial infarct reduces the risk of cardiovascular mortality and fatal reinfarction[233]. In addition, animal studies investigating the effect of exercise training have demonstrated that it could decrease the size of the infarct. It has been suggested that this was as a result of an upregulation of cardiac K_{ATP} receptors[234]. Indeed, these potassium channels have the ability to control the length of the action potential by allowing potassium ions to leave the cell, which causes a hyperpolarization and makes it harder for the cell to reach its threshold voltage. K_{ATP} activation therefore prevents calcium-overload, thereby promoting cell survival and diminishing ischemic injury, resulting in smaller infarct size[235, 236]. In addition, in coronary vessels, K_{ATP} inactivation diminishes coronary blood flow, suggesting that K_{ATP} contribute to the exercise-induced vasodilation[237]. This enhanced blood flow may be explained by a better management of intracellular calcium concentrations[238]. It has been suggested that this mechanism may also be implicated in the beneficial effects of exercise training on AP control. Indeed, calcium channel blockers are already being used as antihypertensive medication[239].

An increase in NO is among the beneficial impacts of exercise training as it decreases vascular tone. Moreover, it does not lead to the production of reactive nitrogen species (RNS)[240] as exercise also stimulates antioxidant defences in the

vasculature[228]. Similarly, SOD and GPx concentrations in skeletal muscle, and glutathione (GSH) in the liver are increased following exercise training[83]. Further supporting this is the observation that circulating lipid peroxides are also diminished with exercise training, due to an improved scavenging ability by the body's antioxidant defences[241]. Thus, this increased antioxidant defense decreases ROS, making NO more available to produce vasodilation. In addition, an increase in the number of mitochondria in skeletal muscles is also observed with exercise training, possibly rendering the body more resilient to oxidative damage, as each mitochondrion has a reduced oxidative load[242, 243]. Also, exercise reduces the oxidative capacity of iron by increasing iron's binding capacity to apotransferrin, thereby shrinking the iron labile pool[244]. Indeed, ferrous iron can have deleterious effects as it can catalyze the Fenton reaction, thereby generating ROS.

Although acute bouts of exercise are known to be pro-inflammatory and induce oxidative stress, regular aerobic exercise has been shown to have anti-inflammatory effects[245-247]. Indeed, long-term adherence to an exercise program leads to an increase in IL-6, which subsequently stimulates the release of IL-10 and IL-1RA (Interleukin-1 receptor antagonist), which are anti-inflammatory cytokines, and inhibits TNF-α production[248]. Platelet-related inflammatory mediators, like P-selectin and soluble CD40 ligand, have also been found to be reduced following regular exercise training[249]. As such, chronic

exercise is recommended to patients with heart failure for these anti-inflammatory effects, [249].

In addition, exercise can reverse endothelial damage that develops following an inflammatory response, as highlighted by the reduction in markers of endothelial dysfunction, like soluble vascular cell adhesion molecule-1 and monocyte chemoattractant protein-1 found among patients with CVD following exercise training[250]. In addition, chronic exercise diminishes the expression of circulating Toll-like receptors (TLR), which are involved in innate immune responses[251]. Downstream signaling pathways stimulated by the binding of lipopolysaccharide to the TLR were also decreased in skeletal muscle with exercise training, as were inflammatory cytokines, like TNF-α[251]. As such, this may by implicated in anti-inflammatory effects of exercise training.

Exercise also upregulates the production of heat shock proteins (HSP), which are cytoprotective chaperones that ensure the proper transport and folding of proteins, and the removal of malfunctioning proteins[252]. Higher levels of HSP70, which have anti-apoptotic effects, have been observed in heart tissues with exercise training[253, 254]. It is interesting to note that higher levels of cardiac heat shock proteins correlate with an improved LV pressure recuperation following an ischemic episode[253]. HSPs may also be implicated in the reduction in TLR4 observed with exercise training[251].

The RAS is heavily involved in AP control, and dysregulation of this system has been implicated in several diseases[25]. Exercise training may modulate the RAS components in healthy individuals, however to date, much of the focus has been on investigating the effects in animal models and patients with CVD. For example, in the paraventricular nucleus and rostral ventrolateral medulla of spontaneously hypertensive rats (SHR), a significant increase in MasR and ACE2, along with a significant reduction in ACE and AT1R protein has been observed with chronic exercise[255]. An increase in Ang-(1-7) and its receptor have also been reported in the heart of SHR, while an rise in MasR could be observed in the aorta (Ang-(1-7) was not investigated), with exercise training[256, 257]. Additionally, exercise is reported to benefit the heart via a reduction in cardiac ACE activity and AngII levels in an animal model of heart failure[258].

Circulating RAS components are also modulated by exercise training. Circulating renin, ACE, AngII and aldosterone were all decreased in rats who trained post-myocardial infarction, compared to their sedentary counterparts, which was associated with a reduced LV end-systolic diameter (LVESD) and improved fractional shortening (FS)[259].

4.2 Exercise training and pregnancy

The American College of Obstetrics and Gynecology(ACOG) recommends at least 30 minutes of moderate intensity exercise daily for healthy pregnant women[260] while the Canadian Society for Exercise Physiology (CSEP) and the Society of

Obstetricians and Gynaecologists of Canada (SOGC) recommend at least 30 minutes of aerobic exercise, 4 days a week[261]. Of course, sports that may cause trauma, such as alpine skiing or hockey, are not recommended whereas recommended activities include, but are not limited to, aerobics, jogging, swimming and Pilates[260]. Many beneficial cardiovascular adaptations ensue as a result of training during pregnancy, including an increased blood volume, SV and CO, and diminished HR and TPR, when compared to their sedentary counterparts[103, 262]. Additionally, the risk of gestational diseases, including diabetes, insulin resistance and possibly hypertension, is diminished with exercise training[105, 114, 263]. Some of these benefits may be related to exercise training's ability to limit gestational weight gain. It is important to note however that fetal growth and development are not known to be compromised[103].

The benefits of exercise training during pregnancy can also be observed at both the fetal and placental level. Indeed, active women are reported to have both a greater total placental (462±18 vs. 414±14 cm^3, p=0.05) and fetal (3.75±0.08 vs. 3.49±0.07 kg, p=0.05) weight, accompanied by a greater placental growth rate (26±2 vs. 21±1cm^3/week, p=0.04) [264, 265]. Improved placental development is proposed to result from the short-lived hypoxic environment that is thought to be produced by bouts of exercise which divert blood away from the placenta and towards the exercising muscles and for skin heat dissipation[266], while improved fetal development results from the placental effects mentioned here-in.

Additionally, the placentas of trained mothers have an improved functional volume (434±19 vs. 367±14 cm^3, p=0.006), along with a diminished non-functional tissue volume (28±4 vs. 45±6 cm^3, p=0.04)[265]. Moreover, one study observed trophoblastic, stromal and endothelial cell proliferation to be greater among active women during normal pregnancy, with a proliferative index of 45±14 mitoses/1000 nuclei vs. 29±10 mitoses/1000 nuclei, compared to their sedentary counterparts ($p<0.008$), allowing for an improved exchange between mother and fetus[267].

Exercise training does not appear to have a detrimental effect on fetal birth weight[211], however a literature review by the Cochrane Collaboration has concluded that insufficient data exists to ascertain fetal benefits or risks[268]. Several studies have however observed a link between an increase in low fetal weight and exercise[211]. The smaller fetuses nevertheless had an enhanced lean body:fat mass ratio. A likely explanation for the discrepancy in the fetal weight data may be an inadequate nutritional intake on the part of the exercising mother. Indeed, exercising pregnant women require an increase in food consumption to compensate for the increased energy expenditure associated with exercise training. With adequate nutritional intake, exercise training does not appear to hinder fetal development, but rather promote it[211].

Exercise training may also benefit endothelial function by altering angiogenic balance and circulating pro-inflammatory cytokines. Indeed, Weissgerber et al

have reported an increase in circulating PlGF [median (interquartile range): 278 (221,647) vs. 268 (159,290) pg/mL, p=0.014] and a reduction in circulating sFlt-1 [4217 (2014, 5481) vs. 5180 (4549, 5834) pg/mL, p=0.005] and sEng [7.8 (6.5, 10.1) vs. 9.1 (7.7, 16.7) ng/ml, p=0.025] in late gestation compared to levels in sedentary counterparts[269]. A reduction in the anti-angiogenic factors sFlt-1 and sEng may suggest that placental development is closer to being accomplished when comparing trained versus sedentary women at the same time point. Additionally, Clapp et al observed a decrease in circulating TNF-α with exercise training, suggesting that exercise may also have anti-inflammatory effects during pregnancy[270].

4.3 Exercise training and PE

As mentioned previously, exercise training reduces the risk of developing CVD in a non-pregnant population. It stands to reason that exercise may also be beneficial for women at risk of PE.

Many epidemiological studies are responsible for prompting the scientific community to investigate the impact of exercise training on gestational diseases. Exercise training has been reported to decrease the risk of developing gestational diabetes mellitus and gestational hypertension[271, 272] as well as been demonstrated to reduce PE risk[271, 273-281]. One of the first studies was carried out by Marcoux and colleagues in 1989 where they reported that women who

performed regular physical activity during the first 20 weeks of pregnancy had a 43% reduction in the risk of developing PE compared to their sedentary counterparts[271]. Moreover, risk reduction was directly related to the amount of time spent exercising. In 2003, Sorensen et al. corroborated these results by showing that exercise during early pregnancy reduced the risk of PE by 35%[273]. Additionally, they found that training prior to and during gestation further reduced the risk of PE compared to inactive women. Similar results were again found by this group in women who exercised strenuously and to maximal exertion[275]. Indeed, in that study, the risk of PE was reduced by 48% and 78% in women categorized into the groups “moderate” and ”strenuous/maximal”, respectively, in comparison to the reference group (i.e. negligible/minimal physical exertion)[275]. In 2004, Saftlas et al. published comparable results in which they observed a reduced PE prevalence, although non-significant, in pregnant women who engaged in any leisure-time activity during gestation compared to inactive women. However, unlike previous studies, this was independent of the level of caloric expenditure (i.e. amount of exercise)[274]. The small sample size (n=44), compared to those in previously mentioned studies (n=172[271] and n=201[273]), likely compromised the evaluation of the effect of exercise training on PE incidence in this study. More recently, Rudra et al. (2008) assessed prospectively the effect of exercise on PE risk by categorizing the subjects based on time spent exercising and energy expenditure (n=224 with PE)[281]. Although their results did not reach statistical significance, recreational activity in the year prior to

pregnancy, associated with or without gestational exercise training, correlated with a reduced risk of PE, compared to inactive women. Conversely, women who were active only during early pregnancy (n=83) tended to have an increased PE risk (n=9) compared to those who were sedentary before and during pregnancy although this was also not significant. The evaluation period for training consisted of only a single week prior to the prenatal visit, thereby biasing the exercise training evaluation. Furthermore, the sample size was again small (n=9) which somewhat undermines the conclusions that can be inferred from these results. Magnus et al. carried out a prospective study to examine the effect of recreational physical activity on the incidence of PE[276]. They too observed a beneficial effect/trend but only among non-obese women, and the extent of the protection was limited (20% reduction in risk). However, in this study, intensity and duration of the physical activity was not taken into consideration. As such, it is likely that obese women who exercised as often as the non-obese may have done so at lower intensities and for shorter bouts, which may explain the lack of effect observed in this group, particularly since others have demonstrated that exercise training benefits typically correlate with the intensity and duration of the activity[271, 273, 275]. Hegaard et al. also found a non-significant reduction in PE risk among women with the highest degree of physical activity in the year prior to pregnancy, especially among overweight women[278]. However, this study failed to acquire information about gestational physical activity, which may have contributed to the non-significance of the physical activity effect reported, as women may have

stopped exercising during pregnancy and thus may not have obtained as much benefits. Tyldum's study again found a non-significant lower risk of PE among women that trained 120 min/week previously to pregnancy but also failed to gather information about gestational exercise patterns[279]. Moreover, the exercise training information was obtained between 9 months and 20 years before pregnancy, and as such this data may not accurately reflect the activity profile of these subjects immediately prior to their pregnancies. Additionally, the reference or control group consisted of women who either never exercised or exercised less than once a week and as such, may not be completely sedentary and may mask a possible effect of training. Recently, Fortner et al. investigated prospectively the effect of pre-pregnancy and early pregnancy activity on PE risk[280]. The authors observed a non-significant lower risk of developing PE during early pregnancy with total activity. This study did not however observe an association between pre-pregnancy total activity and PE risk, unlike previous studies. Again, a small sample size (30 preeclamptic women) may have prevented the detection of a link between PE risk and physical activity.

Yeo et al. investigated the effect of stretching versus walking on PE risk[277]. They observed that stretching tended to reduce the risks of developing PE compared to pregnant walkers (2.6% vs. 14.6%, respectively) although these differences were not significant, likely due to sample size (n=41 and n=38 for walkers and stretchers respectively) [282]. However, it should be noted that

women in the walking group were also reported to work more hours per week. Hence, this may have caused an increased stress, which may have contributed to the increased prevalence of the disease as has been previously reported[88, 126, 127]. In addition, one should be aware that in this study the exercise program began at 18 weeks of gestation in previously sedentary women who were at risk for PE. Hence, the mechanisms involved in the development of the disease may have already been initiated and more vigorous exercise at this time point may have accentuated the development of the disease while lower intensity exercise, such as stretching, may have prevented the disease by favouring antioxidant defences. Indeed, the authors found that the stretching group had significantly more circulating transferrin, an antioxidant marker, at the end of gestation compared to their walking counterparts. Interestingly, in Sorensen's study, the small group of women who were previously inactive before pregnancy tended to have an increased risk for PE when exercise training was initiated at the beginning of pregnancy, although again, not reaching statistical significance[281]. Hence, this suggests that initiating an exercise program during mid-gestation, in previously sedentary women, may enhance the risk of PE.

However, the observation that exercise reduces the risk of PE is not shared by all. Vollebregt et al found no protective effect of physical activity on PE risk[283]. Although this study did not find an association, their evaluation of physical activity was based on a short time-frame (1 week) prior to their first prenatal care

visit. This cannot be used as an adequate evaluation of gestational physical activity, and consequently, caution should be exerted regarding the study's conclusions. Curiously, two studies found a deleterious effect of exercise on PE risk[281, 284]. As mentioned above, Rudra et al observed a non-significant increase in PE with exercise training in women initiating exercise during early pregnancy[281]. Because of the small number of cases however, no clear conclusion can be made. Although Osterdal found that exercise had no protective effect over PE prevalence, they did observe that women who did moderate exercise for more than 270 min/week during early pregnancy were at an increased risk of severe PE[284]. It is important to note that both Vollebregt and Osterdal's studies included women with severe PE or SPE, respectively. These studies suggest that initiating physical activity at the beginning of pregnancy may be deleterious to women at risk of severe PE, such as those who are previously hypertensive, much like what is observed in Yeo et al.'s study[277]. Additionally, both studies investigated the effect of initiating physical activity on PE risk, unlike most previous studies. As such, the time at which exercise is initiated appears to play a primordial role in determining how exercise may modulate PE risk.

Most of the studies investigating the effects of exercise prior to pregnancy observed either a significant[273, 275, 281, 283] or non-significant[278, 279] decrease in prevalence of PE. Limitations within the studies observing a non-significant effect may have been responsible for reducing the detectable impacts of

exercise training on PE risk. For instance, the studies by Saftlas et al. and Fortner et al., did not observe a protective effect of pre-pregnancy activity on PE risk, possibly as a result of the small sample size, which may have lacked the statistical power to demonstrate this association.

To date, exercise training has yet to be recommended in at-risk pregnancies even though retrospective studies have shown a positive link between exercise training and a decreased PE risk. These studies are considered insufficient as they were mainly observational in nature, they are not randomized studies and their samples sizes are too small[285]. Biases can result from such studies, and their results depend solely on the mother' self-evaluation of her exercise pattern before and/or during pregnancy. There is thus no quantitative assessment of the amount of training performed by those enrolled in the studies. As such, large-scale studies will be required to determine the effect of exercise on PE risk, with specific attention to the time frame of initiation of exercise training as well as to the intensity and type of exercise prescribed. Indeed, a link between aerobic fitness and hypertension prevention has been observed[286].

Recently, an animal study from our group was published demonstrating the benefits of exercise training in the prevention of PE-like features, lending support to the albeit inconclusive epidemiological findings mentioned above[287]. The use of an animal model allowed many parameters to be held constant, enabling us to

show the cause and effect relationship between exercise training and PE risk. The transgenic model used in this study consisted of female mice overexpressing human angiotensinogen (hANG), which upon mating with males overexpressing human renin (hREN) developed many PE-like features, including hypertension, proteinuria, cardiac hypertrophy, placental alterations and impaired vascular reactivity. To investigate the effect of exercise training, female hANG mice were placed in exercise cages with free access to a wheel 4 weeks prior to mating and were maintained within these cages for the length of their pregnancy. Interestingly, much like what is observed in women upon becoming pregnant, the pregnant female mice consistently diminished the amount of exercise performed, reaching a minimum 2-3 days prior to delivery. Exercise training prevented the development of proteinuria, pathological cardiac hypertrophy and placental alterations while their AP was no longer significantly different from their non-transgenic counterparts at the end of gestation. Moreover, there were no deleterious effects of exercise observed on the fetus, both in the normal and preeclamptic pregnancies, further supporting exercise as a preventive and possibly therapeutic option for PE.

4.3.1 Potential mechanisms involved in the beneficial impact of exercise of PE

Although exercise training appears to protect women from developing PE, very little is known regarding the mechanisms involved in these effects. Exercise training has been proposed to reduce PE risk by promoting placental growth and development, reducing oxidative damage, improving endothelial function, as well

as immune and inflammatory responses and modulating the RAS components, all of which could contribute to improving pregnancy outcome.

Placental development. Normal placental growth and development requires fine modulation of oxygen tension. Thus the benefits of exercise training may lie primarily in its ability to ensure adequate placentation. Indeed, by temporarily creating a hypoxic environment, exercise promotes placental growth and development, and in doing so, minimizes the placental release of anti-angiogenic factors into the circulation, such as sFlt-1, ROS and pro-inflammatory mediators, such as TNF-α[10, 266, 269, 288, 289]. Further supporting the notion that exercise promotes adequate placental development, our group has demonstrated, using an animal model, that exercise training was associated with normalization of placental VEGF levels and prevention of PE-like features (i.e. placental alterations)[287]. In addition to the role of anti-angiogenic and pro-inflammatory mediators in PE, circulating factors, like AngII and endothelin-1 are heavily implicated in the development of the diffused endothelial cell damage observed during PE[177, 178].

Oxidative stress. An imbalance between ROS production and antioxidant capacities has been observed in PE and has been proposed to be implicated in disease development[199]. As previously mentioned, training promotes antioxidant defences and reduces markers of oxidative stress, such as lipid

peroxide levels[83, 241, 290]. Chronic exercise is likely an important mechanism by protecting mothers from oxidative damage thereby reducing their risk of developing PE[199, 246].

Endothelial function. PE is often characterized by systemic endothelial dysfunction. Exercise training has the potential to prevent and reverse the damage mediated by ROS and anti-inflammatory cytokines, as seen in non-pregnant individuals, and anti-angiogenic mediators, as is observed during normal pregnancy[248, 251, 269, 288]. Importantly, a decrease in these cytokines will consequently reduce the levels of endothelin-1, and thus diminish vasoconstriction[291]. Additionally, increased shear stress may also contribute to the benefits related to exercise training, as it induces endothelial cell proliferation and promotes eNOS and antioxidants expression[227]. Exercise training may reduce circulating levels of AngII, similar to what is observed in an animal model of myocardial infarction, and thus improve endothelial function[292]. Along similar lines, exercise training reduces plasma endothelin-1 in non-pregnant women[231, 293]. Analogous effects on AngII and endothelin-1 are proposed to arise during PE[231, 259]. Indeed, lowering circulating levels of these mediators will diminish their capacity to promote endothelial dysfunction.

Inflammation and immunity. Abnormal immune responses and inflammation have been implicated in the initiation of PE. Although very little is known concerning the effectiveness of exercise training during pregnancy and PE, it is proposed to stimulate anti-inflammatory responses and reverse inflammation-related endothelial cell injury, as alluded to previously.

Renin-angiotensin system. RAS components are dysregulated in many CVD, including PE[22, 25]. The effect of exercise training on RAS components in PE has had limited exposure in the scientific community. Nevertheless, since PE is considered a CVD, exercise training is postulated to have similar effects in preeclamptic patients, as previously mentioned. MasR and ACE2 are proposed to increase while ACE and AT1R are postulated to decrease, with exercise training, much like what has been observed in hypertensive animal models[255]. The increase in ACE2 should yield higher levels of Ang-(1-7), as a result of AngII degradation[256, 257]. These effects are suspected to occur both in circulation and at the tissue level[256, 257, 259].

Many mechanisms are likely implicated in the protective effect of exercise training on PE, since it targets many pathways and systems. Some of these have yet to be investigated specifically during pregnancy and in women at risk of PE. Mechanistic studies, like those performed with animal models, are thus warranted

to better understand the effect of exercise training on PE risk, and should further shed light into the pathogenesis of PE.

Chapter 5: Objectives and Hypotheses

Hypertension is an important risk factor for preeclampsia. Identifying possible preventive therapies or treatments are greatly needed, as to date, there is no cure for this disease, aside from fetal delivery. The purpose of this study is thus to investigate the effect of exercise training as a prophylactic measure to alleviate preeclampsia-like features in an animal model of preeclampsia superimposed on chronic hypertension. In addition, possible mechanisms involved in disease development will be investigated.

Given that exercise training is currently an effective therapy for treating and preventing several cardiovascular diseases, including hypertension and heart failure, we hypothesize that it will prevent the development of superimposed preeclampsia in our animal model. Furthermore, based on previously reported effects of exercise training on normal pregnancy, we suspect that it will have beneficial effects on placental development, and consequently, on maternal symptoms.

Chapter 6: Novel role of the renin-angiotensin system in the beneficial effects of exercise on a preeclampsia model.

Novel role of the renin-angiotensin system in the beneficial effects of exercise on a preeclampsia model.

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Abbreviation list

ACE2: Angiotensin converting enzyme 2

ACOG: American College of Obstetricians and Gynecologists

ACR: Albumin/creatinine ratio

AngII: Angiotensin II

AP: Arterial pressure

AT1R: Angiotensin II type 1 receptor

BNP: Brain natriuretic protein

EF: Ejection fraction

ExT: Exercise training

FS: Fractional shortening

HR: Heart rate

IUGR: Intra-uterine growth restriction

IVS: Interventricular septum

LV: Left ventricle

LVD: Left ventricular diameter

LVEDD: Left ventricular diastolic volume

LVEDV: Left ventricular end-diastolic volume

LVESD: Left ventricular systolic volume.

LVESV: Left ventricular end-systolic volumes.

LVID: Left ventricular internal diameter

LVPW: Left ventricular posterior wall thickness

MAP: Mean arterial pressure

MasR: Angiotensin-(1-7) Mas oncogene receptor

Nab1: NGFI-A binding protein 1

NT: Non-transgenic

PE: Preeclampsia

S16: 40S ribosomal protein S16

sFlt-1: Soluble Fms-like tyrosine kinase-1

SPE: Preeclampsia superimposed on chronic hypertension

VEGF: Vascular endothelial growth factor

Abstract

Hypertension increases preeclampsia prevalence to 15-25%. Our aim is to investigate the protective role of exercise traininig (ExT) on pregnancy outcome in a mouse model of superimposed preeclampsia on chronic hypertension (SPE) as well as the implicated mechanisms in this effect.

Transgenic female mice overexpressing human angiotensinogen and human renin were used as a model of SPE. Trained mothers were placed in cages with access to a running wheel 4 weeks prior to mating, and remained within these cages throughout gestation. Blood pressure was measured by telemetry. Proteinuria and circulating soluble Fms-like tyrosine kinase-1 (sFlt-1) were determined by ELISA.

The blood pressure and proteinuria increase present in pregnant transgenic mice was markedly reduced with ExT whereas placental pathology was absent. Placental VEGF protein, sFlt-1 mRNA, and circulating sFlt-1 were significantly increased in transgenic sedentary mice, while training normalized these markers. In addition, training normalized the significant increase in angiotensin II type 1 receptor and reduction in Angiotensin-(1-7) Mas receptor (MasR) observed in sedentary R^+A^+ placenta. Also, reduced Aortic MasR and angiotensin converting-enzyme 2 in sedentary R^+A^+ mice were increased with training.

This study demonstrates the protective effects of ExT on SPE-like features in an animal model and involves the novel modulation of the renin-angiotensin system.

Keywords: exercise training, superimposed preeclampsia on chronic hypertension, placental markers, renin-angiotensin system, angiogenic balance

Introduction

Preeclampsia (PE) affects 5-7% of all pregnancies in the developed world and far more in developing countries. As such, it is among the leading causes of perinatal mortality and morbidity[1]. PE is diagnosed by the onset of hypertension and proteinuria after 20 weeks of gestation and can deteriorate to maternal end-organ damage and fetal death[2]. Chronic hypertension is an important risk factor for this pathology as 15-25% of hypertensive women develop superimposed PE on chronic hypertension (SPE) during their pregnancy. PE is a multifactorial disease arising from a complex combination of genetic and environmental factors, whose underlying molecular mechanisms are still poorly understood[1, 3, 4]. Delivery remains the only treatment option available to preeclamptic women[5]. Consequently, a high incidence of preterm delivery is associated with this disease[2].

We have recently characterized a new animal model of SPE[6]. These mice overexpress both human angiotensinogen and renin (R^+A^+), and thus, are hypertensive at baseline. During their pregnancy, these mice develop SPE-like features as their blood pressure increases and they develop *de novo* proteinuria. This new model is especially useful as SPE is a clinical reality that affects many women and to our knowledge, it is the only model of its kind. Furthermore, it is particularly relevant as many studies are now pointing to the involvement of the renin-angiotensin system in the development of PE. Indeed, many circulating RAS components are altered and sensitivity to angiotensin II (AngII) is increased during PE compared to normal pregnancy. Interestingly, auto-antibodies against the AngII type 1 receptor (AT1R) have been identified in the circulation of women destined to develop preeclampsia, which, along with AngII, can for instance, stimulate the secretion of soluble Fms-like tyrosine kinase-1 (sFlt-1).

This model therefore will permit a better understanding of the mechanisms involved in the development of SPE. Indeed, mice are an asset in mechanistic studies since experimental parameters can be held constant while trying to elucidate the cause and effect relationship between exercise training (ExT) and SPE-like features. Furthermore, mice share many physiological characteristics with that of a human pregnancy[7]. Importantly, both human and mouse pregnancies are characterized by a hemochorial placentation, a central component for the development of PE[8], and a decrease in blood pressure as a result of pregnancy[9, 10].

The beneficial impact of ExT on the prevention and treatment of cardiovascular diseases has been well documented in men and non-pregnant women[11]. Physical activity has also been suggested to be beneficial during normal pregnancy by improving maternal cardiovascular and metabolic adaptations as well as placental and fetal development[12]. Furthermore, it has been shown to reduce the incidence of gestational diseases such as PE and gestational diabetes[13]. However, for PE, drawing conclusions on the effect of exercise on pregnancy is made difficult by the low number of studies, which are largely observational, and their small sample sizes[14]. Although guidelines from the American College of Obstetricians and Gynecologists (ACOG) recommend that pregnant women participate in moderate intensity exercise on most, if not all days of the week during their pregnancy[15], this does not apply to women at risk of complications given the lack of data, even though they may benefit from regular physical activity. Conversely, we have recently demonstrated in a mouse model of PE that ExT both before and during pregnancy can markedly reduce PE-like features[16]. Moreover, our data suggests that normalization of placental development and angiogenic balance may be implicated in the effects of ExT. Given this and the numerous human studies which support

the beneficial effects of physical activity, more research is warranted to eventually modify the present ACOG guidelines. Therefore, the aim of this study is to determine if SPE-like features can be alleviated or normalized by ExT in R^+A^+ mice.

Results

Similar exercise patterns were observed in both R^+A^+ and R^-A^- trained mice prior to and during pregnancy (Figure S1; see online supplement). The distance travelled daily diminished drastically at day 1 of gestation and continued to steadily decrease as pregnancy progressed in both groups, reaching a minimum around night 18 of gestation. Furthermore, there was no effect of ExT or genotype on length of gestation, baseline bodyweight and gestational weight gain (Table S2; please see online supplement).

As shown previously, R^+A^+ mice were hypertensive at baseline (136.62 vs. 98.91 mmHg in R^+A^+ and non-transgenic respectively, $p< 0.001$), and consequently blood pressure was significantly higher than their non-transgenic littermates prior to and throughout gestation (data not shown). Sedentary R^+A^+ mice experienced a further increase in blood pressure at the end of pregnancy, which was prevented by ExT (Figure 1A). Proteinuria, assessed by the albumin/creatinine ratio (ACR), was significantly increased in R^+A^+ compared to their non-transgenic littermates and was significantly increased with gestation in (Figure 1B). Conversely, trained R^+A^+ had significantly lower levels of ACR although these values were not completely normalised (Figure 1B).

As reported previously, cardiac hypertrophy, characterized by elevated ventricular mass, was observed in R^+A^+ at the end of gestation, but was unaffected by training (Table 1). Interestingly, the right atrium had a significantly increased mass in trained mice independently of genotype whereas the right ventricle was also significantly enlarged by ExT but only in non-transgenic mice. Nab1, a marker of left ventricular dysfunction, was found to be increased in the transgenic mice, as reported previously, while ExT caused a significant reduction, these values were not completely normalized (Figure 2A). Although the marker of pathological cardiac hypertrophy, BNP, was not significantly increased in R^+A^+ mice, a significant reduction in BNP was observed with ExT, independent of genotype (Figure 2B). Echocardiographic analysis demonstrated similar trends, where ejection fraction and fractional shortening were significantly decreased in R^+A^+ and tended to be increased with ExT but this was not significant (Tables S3 and S4; please see online supplement).

As previously published, sedentary R^+A^+ mice presented with an evident placental pathology in comparison to their non-transgenic littermates (Table 2). This was characterized by a significant increase in hyalinization, giant cell island loss and loss of labyrinthine structure. These alterations were supported by our immunohistochemistry results demonstrating a significant increase in placental cytokeratin and histone H3 staining in placentas from our sedentary transgenic mothers (Figures S2 and S3, please see online supplement). Importantly, all these placental parameters were completely normalized with ExT (Table 2 and Figures S2 and S3). This had a functional impact as it translated into a normalization of total fetal and placental weight as well as litter size with ExT in R^+A^+ (Table 3). Indeed, sedentary transgenic mice had decreased placental weight and an increased prevalence of IUGR, in addition to

smaller litters compared to non-transgenic controls. Lower placental weight is likely responsible for the IUGR observed. Interestingly, we also observed an increase in fetal:placental weight ratio(Table 3) with ExT independently of genotype suggesting an improved placental function, even in non-transgenic animals.

Placental VEGF protein was significantly increased in the sedentary transgenic mice compared to their sedentary controls, whereas this was attenuated with ExT (Figure 3A). Placental sFlt-1 mRNA was also significantly increased in sedentary R^+A^+ mice compared to non-transgenic littermates but was completely normalized by ExT (Figure 3B). A similar pattern was observed in the circulation, where circulating sFlt-1 levels were found to be increased in the sedentary transgenic group compared to their non-transgenic controls, but these differences were absent in the trained mice (Figure 3C). As such, the decreased placental sFlt-1 transcription in trained R^+A^+ mice is likely responsible for the reduced circulating sFlt-1 levels observed in this study.

Placental AT1R protein was significantly increased while that of the placental MasR and ACE2 were diminished in the sedentary R^+A^+ mice compared to non-transgenic littermates (Figure 4). Interestingly, ExT normalized both AT1R and Mas receptor expression whereas ACE2 was unaffected by training. In addition, ExT also reduced AT1R in non-transgenic controls. RAS components were also studied in the mouse aorta (Figure 5), to investigate potential mechanisms implicated in blood pressure regulation. Similarly to what we observed in the placenta, aortic AT1R was significantly increased while the MasR and ACE2 were

diminished in the aortas of R^+A^+ mice. In contrast, ExT caused an increase only in the MasR while it had no effect of AT1 and ACE2.

Circulating glucose was also examined in our animal model, as diabetes is a known risk factor for preeclampsia. Although no effect of genotype could be observed on circulating glucose levels, exercise did cause a significant reduction (Figure S4; please see online supplement) as has been previously observed by others[17].

Discussion

In our study, R^+A^+ mice developed many SPE-like features, including an increase in blood pressure, proteinuria, placental alterations and IUGR as previously reported[18]. However, ExT before and during gestation in these mice normalized all of these features with the exception of proteinuria which was reduced but not to the levels of control mice. This strongly supports the beneficial effect of this ExT regimen on SPE features and is in line with the numerous retrospective studies which have demonstrated a protective effect on PE in women[14]. Moreover, our data implicates a novel modulation of the RAS and angiogenic balance by both the disease and ExT. For instance, we observed an increase in both ACE2 and MasR expression in aortic extracts with ExT which may contribute to promoting healthy endothelial function. Indeed, ACE2 converts AngII into Ang-(1-7), a peptide whose effects opposes those of AngII while the MasR is the receptor that transduces the Ang-(1-7) signal[19]. As such, the observed increase in both ACE2 and MasR in the vasculature with ExT could contribute to minimize the AngII-associated vasoconstriction which is often exaggerated in PE. This is line with reports that have demonstrated a stimulation of the Ang-

(1-7) axis with ExT looking at cardiac ACE2 which has been suggested to contribute to the physiological cardiac hypertrophy associated with this type of ExT as well as to prevent ventricular dysfunction in a heart failure mouse model[20]. Increased MasR has also been reported in hearts[21] and aortas[22] of spontaneously hypertensive rats (SHR) rats with ExT.

In our study, ExT may improve endothelial function during pregnancy not only by altering RAS components, but by diminishing circulating levels of glucose and by promoting angiogenic balance. For instance, the ExT normalization of placental sFlt-1 mRNA expression and circulating levels of this anti-angiogenic factor may promote adequate endothelial response. Indeed, circulating sFlt-1 has been shown to antagonize circulating levels of VEGF and placental growth factor, and in doing so promotes endothelial dysfunction. Moreover, as high levels of sFlt-1 are known to inhibit trophoblastic invasion, training-induced decreases in sFlt-1 may also favour placental development.

Consequently, the increased sFlt-1 levels observed in our sedentary R^+A^+ mice may contribute to the development of several SPE-like features observed in our model. Indeed, much like women who suffer from PE[23], our sedentary R^+A^+ dams presented with a significant increase in circulating sFlt-1 and placental sFlt-1 mRNA expression. This is in line with the hypothesis that PE is associated with an anti-angiogenic shift that is initiated in the placenta.

Interestingly, our sedentary transgenic mice also had a significant increase in placental VEGF protein expression, which was reduced with training. Normal pregnancy is characterized by an increase in angiogenic factors, such as VEGF, at the start of pregnancy which usually decrease as pregnancy progresses and placental development nears completion. The presence of high VEGF levels at the end of gestation in our model may result from the inadequate placental development that is present. As such, in response to hypoxia, increased VEGF levels may be

aimed at stimulating adequate placental vascularization. Alternatively, this increase may occur to counter-balance the increase in sFlt-1 associated with our SPE model.

Placental alterations observed in sedentary R^+A^+ mice were completely normalized by ExT. This was further characterized by normalization, in trained transgenic females, of placental immunostaining to histone H3 and cytokeratin, which are markers of mitosis and trophoblastic cells in all phases respectively, which were found to be increased in sedentary transgenic mice. These increased placental markers in conjunction with the increase in labyrinthine structure loss observed by histology, support the premise that trophoblasts in R^+A^+ placentas are highly proliferative and fail to differentiate. This is in line with studies which suggest that PE is produced not only by an inadequate trophoblastic invasion, but also by a highly proliferative and underdeveloped placenta[24]. As such, our data further supports this hypothesis and suggests that our SPE model truly represents a clinical reality. On the other hand, the cytokeratin and histone H3 results support the hypothesis that ExT promotes healthy placental development by increasing placental villous trophoblastic volume and enhancing the exchange of nutrients and oxygen towards the growing fetus. As such, we observed an increase in the ratio of fetal to placental weight with ExT suggesting an improved placental function. In addition, fetal weight and litter size were completely normalized in trained R^+A^+ females suggesting these placental modifications had a functional impact on fetal development. Hence, it is clear that ExT both before and during gestation can be very beneficial to fetal outcome in mice.

In addition to the effects of ExT on the aortic RAS components, local placental RAS was also modified. Indeed, our sedentary R^+A^+ mice had a significant increase in AT1R, which was completely normalized with ExT. AngII, via the AT1R, is known to induce the production and secretion of sFlt-1, along with mediating oxidative stress, inflammation and vasoconstriction[25-27]. As such, the exercise-induced reduction in AT1R may be responsible for the decrease in both placental sFlt-1 mRNA and circulating sFlt-1 but may also contribute to improving many of the SPE-associated features by this mechanism. Similar AT1R reductions with ExT have been observed in other tissues, such as the paraventricular nucleus and rostral ventrolateral medulla, in SHR rats[28]. Moroever, the significant reduction in placental MasR observed in the R^+A^+ sedentary mothers was completely normalized by ExT. This may therefore enhance the Ang-(1-7) mediated vasodilation pathway and minimize under perfusion of the placenta. Conversely, placental ACE2 was found to be decreased in our mouse model with no significant effect of ExT. In line with our results, decreased placental ACE2 has been suggested to be implicated in the development of IUGR, for instance with maternal protein-restriction[29] and in a rat model of PE[30]. Indeed, it is proposed that the resulting reduction in placental Ang-(1-7) decreases local vasodilation and thus impedes placental-fetal blood flow. The effect of ExT on the MasR may thus not only be beneficial for PE but also other conditions which are at risk of IUGR.

As previously reported, sedentary R^+A^+ mice develop pathological cardiac hypertrophy compared to the non-transgenic mice. Although ExT did not have any effect on heart weight, modifications in cardiac pathological markers could be observed suggesting that there may be modifications in the type of hypertrophy present in trained transgenic mice. Indeed, Nab1 and

BNP were both significantly decreased with ExT in transgenic dams although Nab1 was not completely normalized. Fractional shortening and ejection fraction were significantly compromised in the sedentary R^+A^+ mice while ExT tended to increase these parameters but did not reach significance. Given that the blood pressure in R^+A^+ mice is not reduced below baseline with ExT, it is not very surprising that ventricular function is not markedly ameliorated although cardiac markers suggest that there may be a slight improvement.

Cages with free access to an exercise wheel were used to investigate the effect of training in SPE prevention in our study. Of note, the degree of training observed in our mice corresponds to what is reported in the literature[31-33] and did not vary according to genotype. Three weeks of free wheel training is associated with many cardiovascular benefits in non-pregnant rodents, including an increase in VO_2max[34-36]. As similar improvements are observed in non-pregnant women following a moderate aerobic ExT regimen[37, 38], we suspect that the beneficial effects of this type of ExT on pregnancy outcome in our mouse model may also translate to pregnant women. This would be in line with the well-known use of ExT as an effective therapy for preventing and treating many cardiovascular diseases in non-pregnant patients[39].

This study has advanced our understanding of the role of ExT in the prevention of many hallmark features of PE. ExT attenuated many of the SPE-associated features in our animal model, including the rise in blood pressure and proteinuria, placental alterations and IUGR. Preeclampsia is suspected to arise due to abnormalities in placentation, either as a result of an inadequate trophoblast invasion or hyperproliferation, which gives rise to the release of anti-

angiogenic factors into the circulation. We propose that ExT restores angiogenic balance by rehabilitating the placental *milieu*, and in doing so, promotes placental development. Therefore, the healthy placenta no longer releases anti-angiogenic mediators which would favour endothelial dysfunction. Additionally, modulation of the RAS may be implicated in the ExT SPE risk reduction by minimizing the effects of Angiotensin II via modulation of AT1R but also by stimulating the Ang-(1-7) pathway. This preventive and therapeutic approach, if proven effective, could minimize the prevalence of future cardiovascular diseases, deficits associated with preterm births, and perinatal mortality and morbidity. Indeed, in contrast to pharmacological treatments inhibiting the RAS which are teratogenic, ExT may efficiently modulate this system to obtain the required benefits such as decreased blood pressure and improved placental development without the adverse fetal effects. However, large-scale randomized studies are needed to confirm these effects in women and eventually lead to modifications of the ACOG guidelines, which presently do not support the prescription of ExT to women at risk of gestational complications.

Materials and Methods

Animals. R^+A^+ transgenic mice were produced by breeding heterozygous human renin mice (Ren9 line)[40] with heterozygous human angiotensinogen mice (204/1 line)[41]. Both single transgenic lines were a generous gift from Dr. Curt D. Sigmund of the University of Iowa, and were maintained in our animal facility by backcrossing with C57BL/6 mice (STRAIN CODE 027; Charles River, St-Constant, QC, Canada). The genotypes were determined as described previously[40, 41]. The animals were kept on a 12h light/dark cycle with water and standard laboratory chow *ad libitum* (2018; Teklab Premier Laboratory Diets, Madison, WI). Female mice in these experiments were 8-10 weeks of age, and were

separated into 4 groups (Sedentary R^+A^+ and non-transgenic (R^-A^-); Trained R^+A^+ and R^-A^-). Their care met the standards set forth by the Canadian Council on Animal Care for the use of experimental animals. All procedures were approved by the Animal Care Committee of the CHUM Research Centre.

ExT. Mice were placed in cages with free access to a running wheel, for one month before mating and remained in these cages throughout gestation as done previously in a different mouse model of PE where we have shown significant improvement in maternal outcomes this type of Ext regimen[16]. The use of voluntary exercise was chosen to prevent the stress that is usually associated with treadmill running[42]. ExT was measured, as each cage was connected to a computer and, the number of revolutions could be recorded and used to confirm training status (Compte-tour5, Aquila, Boucherville, Qc, Canada) as done previously[16].

Arterial pressure measurement. Arterial pressure (AP) and heart rate (HR) were quantified in the carotid artery by telemetry using TA11PA-C10 probes (Data Sciences International, St. Paul, MN) as done previously[43, 44]. After 7-10 days of recovery post-surgery, AP and HR were recorded for 3 consecutive days to represent baseline values. The mice were then put in cages with access to an exercise wheel for 1 month. Sedentary animals were placed in standard cages. After this period, male mice were introduced into the cages for timed-mating of trained animals, while the reverse was performed for sedentary females. Gestation was confirmed by the presence of a vaginal plug, which was recorded as day 1 of pregnancy. AP and HR measurements were recorded every 2 days, beginning on day 1 up to day 19, and then measurements were collected continuously until 48 h post-partum. Since mice normally give

birth between day 19 and 21 of gestation, the day prior to delivery was considered as "end of gestation" to permit an adequate comparison.

Proteinuria: Urine samples were collected on day 0 (prior to mating) and on day 18 (prior to sacrifice) and albumin and creatinine were assayed as done previously[16, 18].

Echocardiography. Transthoracic echocardiographic studies were performed prior to mating and at the end of pregnancy by high-resolution ultrasound biomicroscopy (Vevo660; Visualsonics, Toronto, ON, Canada) equipped with a 25-55 MHz probe, as previously described[18].

Tissue collection and histology. On day 18 of gestation, mice were anesthetized by isoflurane 2-3% in oxygen, and then maintained on 2% isoflurane. Blood was collected by intrathoracic cardiac puncture and placed in a chilled 1.5 ml tube containing EDTA (EMD, Gibbstown, NJ, USA). Plasma was separated by centrifugation and samples were snap frozen in liquid nitrogen, and stored at -80^{o}C until assayed. The pups were weighed, and their tails were cut and kept for genotyping. Heart and placentas were all collected, weighed, and either snap-frozen in liquid nitrogen or fixed for future experiments as done previously[16, 18].

Immunohistochemistry. Immunochemical analysis was assessed from 3 different placentas issued from 3 different mothers/group as done previously[16]. Paraffin-embedded placentas were sectioned at 4 μm and deparaffinised to assess cytokeratin (1:800; ab9377, Abcam,

Cambridge, MA) and Histone H3 (1:1600; ab5176-100, Abcam) to evaluate trophoblasts at all stages and mitosis, respectively[45, 46].

Western Blot. Frozen placentas and aortas were lyophilized and subsequently homogenised in lysis buffer. Total protein content was measured in supernatants by standard Bradford assay. 50μg of protein were loaded on 10% or 12% sodium dodecyl sulfate (SDS)-polyacrylamide gel, depending on the molecular weight of the protein studied, and separated by electrophoresis. Proteins were transferred on a nitrocellulose membrane (Amersham, Baie d'Urfe, QC). The following antibodies and concentrations were used: vascular endothelial growth factor (VEGF) (1:1000, ab46154, Abcam, Cambridge, MA); angiotensin converting enzyme 2 (ACE2) (1:500, SC-20998, Santa Cruz Biotechnology, Santa Cruz, CA); Angiotensin-(1-7)-Mas receptor (MasR) (1:2000, AAR-013, Alamone Labs, Jerusalem, Isreal); AT1R (1:2000, SC-578, Santa Cruz Biotechnology, Santa Cruz, CA); glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (1:2000, SC-20357, Santa Cruz Biotechnology, Santa Cruz, CA). Each protein signal was normalized to their respective GAPDH band.

Real-time PCR. Total RNA was extracted from frozen samples using Trizol (Invitrogen, Burlington, ON, Canada) according to the manufacturer's protocol. Removal of genomic DNA, reverse transcriptase reaction and real-time PCR were conducted as previously reported(18). In brief, real-time PCR was done using SYBR green chemistry and the following genes were investigated: 40S ribosomal protein S16 (S16), sFlt-1, NGFI-A-binding protein 1 (Nab1) and brain natriuretic peptide (BNP). Primer sequences are described in Table S1

(please see online supplement). Each sample was run and analyzed in duplicate, and is expressed as relative to s16 mRNA.

Plasma sFlt-1 levels: Circulating sFlt-1concentrations were measured using a commercial ELISA kit (R&D-Quantikine, Minneapolis, MN). Plasma samples were diluted 1:20 using the manufacturer's dilutor prior to the experiment and each sample was measured in duplicate.

Plasma glucose levels: Circulating glucose levels were determined using a commercially available enzymatic kit (Autokit Glucose, WAKO Diagnositics, Richmond, VA), following the manufacturer's instructions. Each sample was measured in duplicate.

Statistical analysis. All values are expressed as means ± SE. A p-value of ≤ 0.05 was considered significant. Differences in tissue weights and ratios, number of pups, circulating glucose and sFlt-1 levels, as well as data obtained by real-time PCR, Western blot and immunohistochemistry were computed by 2-way ANOVA. 2-way repeated measures ANOVA was used to analyze echocardiography parameters, blood pressure and albumin/creatinine ratio. These analyses were all followed by Tukey's post-hoc test if an interaction was detected. Placental alterations were analyzed using a non-parametric Mann-Whitney Rank Sum Test.

A more detailed methods section can be found in the online supplement. Also present in this supplemental section are the results regarding the effects of exercise training and genotype on distance travelled prior to and throughout gestation, placental cytokeratin and histone H3

immunostaining, circulating glucose levels, maternal characteristics and cardiac parameters calculated and measured following echocardiography.

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Conflict of Interest

None.

References

1. Sibai,B., G.Dekker, and M.Kupferminc. 2005. Pre-eclampsia. *Lancet* 365:785-799.

2. Moutquin,J.M., P.R.Garner, R.F.Burrows, E.Rey, M.E.Helewa, I.R.Lange, and S.W.Rabkin. 1997. Report of the Canadian Hypertension Society Consensus Conference: 2. Nonpharmacologic management and prevention of hypertensive disorders in pregnancy. *CMAJ.* 157:907-919.

3. Cross,J.C. 2003. The genetics of pre-eclampsia: a feto-placental or maternal problem? *Clin.Genet.* 64:96-103.

4. Lachmeijer,A.M., G.A.Dekker, G.Pals, J.G.Aarnoudse, L.P.ten Kate, and R.Arngrimsson. 2002. Searching for preeclampsia genes: the current position. *Eur.J.Obstet.Gynecol.Reprod.Biol.* 105:94-113.

5. Roberts,J.M. and K.Y.Lain. 2002. Recent Insights into the pathogenesis of pre-eclampsia. *Placenta* 23:359-372.

6. Falcao,S., E.Stoyanova, G.Cloutier, R.L.Maurice, J.Gutkowska, and J.L.Lavoie. 2009. Mice overexpressing both human angiotensinogen and human renin as a model of superimposed preeclampsia on chronic hypertension. *Hypertension* 54:1401-1407.

7. Rossant,J. and J.C.Cross. 2001. Placental development: lessons from mouse mutants. *Nat.Rev.Genet.* 2:538-548.

8. Malassine,A., J.L.Frendo, and D.Evain-Brion. 2003. A comparison of placental development and endocrine functions between the human and mouse model. *Hum.Reprod.Update.* 9:531-539.

9. Davisson,R.L., D.S.Hoffmann, G.M.Butz, G.Aldape, G.Schlager, D.C.Merrill, S.Sethi, R.M.Weiss, and J.N.Bates. 2002. Discovery of a spontaneous genetic mouse model of preeclampsia. *Hypertension* 39:337-342.

10. Wong,A.Y., S.Kulandavelu, K.J.Whiteley, D.Qu, B.L.Langille, and S.L.Adamson. 2002. Maternal cardiovascular changes during pregnancy and postpartum in mice. *Am.J.Physiol Heart Circ.Physiol* 282:H918-H925.

11. Peterson,J.A. 2007. Get moving! Physical activity counseling in primary care. *J Am Acad Nurse Pract.* 19:349-357.

12. Melzer,K., Y.Schutz, M.Boulvain, and B.Kayser. 2010. Physical activity and pregnancy: cardiovascular adaptations, recommendations and pregnancy outcomes. *Sports Med.* 40:493-507.

13. Weissgerber,T.L., L.A.Wolfe, G.A.Davies, and M.F.Mottola. 2006. Exercise in the prevention and treatment of maternal-fetal disease: a review of the literature. *Appl.Physiol Nutr.Metab* 31:661-674.

14. Genest,D.S., S.Falcao, J.Gutkowska, and J.L.Lavoie. 2012. Impact of exercise training on preeclampsia: potential preventive mechanisms. *Hypertension*.

15. Olson,D., R.S.Sikka, J.Hayman, M.Novak, and C.Stavig. 2009. Exercise in pregnancy. *Curr.Sports Med.Rep.* 8:147-153.

16. Falcao,S., S.Bisotto, C.Michel, A.A.Lacasse, C.Vaillancourt, J.Gutkowska, and J.L.Lavoie. 2010. Exercise training can attenuate preeclampsia-like features in an animal model. *J.Hypertens.* 28:2446-2453.

17. Goodyear,L.J. and B.B.Kahn. 1998. Exercise, glucose transport, and insulin sensitivity. *Annu.Rev.Med.* 49:235-261.

18. Falcao,S., E.Stoyanova, G.Cloutier, R.L.Maurice, J.Gutkowska, and J.L.Lavoie. 2009. Mice overexpressing both human angiotensinogen and human renin as a model of superimposed preeclampsia on chronic hypertension. *Hypertension* 54:1401-1407.

19. Fyhrquist,F. and O.Saijonmaa. 2008. Renin-angiotensin system revisited. *J.Intern.Med.* 264:224-236.

20. Fernandes,T., N.Y.Hashimoto, F.C.Magalhaes, F.B.Fernandes, D.E.Casarini, A.K.Carmona, J.E.Krieger, M.I.Phillips, and E.M.Oliveira. 2011. Aerobic exercise training-induced left ventricular hypertrophy involves regulatory MicroRNAs, decreased angiotensin-converting enzyme-angiotensin ii, and synergistic regulation of angiotensin-converting enzyme 2-angiotensin (1-7). *Hypertension* 58:182-189.

21. Filho,A.G., A.J.Ferreira, S.H.Santos, S.R.Neves, E.R.Silva Camargos, L.K.Becker, H.A.Belchior, M.F.Dias-Peixoto, S.V.Pinheiro, and R.A.Santos. 2008. Selective increase of angiotensin(1-7) and its receptor in hearts of spontaneously hypertensive rats subjected to physical training. *Exp.Physiol* 93:589-598.

22. Silva,D.M., A.Gomes-Filho, V.C.Olivon, T.M.Santos, L.K.Becker, R.A.Santos, and V.S.Lemos. 2011. Swimming training improves the vasodilator effect of angiotensin-(1-7) in the aorta of spontaneously hypertensive rat. *J.Appl.Physiol* 111:1272-1277.

23. Maynard,S., F.H.Epstein, and S.A.Karumanchi. 2008. Preeclampsia and angiogenic imbalance. *Annu.Rev Med.* 59:61-78.

24. Huppertz,B., J.Kingdom, I.Caniggia, G.Desoye, S.Black, H.Korr, and P.Kaufmann. 2003. Hypoxia favours necrotic versus apoptotic shedding of placental syncytiotrophoblast into the maternal circulation. *Placenta* 24:181-190.

25. Zhou,C.C., S.Ahmad, T.Mi, L.Xia, S.Abbasi, P.W.Hewett, C.Sun, A.Ahmed, R.E.Kellems, and Y.Xia. 2007. Angiotensin II induces soluble fms-Like tyrosine kinase-1 release via calcineurin signaling pathway in pregnancy. *Circ.Res.* 100:88-95.

26. Mehta,P.K. and K.K.Griendling. 2007. Angiotensin II cell signaling: physiological and pathological effects in the cardiovascular system. *Am.J.Physiol Cell Physiol* 292:C82-C97.

27. Dechend,R., C.Viedt, D.N.Muller, B.Ugele, R.P.Brandes, G.Wallukat, J.K.Park, J.Janke, P.Barta, J.Theuer, A.Fiebeler, V.Homuth, R.Dietz, H.Haller, J.Kreuzer, and F.C.Luft. 2003. AT1 receptor agonistic antibodies from preeclamptic patients stimulate NADPH oxidase. *Circulation* 107:1632-1639.

28. Agarwal,D., M.A.Welsch, J.N.Keller, and J.Francis. 2011. Chronic exercise modulates RAS components and improves balance between pro- and anti-inflammatory cytokines in the brain of SHR. *Basic Res.Cardiol.* 106:1069-1085.

29. Gao,H., U.Yallampalli, and C.Yallampalli. 2012. Maternal protein restriction reduces expression of angiotensin I-converting enzyme 2 in rat placental labyrinth zone in late pregnancy. *Biol.Reprod.* 86:31.

30. Neves,L.A., K.Stovall, J.Joyner, G.Valdes, P.E.Gallagher, C.M.Ferrario, D.C.Merrill, and K.B.Brosnihan. 2008. ACE2 and ANG-(1-7) in the rat uterus during early and late gestation. *Am.J.Physiol Regul.Integr.Comp Physiol* 294:R151-R161.

31. Dohm,M.R., C.S.Richardson, and T.Garland, Jr. 1994. Exercise physiology of wild and random-bred laboratory house mice and their reciprocal hybrids. *Am.J.Physiol* 267:R1098-R1108.

32. Eisele,J.C., I.M.Schaefer, N.J.Randel, H.Post, D.Liebetanz, A.Bruel, and C.Muhlfeld. 2008. Effect of voluntary exercise on number and volume of cardiomyocytes and their mitochondria in the mouse left ventricle. *Basic Res.Cardiol.* 103:12-21.

33. Russell,J.C., W.F.Epling, D.Pierce, R.M.Amy, and D.P.Boer. 1987. Induction of voluntary prolonged running by rats. *J.Appl.Physiol* 63:2549-2553.

34. Allen,D.L., B.C.Harrison, A.Maass, M.L.Bell, W.C.Byrnes, and L.A.Leinwand. 2001. Cardiac and skeletal muscle adaptations to voluntary wheel running in the mouse. *J.Appl.Physiol* 90:1900-1908.

35. Haubold,K.W., D.L.Allen, Y.Capetanaki, and L.A.Leinwand. 2003. Loss of desmin leads to impaired voluntary wheel running and treadmill exercise performance. *J.Appl.Physiol* 95:1617-1622.

36. Danson,E.J. and D.J.Paterson. 2003. Enhanced neuronal nitric oxide synthase expression is central to cardiac vagal phenotype in exercise-trained mice. *J.Physiol* 546:225-232.

37. Lambert,M.I. and T.D.Noakes. 1990. Spontaneous running increases VO2max and running performance in rats. *J.Appl.Physiol* 68:400-403.

38. Meka,N., S.Katragadda, B.Cherian, and R.R.Arora. 2008. Endurance exercise and resistance training in cardiovascular disease. *Ther.Adv.Cardiovasc.Dis.* 2:115-121.

39. Myers,J. 2003. Cardiology patient pages. Exercise and cardiovascular health. *Circulation* 107:e2-e5.

40. Sigmund,C.D., C.A.Jones, C.M.Kane, C.Wu, J.A.Lang, and K.W.Gross. 1992. Regulated tissue- and cell-specific expression of the human renin gene in transgenic mice. *Circ.Res.* 70:1070-1079.

41. Yang,G., D.C.Merrill, M.W.Thompson, J.E.Robillard, and C.D.Sigmund. 1994. Functional expression of the human angiotensinogen gene in transgenic mice. *J.Biol.Chem.* 269:32497-32502.

42. Moraska,A., T.Deak, R.L.Spencer, D.Roth, and M.Fleshner. 2000. Treadmill running produces both positive and negative physiological adaptations in Sprague-Dawley rats. *Am.J.Physiol Regul.Integr.Comp Physiol* 279:R1321-R1329.

43. Falcao,S., S.Bisotto, J.Gutkowska, and J.L.Lavoie. 2009. Hyperhomocysteinemia is not sufficient to cause preeclampsia in an animal model: the importance of folate intake. *Am.J.Obstet.Gynecol.* 200:198.e1-198.e5.

44. Falcao,S., C.Solomon, C.Monat, J.Berube, J.Gutkowska, and J.L.Lavoie. 2009. Impact of diet and stress on the development of preeclampsia-like symptoms in p57kip2 mice. *Am J Physiol Heart Circ.Physiol* 296:H119-H126.

45. Daya,D. and L.Sabet. 1991. The use of cytokeratin as a sensitive and reliable marker for trophoblastic tissue. *Am.J.Clin.Pathol.* 95:137-141.

46. Colman,H., C.Giannini, L.Huang, J.Gonzalez, K.Hess, J.Bruner, G.Fuller, L.Langford, C.Pelloski, J.Aaron, P.Burger, and K.Aldape. 2006. Assessment and prognostic significance of mitotic index using the mitosis marker phospho-histone H3 in low and intermediate-grade infiltrating astrocytomas. *Am.J.Surg.Pathol.* 30:657-664.

Figure legends

Figure 1. Effect of training and genotype on mean arterial pressure (MAP) and proteinuria.

The MAP (A) increase observed in the sedentary R^+A^+ mice at the end of gestation (n=7) was prevented by ExT (n=7). R^+A^+ mice also presented with a significant increase in albumin/creatinine ratio (ACR), compared with their non-transgenic counterparts (n=13), which was further worsened with pregnancy (B). The ACR of trained transgenic mice (n=14) was significantly reduced compared to sedentary R^+A^+ mice (n=11). Values are expressed as means ± SE. ‡ $p<0.05$ significantly different from sedentary animals. ‖ $p \leq 0.05$ and # $p \leq 0.001$, statistically different from before pregnancy (Day 0); ** $p < 0.001$, statistically different from R-A- mice. MAP, mean arterial pressure; END, end of pregnancy.

Figure 2. Left ventricular gene expression of Nab1 and BNP.

Nab1 and BNP gene expression were significantly decreased with ExT in both R^-A^- and R^+A^+ mice compared to their sedentary counterparts. Nab1 expression however was also significantly greater among R^+A^+ mice, compared to non-transgenic mice. Values are expressed as means ± SE. ‡ $p<0.05$ and § $p<0.005$ statistically different from sedentary mice; * $p<0.05$ statistically different from R^-A^-. For Nab1, sedentary R^-A^- and trained R^+A^+, n=10; trained R^-A^-, n=8; sedentary R^+A^+, n=14. For BNP, sedentary R, n=11; trained r-, n=9; sedentary R+, n=14; trained R+, n=10.

Figure 3. Effect of ExT and SPE on angiogenic balance.

Sedentary R^+A^+ mice presented with significant increase in placental VEGF protein (A) and sFlt-1 mRNA (B) compared to their non-transgenic littermates, which was significantly decreased with ExT. Comparably, a significant increase in circulating sFlt-1(C) was observed among sedentary R^+A^+ mice, which was absent in trained transgenic mice. Placental VEGF: Sedentary R^-A^-, n=5; trained R^-A^-, n=6; sedentary R^+A^+, n=5; trained R^+A^+, n=6. Placental sFlt-1 mRNA: Sedentary R^-A^-, n=6; trained R^-A^-, n=8; sedentary R^+A^+, n=7; trained R^+A^+, n=6. Circulating sFlt-1: Sedentary R^-A^-, n=8; trained R^-A^-, n=9; sedentary R^+A^+, n=11; trained R^+A^+, n=9. Values are expressed as mean ± SE.* $p<0.05$, † $p<0.01$, ** $p<0.001$ significantly different than R^-A^- mice; ‡ $p<0.05$, § $p<0.001$ significantly different from sedentary mice.

Figure 4. Modulation of placental AT1R, MasR and ACE2 protein expression by a SPE and ExT.

A significant increase in placental AT1R (A) could be observed in sedentary R^+A^+ mice, along with a significant reduction in MasR (B) and ACE2 (C). Both MasR and AT1 were normalized with ExT and AT1R was even decreased in non-transgenic mice. Conversely, ACE2 was unaffected by ExT. Values are expressed as mean ± SE. ‡ $p<0.05$ and § $p<0.005$ statistically different from sedentary mice; * $p < 0.05$ and † $p < 0.01$ statistically different fron R^-A^-. Placental AT1R: Sedentary R^-A^-, n=3; trained R^-A^-, n=6; sedentary R^+A^+, n=7; trained R^+A^+, n=5. Placental MasR: Sedentary R^-A^-, n=5; trained R^-A^-, n=6; sedentary R^+A^+, n=7; trained R^+A^+, n=5. Placental ACE2: Sedentary R^-A^-, n=6; trained R^-A^-, n=8; sedentary R^+A^+, n=6; trained R^+A^+, n=6.

Figure 5. Effect of SPE-like phenotype and exercise on aortic AT1R, MasR and ACE2 protein expression, respectively.

Aortic AT1R (A) expression was significant increased, while MasR (B) and ACE2 (C) expression were significantly reduced among R^+A^+ mice. Conversely, MasR (B) was significantly increased with ExT. Values are expressed as mean ± SE. ‡ $p<0.05$ statistically different than sedentary mice; * $p < 0.05$ statistically different than R^-A^-. Aortic AT1R: Sedentary R^-A^-, n=6; trained R^-A^-, n=7; sedentary R^+A^+, n=6; trained R^+A^+, n=5. Aortic MasR: Sedentary R^-A^-, n=5; trained R^-A^-, n=7; sedentary R^+A^+, n=6; trained R^+A^+, n=8. Aortic ACE2: Sedentary R^-A^-, n=6; trained R^-A^-, n=5; sedentary R^+A^+, n=6; trained R^+A^+, n=7.

	Mother's genotype	N	Heart/tibia ratio	LV/tibia ratio	RV/tibia ratio	LA/tibia ratio	RA/tibia ratio
Sedentary	R-/A-	5	7.13 ± 0.32	5.27 ± 0.29	1.21 ± 0.07	0.18 ± 0.01	0.17 ± 0.02
	R+/A+	5	9.54 ± 0.23†	7.35 ± 0.28†	1.47 ± 0.04*	0.20 ± 0.02	0.19 ± 0.02‡
Trained	R-/A-	7	7.73 ± 0.18	5.17 ± 0.34	1.47 ± 0.07‡	0.18 ± 0.01	0.21 ± 0.01
	R+/A+	6	9.86 ± 0.36†	7.18 ± 0.26†	1.41 ± 0.04	0.22 ± 0.03	0.24 ± 0.02‡

Table 1: Effect of ExT and SPE on ratio of the whole heart and its compartments to tibia length at the end of pregnancy.

Transgenic mice were found to have a statistically larger hearts, left ventricles, right ventricles and right atria, when corrected to left tibia length in comparison with their non-transgenic littermates without any effect of ExT. Right ventricles were also increased among the trained non-transgenic animals. Values are expressed as mean ± SE. * $p \leq 0.05$, † $p \leq 0.001$, significantly different from R-/A- mice; ‡ $p \leq 0.05$ significantly different than sedentary mice. N, number; LV, left ventricle; RV, right ventricle; LA, left atrium; RA, right atrium.

	Mother's genotype	N	Necrosis		Hyalinization		Microcal.		GCIL		LLTS		Total	
			Mdn	75%	Mdn	75%	Mdn	75%	Mdn	75%	Mdn	75%	Mdn	75%
Sedentary	R^-A	22	0.0	1.0	0.5	1.0	0.0	0.0	0.0	0.0	0.0	1.0	1.0	2.0
	R^+A^+	21	1.0	2.0	2.0*	2.0	0.0	0.0	1.0*	1.0	1.0*	2.0	4.0*	6.0
Trained	R^+A^+	25	1.0	1.0	0.0‡	1.0	0.0	0.0	0.0	0.25	1.0‡	1.0	2.0‡	3.0

Table 2: Characterization of placental pathology.

Results are expressed as the median (Mdn) and the 75th percentile of the score given in histology. ‡ $p \leq 0.05$ significantly different from sedentary mice. * $p \leq 0.05$ significantly from non-transgenic mice. GCIL, Giant cell island loss; LLTS, Loss of labyrinthine trophoblast structure; Mdn, Median; Microcal., Microcalcification.

	Mother's phenotype	Total fetal weight (g)	Total placental weight (g)	Fetal/placental weight ratio	Litter size
Sedentary	R^-A^-	6.41 ± 0.42	0.92 ± 0.08	7.14 ± 0.15	8.9 ± 0.6
	R^+A^+	5.23 ± 0.25*	0.73 ± 0.04*	7.38 ± 0.18	7.0 ± 0.4†
Trained	R^-A^-	6.41 ± 0.45	0.82 ± 0.05	7.96 ± 0.12§	8.1 ± 0.6
	R^+A^+	7.07 ± 0.32§	0.92 ± 0.05‡	7.73 ± 0.16§	9.6 ± 0.4§

Table 3. Fetal consequences of SPE-like phenotype and ExT.

Training was found to significantly increase total fetal and placenta weight, fetal/placental weight ratio and litter size in R^+A^+ mice compared to their sedentary littermates. Conversely, sedentary transgenic mice has a significant decrease in all of these parameters. Fetal/placental weigth ration was also found to be increased with ExT in the non-transgenic mice. 8 litters were used for each of the groups studied. Values are expressed as mean ± SE. ‡ $p<0.05$ and § $p<0.005$ statistically different from sedentary mice; * $p<0.05$ and † $p<0.01$ statistically different from R-A- mice.

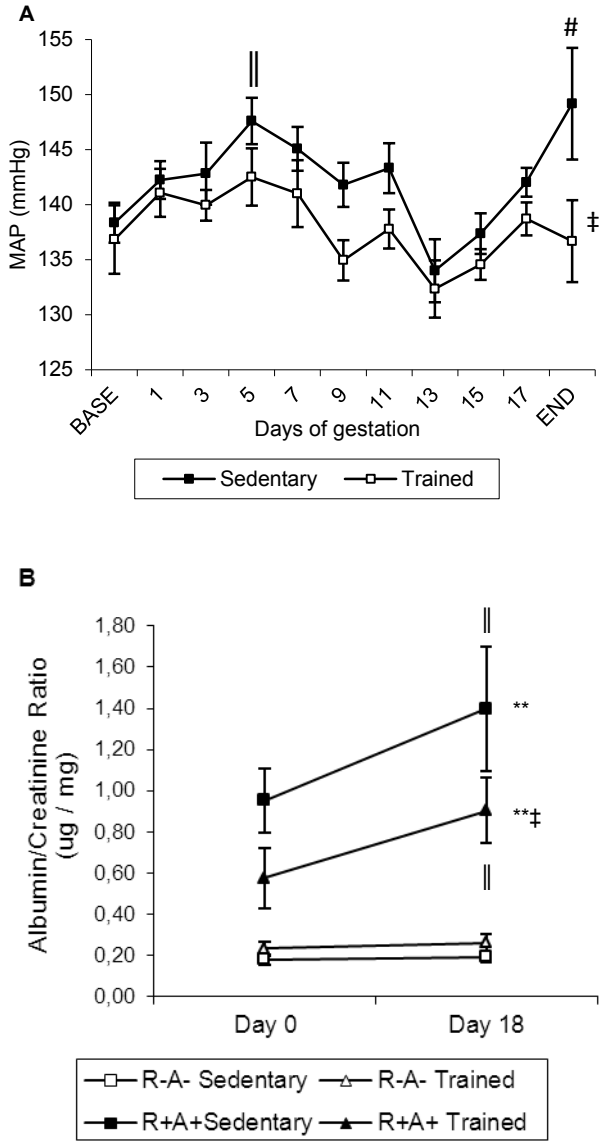

Figure 1. Effect of training and genotype on mean arterial pressure (MAP) and proteinuria.

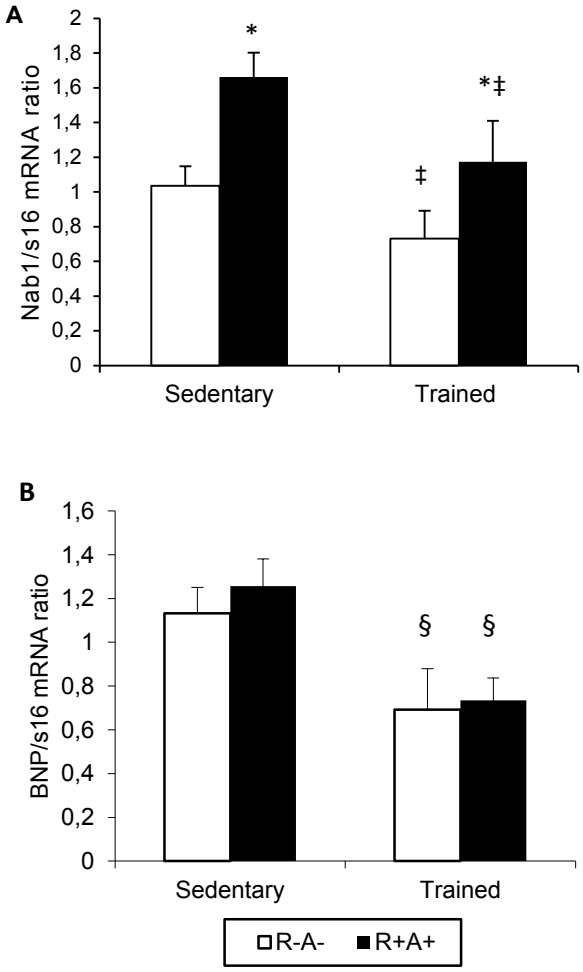

Figure 2. Left ventricular gene expression of Nab1 and BNP.

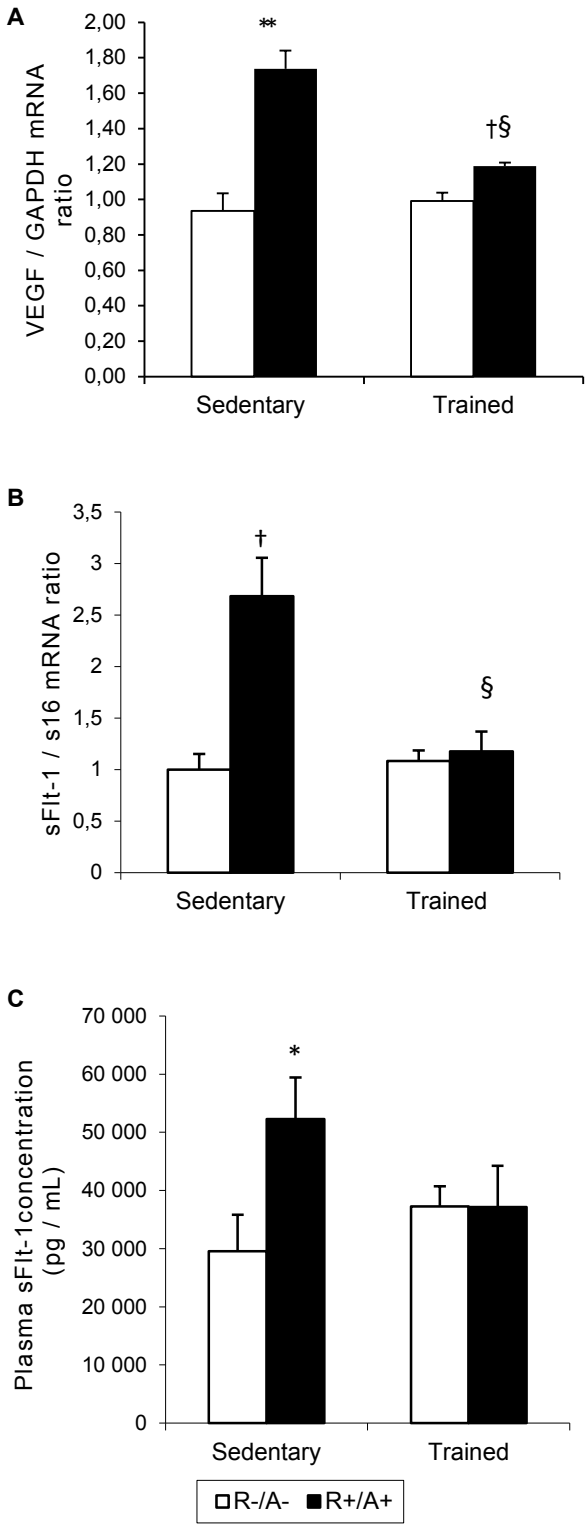

Figure 3. Effect of ExT and SPE on angiogenic balance.

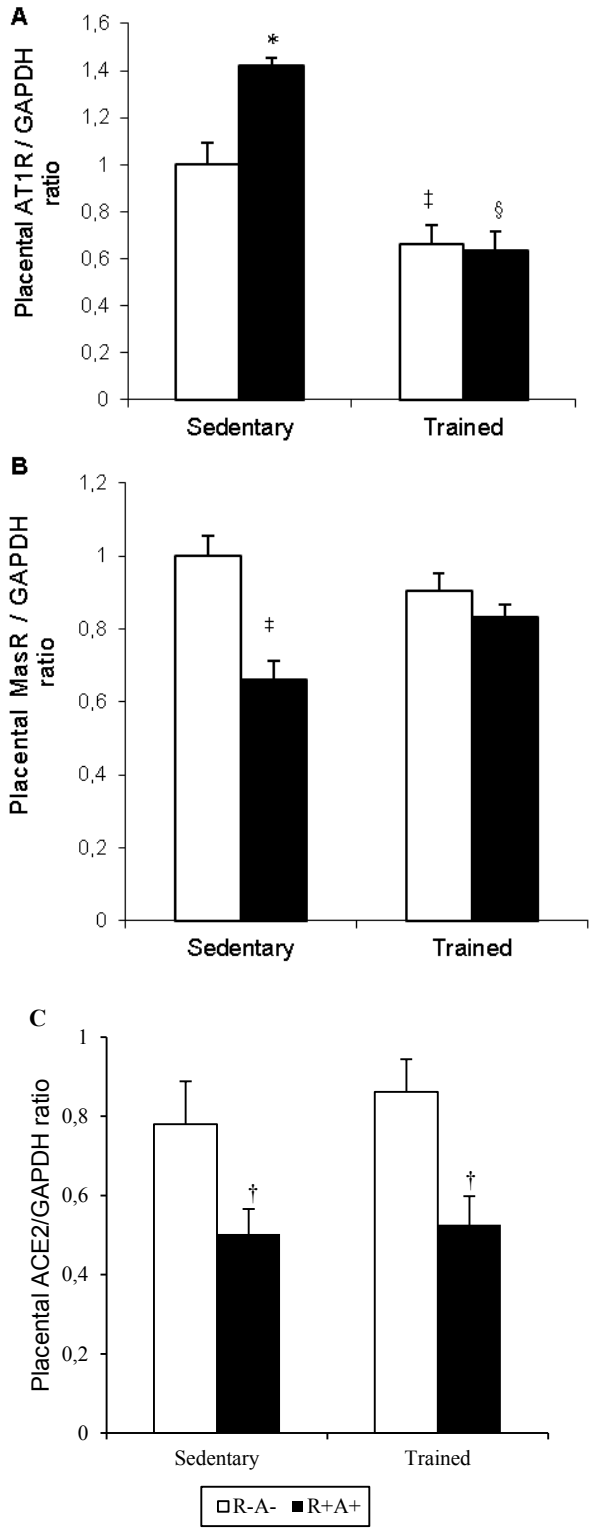

Figure 4. Modulation of placental AT1R, MasR and ACE2 protein expression by a SPE and ExT.

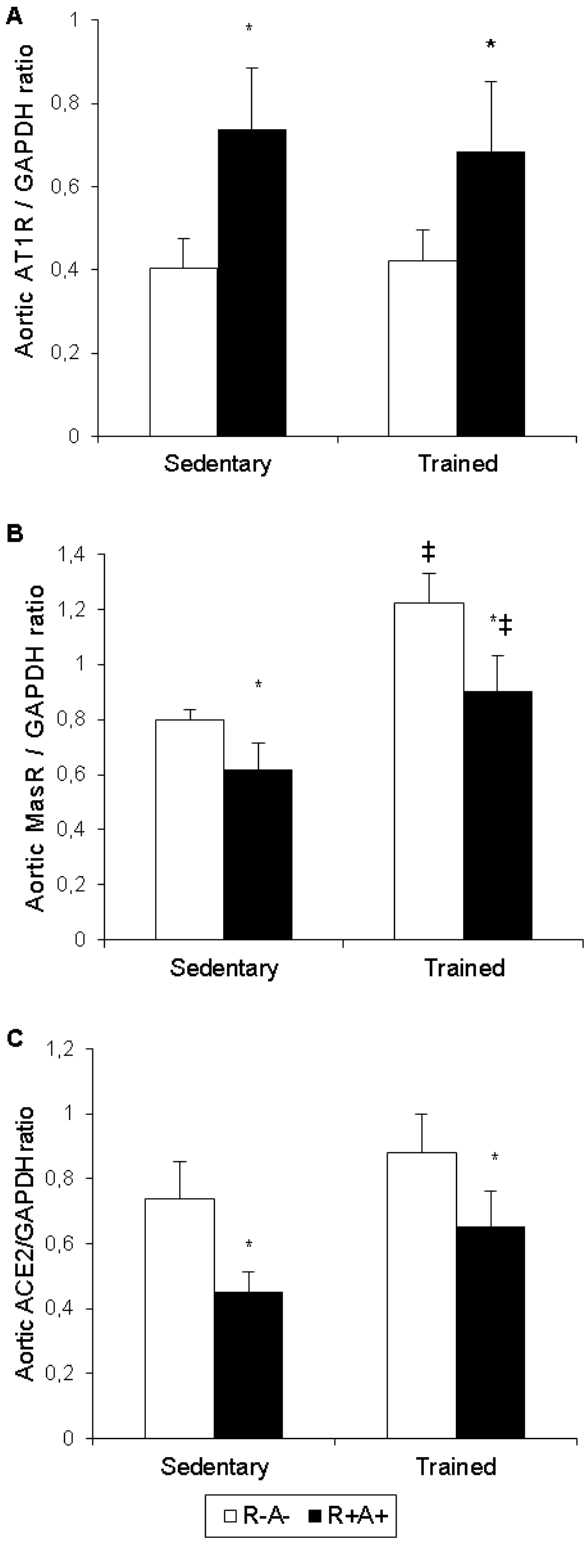

Figure 5. Effect of SPE-like phenotype and exercise on aortic AT1R, MasR and ACE2 protein expression, respectively.

SUPPLEMENTAL MATERIAL

Detailed Methods

Animals. R^+A^+ transgenic mice were produced by breeding heterozygous human renin mice (Ren9 line)[1] with heterozygous human angiotensinogen mice (204/1 line)[2]. Both single transgenic lines were originally obtained from Dr. Curt D. Sigmund of the University of Iowa, and were maintained in our animal facility by backcrossing with C57BL/6 mice (strain code 027, Charles River, St-Constant, QC, Canada) for over 20 generations. The genotypes were determined by performing a polymerase chain reaction on genomic DNA obtained from tail biopsies using primers specific to hREN and hANG (see Table S1 for sequence). Each reaction contained 1µl 10X buffer; 0.2 µl 10mM dNTP, 0.1 µl of each primer, 6.4 µl of water, 0.5 µl taq polymerase (Feldan, Québec, QC, Canada) and 2 µl of genomic DNA. The PCR products were subsequently analyzed by southern blot using a 1% agarose gel. The animals were kept on a 12h light/dark cycle with water and standard laboratory chow (2018; Teklab Premier Laboratory Diets, Madison, WI) *ad libitum*. Female mice in these experiments were 8-10 weeks of age, and were separated into 4 groups (Sedentary R^+A^+ and non-transgenic (R^-A^-); Trained R^+A^+ and R^-A^-). R^+A^+ mice were bred with non-transgenic (NT) males. Control mice corresponded to NT littermates. The care of all mice met the standards set forth by the Canadian Council on Animal Care for the use of experimental animals. All procedures were approved by the Animal Care Committee of the CHUM Research Centre.

Exercise training. Mice were placed in cages with free access to a running wheel, for one month and remained in these cages throughout gestation. In fact, studies have shown

significant improvements in aerobic capacity with only 3 weeks of exercise training (ExT) with a running wheel[3, 4]. In addition, we have shown significant improvement in maternal outcomes in a different mouse model with this type of Ext regimen[5]. The use of voluntary exercise was chosen to prevent the stress that is usually associated with treadmill running[6]. ExT was measured, as each cage was connected to a computer and, the number of revolutions were recorded and used to confirm training status (Compte-tour5, Aquila, Boucherville, Qc, Canada).

Arterial pressure measurement. Arterial pressure (AP) and heart rate (HR) were quantified in the carotid artery by telemetry using TA11PA-C10 probes (Data Sciences International, St. Paul, MN) [7, 8]. The probe's catheter was inserted into the left carotid artery of female mice anesthetized by inhalation of isoflurane 2-3% in oxygen, and maintained on 2% isoflurane. After 7-10 days of recovery post-surgery, AP and HR were recorded for 3 consecutive days to represent baseline values. The mice were then put in cages with access to an exercise wheel for 1 month. Sedentary animals were placed in standard cages. After this period, male mice were introduced into the cages for timed-mating of trained animals, while the reverse was performed for sedentary females. Gestation was confirmed by the presence of a vaginal plug, which was recorded as day 1 of pregnancy. AP and HR measurements were recorded every 2 days, beginning on day 1 up to day 19. Since mice normally give birth between day 19 and 21 of gestation, the day prior to delivery was considered as "end of gestation" to permit an adequate comparison.

Proteinuria. Urine samples were collected on day 0 (prior to mating) and on day 18 (prior to sacrifice). In order to do so, the mice were momentarily restrained and their urine was directly collected in a 1.5mL tube. Collecting urine via this method is associated with minimal amount of stress, which is far less than that associated with placing mice in metabolic cages for 24h[9]. Once collected, the urine samples were maintained at -80^{o}C until assayed. Albuwell and Creatinine companion ELISA kits were used to determine urine albumin and creatinine clearance, respectively (Exocell, Philadelphia, PA, United States). Urine samples were diluted 1:10, and each sample was done in duplicate. Proteinuria was assessed by calculating the albumin/creatinine ratio (ACR).

Echocardiography. Transthoracic echocardiographic studies were performed prior to training and mating, and at the end of pregnancy. The mice were anesthetized by inhalation of isoflurane 2-3% in oxygen, and maintained on 2% isoflurane and ultrasound transmission gel (Ecogel 200, EcoMed Inc., Mississauga, ON) was placed over the cardiothoracic region to provide an acoustic coupling medium between the probe and the animal. Their heart were investigated by high-resolution ultrasound biomicroscopy (Vevo660; Visualsonics, Toronto, ON, Canada) equipped with a 25-55 MHz probe. Positioning of the M-lines was guided by B-mode echocardiography. The parasternal long-axis view served to capture M-mode tracings through the anterior and posterior left ventricular (LV) walls at the level of the papillary muscle. The ejection fraction (EF) was estimated by the following formula: EF = (LVEDV – LVESV) × 100 / LVEDV, where LVEDV and LVESV are respectively LV end-diastolic and end-systolic volumes. LV fractional shortening (FS) was given by (LVEDD – LVESD) × 100 / LVEDD. Lastly, LV volumes during diastole and systole were determined as 7 × LVD^3 / (2.4

+ LVD), where left ventricular diameter (LVD) is substituted by LVEDD for LV diastolic volume or LVESD for LV systolic volume, respectively.

Tissue collection and histology. On day 18 of gestation, mice were anesthetized by isoflurane 2-3% in oxygen, and then maintained on 2% isoflurane. Blood was collected by intrathoracic cardiac puncture and placed in a chilled 1.5 ml tube containing 15ul of 500mM EDTA (pH: 8.0) (EMD, Gibbstown, NJ, USA). Plasma was separated by centrifugation and samples were snap frozen in liquid nitrogen, and stored at -80^{o}C until assayed. The pups were weighed, and their tails were cut and kept for genotyping. Kidneys, heart, and placentas were all collected, weighed, and either snap-frozen in liquid nitrogen or placed overnight in 4% paraformaldehyde for fixation. 24h after the tissues had been fixed, they were washed with phosphate buffer and subsequently embedded in paraffin. Sections were obtained by cross-sectionally cutting the fixed tissue using a microtome. To evaluate placental morphology, the sections were stained with hematoxylin phloxine saffron (HPS) and evaluated by light microscopy. Embedding, sectioning and staining were performed by the histology platform of the Research Institute in Immunology and Cancerology at the Université de Montréal.

Placental alterations were characterized by 5 criteria: necrosis, hyalinization, microcalcification, giant cell island loss and labyrinthine trophoblast structure loss. The latter two are analogous to human extravillous cytotrophoblasts cells and chorionic villi structure, respectively[10]. A score from 0 to 3 was assigned for each criterion: 0 for no change, 1 for mild, 2 for moderate, and 3 for severe alteration. All scores were then summed up for total evaluation of the placental alterations. To avoid any bias, the investigator scoring the tissues was blinded to the genotype of both mother and pups.

Real-time PCR. Total RNA was extracted from frozen samples using Trizol (Invitrogen, Burlington, ON, Canada) according to the manufacturer's protocol. To remove genomic DNA, RNA samples were incubated with 2 U deoxyribonuclease I (DNase I; Invitrogen)/ug RNA for 30 min at 37°C. Single-stranded cDNA was synthesized by reverse-transcriptase reaction with Moloney Murine Leukemia Virus (M-MLV) (Invitrogen). PCR was undertaken in the iCycler IQ Real Time PCR detection System (Bio-Rad Laboratories, Hercules, CA), using SYBR® green chemistry[11]. In brief, 2 µl of diluted cDNA was added to an 18 µl reaction mixture containing 1X iQ SYBR Green Supermix (Bio-Rad Laboratories) and 200 nM forward and reverse primers (Invitrogen). The following genes were investigated: 40S ribosomal protein S16 (S16), sFlt-1, NGFI-A-binding protein 1 (Nab1) and brain natriuretic peptide (BNP). Primer sequences are described in Table 1. Each placental and aortic sample was run and analyzed in duplicate. mRNA levels are expressed as values relative to s16 mRNA.

Immunohistochemistry. Immunochemical analysis was assessed on 3 different placentas from 3 different litters. Paraffin-embedded tissues were sectioned at 4 μm and deparaffinised in citrisolv (Fisher Scientific, Ottawa, ON, Canada). Antigen retrieval was performed by boiling the sections in sodium citrate buffer (10 nM, pH 6.0) for 3min. Immunostaining was then carried out with Catalyzed Signal Amplification System (Dako, Carpinteria, CA), according to the manufacturer's instructions. The following modification were used: Peroxidase activity was blocked for 15min at room temperature with peroxidase block solution. Samples were rinsed in 3 baths for 3min in TBS-T (0.05 M tris-HCL, 0.3 M NaCl, 0.1% tween 20, pH 7.6). The sections were then incubated with endogenous avidin and biotin blocking solutions

(Thermo Scientific, Ottawa, ON, Canada) for 15min at room temperature. Non-specific antigen binding was blocked by incubation in protein block solution for 30min at room temperature. The sections were incubated overnight at 4ºC with specific immunohistochemical primary antibodies diluted in TBS with 1% bovine serum albumin at the following concentrations: anti-pan-cytokeratin (1:800; ab9377, Abcam, Cambridge, MA) and histone H3 (1:1600; ab5176-100, Abcam)to evaluate the presence of trophoblast and mitosis, respectively[12, 13]. Tissue sections were incubated with secondary antibody conjugated to biotin (1:5000, donkey anti-rabbit; AP182B, Chemicon international, Millipore, Billerica, MA) solution for 1h at room temperature. Primary antibodies were omitted in the negative control. Samples were incubated with streptavidin-biotin complex for 15min and subsequently, with amplification reagent for 15min at room temperature. The sections were then incubated with a streptavidin-peroxidase for 15 min. Lastly, staining with substrate-chromogen solution for 4min and counterstaining with Mayer's hematoxylin (Sigma-Aldrich, Oakville, ON, Canada) blue in 0.3% ammonia water were performed. Sections were viewed and photographed with a Leitz Diaplan microscope equipped with a Nikon CoolPix 990 camera (Nikon Instruments, Melville, NY).

Western Blot. Frozen placentas and aortas were lyophilized and subsequently homogenised in lysis buffer (50mM HEPES pH 7.5, 137mM NaCl, 1mM $MgCl_2$, 1mM $CaCl_2$, 2mM Na_3VO_4, 10mM Na pyrophosphate, 10mM NaF, 2mM EDTA, 1% NP-40, 10% glycerol, 34mg/L PMSF along with a protease inhibitor cocktail (Roche, Mississauga, ON, Canada)). Total protein content was measured in supernatants by standard Bradford assay. Samples containing 50µg of protein were loaded on 10% or 12% sodium dodecyl sulfate (SDS)-polyacrylamide gel,

depending on the molecular weight of the protein studied, and separated by electrophoresis. Proteins were transferred on a nitrocellulose membrane (Amersham, Baie d'Urfe, QC). Placental and aortic non-specific sites were blocked overnight at 4^{o}C in SuperBlock buffer (Thermo Fisher Scientific, Rockford, IL) or in Bløk^{TM}-Chemiluminescent blocker (Millipore, Temecula, MA), respectively. Membranes were then incubated with the primary antibody in 10% Superblock and 0.1% Tween 20 (Fisher Scientific, Ottawa, ON, Canada) in tris buffered solution (TBS) (Abcam, Cambridge, MA) overnight at 4˚C. The following antibodies and concentrations were used: vascular endothelial growth factor (VEGF) (1:1000, ab46154, Abcam, Cambridge, MA); angiotensin converting enzyme 2 (ACE2) (1:500, SC-20998, Santa Cruz Biotechnology, Santa Cruz, CA); Angiotensin-(1-7)-Mas receptor (MasR) (1:2000, AAR-013, Alamone Labs, Jerusalem, Israel); AT1R (1:2000, SC-578, Santa Cruz Biotechnology, Santa Cruz, CA); glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (1:2000, SC-20357, Santa Cruz Biotechnology, Santa Cruz, CA). The membranes were subsequently washed with 0.1% TBS-T for 30min and incubated for 1h at room temperature with their respective horseradish peroxidase conjugated secondary antibody (1:3000, 1:10 Superblock in 0.1% Tween 20 in TBS) independent of primary antibody used. The GAPDH antibody was already linked to horseradish peroxidise, and thus, a secondary antibody was not use for this experiment. Bands were revealed using the ECL West Pico kit (Pierce, Rockford, IL). Each protein signal was normalized to its respective GAPDH band.

Plasma sFlt-1 levels: Circulating sFlt-1concentrations were measured using a commercial ELISA kit (R&D-Quantikine, Minneapolis, MN). Plasma samples were diluted 1:20 using the manufacturer's dilutor prior to the experiment and each sample was measured in duplicate.

Plasma glucose levels: Circulating glucose levels were determined using a commercially available enzymatic kit (Autokit Glucose, WAKO Diagnositics, Richmond, VA), following the manufacturer's instructions. Each sample was measured in duplicate.

Statistical analysis. All values are expressed as means ± SE. A p-value of ≤ 0.05 was considered significant. Differences in tissue weights and ratios, number of pups, circulating glucose and sFlt-1 levels, as well as data obtained by real-time PCR, Western blot and immunohistochemistry were computed by 2-way ANOVA. 2-way repeated measures ANOVA was used to analyze echocardiography parameters, blood pressure and albumin/creatinine ratio. These analyses were all followed by Tukey's post-hoc test if an interaction was detected. Placental alterations were analyzed using a non-parametric Mann-Whitney Rank Sum Test.

Supplemental Figures

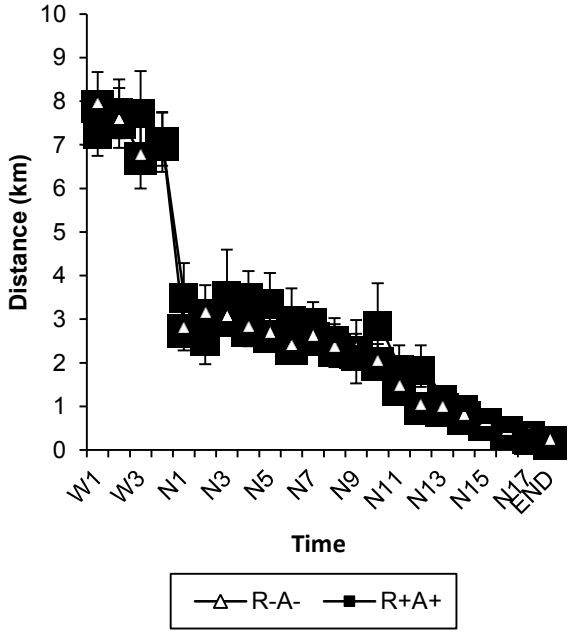

Figure S1: Distance travelled prior to and throughout gestation.

Exercise training patterns were similar regardless of genotype (R^-A^- (n=10) and R^+A^+ (n=11)). Training was greater prior to pregnancy in both groups, and decreased progressively as pregnancy advanced, reaching a nadir prior to sacrifice. W, average distance travelled weekly; N, distance travelled during nighttime; END, end of pregnancy.

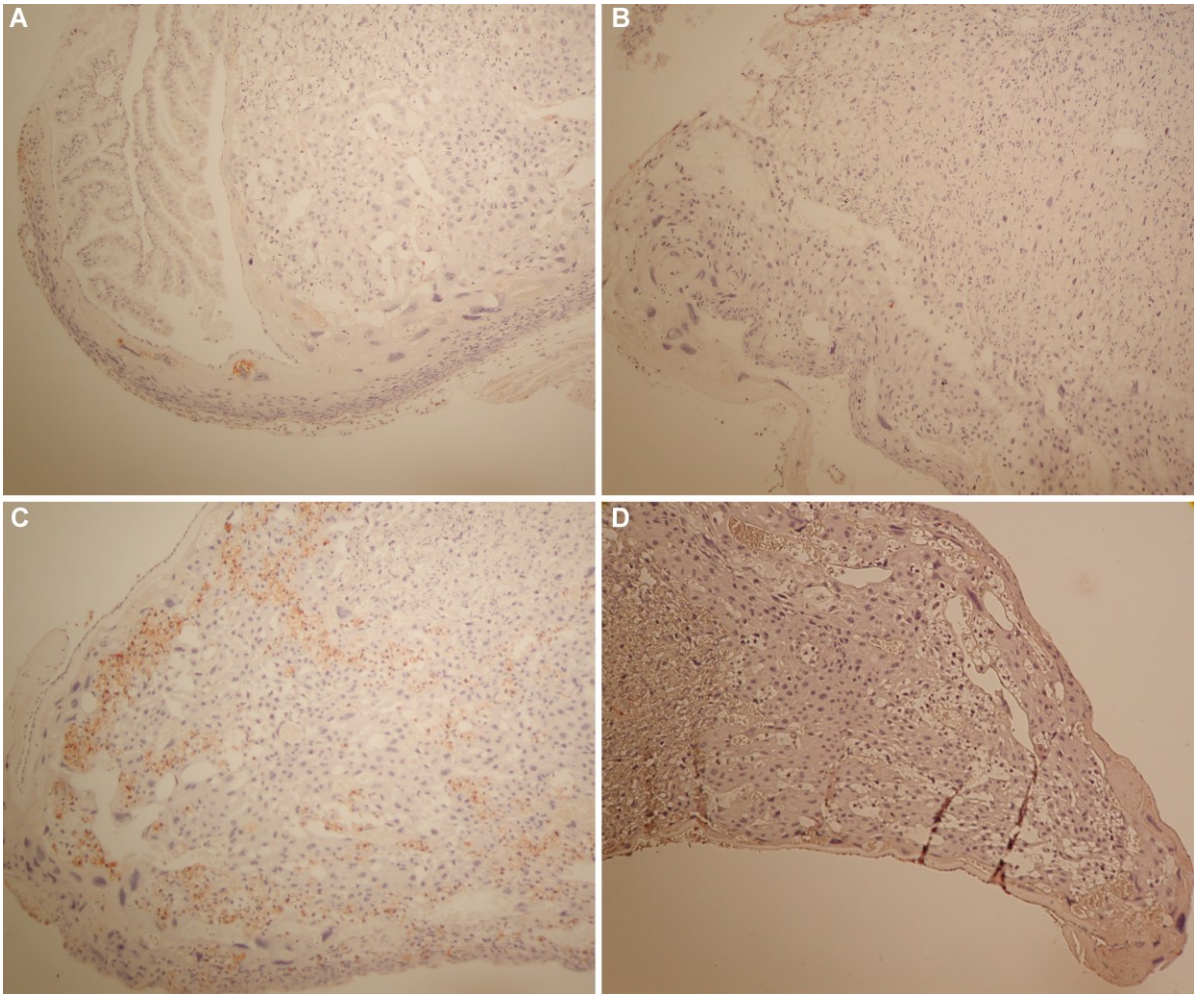

Figure S2: Effects of exercise training and SPE on placental cytokeratin immunostaining.

Cytokeratin is a marker of trophoblast proliferation at all stages of gestation. Histological analysis revealed a significant increase in cytokeratin positive trophoblastic cells among sedentary transgenic mice (Image C, n=3), compared to their nontransgenic counterparts (Image A, n=3). Interestingly, training led to a significant reduction in trophoblast positive staining among R^+A^+ mice (Image D, n=3). A small increase in cytokeratin was observed among trained R^-A^- (Image B, n=3), compared to their sedentary counterparts (Image A, n=3). These results suggest that both phenotype and training may affect trophoblast function, and, consequently, fetal development. Additionally, these results are in line with studies demonstrating an increase in placental villous area and perfusion with exercise training. Magnification 200 X.

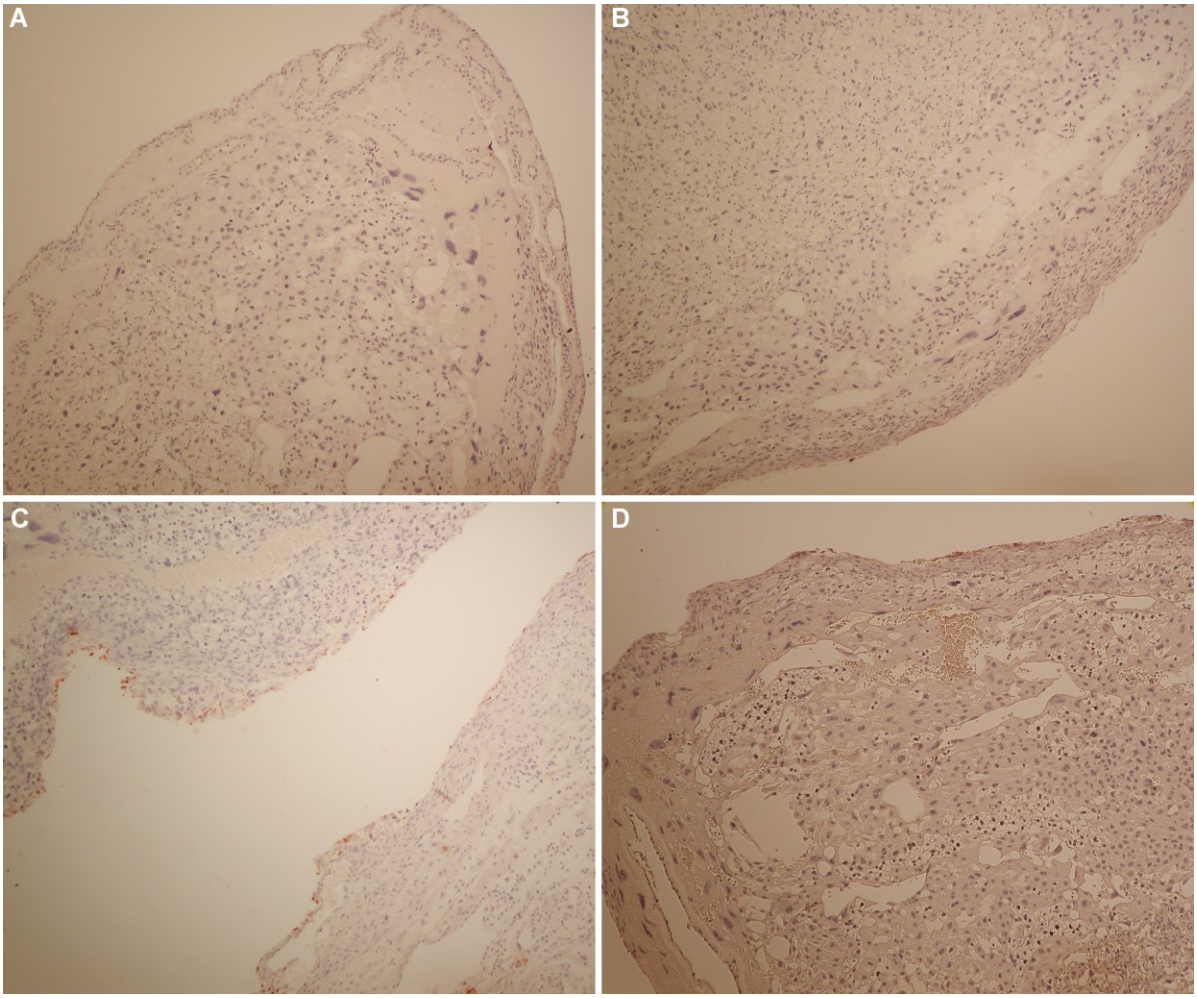

Figure S3: Effects of exercise training and SPE on placental histone H3 immunostaining.

Histone H3-phospho-immunostaining is a marker of mitosis, which allows distinguishing proliferating from endoreplicating cells (a phenomenon specific to trophoblastic giant cell). A weak increase in histone H3 staining was observed with training among the R^-A^- mice (Image B, n=3), compared to sedentary R^-A^- mothers (Image A, n=3). Sedentary transgenic dams has significantly more histone H3 immunostaining (Image C, n=3), which disappeared with training. In sedentary R^+A^+ mice, the staining was primarily localized in peripheral trophoblasts, demonstrating an increase in number of cells undergoing endoreplication. Magnification 200 X.

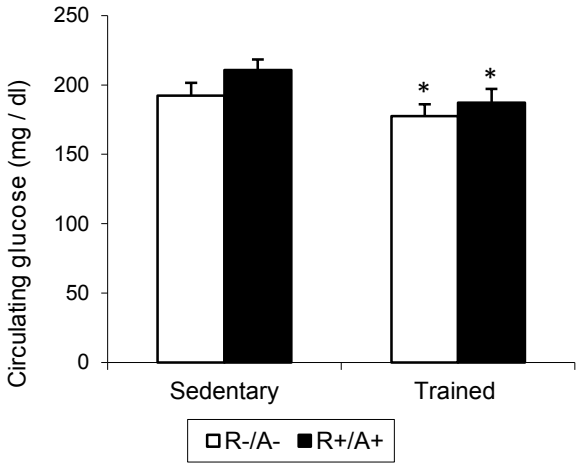

Figure S4: Changes in circulating glucose levels with ExT and SPE-like phenotype.

Circulating glucose levels were consistently lower among trained animals compared with their sedentary counterparts, irrespective of genotype (n=10, per group). Values are expressed as means ± SE. * $p<0.05$ sig

Supplemental Tables:

Primer	*Forward*	*Reverse*
s16	5'-ATCTCAAAGGCCCTGGTCGC-3'	5'-ACAAAGGTAAACCCCGATCC-3'
sFlt-1	5'- AGGTGAGCACTGCGGCA-3'	5'-ATGAGTCCTTTAATGTTTGA-3'
Nab1	5'-CTGGCCAGGGTTTCTC-3'	5'-TGGCACAGATTCCTGGAAGTC-3'
BNP	5'-AATTCCAAGGTGACACATATC TC-3'	5'-GGTCTTCCTACAACAACTTCAG-3'
hREN	5'-TGACACTGGTTCGTCCAATG-3'	5'-ATA GCG GAG GGT GAG TTC TG-3'
hANG	5'-TGGTGCTAGTCGCTGCAAAACTTGACACCG-3'	5'-CAGGGAGCAGCCAGTCTTCCATCCTGTCAC-3'

Table S1: Primer sequences used for real-time PCR.

Mother's genotype	*N*	*Baseline weight (grams)*	*Weight gain (grams)*	*Length of pregnancy (days)*
Sedentary				
R^-A^-	11	21.67 ± 0.23	13.9 ± 0.68	19.0 ± 0.0
R^+A^+	10	22.54 ± 0.42	13.95 ± 0.84	19.1 ± 0.01
Trained				
R^-A^-	10	21.14 ± 0.59	12.61 ± 0.80	19.0 ± 0.0
R^+A^+	11	21.32 ± 0.43	14.44 ± 0.52	19.2 ± 0.2

Table S2: Maternal characteristics.

No statistical significance could be detected on baseline body weight, weight gain and length of pregnancy. Values are expressed as mean ± SE. N, number; BW, body weight; LV, left ventricle; LK, left kidney.

	Mother's genotype	*N*	*LV diastolic volume (µl)*	*LV systolic volume (µl)*	*FS (%)*	*EF (%)*
Before Pregnancy						
Sedentary	R-/A-	8	62.31±4.52	29.38±1.56	26.46±1.00	52.28±1.52
	R+/A+	8	59.66±3.03	30.96±3.01	23.02±1.46*	46.59±2.40*
Trained	R-/A-	9	61.87±3.83	29.27±2.12	26.14±0.70	51.83±1.12
	R+/A+	12	66.98±3.68	34.80±3.60	24.98±2.13*	48.67±3.27*
End of Pregnancy						
Sedentary	R-/A-	8	67.71±4.07‖	31.85±4.36	28.01±2.47	54.16±3.91
	R+/A+	8	70.54±2.32‖	34.61±2.82	25.93±1.97*	50.99±3.13*
Trained	R-/A-	9	73.81±5.17‖	35.73±2.88	28.81±1.74	55.32±2.63
	R+/A+	12	74.38±4.72‖	35.92±3.83	26.98±1.84*	51.93±2.72*

Table S3: Cardiac parameters calculated following echocardiography.

Left ventricular diastolic volume was increased at the end of pregnancy in all groups among the sedentary R^+A^+ both before and at the end of pregnancy. There was a non-significant decrease in fractional shortening and ejection fraction, accompanied by an increase. Similarly, a non-significant increase in left ventricular systolic volume was observed among sedentary transgenic dams, in comparison with their sedentary and nontransgenic counterparts. Values are expressed as means ± SE.‖ $p \leq 0.05$, statistically different from the non-pregnant state, and * $p \leq 0.05$, statistically different from the R^-A^- genotype. N, number; LV, left ventricular; FS, Fractional shortening; EF, Ejection fraction.

	Mother's genotype	*N*	*Systolic LVID (mm)*	*Diastolic LVID (mm)*	*Systolic LVPW (mm)*	*Diastolic LVPW (mm)*	*Systolic IVS(mm)*	*Diastolic IVS(mm)*
Before Pregnancy								
Sedentary	R-/A-	8	2.78 ± 0.06	3.79 ± 0.11	1.60 ± 0.10	1.45 ± 0.15	1.18 ± 0.03	0.82 ± 0.02
	R+/A+	8	2.82 ± 0.12	3.72 ± 0.08	1.84 ± 0.08	1.69 ± 0.08	1.35 ± 0.08	1.00 ± 0.05*
Trained	R-/A-	9	2.77 ± 0.09	3.78 ± 0.10	1.85 ± 0.15	1.78 ± 0.16	1.18 ± 0.05	0.88 ± 0.07
	R+/A+	12	2.94 ± 0.13	3.91 ± 0.09	1.88 ± 0.06	1.69 ± 0.07	1.35 ± 0.09	1.02 ± 0.06*
End of Pregnancy								
Sedentary	R-/A-	8	2.84 ± 0.16	3.93 ± 0.10 ‖	1.95 ± 0.19	1.79 ± 0.16	1.36 ±0.09 ‖	0.95 ± 0.07 ‖
	R+/A+	8	2.97 ± 0.10	4.01 ± 0.06 ‖	2.02 ± 0.13	1.89 ± 0.19	1.46 ± 0.09 ‖	1.05 ± 0.05 ‖ *
Trained	R-/A-	9	2.88 ± 0.13	4.04 ± 0.16 ‖	1.64 ± 0.14	1.57 ± 0.16	1.44 ± 0.08 ‖	1.08 ± 0.14 ‖
	R+/A+	12	2.99 ± 0.15	4.08 ± 0.12 ‖	1.91 ± 0.16	1.77 ± 0.17	1.39 ± 0.08 ‖	1.07 ± 0.06 ‖ *

Table S4: Cardiac parameters calculated following echocardiography.

A significant increase in diastolic LVID was observed at the end of pregnancy, independent of genotype and training. Additionally, systolic IVS was significantly increased at the end of pregnancy among nontransgenic animals. The above values are expressed as mean ± SE. ‖ $p \leq 0.05$, statistically different from the non-pregnant state, and * $p \leq 0.05$, statistically different from the R^-A^- genotype. N, number; LVID, left ventricular internal diameter; LVPW, left ventricular posterior wall thickness; IVS, interventricular septum.

References

[1] Sigmund CD, Jones CA, Kane CM, Wu C, Lang JA, Gross KW. Regulated tissue- and cell-specific expression of the human renin gene in transgenic mice. *Circ Res* 1992;70:1070-9.

[2] Yang G, Merrill DC, Thompson MW, Robillard JE, Sigmund CD. Functional expression of the human angiotensinogen gene in transgenic mice. *J Biol Chem* 1994;269(51):32497-502.

[3] Haubold KW, Allen DL, Capetanaki Y, Leinwand LA. Loss of desmin leads to impaired voluntary wheel running and treadmill exercise performance. *J Appl Physiol* 2003 October;95(4):1617-22.

[4] Danson EJ, Paterson DJ. Enhanced neuronal nitric oxide synthase expression is central to cardiac vagal phenotype in exercise-trained mice. *J Physiol* 2003 January 1;546(Pt 1):225-32.

[5] Falcao S, Bisotto S, Michel C, Lacasse AA, Vaillancourt C, Gutkowska J, Lavoie JL. Exercise training can attenuate preeclampsia-like features in an animal model. *J Hypertens* 2010 December;28(12):2446-53.

[6] Moraska A, Deak T, Spencer RL, Roth D, Fleshner M. Treadmill running produces both positive and negative physiological adaptations in Sprague-Dawley rats. *Am J Physiol Regul Integr Comp Physiol* 2000 October;279(4):R1321-R1329.

[7] Falcao S, Solomon C, Monat C, Berube J, Gutkowska J, Lavoie JL. Impact of diet and stress on the development of preeclampsia-like symptoms in p57kip2 mice. *Am J Physiol Heart Circ Physiol* 2009 January;296(1):H119-H126.

[8] Falcao S, Bisotto S, Gutkowska J, Lavoie JL. Hyperhomocysteinemia is not sufficient to cause preeclampsia in an animal model: the importance of folate intake. *Am J Obstet Gynecol* 2009 February;200(2):198.e1-198.e5.

[9] Hoppe CC, Moritz KM, Fitzgerald SM, Bertram JF, Evans RG. Transient hypertension and sustained tachycardia in mice housed individually in metabolism cages. *Physiol Res* 2008 January 17.

[10] Cross JC. The genetics of pre-eclampsia: a feto-placental or maternal problem? *Clin Genet* 2003 August;64(2):96-103.

[11] Jankowski M, Wang D, Mukaddam-Daher S, Gutkowska J. Pregnancy alters nitric oxide synthase and natriuretic peptide systems in the rat left ventricle. *J Endocrinol* 2005 January;184(1):209-17.

[12] Daya D, Sabet L. The use of cytokeratin as a sensitive and reliable marker for trophoblastic tissue. *Am J Clin Pathol* 1991 February;95(2):137-41.

[13] Colman H, Giannini C, Huang L, Gonzalez J, Hess K, Bruner J, Fuller G, Langford L, Pelloski C, Aaron J, Burger P, Aldape K. Assessment and prognostic significance of mitotic index using the mitosis marker phospho-histone H3 in low and intermediate-grade infiltrating astrocytomas. *Am J Surg Pathol* 2006 May;30(5):657-64.

Chapter 7: Discussion

This animal study is the first of its kind to demonstrate the protective effect of exercise training on an SPE model in addition to elucidating potential mechanisms implicated in these effects. The R^+A^+ animal model used in this study has recently been characterized in our laboratory[294]. As previously shown, the R^+A^+ pregnant mice developed a further increase in AP, proteinuria, placental alterations and cardiac hypertrophy while the pups developed IUGR[294]. Although clinical features have been characterized, it is essential to identify mechanisms implicated in disease development since the etiology appears to be complex and multifactorial, and little is known about this specific type of PE (i.e. SPE).

Although the amount of exercise performed by our trained mice may seem impressive, the cardiovascular benefits are comparable to those observed in humans who train 45 minutes, 3-4 times weekly[295-297]. Moreover, much like what women do instinctively, our trained mice significantly reduced their exercise volume upon conceiving, and continued to do so progressively during pregnancy, reaching a minimum around day 18, at which point the animals were sacrificed. As such, we feel that this form of exercise training is ideal for this type of study as it best represents what would be observed in pregnant women. Moreover, in our study, this type of moderate and voluntary exercise training before and during pregnancy attenuated a number of PE-like features, including the increase in AP seen at term.

Interestingly, we have identified the Ang-(1-7) axis as a likely player implicated in AP regulation during pregnancy. Indeed, we observed a significant increase in aortic MasR and a non-significant trend for an elevation in aortic ACE2 with exercise training, suggesting an enhanced Ang-(1-7) axis. Moreover, it may potentially produce a reduction in AngII signalling as ACE2 promotes the conversion of AngII into Ang-(1-7), thereby further promoting the beneficial effects of Ang-(1-7) and diminishing the deleterious effects mediated by AngII. Taken together, the overall effect would be an increased vasodilation through the stimulation of the MasR and a reduced effect of the AT1R. Other mechanisms aimed at promoting endothelial function are likely involved in preventing this rise in AP, including an enhanced production of NO with exercise training[231]. Along the same line, an increase in NO may explain the reduction in proteinuria observed in trained R^+A^+, compared to their sedentary counterparts.

The kidney is also equipped with a local renin-angiotensin system. Hence, given the results obtained in the aorta regarding the RAS as well as the lack of increase in proteinuria with exercise training in our animal model, an investigation of the renal RAS components, including ACE, ACE2, AT1R and MasR, would be interesting to conduct in R^+A^+ mice, since modulation of this local system has been implicated in causing renal disease[25]. Moreover, a downregulation in renal AT1R has also been observed among SHR that experience a decrease in AP in response to chronic exercise training[298]. It is thus possible that a local

modulation of the renal RAS may be involved in the development of the disease as well as in the prevention of PE-like features by exercise training.

Abnormal placentation is proposed to be the initiating factor in many PE cases. Our sedentary transgenic mothers have a significant increase in placental alterations, yet these are completely normalized with exercise training. Immunostaining against histone H3 and cytokeratin, which are markers of mitosis and trophoblast proliferation respectively, were also normalized with exercise training, as was placental expression of VEGF. Hypoxia is known to induce cell proliferation and inhibit cell differentiation, in addition to stimulating the production of VEGF[10, 11, 299]. Therefore, these results suggest that exercise prevents hyperproliferation of placental cytotrophoblasts and consequently, placental hypoxia.

This placental hypoxia is likely responsible for the hindered fetal growth and development present in our animal model. Indeed, we observed a reduction in total fetal and total placental weight in sedentary R^+A^+ mice which was completely normalized by exercise training. This suggests that the placentas from the R^+A^+ sedentary females are less effective at nourishing the fetus, but that this was normalized by exercise training. In addition, exercise training was found to significantly increase fetal/placental weight ratio in both transgenic and non-transgenic mice further suggesting that it improves the placenta's capacity to

supply the fetus with oxygen and nutrients. This is thus comparable to what is observed in normal human pregnancy, where exercise training is known to improve placental functional volume[264]. Moreover, this explains why total fetal weight is increased with exercise training. As such, exercise training may not only be effective at preventing IUGR in PE but it may also improve fetal growth in association with other diseases. Interestingly, sedentary R^+A^+ mice have significantly smaller litter size, which is normalized with exercise training. The benefits of exercise training may thus extend beyond cardiovascular and hemodynamics adaptations, to modulating the implantation process. Therefore, it may be very interesting to explore the impact of exercise training on fertility, as these seem to be more prevalent in our society particularly given the increasing maternal age at fertilization[50, 52] .

Another mechanism that was identified was the modulation of the uteroplacental RAS components by the R+A+ genotype and exercise training. Indeed, we found that placental AT1R were significantly increased in our sedentary transgenic model and significantly reduced with exercise training. As such, this may be implicated in the modulation of blood flow to the fetus. Moreover, activation of the AT1R by AngII has also been found to inhibit trophoblastic invasion *in vitro* via the action of the plasminogen activating inhibitor (PAI)-1[300]. A PE-associated increase in PAI-1 is also implicated in preventing fibrinolysis, thereby contributing to the development of microvascular thrombi[301]. This provides

further support to the deleterious effects of RAS overstimulation during pregnancy. Interestingly, placental MasR was significantly reduced in our sedentary R^+A^+ mice, suggesting that reduced Ang-(1-7) vasodilation may be involved in further compromising fetal delivery of nutrients and gases. Conversely, this was normalized with exercise training, which contributed to the normalisation of total fetal weight. Upregulation of the Ang-(1-7) axis thus appears to positively impact placental development and subsequently fetal development. It would be of interest to study the impact of Ang (1-7) administration on PE-like features in this model to see if it can also prevent the disease, thereby providing a novel potential therapeutic avenue.

In addition, we found that exercise training significantly reduced placental sFlt-1 mRNA and circulating sFlt-1, a protein capable of antagonizing circulating levels of VEGF and PlGF, suggesting that angiogenic balance is improved. Indeed, PE is often associated with increased levels of sFlt-1, in addition to sEng. This increase in anti-angiogenic factors has been suggested to contribute to the development of widespread endothelial dysfunction by antagonizing VEGF and TGF-β1[217]. Indeed, evidence has shown that injection of sFlt-1 and sEng into pregnant rats causes hypertension, proteinuria and the HELLP syndrome, and reduces fetal weight[144]. In non-pregnant rats, hypertension and proteinuria are also observed. Moreover, given that AngII has been shown to promote the placental production and secretion of sFlt-1 through the stimulation of the AT1R, this further reinforces

our data suggesting that exercise training reduces PE-like features by decreasing the placental AngII axis in favour of the Ang-(1-7) axis[78, 145].

We did not however investigate the effects of exercise training on sEng, nor its ligand (TGFβ3), which have also been involved in the development of PE. Consequently, it would be of value to test if exercise training also modulates these components, as well as if they are implicated in the development of PE-like features in our animal model, similar to what is observed in the human condition.

Future research should also explore the presence of AT1-AA should be explored in the R^+A^+ model, as they have been found in the hANG rat model of PE[302]. The hANG rat corresponds to a model of *de novo* PE, in which a female rat overexpressing hANG is crossed with a male overexpressing hREN. Because of the species specificity of the RAS, the PE-like features develop upon mating and give rise to *de novo* hypertension and proteinuria, in addition to placental alterations and pathological cardiac hypertrophy. The identification of AT1-AA in this animal model, in addition to its presence in the sera of some PE patients, has suggested to be yet another mechanism implicated in the pathogenesis of the disease[302, 303]. How these antibodies develop is not completely understood, but infusion of IL-17 into pregnant rats has been shown to cause a significant increase in AT1-AA, via the intermediary of oxidative damage[304]. In the circulation, the AT1-AA have strong affinity for the AT1 receptor, and as such can activate the downstream signalling pathways of this receptor, producing vasoconstriction,

oxidative stress, hypertrophy, proliferation, and inhibiting fibrinolysis[65, 180]. Determining how these AT1-AA develop requires further investigation and necessitates the use of animal models of PE. Investigating whether exercise training can alter circulating levels of AT1-AA will provide a more complete understanding of the impact of exercise training in PE prevention.

Cardiac hypertrophy was evident in our sedentary R^+A^+ mice, and this was associated with a significant increase in Nab1, which is a marker of pathological cardiac hypertrophy[305]. Interestingly, a significant reduction in Nab1 was observed with exercise training, although these values were not completely normalised. Moreover, BNP, a marker of LV dysfunction, was unaffected by pregnancy in our transgenic animal model, but was found to be decreased with exercise training[306]. Echocardiographic analysis were in accordance with these results as we observed a significant reduction in the EF and FS of sedentary R^+A^+ mice at the end of gestation, compared to their non-transgenic counterparts which indicates that these animals had a decrease in cardiac function. Both parameters tended to be normalised with exercise training however this did not reach statistical significance. This is not that surprising since these mice remained hypertensive even with the exercise training and as such, the increased afterload surely contributes to the development of this pathological cardiac hypertrophy.

Conversely, RAS overstimulation from birth may have caused the development of compensatory mechanisms to minimize cardiac injury[307]. It would thus be

interesting to evaluate the expression of local RAS components to determine their potential implication. Increases in ACE and AT1R, leading to activation of AngII-mediated pathways, and decreases in MasR and ACE2, leading to depression of those mediated by Ang-(1-7), in sedentary R^+A^+ mice may explain why heart function is further compromised. Normalisation in these parameters may be implicated in the absence of worsening effects on cardiac hypertrophy and the observed trend towards an improvement in FS and EF with exercise training in the transgenic animal model.

It is important to note that pregnancy in mice does differ in some points from that of the human gestation in that human trophoblastic invasion is deep while murine invasion is generally more superficial[308]. PE is characterized by inadequate, and thus superficial, trophoblastic invasion[309]. This difference in the placentation may limit the conclusions that can be made from mice models of PE. Nevertheless, it is important to mention that there are important similarities between murine and human pregnancy. In fact, both species necessitate a hemochorial placentation and require similar cardiovascular adaptations for pregnancy to ensue[2, 310]. Additionally, features that develop during gestation in our mouse model do resemble those that are observed in women who suffer from SPE[294]. Indeed, these mice develop a further increase in AP, proteinuria, cardiac hypertrophy and placental abnormalities, and their fetuses suffer from IUGR. Hence, we feel this animal model will be very useful to understand the mechanism involved in the

pathogenesis of these features and will provide a great deal of insight into the causes of SPE. Indeed, the use of animal models permits us to investigate the singular effect of exercise training on PE risk, as it is possible to hold most parameters constant, including environment and genetic background, and is thus an asset for any research. Moreover, invasive and timeline studies are also possible and can contribute to the investigation of potential therapeutic agents.

The diverse beneficial effects of exercise training in healthy individuals are well known[82, 224, 225]. Identifying whether all the mechanisms implicated in these effects are also involved in protecting against PE will need to be investigated. Moreover, the determination of the factors which are implicated in the beneficial effects of exercise training may give us some new insights into the pathophysiology of the disease as well as suggest potential novel therapeutics.

Currently, inflammation, immune response, oxidative stress and endothelial dysfunction are postulated PE etiologies[62]. As such, it would be interesting to investigate the effects of exercise training during normal and preeclamptic pregnancies on these systems in several tissues, including the vasculature, the placenta, the heart and the kidneys. Moreover, temporal analysis of associate markers during pregnancy will likely shed light on the progression of PE-like features and may suggest critical time points for the administration of novel treatments. For instance, iNOS expression is augmented in placentas of preeclamptic women and may be implicated in the production of oxidative stress,

via peroxynitrite formation[39, 212]. It would thus be interesting to investigate whether an increase in iNOS, normally expressed under pathological conditions, and/or a decrease in eNOS, expressed under physiological conditions, are observed in our sedentary transgenic mothers in the vasculature, the heart, the placenta and the kidney[312, 313]. This may contribute to the development of the numerous symptoms associated with PE as a result of the increased oxidative stress, as chronically elevated levels of NO, resulting from iNOS, can react with ROS to form peroxynitrite. Moreover, this increased production of ROS would be compounded by the decrease in antioxidant defense [314]. Indeed, PE is often associated with a decrease in antioxidant defences, such as catalase, GPx, SOD and glutathione S-transferase [118, 199]. In the general population, exercise training has been shown to enhance the expression of the antioxidants SOD and GPx[245-247]. It stands to reason that exercise training may also enhance their expression during PE and thus protect the mother from oxidative damage.

Moreover, an immune reaction against paternal antigens on the fetus is considered a potential disease-initiating entity in some cases of PE. A preponderance of pro-inflammatory reactions, as is the case during PE, has deleterious effects on the body, for instance causing endothelial dysfunction by targeting the coagulation pathway, vascular relaxation and oxidative stress[215, 216]. Minimizing circulating mediators like IFN-γ and IL-18, may reduce the prevalence and/or severity of PE. On the other hand, exercise training has been shown to produce anti-inflammatory effects, for instance by favouring the production of IL-6, IL-10

and IL-1RA and inhibiting that of TNF-α[248]. As such, exercise training may thus provide protection by minimizing the pro-inflammatory effects associated with PE and thus, measuring inflammatory mediators in the circulation of trained animals would be of interest[245].

The effects of exercise training on PE prevention has currently been investigated in only two animal models, both of which have been published by our laboratory[287]. In both, the effects of exercise training before and during pregnancy were explored. Determining whether these benefits arise when exercise training is initiated during pregnancy needs to be addressed as there is data which suggests that it may have negative impacts. Indeed, Yeo et al. (2008) carried out a prospective study in which the effect on PE risk of two types of exercise was investigated when initiated at 18 weeks of pregnancy[277]. Although non-significant, the walking women were more likely to develop PE then the women who followed a stretching program. Moreover, some studies have found PE risk to be increased with higher intensity exercise training that was initiated during pregnancy[273, 284]. Therefore, identifying the optimal intensity, type of exercise and frequency of training, if at all, are mandatory to determine what is beneficial for women at risk of the disease.

Several studies have found that preeclamptic women are at an increased risk of developing CVD later in life[66, 67]. It would be of value to determine if this is

the case in our animal models. Subsequently, identifying which, if any, disease promoting entities, like for example the decrease in aortic MasR and ACE2 observed among sedentary R^+A^+ mice, persist post-partum may shed light into why PE patients are more susceptible to CVD. Protecting these at-risk women from prospective CVD is a major concern that must be examined.

To date, the ACOG does not recommend that at-risk women take part in aerobic training during pregnancy[262]. Although several epidemiological studies have observed a protective effect of training on PE risk, these are considered insufficient as they are not randomized studies, the cohorts are too small and the mechanisms are ill-understood[285]. Conversely, our animal study has demonstrated that exercise training prevents the development of gestational features similar to those observed during SPE. Future studies are thus warranted to better determine the effect of exercise training in this target population. Additionally, animal studies are needed to corroborate our findings, and provide mechanistic insight into the benefits of exercise training. A clear understanding of their benefits on PE prevention will further entice research groups to carry out controlled large randomized studies on the effect of exercise training on PE risk. Without further investigation, the ACOG will not modify their guidelines concerning their exercise recommendations for at risk women.

Chapter 8 - Conclusion

Our study investigating the effects of exercise training on SPE risk is the first animal study to demonstrate the protective effect of exercise training in a known at-risk population. This animal study allowed us to solely investigate the cause and effect relationship between exercise training and PE risk, as it was possible to hold many parameters constant, including environment and genetic background. Our results have helped elucidate potential mechanisms implicated in PE as well as those involved as a result of exercise training. This study represents a milestone for the future investigation of the effects of exercise training on PE risk in humans studies.

To date, the ACOG does not recommend at-risk women to take part in aerobic training. This animal study warrants future studies be carried out to better determine the effect of training in at risk populations. The use of animals is by no means over. Investigating the mechanisms implicated, as well as the effect of exercise training during pregnancy alone are also needed to further support the recommendation of exercise training during PE. Several etiologies have been suggested to contribute to PE, and thus, each ought to be investigated to acquire a better understanding of the complexity of this disease with no known cure. Investigating the effects of exercise training in other animal models, as well as the mechanisms implicated, will also be important because of the multiple etiologies.

Reference List

1. Hossain, P., B. Kawar, and M. El Nahas, *Obesity and diabetes in the developing world--a growing challenge.* N Engl J Med, 2007. **356**(3): p. 213-5.

2. Osol, G. and M. Mandala, *Maternal uterine vascular remodeling during pregnancy.* Physiology (Bethesda), 2009. **24**: p. 58-71.

3. Demir, R., et al., *Fetal vasculogenesis and angiogenesis in human placental villi.* Acta Anat (Basel), 1989. **136**(3): p. 190-203.

4. Castellucci, M., et al., *Villous sprouting: fundamental mechanisms of human placental development.* Hum Reprod Update, 2000. **6**(5): p. 485-94.

5. Zhou, Y., et al., *Human cytotrophoblasts adopt a vascular phenotype as they differentiate. A strategy for successful endovascular invasion?* J Clin Invest, 1997. **99**(9): p. 2139-51.

6. Kaufmann, P., S. Black, and B. Huppertz, *Endovascular trophoblast invasion: implications for the pathogenesis of intrauterine growth retardation and preeclampsia.* Biol Reprod, 2003. **69**(1): p. 1-7.

7. Salamonsen, L.A., et al., *Complex regulation of decidualization: a role for cytokines and proteases--a review.* Placenta, 2003. **24 Suppl A**: p. S76-85.

8. Palmer, S.K., et al., *Quantitative estimation of human uterine artery blood flow and pelvic blood flow redistribution in pregnancy.* Obstet Gynecol, 1992. **80**(6): p. 1000-6.

9. Khong, T.Y., et al., *Inadequate maternal vascular response to placentation in pregnancies complicated by pre-eclampsia and by small-for-gestational age infants.* Br J Obstet Gynaecol, 1986. **93**(10): p. 1049-59.

10. Genbacev, O., et al., *Regulation of human placental development by oxygen tension.* Science, 1997. **277**(5332): p. 1669-72.

11. Genbacev, O., et al., *Hypoxia alters early gestation human cytotrophoblast differentiation/invasion in vitro and models the placental defects that occur in preeclampsia.* J Clin Invest, 1996. **97**(2): p. 540-50.

12. Rodesch, F., et al., *Oxygen measurements in endometrial and trophoblastic tissues during early pregnancy.* Obstet Gynecol, 1992. **80**(2): p. 283-5.

13. Datto, M.B., et al., *Transforming growth factor beta induces the cyclin-dependent kinase inhibitor p21 through a p53-independent mechanism.* Proc Natl Acad Sci U S A, 1995. **92**(12): p. 5545-9.

14. Caniggia, I., et al., *Oxygen and placental development during the first trimester: implications for the pathophysiology of pre-eclampsia.* Placenta, 2000. **21 Suppl A**: p. S25-30.

15. Hoffmann, P., J.J. Feige, and N. Alfaidy, *Expression and oxygen regulation of endocrine gland-derived vascular endothelial growth factor/prokineticin-1 and its receptors in human placenta during early pregnancy.* Endocrinology, 2006. **147**(4): p. 1675-84.

16. Jaffe, R., *First trimester utero-placental circulation: maternal-fetal interaction.* J Perinat Med, 1998. **26**(3): p. 168-74.

17. Wang, G.L. and G.L. Semenza, *General involvement of hypoxia-inducible factor 1 in transcriptional response to hypoxia.* Proc Natl Acad Sci U S A, 1993. **90**(9): p. 4304-8.

18. Dvorak, H.F., et al., *Vascular permeability factor/vascular endothelial growth factor, microvascular hyperpermeability, and angiogenesis.* Am J Pathol, 1995. **146**(5): p. 1029-39.

19. Gale, N.W. and G.D. Yancopoulos, *Growth factors acting via endothelial cell-specific receptor tyrosine kinases: VEGFs, angiopoietins, and ephrins in vascular development.* Genes Dev, 1999. **13**(9): p. 1055-66.

20. Ahmed, A., et al., *Regulation of placental vascular endothelial growth factor (VEGF) and placenta growth factor (PIGF) and soluble Flt-1 by oxygen--a review.* Placenta, 2000. **21 Suppl A**: p. S16-24.

21. Cooper, J.C., et al., *VEGF mRNA levels in placentae from pregnancies complicated by pre-eclampsia.* Br J Obstet Gynaecol, 1996. **103**(12): p. 1191-6.

22. Shah, D.M., *Role of the renin-angiotensin system in the pathogenesis of preeclampsia.* Am J Physiol Renal Physiol, 2005. **288**(4): p. F614-25.

23. Gilson, G.J., et al., *Changes in hemodynamics, ventricular remodeling, and ventricular contractility during normal pregnancy: a longitudinal study.* Obstet Gynecol, 1997. **89**(6): p. 957-62.

24. Ohkuma, H., et al., *Role of a decreased expression of the local renin-angiotensin system in the etiology of cerebral aneurysms.* Circulation, 2003. **108**(7): p. 785-7.

25. Kobori, H., et al., *The intrarenal renin-angiotensin system: from physiology to the pathobiology of hypertension and kidney disease.* Pharmacol Rev, 2007. **59**(3): p. 251-87.

26. Hagemann, A., A.H. Nielsen, and K. Poulsen, *The uteroplacental renin-angiotensin system: a review.* Exp Clin Endocrinol, 1994. **102**(3): p. 252-61.

27. Longo, L.D., *Maternal blood volume and cardiac output during pregnancy: a hypothesis of endocrinologic control.* Am J Physiol, 1983. **245**(5 Pt 1): p. R720-9.

28. Chapman, A.B., et al., *Temporal relationships between hormonal and hemodynamic changes in early human pregnancy.* Kidney Int, 1998. **54**(6): p. 2056-63.

29. Robson, S.C., et al., *Serial study of factors influencing changes in cardiac output during human pregnancy.* Am J Physiol, 1989. **256**(4 Pt 2): p. H1060-5.

30. Krane, N.K. and M. Hamrahian, *Pregnancy: kidney diseases and hypertension.* Am J Kidney Dis, 2007. **49**(2): p. 336-45.

31. Clapp, J.F., 3rd and E. Capeless, *Cardiovascular function before, during, and after the first and subsequent pregnancies.* Am J Cardiol, 1997. **80**(11): p. 1469-73.

32. Massicotte, G., et al., *Decreased in vitro responses to vasoconstrictors during gestation in normotensive and spontaneously hypertensive rats.* Can J Physiol Pharmacol, 1987. **65**(12): p. 2466-71.

33. Paller, M.S., *Mechanism of decreased pressor responsiveness to ANG II, NE, and vasopressin in pregnant rats.* Am J Physiol, 1984. **247**(1 Pt 2): p. H100-8.

34. Brosnihan, K.B., et al., *Enhanced renal immunocytochemical expression of ANG-(1-7) and ACE2 during pregnancy.* Hypertension, 2003. **42**(4): p. 749-53.

35. Yagil, Y. and C. Yagil, *Hypothesis: ACE2 modulates blood pressure in the mammalian organism.* Hypertension, 2003. **41**(4): p. 871-3.

36. Valdes, G., et al., *Distribution of angiotensin-(1-7) and ACE2 in human placentas of normal and pathological pregnancies.* Placenta, 2006. **27**(2-3): p. 200-7.

37. Fyhrquist, F. and O. Saijonmaa, *Renin-angiotensin system revisited.* J Intern Med, 2008. **264**(3): p. 224-36.

38. Santos, R.A., A.J. Ferreira, and E.S.A.C. Simoes, *Recent advances in the angiotensin-converting enzyme 2-angiotensin(1-7)-Mas axis.* Exp Physiol, 2008. **93**(5): p. 519-27.

39. Merrill, D.C., et al., *Angiotensin-(1-7) in normal and preeclamptic pregnancy.* Endocrine, 2002. **18**(3): p. 239-45.

40. Valdes, G., et al., *Utero-placental expression of angiotensin-(1-7) and ACE2 in the pregnant guinea-pig.* Reprod Biol Endocrinol, 2013. **11**: p. 5.

41. Crackower, M.A., et al., *Angiotensin-converting enzyme 2 is an essential regulator of heart function.* Nature, 2002. **417**(6891): p. 822-8.

42. Tikellis, C., et al., *Developmental expression of ACE2 in the SHR kidney: a role in hypertension?* Kidney Int, 2006. **70**(1): p. 34-41.

43. Gurley, S.B., et al., *Altered blood pressure responses and normal cardiac phenotype in ACE2-null mice.* J Clin Invest, 2006. **116**(8): p. 2218-25.

44. Sampaio, W.O., et al., *Angiotensin-(1-7) through receptor Mas mediates endothelial nitric oxide synthase activation via Akt-dependent pathways.* Hypertension, 2007. **49**(1): p. 185-92.

45. Yamaleyeva, L.M., et al., *AT1, AT2, and AT1-7 receptor expression in the uteroplacental unit of normotensive and hypertensive rats during early and late pregnancy.* Placenta, 2013. **34**(6): p. 497-502.

46. Knowles, R.G. and S. Moncada, *Nitric oxide synthases in mammals.* Biochem J, 1994. **298 (Pt 2)**: p. 249-58.

47. Hayashi, T., et al., *Basal release of nitric oxide from aortic rings is greater in female rabbits than in male rabbits: implications for atherosclerosis.* Proc Natl Acad Sci U S A, 1992. **89**(23): p. 11259-63.

48. Cockell, A.P. and L. Poston, *Flow-mediated vasodilatation is enhanced in normal pregnancy but reduced in preeclampsia.* Hypertension, 1997. **30**(2 Pt 1): p. 247-51.

49. Chen, Z., et al., *Estrogen receptor alpha mediates the nongenomic activation of endothelial nitric oxide synthase by estrogen.* J Clin Invest, 1999. **103**(3): p. 401-6.

50. Zamudio, S., et al., *Low serum estradiol and high serum progesterone concentrations characterize hypertensive pregnancies at high altitude.* J Soc Gynecol Investig, 1994. **1**(3): p. 197-205.

51. Zeisler, H., et al., *Concentrations of estrogens in patients with preeclampsia.* Wien Klin Wochenschr, 2002. **114**(12): p. 458-61.

52. Nabulsi, A.A., et al., *Association of hormone-replacement therapy with various cardiovascular risk factors in postmenopausal women. The Atherosclerosis Risk in Communities Study Investigators.* N Engl J Med, 1993. **328**(15): p. 1069-75.

53. Ganzevoort, W., et al., *Plasma volume and blood pressure regulation in hypertensive pregnancy.* J Hypertens, 2004. **22**(7): p. 1235-42.

54. Wallis, M.G., M.F. Lankford, and S.R. Keller, *Vasopressin is a physiological substrate for the insulin-regulated aminopeptidase IRAP.* Am J Physiol Endocrinol Metab, 2007. **293**(4): p. E1092-102.

55. Albiston, A.L., et al., *Evidence that the angiotensin IV (AT(4)) receptor is the enzyme insulin-regulated aminopeptidase.* J Biol Chem, 2001. **276**(52): p. 48623-6.

56. Nomura, S., et al., *Gene regulation and physiological function of placental leucine aminopeptidase/oxytocinase during pregnancy.* Biochim Biophys Acta, 2005. **1751**(1): p. 19-25.

57. Mizutani, S., et al., *Human placental leucine aminopeptidase (P-LAP) as a hypotensive agent.* Experientia, 1982. **38**(7): p. 821-2.

58. Moutquin, J.M., et al., *Report of the Canadian Hypertension Society Consensus Conference: 2. Nonpharmacologic management and prevention of hypertensive disorders in pregnancy.* CMAJ, 1997. **157**(7): p. 907-19.

59. Sibai, B.M., *Diagnosis and management of gestational hypertension and preeclampsia.* Obstet Gynecol, 2003. **102**(1): p. 181-92.

60. Irgens, H.U., et al., *Long term mortality of mothers and fathers after pre-eclampsia: population based cohort study.* BMJ, 2001. **323**(7323): p. 1213-7.

61. Brown, M.A., et al., *The classification and diagnosis of the hypertensive disorders of pregnancy: statement from the International Society for the Study of Hypertension in Pregnancy (ISSHP).* Hypertens Pregnancy, 2001. **20**(1): p. IX-XIV.

62. Sibai, B., G. Dekker, and M. Kupferminc, *Pre-eclampsia.* Lancet, 2005. **365**(9461): p. 785-99.

63. Visser, W. and H.C. Wallenburg, *Central hemodynamic observations in untreated preeclamptic patients.* Hypertension, 1991. **17**(6 Pt 2): p. 1072-7.

64. Frey, N. and E.N. Olson, *Cardiac hypertrophy: the good, the bad, and the ugly.* Annu Rev Physiol, 2003. **65**: p. 45-79.

65. Simmons, L.A., A.G. Gillin, and R.W. Jeremy, *Structural and functional changes in left ventricle during normotensive and preeclamptic pregnancy.* Am J Physiol Heart Circ Physiol, 2002. **283**(4): p. H1627-33.

66. Sattar, N. and I.A. Greer, *Pregnancy complications and maternal cardiovascular risk: opportunities for intervention and screening?* BMJ, 2002. **325**(7356): p. 157-60.

67. Bellamy, L., et al., *Pre-eclampsia and risk of cardiovascular disease and cancer in later life: systematic review and meta-analysis.* BMJ, 2007. **335**(7627): p. 974.

68. Moran, P., M.D. Lindheimer, and J.M. Davison, *The renal response to preeclampsia.* Semin Nephrol, 2004. **24**(6): p. 588-95.

69. Seitchik, J., *The metabolism of urate in pre-eclampsia.* Am J Obstet Gynecol, 1956. **72**(1): p. 40-7.

70. Kang, D.H., et al., *A role for uric acid in the progression of renal disease.* J Am Soc Nephrol, 2002. **13**(12): p. 2888-97.

71. Johnson, R.J., et al., *Is there a pathogenetic role for uric acid in hypertension and cardiovascular and renal disease?* Hypertension, 2003. **41**(6): p. 1183-90.

72. Karumanchi, S.A., et al., *Preeclampsia: a renal perspective.* Kidney Int, 2005. **67**(6): p. 2101-13.

73. Sibai, B.M., *Diagnosis, controversies, and management of the syndrome of hemolysis, elevated liver enzymes, and low platelet count.* Obstet Gynecol, 2004. **103**(5 Pt 1): p. 981-91.

74. Leduc, L., et al., *Coagulation profile in severe preeclampsia.* Obstet Gynecol, 1992. **79**(1): p. 14-8.

75. Martin, J.N., Jr., et al., *The spectrum of severe preeclampsia: comparative analysis by HELLP (hemolysis, elevated liver enzyme levels, and low*

platelet count) syndrome classification. Am J Obstet Gynecol, 1999. **180**(6 Pt 1): p. 1373-84.

76. Saftlas, A.F., et al., *Epidemiology of preeclampsia and eclampsia in the United States, 1979-1986.* Am J Obstet Gynecol, 1990. **163**(2): p. 460-5.

77. Cunningham, F.G. and D. Twickler, *Cerebral edema complicating eclampsia.* Am J Obstet Gynecol, 2000. **182**(1 Pt 1): p. 94-100.

78. Altman, D., et al., *Do women with pre-eclampsia, and their babies, benefit from magnesium sulphate? The Magpie Trial: a randomised placebo-controlled trial.* Lancet, 2002. **359**(9321): p. 1877-90.

79. Chappell, S. and L. Morgan, *Searching for genetic clues to the causes of pre-eclampsia.* Clin Sci (Lond), 2006. **110**(4): p. 443-58.

80. Myatt, L., *Placental adaptive responses and fetal programming.* J Physiol, 2006. **572**(Pt 1): p. 25-30.

81. Duley, L., *Pre-eclampsia and the hypertensive disorders of pregnancy.* Br Med Bull, 2003. **67**: p. 161-76.

82. Seckl, J.R. and M.C. Holmes, *Mechanisms of disease: glucocorticoids, their placental metabolism and fetal 'programming' of adult pathophysiology.* Nat Clin Pract Endocrinol Metab, 2007. **3**(6): p. 479-88.

83. Kearney, P.M., et al., *Global burden of hypertension: analysis of worldwide data.* Lancet, 2005. **365**(9455): p. 217-23.

84. Vatten, L.J. and R. Skjaerven, *Is pre-eclampsia more than one disease?* BJOG, 2004. **111**(4): p. 298-302.

85. Macgillivray, I., *Some observations on the incidence of pre-eclampsia.* J Obstet Gynaecol Br Emp, 1958. **65**(4): p. 536-9.

86. Redman, C.W., *Immunology of preeclampsia.* Semin Perinatol, 1991. **15**(3): p. 257-62.

87. Sibai, B.M., et al., *Risk factors for preeclampsia in healthy nulliparous women: a prospective multicenter study. The National Institute of Child Health and Human Development Network of Maternal-Fetal Medicine Units.* Am J Obstet Gynecol, 1995. **172**(2 Pt 1): p. 642-8.

88. Eskenazi, B., L. Fenster, and S. Sidney, *A multivariate analysis of risk factors for preeclampsia.* JAMA, 1991. **266**(2): p. 237-41.

89. Seidman, D.S., et al., *The effect of abortion on the incidence of pre-eclampsia.* Eur J Obstet Gynecol Reprod Biol, 1989. **33**(2): p. 109-14.

90. Robillard, P.Y., G.A. Dekker, and T.C. Hulsey, *Revisiting the epidemiological standard of preeclampsia: primigravidity or primipaternity?* Eur J Obstet Gynecol Reprod Biol, 1999. **84**(1): p. 37-41.

91. Klonoff-Cohen, H.S., et al., *An epidemiologic study of contraception and preeclampsia.* JAMA, 1989. **262**(22): p. 3143-7.

92. Feeney, J.G. and J.S. Scott, *Pre-eclampsia and changed paternity.* Eur J Obstet Gynecol Reprod Biol, 1980. **11**(1): p. 35-8.

93. Lie, R.T., et al., *Fetal and maternal contributions to risk of pre-eclampsia: population based study.* BMJ, 1998. **316**(7141): p. 1343-7.

94. Esplin, M.S., et al., *Paternal and maternal components of the predisposition to preeclampsia.* N Engl J Med, 2001. **344**(12): p. 867-72.

95. Chobanian, A.V., et al., *The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure: the JNC 7 report.* JAMA, 2003. **289**(19): p. 2560-72.

96. Chalmers, J., et al., *1999 World Health Organization-International Society of Hypertension Guidelines for the management of hypertension. Guidelines sub-committee of the World Health Organization.* Clin Exp Hypertens, 1999. **21**(5-6): p. 1009-60.

97. Collins, R. and S. MacMahon, *Blood pressure, antihypertensive drug treatment and the risks of stroke and of coronary heart disease.* Br Med Bull, 1994. **50**(2): p. 272-98.

98. Turnbull, F. and C. Blood Pressure Lowering Treatment Trialists, *Effects of different blood-pressure-lowering regimens on major cardiovascular events: results of prospectively-designed overviews of randomised trials.* Lancet, 2003. **362**(9395): p. 1527-35.

99. Rey, E. and A. Couturier, *The prognosis of pregnancy in women with chronic hypertension.* Am J Obstet Gynecol, 1994. **171**(2): p. 410-6.

100. Zetterstrom, K., et al., *Maternal complications in women with chronic hypertension: a population-based cohort study.* Acta Obstet Gynecol Scand, 2005. **84**(5): p. 419-24.

101. August, P., et al., *Longitudinal study of the renin-angiotensin-aldosterone system in hypertensive pregnant women: deviations related to the*

development of superimposed preeclampsia. Am J Obstet Gynecol, 1990. **163**(5 Pt 1): p. 1612-21.

102. Chaturvedi, S., *The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC 7): is it really practical?* Natl Med J India, 2004. **17**(4): p. 227.

103. Diav-Citrin, O., et al., *Pregnancy outcome after in utero exposure to angiotensin converting enzyme inhibitors or angiotensin receptor blockers.* Reprod Toxicol, 2011. **31**(4): p. 540-5.

104. Kessel, I., I.R. Makhoul, and P. Sujov, *Congenital hypothyroidism and nonimmune hydrops fetalis: associated?* Pediatrics, 1999. **103**(1): p. E9.

105. Kajii, T. and K. Ohama, *Androgenetic origin of hydatidiform mole.* Nature, 1977. **268**(5621): p. 633-4.

106. Sutherland, A., et al., *The indicence of severe pre-eclampsia amongst mothers and mothers-in-law of pre-eclamptics and controls.* Br J Obstet Gynaecol, 1981. **88**(8): p. 785-91.

107. Arngrimsson, R., et al., *Genetic and familial predisposition to eclampsia and pre-eclampsia in a defined population.* Br J Obstet Gynaecol, 1990. **97**(9): p. 762-9.

108. Adams, E.M. and A. Finlayson, *Familial aspects of pre-eclampsia and hypertension in pregnancy.* Lancet, 1961. **2**(7217): p. 1375-8.

109. Skjaerven, R., et al., *Recurrence of pre-eclampsia across generations: exploring fetal and maternal genetic components in a population based cohort.* BMJ, 2005. **331**(7521): p. 877.

110. Astin, M., J.R. Scott, and R.J. Worley, *Pre-eclampsia/eclampsia: a fatal father factor.* Lancet, 1981. **2**(8245): p. 533.

111. Falcao, S., et al., *Impact of diet and stress on the development of preeclampsia-like symptoms in p57kip2 mice.* Am J Physiol Heart Circ Physiol, 2009. **296**(1): p. H119-26.

112. Lachmeijer, A.M., et al., *Searching for preeclampsia genes: the current position.* Eur J Obstet Gynecol Reprod Biol, 2002. **105**(2): p. 94-113.

113. Tewksbury, D.A. and R.A. Dart, *High molecular weight angiotensinogen levels in hypertensive pregnant women.* Hypertension, 1982. **4**(5): p. 729-34.

114. Ward, K., et al., *A molecular variant of angiotensinogen associated with preeclampsia.* Nat Genet, 1993. **4**(1): p. 59-61.

115. Tempfer, C.B., et al., *An endothelial nitric oxide synthase gene polymorphism is associated with preeclampsia.* Hypertens Pregnancy, 2001. **20**(1): p. 107-18.

116. Arngrimsson, R., et al., *Evidence for a familial pregnancy-induced hypertension locus in the eNOS-gene region.* Am J Hum Genet, 1997. **61**(2): p. 354-62.

117. de Luca Brunori, I., et al., *Increased HLA-DR homozygosity associated with pre-eclampsia.* Hum Reprod, 2000. **15**(8): p. 1807-12.

118. Zusterzeel, P.L., et al., *Polymorphism in the glutathione S-transferase P1 gene and risk for preeclampsia.* Obstet Gynecol, 2000. **96**(1): p. 50-4.

119. Redman, C.W. and I.L. Sargent, *Latest advances in understanding preeclampsia.* Science, 2005. **308**(5728): p. 1592-4.

120. Saito, S., et al., *The role of the immune system in preeclampsia.* Mol Aspects Med, 2007. **28**(2): p. 192-209.

121. Poston, L., et al., *Vitamin C and vitamin E in pregnant women at risk for pre-eclampsia (VIP trial): randomised placebo-controlled trial.* Lancet, 2006. **367**(9517): p. 1145-54.

122. Villar, J., et al., *World Health Organization randomized trial of calcium supplementation among low calcium intake pregnant women.* Am J Obstet Gynecol, 2006. **194**(3): p. 639-49.

123. Haugen, M., et al., *Vitamin D supplementation and reduced risk of preeclampsia in nulliparous women.* Epidemiology, 2009. **20**(5): p. 720-6.

124. Cohen, S., D. Janicki-Deverts, and G.E. Miller, *Psychological stress and disease.* JAMA, 2007. **298**(14): p. 1685-7.

125. Knackstedt, M.K., E. Hamelmann, and P.C. Arck, *Mothers in stress: consequences for the offspring.* Am J Reprod Immunol, 2005. **54**(2): p. 63-9.

126. Klonoff-Cohen, H.S., J.L. Cross, and C.F. Pieper, *Job stress and preeclampsia.* Epidemiology, 1996. **7**(3): p. 245-9.

127. Klebanoff, M.A., P.H. Shiono, and G.G. Rhoads, *Outcomes of pregnancy in a national sample of resident physicians.* N Engl J Med, 1990. **323**(15): p. 1040-5.

128. Cnattingius, S., *The epidemiology of smoking during pregnancy: smoking prevalence, maternal characteristics, and pregnancy outcomes.* Nicotine Tob Res, 2004. **6 Suppl 2**: p. S125-40.

129. Bainbridge, S.A., E.H. Sidle, and G.N. Smith, *Direct placental effects of cigarette smoke protect women from pre-eclampsia: the specific roles of carbon monoxide and antioxidant systems in the placenta.* Med Hypotheses, 2005. **64**(1): p. 17-27.

130. Belgore, F.M., G.Y. Lip, and A.D. Blann, *Vascular endothelial growth factor and its receptor, Flt-1, in smokers and non-smokers.* Br J Biomed Sci, 2000. **57**(3): p. 207-13.

131. Chun, D., et al., *Clinical Observations on Some Aspects of Hydatidiform Moles.* J Obstet Gynaecol Br Commonw, 1964. **71**: p. 180-4.

132. Wen, S.W., et al., *Maternal morbidity and obstetric complications in triplet pregnancies and quadruplet and higher-order multiple pregnancies.* Am J Obstet Gynecol, 2004. **191**(1): p. 254-8.

133. Roberts, D.J. and M.D. Post, *The placenta in pre-eclampsia and intrauterine growth restriction.* J Clin Pathol, 2008. **61**(12): p. 1254-60.

134. Khong, T.Y., *Acute atherosis in pregnancies complicated by hypertension, small-for-gestational-age infants, and diabetes mellitus.* Arch Pathol Lab Med, 1991. **115**(7): p. 722-5.

135. Khong, T.Y., *Placental vascular development and neonatal outcome.* Semin Neonatol, 2004. **9**(4): p. 255-63.

136. Karumanchi, S.A. and Y. Bdolah, *Hypoxia and sFlt-1 in preeclampsia: the "chicken-and-egg" question.* Endocrinology, 2004. **145**(11): p. 4835-7.

137. Granger, J.P., et al., *Pathophysiology of pregnancy-induced hypertension.* Am J Hypertens, 2001. **14**(6 Pt 2): p. 178S-185S.

138. Podjarny, E., G. Losonczy, and C. Baylis, *Animal models of preeclampsia.* Semin Nephrol, 2004. **24**(6): p. 596-606.

139. Gant, N.F., et al., *A study of angiotensin II pressor response throughout primigravid pregnancy.* J Clin Invest, 1973. **52**(11): p. 2682-9.

140. Taylor, R.N., et al., *Women with preeclampsia have higher plasma endothelin levels than women with normal pregnancies.* J Clin Endocrinol Metab, 1990. **71**(6): p. 1675-7.

141. Walsh, S.W. and V.M. Parisi, *The role of arachidonic acid metabolites in preeclampsia.* Semin Perinatol, 1986. **10**(4): p. 334-55.

142. Zhou, Y., C.H. Damsky, and S.J. Fisher, *Preeclampsia is associated with failure of human cytotrophoblasts to mimic a vascular adhesion phenotype. One cause of defective endovascular invasion in this syndrome?* J Clin Invest, 1997. **99**(9): p. 2152-64.

143. Levine, R.J., et al., *Circulating angiogenic factors and the risk of preeclampsia.* N Engl J Med, 2004. **350**(7): p. 672-83.

144. Venkatesha, S., et al., *Soluble endoglin contributes to the pathogenesis of preeclampsia.* Nat Med, 2006. **12**(6): p. 642-9.

145. Zhou, C.C., et al., *Angiotensin II induces soluble fms-Like tyrosine kinase-1 release via calcineurin signaling pathway in pregnancy.* Circ Res, 2007. **100**(1): p. 88-95.

146. Lockwood, C.J., et al., *Decidual cell-expressed tissue factor in human pregnancy and its involvement in hemostasis and preeclampsia-related angiogenesis.* Ann N Y Acad Sci, 2008. **1127**: p. 67-72.

147. Maynard, S., F.H. Epstein, and S.A. Karumanchi, *Preeclampsia and angiogenic imbalance.* Annu Rev Med, 2008. **59**: p. 61-78.

148. Ng, Y.S., et al., *Identification of genes involved in VEGF-mediated vascular morphogenesis using embryonic stem cell-derived cystic embryoid bodies.* Lab Invest, 2004. **84**(9): p. 1209-18.

149. Nagamatsu, T., et al., *Cytotrophoblasts up-regulate soluble fms-like tyrosine kinase-1 expression under reduced oxygen: an implication for the placental vascular development and the pathophysiology of preeclampsia.* Endocrinology, 2004. **145**(11): p. 4838-45.

150. Shore, V.H., et al., *Vascular endothelial growth factor, placenta growth factor and their receptors in isolated human trophoblast.* Placenta, 1997. **18**(8): p. 657-65.

151. Zhou, Y., et al., *Vascular endothelial growth factor ligands and receptors that regulate human cytotrophoblast survival are dysregulated in severe preeclampsia and hemolysis, elevated liver enzymes, and low platelets syndrome.* Am J Pathol, 2002. **160**(4): p. 1405-23.

152. Hirashima, M., et al., *Trophoblast expression of fms-like tyrosine kinase 1 is not required for the establishment of the maternal-fetal interface in the mouse placenta.* Proc Natl Acad Sci U S A, 2003. **100**(26): p. 15637-42.

153. Maynard, S.E., et al., *Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia.* J Clin Invest, 2003. **111**(5): p. 649-58.

154. Lu, F., et al., *The effect of over-expression of sFlt-1 on blood pressure and the occurrence of other manifestations of preeclampsia in unrestrained conscious pregnant mice.* Am J Obstet Gynecol, 2007. **196**(4): p. 396 e1-7; discussion 396 e7.

155. Solomon, C.G. and E.W. Seely, *Preeclampsia -- searching for the cause.* N Engl J Med, 2004. **350**(7): p. 641-2.

156. Toporsian, M., et al., *A role for endoglin in coupling eNOS activity and regulating vascular tone revealed in hereditary hemorrhagic telangiectasia.* Circ Res, 2005. **96**(6): p. 684-92.

157. Santibanez, J.F., et al., *Endoglin increases eNOS expression by modulating Smad2 protein levels and Smad2-dependent TGF-beta signaling.* J Cell Physiol, 2007. **210**(2): p. 456-68.

158. ten Dijke, P., M.J. Goumans, and E. Pardali, *Endoglin in angiogenesis and vascular diseases.* Angiogenesis, 2008. **11**(1): p. 79-89.

159. Irani, R.A., et al., *The detrimental role of angiotensin receptor agonistic autoantibodies in intrauterine growth restriction seen in preeclampsia.* J Exp Med, 2009. **206**(12): p. 2809-22.

160. Deanfield, J.E., J.P. Halcox, and T.J. Rabelink, *Endothelial function and dysfunction: testing and clinical relevance.* Circulation, 2007. **115**(10): p. 1285-95.

161. Feletou, M. and P.M. Vanhoutte, *Endothelial dysfunction: a multifaceted disorder (The Wiggers Award Lecture).* Am J Physiol Heart Circ Physiol, 2006. **291**(3): p. H985-1002.

162. Baxter, R.C. and J.L. Martin, *Radioimmunoassay of growth hormone-dependent insulinlike growth factor binding protein in human plasma.* J Clin Invest, 1986. **78**(6): p. 1504-12.

163. Greer, I.A., et al., *Increased concentrations of cytokines interleukin-6 and interleukin-1 receptor antagonist in plasma of women with preeclampsia: a mechanism for endothelial dysfunction?* Obstet Gynecol, 1994. **84**(6): p. 937-40.

164. Yap, S.H., et al., *Tumor necrosis factor (TNF) inhibits interleukin (IL)-1 and/or IL-6 stimulated synthesis of C-reactive protein (CRP) and serum*

amyloid A (SAA) in primary cultures of human hepatocytes. Biochim Biophys Acta, 1991. **1091**(3): p. 405-8.

165. Grimble, R.F., *Nutritional modulation of cytokine biology.* Nutrition, 1998. **14**(7-8): p. 634-40.

166. Gerretsen, G., H.J. Huisjes, and J.D. Elema, *Morphological changes of the spiral arteries in the placental bed in relation to pre-eclampsia and fetal growth retardation.* Br J Obstet Gynaecol, 1981. **88**(9): p. 876-81.

167. Brosens, I.A., W.B. Robertson, and H.G. Dixon, *The role of the spiral arteries in the pathogenesis of preeclampsia.* Obstet Gynecol Annu, 1972. **1**: p. 177-91.

168. Brosens, J.J., R. Pijnenborg, and I.A. Brosens, *The myometrial junctional zone spiral arteries in normal and abnormal pregnancies: a review of the literature.* Am J Obstet Gynecol, 2002. **187**(5): p. 1416-23.

169. Reuvekamp, A., et al., *Selective deficit of angiogenic growth factors characterises pregnancies complicated by pre-eclampsia.* Br J Obstet Gynaecol, 1999. **106**(10): p. 1019-22.

170. Lyall, F., et al., *Suppression of serum vascular endothelial growth factor immunoreactivity in normal pregnancy and in pre-eclampsia.* Br J Obstet Gynaecol, 1997. **104**(2): p. 223-8.

171. Kupferminc, M.J., et al., *Vascular endothelial growth factor is increased in patients with preeclampsia.* Am J Reprod Immunol, 1997. **38**(4): p. 302-6.

172. Baker, P.N., et al., *Elevated serum levels of vascular endothelial growth factor in patients with preeclampsia.* Obstet Gynecol, 1995. **86**(5): p. 815-21.

173. VanWijk, M.J., et al., *Vascular function in preeclampsia.* Cardiovasc Res, 2000. **47**(1): p. 38-48.

174. Anthony, F.W., et al., *Variation in detection of VEGF in maternal serum by immunoassay and the possible influence of binding proteins.* Ann Clin Biochem, 1997. **34 (Pt 3)**: p. 276-80.

175. Kamba, T. and D.M. McDonald, *Mechanisms of adverse effects of anti-VEGF therapy for cancer.* Br J Cancer, 2007. **96**(12): p. 1788-95.

176. Knight, M., et al., *Shedding of syncytiotrophoblast microvilli into the maternal circulation in pre-eclamptic pregnancies.* Br J Obstet Gynaecol, 1998. **105**(6): p. 632-40.

177. Maynard, S.E., et al., *Soluble Fms-like tyrosine kinase 1 and endothelial dysfunction in the pathogenesis of preeclampsia.* Pediatr Res, 2005. **57**(5 Pt 2): p. 1R-7R.

178. Roberts, J.M., *Endothelial dysfunction in preeclampsia.* Semin Reprod Endocrinol, 1998. **16**(1): p. 5-15.

179. Poston, L., *Endothelial dysfunction in pre-eclampsia.* Pharmacol Rep, 2006. **58 Suppl**: p. 69-74.

180. Mehta, P.K. and K.K. Griendling, *Angiotensin II cell signaling: physiological and pathological effects in the cardiovascular system.* Am J Physiol Cell Physiol, 2007. **292**(1): p. C82-97.

181. Conrad, K.P. and D.F. Benyo, *Placental cytokines and the pathogenesis of preeclampsia.* Am J Reprod Immunol, 1997. **37**(3): p. 240-9.

182. Giardina, J.B., et al., *TNF-alpha enhances contraction and inhibits endothelial NO-cGMP relaxation in systemic vessels of pregnant rats.* Am J Physiol Regul Integr Comp Physiol, 2002. **283**(1): p. R130-43.

183. Frey, R.S., et al., *PKCzeta regulates TNF-alpha-induced activation of NADPH oxidase in endothelial cells.* Circ Res, 2002. **90**(9): p. 1012-9.

184. Marsden, P.A. and B.M. Brenner, *Transcriptional regulation of the endothelin-1 gene by TNF-alpha.* Am J Physiol, 1992. **262**(4 Pt 1): p. C854-61.

185. Poston, L., A.L. McCarthy, and J.M. Ritter, *Control of vascular resistance in the maternal and feto-placental arterial beds.* Pharmacol Ther, 1995. **65**(2): p. 215-39.

186. Pascoal, I.F., et al., *Preeclampsia selectively impairs endothelium-dependent relaxation and leads to oscillatory activity in small omental arteries.* J Clin Invest, 1998. **101**(2): p. 464-70.

187. McCarthy, A.L., et al., *Abnormal endothelial cell function of resistance arteries from women with preeclampsia.* Am J Obstet Gynecol, 1993. **168**(4): p. 1323-30.

188. Knock, G.A. and L. Poston, *Bradykinin-mediated relaxation of isolated maternal resistance arteries in normal pregnancy and preeclampsia.* Am J Obstet Gynecol, 1996. **175**(6): p. 1668-74.

189. Mills, J.L., et al., *Prostacyclin and thromboxane changes predating clinical onset of preeclampsia: a multicenter prospective study.* JAMA, 1999. **282**(4): p. 356-62.

190. Cockell, A.P., et al., *Human placental syncytiotrophoblast microvillous membranes impair maternal vascular endothelial function.* Br J Obstet Gynaecol, 1997. **104**(2): p. 235-40.

191. Savvidou, M.D., et al., *Endothelial dysfunction and raised plasma concentrations of asymmetric dimethylarginine in pregnant women who subsequently develop pre-eclampsia.* Lancet, 2003. **361**(9368): p. 1511-7.

192. Agarwal, A., S. Gupta, and R.K. Sharma, *Role of oxidative stress in female reproduction.* Reprod Biol Endocrinol, 2005. **3**: p. 28.

193. Schonfeld, P. and L. Wojtczak, *Fatty acids as modulators of the cellular production of reactive oxygen species.* Free Radic Biol Med, 2008. **45**(3): p. 231-41.

194. Chirico, S., et al., *Lipid peroxidation in hyperlipidaemic patients. A study of plasma using an HPLC-based thiobarbituric acid test.* Free Radic Res Commun, 1993. **19**(1): p. 51-7.

195. Walsh, S.W. and Y. Wang, *Secretion of lipid peroxides by the human placenta.* Am J Obstet Gynecol, 1993. **169**(6): p. 1462-6.

196. Wang, Y., S.W. Walsh, and H.H. Kay, *Placental lipid peroxides and thromboxane are increased and prostacyclin is decreased in women with preeclampsia.* Am J Obstet Gynecol, 1992. **167**(4 Pt 1): p. 946-9.

197. Wojtczak, L. and P. Schonfeld, *Effect of fatty acids on energy coupling processes in mitochondria.* Biochim Biophys Acta, 1993. **1183**(1): p. 41-57.

198. Jonk, A.M., et al., *Microvascular dysfunction in obesity: a potential mechanism in the pathogenesis of obesity-associated insulin resistance and hypertension.* Physiology (Bethesda), 2007. **22**: p. 252-60.

199. Loverro, G., et al., *Lipoperoxidation and antioxidant enzymes activity in pregnancy complicated with hypertension.* Eur J Obstet Gynecol Reprod Biol, 1996. **70**(2): p. 123-7.

200. Walsh, S.W. and Y. Wang, *Deficient glutathione peroxidase activity in preeclampsia is associated with increased placental production of thromboxane and lipid peroxides.* Am J Obstet Gynecol, 1993. **169**(6): p. 1456-61.

201. Poranen, A.K., et al., *Lipid peroxidation and antioxidants in normal and pre-eclamptic pregnancies.* Placenta, 1996. **17**(7): p. 401-5.

202. Hoffmann, D.S., et al., *Chronic tempol prevents hypertension, proteinuria, and poor feto-placental outcomes in BPH/5 mouse model of preeclampsia.* Hypertension, 2008. **51**(4): p. 1058-65.

203. Hubel, C.A., *Oxidative stress in the pathogenesis of preeclampsia.* Proc Soc Exp Biol Med, 1999. **222**(3): p. 222-35.

204. Roberts, J.M. and C.A. Hubel, *Is oxidative stress the link in the two-stage model of pre-eclampsia?* Lancet, 1999. **354**(9181): p. 788-9.

205. Anton, L. and K.B. Brosnihan, *Systemic and uteroplacental renin--angiotensin system in normal and pre-eclamptic pregnancies.* Ther Adv Cardiovasc Dis, 2008. **2**(5): p. 349-62.

206. AbdAlla, S., et al., *Increased AT(1) receptor heterodimers in preeclampsia mediate enhanced angiotensin II responsiveness.* Nat Med, 2001. **7**(9): p. 1003-9.

207. Wallukat, G., et al., *Patients with preeclampsia develop agonistic autoantibodies against the angiotensin AT1 receptor.* J Clin Invest, 1999. **103**(7): p. 945-52.

208. Dechend, R., et al., *AT1 receptor agonistic antibodies from preeclamptic patients stimulate NADPH oxidase.* Circulation, 2003. **107**(12): p. 1632-9.

209. Xia, Y., et al., *Maternal autoantibodies from preeclamptic patients activate angiotensin receptors on human trophoblast cells.* J Soc Gynecol Investig, 2003. **10**(2): p. 82-93.

210. Xia, Y., S.M. Ramin, and R.E. Kellems, *Potential roles of angiotensin receptor-activating autoantibody in the pathophysiology of preeclampsia.* Hypertension, 2007. **50**(2): p. 269-75.

211. Ferraro, Z.M., L. Gaudet, and K.B. Adamo, *The potential impact of physical activity during pregnancy on maternal and neonatal outcomes.* Obstet Gynecol Surv, 2012. **67**(2): p. 99-110.

212. Neves, L.A., et al., *ACE2 and ANG-(1-7) in the rat uterus during early and late gestation.* Am J Physiol Regul Integr Comp Physiol, 2008. **294**(1): p. R151-61.

213. Dugani, C.B. and A. Klip, *Glucose transporter 4: cycling, compartments and controversies.* EMBO Rep, 2005. **6**(12): p. 1137-42.

214. Shibata, K., et al., *P-LAP/IRAP-induced cell proliferation and glucose uptake in endometrial carcinoma cells via insulin receptor signaling.* BMC Cancer, 2007. **7**: p. 15.

215. Challis, J.R., et al., *Inflammation and pregnancy.* Reprod Sci, 2009. **16**(2): p. 206-15.

216. Schiessl, B., *Inflammatory response in preeclampsia.* Mol Aspects Med, 2007. **28**(2): p. 210-9.

217. Powe, C.E., R.J. Levine, and S.A. Karumanchi, *Preeclampsia, a disease of the maternal endothelium: the role of antiangiogenic factors and implications for later cardiovascular disease.* Circulation, 2011. **123**(24): p. 2856-69.

218. Hiby, S.E., et al., *Combinations of maternal KIR and fetal HLA-C genes influence the risk of preeclampsia and reproductive success.* J Exp Med, 2004. **200**(8): p. 957-65.

219. Parham, P., *NK cells and trophoblasts: partners in pregnancy.* J Exp Med, 2004. **200**(8): p. 951-5.

220. Hanna, J., et al., *Decidual NK cells regulate key developmental processes at the human fetal-maternal interface.* Nat Med, 2006. **12**(9): p. 1065-74.

221. Smarason, A.K., et al., *The effect of placental syncytiotrophoblast microvillous membranes from normal and pre-eclamptic women on the growth of endothelial cells in vitro.* Br J Obstet Gynaecol, 1993. **100**(10): p. 943-9.

222. von Dadelszen, P., G. Hurst, and C.W. Redman, *Supernatants from co-cultured endothelial cells and syncytiotrophoblast microvillous membranes activate peripheral blood leukocytes in vitro.* Hum Reprod, 1999. **14**(4): p. 919-24.

223. Aly, A.S., et al., *Neutrophils are stimulated by syncytiotrophoblast microvillous membranes to generate superoxide radicals in women with preeclampsia.* Am J Obstet Gynecol, 2004. **190**(1): p. 252-8.

224. Whelton, S.P., et al., *Effect of aerobic exercise on blood pressure: a meta-analysis of randomized, controlled trials.* Ann Intern Med, 2002. **136**(7): p. 493-503.

225. Fletcher, G.F., et al., *Statement on exercise: benefits and recommendations for physical activity programs for all Americans. A statement for health professionals by the Committee on Exercise and Cardiac Rehabilitation of the Council on Clinical Cardiology, American Heart Association.* Circulation, 1996. **94**(4): p. 857-62.

226. Peterson, J.A., *Get moving! Physical activity counseling in primary care.* J Am Acad Nurse Pract, 2007. **19**(7): p. 349-57.

227. Malek, A.M., S.L. Alper, and S. Izumo, *Hemodynamic shear stress and its role in atherosclerosis.* JAMA, 1999. **282**(21): p. 2035-42.

228. Leung, F.P., et al., *Exercise, vascular wall and cardiovascular diseases: an update (Part 1).* Sports Med, 2008. **38**(12): p. 1009-24.

229. Wasserman, S.M., et al., *Gene expression profile of human endothelial cells exposed to sustained fluid shear stress.* Physiol Genomics, 2002. **12**(1): p. 13-23.

230. Paffenbarger, R.S., Jr., et al., *Physical activity and incidence of hypertension in college alumni.* Am J Epidemiol, 1983. **117**(3): p. 245-57.

231. Maeda, S., et al., *Effects of exercise training of 8 weeks and detraining on plasma levels of endothelium-derived factors, endothelin-1 and nitric oxide, in healthy young humans.* Life Sci, 2001. **69**(9): p. 1005-16.

232. Smith, J.K., et al., *Long-term exercise and atherogenic activity of blood mononuclear cells in persons at risk of developing ischemic heart disease.* JAMA, 1999. **281**(18): p. 1722-7.

233. O'Connor, G.T., et al., *An overview of randomized trials of rehabilitation with exercise after myocardial infarction.* Circulation, 1989. **80**(2): p. 234-44.

234. Brown, D.A., et al., *Cardioprotection afforded by chronic exercise is mediated by the sarcolemmal, and not the mitochondrial, isoform of the KATP channel in the rat.* J Physiol, 2005. **569**(Pt 3): p. 913-24.

235. Hanley, P.J. and J. Daut, *K(ATP) channels and preconditioning: a re-examination of the role of mitochondrial K(ATP) channels and an overview of alternative mechanisms.* J Mol Cell Cardiol, 2005. **39**(1): p. 17-50.

236. Lascano, E.C., J.A. Negroni, and H.F. del Valle, *Ischemic shortening of action potential duration as a result of KATP channel opening attenuates myocardial stunning by reducing calcium influx.* Mol Cell Biochem, 2002. **236**(1-2): p. 53-61.

237. Duncker, D.J., et al., *Role of K+ ATP channels and adenosine in the regulation of coronary blood flow during exercise with normal and restricted coronary blood flow.* J Clin Invest, 1996. **97**(4): p. 996-1009.

238. Brown, D.A. and R.L. Moore, *Perspectives in innate and acquired cardioprotection: cardioprotection acquired through exercise.* J Appl Physiol, 2007. **103**(5): p. 1894-9.

239. Brown, M.J., et al., *Morbidity and mortality in patients randomised to double-blind treatment with a long-acting calcium-channel blocker or diuretic in the International Nifedipine GITS study: Intervention as a Goal in Hypertension Treatment (INSIGHT).* Lancet, 2000. **356**(9227): p. 366-72.

240. Sessa, W.C., et al., *Chronic exercise in dogs increases coronary vascular nitric oxide production and endothelial cell nitric oxide synthase gene expression.* Circ Res, 1994. **74**(2): p. 349-53.

241. Alessio, H.M. and A.H. Goldfarb, *Lipid peroxidation and scavenger enzymes during exercise: adaptive response to training.* J Appl Physiol, 1988. **64**(4): p. 1333-6.

242. Moller, P., H. Wallin, and L.E. Knudsen, *Oxidative stress associated with exercise, psychological stress and life-style factors.* Chem Biol Interact, 1996. **102**(1): p. 17-36.

243. Holloszy, J.O. and E.F. Coyle, *Adaptations of skeletal muscle to endurance exercise and their metabolic consequences.* J Appl Physiol, 1984. **56**(4): p. 831-8.

244. Hubel, C.A., et al., *Decreased transferrin and increased transferrin saturation in sera of women with preeclampsia: implications for oxidative stress.* Am J Obstet Gynecol, 1996. **175**(3 Pt 1): p. 692-700.

245. Kasapis, C. and P.D. Thompson, *The effects of physical activity on serum C-reactive protein and inflammatory markers: a systematic review.* J Am Coll Cardiol, 2005. **45**(10): p. 1563-9.

246. Sen, C.K., et al., *Skeletal muscle and liver glutathione homeostasis in response to training, exercise, and immobilization.* J Appl Physiol, 1992. **73**(4): p. 1265-72.

247. Urso, M.L. and P.M. Clarkson, *Oxidative stress, exercise, and antioxidant supplementation.* Toxicology, 2003. **189**(1-2): p. 41-54.

248. Petersen, A.M. and B.K. Pedersen, *The anti-inflammatory effect of exercise.* J Appl Physiol, 2005. **98**(4): p. 1154-62.

249. Bjornstad, H.H., et al., *Exercise training decreases plasma levels of soluble CD40 ligand and P-selectin in patients with chronic heart failure.* Eur J Cardiovasc Prev Rehabil, 2008. **15**(1): p. 43-8.

250. Adamopoulos, S., et al., *Physical training reduces peripheral markers of inflammation in patients with chronic heart failure.* Eur Heart J, 2001. **22**(9): p. 791-7.

251. Flynn, M.G. and B.K. McFarlin, *Toll-like receptor 4: link to the anti-inflammatory effects of exercise?* Exerc Sport Sci Rev, 2006. **34**(4): p. 176-81.

252. Melling, C.W., et al., *Exercise-mediated regulation of Hsp70 expression following aerobic exercise training.* Am J Physiol Heart Circ Physiol, 2007. **293**(6): p. H3692-8.

253. Locke, M., et al., *Enhanced postischemic myocardial recovery following exercise induction of HSP 72.* Am J Physiol, 1995. **269**(1 Pt 2): p. H320-5.

254. Saleh, A., et al., *Negative regulation of the Apaf-1 apoptosome by Hsp70.* Nat Cell Biol, 2000. **2**(8): p. 476-83.

255. Agarwal, D., et al., *Chronic exercise modulates RAS components and improves balance between pro- and anti-inflammatory cytokines in the brain of SHR.* Basic Res Cardiol, 2011. **106**(6): p. 1069-85.

256. Silva, D.M., et al., *Swimming training improves the vasodilator effect of angiotensin-(1-7) in the aorta of spontaneously hypertensive rat.* J Appl Physiol, 2011. **111**(5): p. 1272-7.

257. Filho, A.G., et al., *Selective increase of angiotensin(1-7) and its receptor in hearts of spontaneously hypertensive rats subjected to physical training.* Exp Physiol, 2008. **93**(5): p. 589-98.

258. Pereira, M.G., et al., *Exercise training reduces cardiac angiotensin II levels and prevents cardiac dysfunction in a genetic model of sympathetic hyperactivity-induced heart failure in mice.* Eur J Appl Physiol, 2009. **105**(6): p. 843-50.

259. Wan, W., et al., *Effect of post-myocardial infarction exercise training on the renin-angiotensin-aldosterone system and cardiac function.* Am J Med Sci, 2007. **334**(4): p. 265-73.

260. American College of, O. and Gynecologists, *Exercise during pregnancy and the postpartum period.* Clin Obstet Gynecol, 2003. **46**(2): p. 496-9.

261. Davies, G.A., et al., *Joint SOGC/CSEP clinical practice guideline: exercise in pregnancy and the postpartum period.* Can J Appl Physiol, 2003. **28**(3): p. 330-41.

262. Artal, R. and M. O'Toole, *Guidelines of the American College of Obstetricians and Gynecologists for exercise during pregnancy and the postpartum period.* Br J Sports Med, 2003. **37**(1): p. 6-12; discussion 12.

263. Clapp, J.F., *Effects of Diet and Exercise on Insulin Resistance during Pregnancy.* Metab Syndr Relat Disord, 2006. **4**(2): p. 84-90.

264. Clapp, J.F., *Influence of endurance exercise and diet on human placental development and fetal growth.* Placenta, 2006. **27**(6-7): p. 527-34.

265. Clapp, J.F., 3rd, et al., *Beginning regular exercise in early pregnancy: effect on fetoplacental growth.* Am J Obstet Gynecol, 2000. **183**(6): p. 1484-8.

266. Clapp, J.F., 3rd, *The effects of maternal exercise on fetal oxygenation and feto-placental growth.* Eur J Obstet Gynecol Reprod Biol, 2003. **110 Suppl 1**: p. S80-5.

267. Bergmann, A., M. Zygmunt, and J.F. Clapp, 3rd, *Running throughout pregnancy: effect on placental villous vascular volume and cell proliferation.* Placenta, 2004. **25**(8-9): p. 694-8.

268. Kramer, M.S. and S.W. McDonald, *Aerobic exercise for women during pregnancy.* Cochrane Database Syst Rev, 2006(3): p. CD000180.

269. Weissgerber, T.L., G.A. Davies, and J.M. Roberts, *Modification of angiogenic factors by regular and acute exercise during pregnancy.* J Appl Physiol, 2010. **108**(5): p. 1217-23.

270. Clapp, J.F., 3rd and W. Kiess, *Effects of pregnancy and exercise on concentrations of the metabolic markers tumor necrosis factor alpha and leptin.* Am J Obstet Gynecol, 2000. **182**(2): p. 300-6.

271. Marcoux, S., J. Brisson, and J. Fabia, *The effect of leisure time physical activity on the risk of pre-eclampsia and gestational hypertension.* J Epidemiol Community Health, 1989. **43**(2): p. 147-52.

272. Hegaard, H.K., et al., *Leisure time physical activity during pregnancy and impact on gestational diabetes mellitus, pre-eclampsia, preterm delivery and birth weight: a review.* Acta Obstet Gynecol Scand, 2007. **86**(11): p. 1290-6.

273. Sorensen, T.K., et al., *Recreational physical activity during pregnancy and risk of preeclampsia.* Hypertension, 2003. **41**(6): p. 1273-80.

274. Saftlas, A.F., et al., *Work, leisure-time physical activity, and risk of preeclampsia and gestational hypertension.* Am J Epidemiol, 2004. **160**(8): p. 758-65.

275. Rudra, C.B., et al., *Perceived exertion during prepregnancy physical activity and preeclampsia risk.* Med Sci Sports Exerc, 2005. **37**(11): p. 1836-41.

276. Magnus, P., et al., *Recreational physical activity and the risk of preeclampsia: a prospective cohort of Norwegian women.* Am J Epidemiol, 2008. **168**(8): p. 952-7.

277. Yeo, S., et al., *A comparison of walking versus stretching exercises to reduce the incidence of preeclampsia: a randomized clinical trial.* Hypertens Pregnancy, 2008. **27**(2): p. 113-30.

278. Hegaard, H.K., et al., *The association between leisure time physical activity in the year before pregnancy and pre-eclampsia.* J Obstet Gynaecol, 2010. **30**(1): p. 21-4.

279. Tyldum, E.V., P.R. Romundstad, and S.A. Slordahl, *Pre-pregnancy physical activity and preeclampsia risk: a prospective population-based cohort study.* Acta Obstet Gynecol Scand, 2010. **89**(3): p. 315-20.

280. Fortner, R.T., et al., *Physical activity and hypertensive disorders of pregnancy among Hispanic women.* Med Sci Sports Exerc, 2011. **43**(4): p. 639-46.

281. Rudra, C.B., et al., *A prospective analysis of recreational physical activity and preeclampsia risk.* Med Sci Sports Exerc, 2008. **40**(9): p. 1581-8.

282. Yeo, S., *Adherence to walking or stretching, and risk of preeclampsia in sedentary pregnant women.* Res Nurs Health, 2009. **32**(4): p. 379-90.

283. Vollebregt, K.C., et al., *Does physical activity in leisure time early in pregnancy reduce the incidence of preeclampsia or gestational hypertension?* Acta Obstet Gynecol Scand, 2010. **89**(2): p. 261-7.

284. Osterdal, M.L., et al., *Does leisure time physical activity in early pregnancy protect against pre-eclampsia? Prospective cohort in Danish women.* BJOG, 2009. **116**(1): p. 98-107.

285. Meher, S. and L. Duley, *Exercise or other physical activity for preventing pre-eclampsia and its complications.* Cochrane Database Syst Rev, 2006(2): p. CD005942.

286. Lindheimer, M.D., et al., *Chesley's hypertensive disorders in pregnancy*. 2nd ed. 1999, Stamford, CT: Appleton & Lange. xiv, 654 p.

287. Falcao, S., et al., *Exercise training can attenuate preeclampsia-like features in an animal model.* J Hypertens, 2010. **28**(12): p. 2446-53.

288. Ji, L.L., *Antioxidants and oxidative stress in exercise.* Proc Soc Exp Biol Med, 1999. **222**(3): p. 283-92.

289. Tsukui, S., et al., *Moderate-intensity regular exercise decreases serum tumor necrosis factor-alpha and HbA1c levels in healthy women.* Int J Obes Relat Metab Disord, 2000. **24**(9): p. 1207-11.

290. Chiarelli, P., B. Murphy, and J. Cockburn, *Acceptability of a urinary continence promotion programme to women in postpartum.* BJOG, 2003. **110**(2): p. 188-96.

291. Ishizuka, T., et al., *Endothelin-1 enhances vascular cell adhesion molecule-1 expression in tumor necrosis factor alpha-stimulated vascular endothelial cells.* Eur J Pharmacol, 1999. **369**(2): p. 237-45.

292. Liu, J.L., et al., *Chronic exercise reduces sympathetic nerve activity in rabbits with pacing-induced heart failure: A role for angiotensin II.* Circulation, 2000. **102**(15): p. 1854-62.

293. Maeda, S., et al., *Aerobic exercise training reduces plasma endothelin-1 concentration in older women.* J Appl Physiol, 2003. **95**(1): p. 336-41.

294. Falcao, S., et al., *Mice overexpressing both human angiotensinogen and human renin as a model of superimposed preeclampsia on chronic hypertension.* Hypertension, 2009. **54**(6): p. 1401-7.

295. Lambert, M.I. and T.D. Noakes, *Spontaneous running increases VO2max and running performance in rats.* J Appl Physiol, 1990. **68**(1): p. 400-3.

296. MacNeil, B. and L. Hoffman-Goetz, *Chronic exercise enhances in vivo and in vitro cytotoxic mechanisms of natural immunity in mice.* J Appl Physiol, 1993. **74**(1): p. 388-95.

297. Meka, N., et al., *Endurance exercise and resistance training in cardiovascular disease.* Ther Adv Cardiovasc Dis, 2008. **2**(2): p. 115-21.

298. Ciampone, S., et al., *Long-term exercise attenuates blood pressure responsiveness and modulates kidney angiotensin II signalling and urinary sodium excretion in SHR.* J Renin Angiotensin Aldosterone Syst, 2011. **12**(4): p. 394-403.

299. Semenza, G., *Signal transduction to hypoxia-inducible factor 1.* Biochem Pharmacol, 2002. **64**(5-6): p. 993-8.

300. Xia, Y., H.Y. Wen, and R.E. Kellems, *Angiotensin II inhibits human trophoblast invasion through AT1 receptor activation.* J Biol Chem, 2002. **277**(27): p. 24601-8.

301. Dusse, L.M., et al., *Pre-eclampsia: relationship between coagulation, fibrinolysis and inflammation.* Clin Chim Acta, 2011. **412**(1-2): p. 17-21.

302. Dechend, R., et al., *Agonistic autoantibodies to the AT1 receptor in a transgenic rat model of preeclampsia.* Hypertension, 2005. **45**(4): p. 742-6.

303. Walther, T., et al., *Angiotensin II type 1 receptor agonistic antibodies reflect fundamental alterations in the uteroplacental vasculature.* Hypertension, 2005. **46**(6): p. 1275-9.

304. Dhillion, P., et al., *IL-17-mediated oxidative stress is an important stimulator of AT1-AA and hypertension during pregnancy.* Am J Physiol Regul Integr Comp Physiol, 2012. **303**(4): p. R353-8.

305. Buitrago, M., et al., *The transcriptional repressor Nab1 is a specific regulator of pathological cardiac hypertrophy.* Nat Med, 2005. **11**(8): p. 837-44.

306. Maeda, K., et al., *Plasma brain natriuretic peptide as a biochemical marker of high left ventricular end-diastolic pressure in patients with symptomatic left ventricular dysfunction.* Am Heart J, 1998. **135**(5 Pt 1): p. 825-32.

307. Heineke, J. and J.D. Molkentin, *Regulation of cardiac hypertrophy by intracellular signalling pathways.* Nat Rev Mol Cell Biol, 2006. **7**(8): p. 589-600.

308. Carter, A.M., *Animal models of human placentation--a review.* Placenta, 2007. **28 Suppl A**: p. S41-7.

309. Lim, K.H., et al., *Human cytotrophoblast differentiation/invasion is abnormal in pre-eclampsia.* Am J Pathol, 1997. **151**(6): p. 1809-18.

310. Wong, A.Y., et al., *Maternal cardiovascular changes during pregnancy and postpartum in mice.* Am J Physiol Heart Circ Physiol, 2002. **282**(3): p. H918-25.

311. Krane, V. and C. Wanner, *Dyslipidaemia in chronic kidney disease.* Minerva Urol Nefrol, 2007. **59**(3): p. 299-316.

312. Kroncke, K.D., K. Fehsel, and V. Kolb-Bachofen, *Inducible nitric oxide synthase in human diseases.* Clin Exp Immunol, 1998. **113**(2): p. 147-56.

313. Moncada, S. and E.A. Higgs, *Endogenous nitric oxide: physiology, pathology and clinical relevance.* Eur J Clin Invest, 1991. **21**(4): p. 361-74.

314. Matsubara, K., et al., *Role of nitric oxide and reactive oxygen species in the pathogenesis of preeclampsia.* J Obstet Gynaecol Res, 2010. **36**(2): p. 239-47.