ASSESSING THE EFFECT OF EXERCISE DURING PREGNANCY ON MYOKINE RESPONSE AND PLACENTAL GROWTH AND FUNCTION *IN VITRO*

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ABSTRACT

Background: It is well established throughout the literature that regularly engaging in physical activity throughout pregnancy is associated with optimized health outcomes for both the mother and the fetus. The mediators and mechanistic pathways through which these observed exercise-induced outcomes are achieved are largely unknown. This thesis attempts to address this gap in knowledge.

Methods: The objective of the first study was to develop an exercise protocol based on the recommendations from the '2019 Canadian guideline for physical activity throughout pregnancy' and to subsequently evaluate the myokine response post-exercise. Pregnant (n=13) and non-pregnant (n=17) women performed a moderate-intensity bout of treadmill walking following which pre- and post-exercise serum for a panel of ten well-characterized myokines was analyzed. The objective of the second study was to evaluate whether acute and/or chronic exercise elicited changes in metrics of placental growth and development – thereby proposing possible mechanisms through which physical activity may be conferring health benefits to the fetus. Serum (pre- and post-exercise) collected from the first study was used to treat placental cell lines to assess the effect of acute exercise on cellular proliferation as well as nutrient transporter (GLUT1, SNAT1, FATP4) expression and localization. Term placental tissue collected from active (n=10) and non-active (n=10) participants in the PLACENTA study were used to evaluate the role of chronic exercise on changes in nutrient transporter (GLUT1, SNAT1, FATP4) expression and localization.

Results: Pregnant women from the first study exhibited higher levels of four myokines post- versus pre-exercise: FGF21, EPO, BDNF and IL-15. As for the second study, BeWo cell lines treated with serum collected from pregnant women yielded higher GLUT1 expression compared to non-pregnant serum, independently of exercise. Lastly, FATP4 expression was found to be higher in term placentas of active compared to non-active pregnant women.

Conclusion: This thesis identified four myokines that are elevated in the serum of pregnant women following a bout of acute exercise. The role of these myokines in pregnancy remains to be elucidated. Further, chronic and acute exercise are shown to alter expression of key placental macronutrient transporters.

RÉSUMÉ

Contexte: Il est bien établi dans la littérature que s'engager à faire de l'activité physique tout au long de la grossesse est associé à des résultats optimaux pour la santé de la mère et du fœtus. Les médiateurs et les mécanismes cellulaires par lesquels ces résultats, induits par l'exercice, sont observés sont en grande partie inconnus.

Méthodes: L'objectif de la première étude était de développer un protocole d'exercice basé sur les recommandations du '2019 Canadian guideline for physical activity throughout pregnancy' et d'analyser le sérum avant et après exercice pour un groupe de dix myokines bien caractérisées dans la littérature. Les femmes enceintes (n = 13) et les femmes non enceintes (n = 17) ont effectué une marche d'intensité modérée sur un tapis roulant. La deuxième étude avait comme objectif d'évaluer si l'exercice aigu et/ou chronique entraîne des modifications de certaines mesures de croissance et de développement placentaires – proposant ainsi des mécanismes par lesquels l'activité physique pourrait conférer des avantages pour la santé du fœtus. Le sérum (pré et post-exercice) recueilli lors de la première étude a été utilisé pour traiter des cellules dérivées du placenta afin d'évaluer les effets d'une session d'exercice aigu sur la prolifération cellulaire, l'expression et la localisation de transporteurs de nutriments (GLUT1, SNAT1, FATP4). Les tissus placentaires à terme recueillis auprès des participantes actives (n = 10) et non actives (n = 10) de l'étude PLACENTA ont été évalués pour déterminer des changements dans l'expression et la localisation de transporteurs de nutriments (GLUT1, SNAT1, FATP4).

Résultats: Les femmes enceintes de la première étude ont présenté des taux plus élevés de quatre myokines, FGF21, EPO, BDNF et IL-15, après comparé à avant l'exercice. En ce qui concerne la deuxième étude, les lignées cellulaires BeWo traitées avec du sérum prélevé chez des femmes enceintes ont produit une expression de GLUT1 plus élevée que celle du sérum non-enceinte, indépendamment de l'exercice. Enfin, il a été constaté que l'expression de FATP4 était plus élevée dans les placentas à terme des femmes enceintes actives par rapport aux femmes enceintes non actives.

Conclusion: Cette thèse identifie quatre myokines qui sont élevées dans le sérum des femmes enceintes après une session d'exercice à intensité modérée. Le rôle de ces myokines pendant la grossesse demeure à être évalué. De plus, il a été démontré que l'exercice chronique et aigu modifient l'expression de certains transporteurs de macronutriments du placenta.

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ABBREVIATIONS

AGA	Appropriate-for-gestational-age
ALA	Alpha-linolenic acid
ARA	Arachidonic acid
BM	Basal plasma membrane
BMI	Body mass index
BDNF	Brain-derive neurotropic factor
CT	Cytotrophoblast
DHA	Docosahexaenoic acid
DOHaD	Developmental origins of health and disease
EFA	Essential fatty acid
EVT	Extravillous trophoblast
FFA	Free fatty acids
GDM	Gestational diabetes mellitus
GH	Gestational hypertension
GWG	Gestational weight gain
IL-6	Interleukin-6
IL-15	Interleukin-15
IUGR	Intrauterine growth restriction
LA	Linoleic acid
LCPUFA	Long chain polyunsaturated fatty acids
LGA	Large-for-gestational-age
MVM	Microvillous plasma membrane
PA	Physical activity
PE	Pre-eclampsia
SPARC	Secreted protein acidic rich in cysteine
ST	Syncytiotrophoblast
TG	Triglycerides

CHAPTER 1 – THESIS OVERVIEW

1.1 INTRODUCTION AND LITERATURE REVIEW

1.1.1 PHYSICAL ACTIVITY DURING PREGNANCY

The literature consistently recommends habitual physical activity (PA) in the non-pregnant population for its various health benefits including improved cardiovascular and mental health as well as a decreased risk of developing chronic diseases, namely, diabetes mellitus, cancer and obesity [1][2]. Although there has been much debate in the past regarding the safety and risks, such as miscarriage and pre-term birth, associated with PA during pregnancy, these concerns have been unsubstantiated.

Current research strongly recommends that women engage in exercise throughout their pregnancies regardless of PA levels and body mass index (BMI) before becoming pregnant [3]. In fact, the 2019 Canadian Guideline for PA throughout pregnancy recommends that pregnant women accumulate 150 min of weekly moderate-intensity PA spread over at least three days per week [3]. The 2019 Guideline further expands on the type of PA emphasizing a combination of both aerobic and resistance training to achieve greater benefits [3]. Only women with pregnancy-related complications, some of which include pre-eclampsia (PE) and intrauterine growth restriction (IUGR), should take precaution and consult a physician before starting an exercise regimen [3]. Committing to PA is not only a modifiable risk factor for adverse pregnancy-related diseases and complications but also optimizes fetal-placental health.

1.1.1.1 IMPLICATIONS FOR THE MOTHER

Maternal health outcomes associated with PA engagement are two-fold: decreased risk of developing pregnancy-related pain or diseases and increased odds or likeliness of appropriate gestational weight gain.

Gestational diabetes mellitus (GDM), gestational hypertension (GH) and PE are some of the most well-characterized pregnancy-related disorders. Importantly, such disorders have significant implications for the health of the mother. GDM is described as the onset of hyperglycaemia at any point during pregnancy [4]. Women that acquire GDM are at an increased risk of developing type 2 diabetes mellitus and other cardiovascular morbidities post-pregnancy [5][6]. In contrast, GH and PE are classified as hypertensive disorders of pregnancy [7]. PE, defined as the onset of hypertension and proteinuria [8], occurs in 3-5% of pregnancies in developed countries [8][9]. While in Western societies we can generally attend to complications of PE and accommodate early delivery, in lower-income countries PE is said to be one of the leading causes of maternal and fetal mortality, revealing the true threat of this disease [8]. In line with findings relating to GDM, a review by Bellamy and colleagues [9] displays the increased risk for the development of cardiovascular disorders later in life for pregnant women with PE. The severity and health implications linked to pregnancy-related disorders warrants careful evaluation of one's lifestyle and subsequent adoption of protective behaviours. Research establishes the importance of PA as a protective and modifiable risk factor against GDM, GH and PE [7][10][11]. In terms of pregnancy complications, physically active pregnant women report less low back, pelvic girdle and lumbopelvic pain than their non-active counterparts, throughout pregnancy [12].

Second, engaging in regular PA while pregnant can assist in the management of excessive gestational weight gain (GWG), post-partum weight retention and subsequently decrease the risk of becoming overweight after delivery [13][14]. In 2009, the Institute of Medicine (IOM) created GWG guidelines adapted for different BMI classes [15]. Since then, research has shown that gaining above or below the recommendations, independently of pre-pregnancy BMI, can lead to adverse outcomes for the mother and the fetus. The health implications for women with discordant GWG can be grouped into two categories: labour and delivery complications and increased risk of specific disorders. For example, research shows that women who gain weight in excess during pregnancy are at an increased risk of caesarian section in addition to having delivery complications necessitating forceps or vacuum [16][17]. Furthermore, in women with a normal pre-pregnancy BMI, the duration of labour has been reported to be longer for those that gain excess weight [18]. Moreover, excessive GWG early in pregnancy can increase the risk of developing GDM and hypertensive disorders throughout gestation [19][20].

In summary, committing to PA during pregnancy has important implications for the mother, including a decreased risk of developing pregnancy-related disorders and increased odds of gaining in accordance with GWG recommendations which in turn can decrease the risk of labour and delivery complications.

1.1.1.1 IMPLICATIONS FOR THE FETUS

Maternal behaviour and lifestyle during pregnancy strongly influence the fetus' growth and development *in utero*, which successively dictates long-term health and disease risk– a concept in line with the developmental origins of health and disease (DOHaD) hypothesis. Strong

proponents of the DOHaD hypothesis emphasize the importance of an active pregnancy to optimize the life-long health of the fetus.

As explained in the previous section, PA can help women adhere to GWG guidelines. Gaining above the GWG recommendations has important implications not only for the mother but also for the fetus. Schack-Nielsen and colleagues [21] report that fetuses born to mothers who exceed GWG guidelines have a higher BMI in childhood throughout adulthood and are at greater risk of becoming obese. In parallel, research shows that fetuses whose mothers gain excess weight during pregnancy are more likely to be born large-for-gestational-age (LGA) [22] – perpetuating the intergenerational cycle of obesity [23]. Being born LGA, compared to appropriate-for-gestational-age (AGA), has been associated with higher neo-natal morbidities, such as low Apgar scores, respiratory disorders, hyperbilirubinemia, shoulder dystocia, hypoglycemia in addition to longer hospitalization time [24][25].

Besides being a factor that can influence the weight of the fetus at the time of delivery, habitual PA throughout pregnancy also contributes to its development. Horvath Marques and colleagues [26] suggest that PA during pregnancy may contribute to the optimal development of the fetus' immune and central nervous systems, thereby decreasing the risk of developing neurodevelopmental and psychiatric disorders. Relatedly, a different study conducted by May and colleagues [27] shows that infants born to mothers who engage in regular PA during pregnancy demonstrate a higher heart rate variability one-month post-birth, indicative of better autonomic cardiac function.

The fetal benefits of maternal participation in PA while pregnant are numerous and long-lasting. The main gap in the literature at present is in identifying the mechanisms through which PA acts on the fetus. It would be logical that the pathways and molecules at play as a result of

PA are shaping and interacting with the placenta, which in turn is optimizing fetal growth and health outcomes.

1.1.2 THE HUMAN PLACENTA

The placenta is the interface between mother and fetus. Among its many roles, is transportation of nutrients and oxygen to the fetus for survival, elimination of waste, and production of hormones to support pregnancy [28]. It has both a fetal- and maternal-derived surface (Figure 1A). The maternal surface is embedded in the uterine wall while the fetal surface communicates with the fetus via the umbilical arteries and vein encased within the umbilical cord [28]. For the placenta to function optimally such that it serves the fetus' needs, its development is crucial.

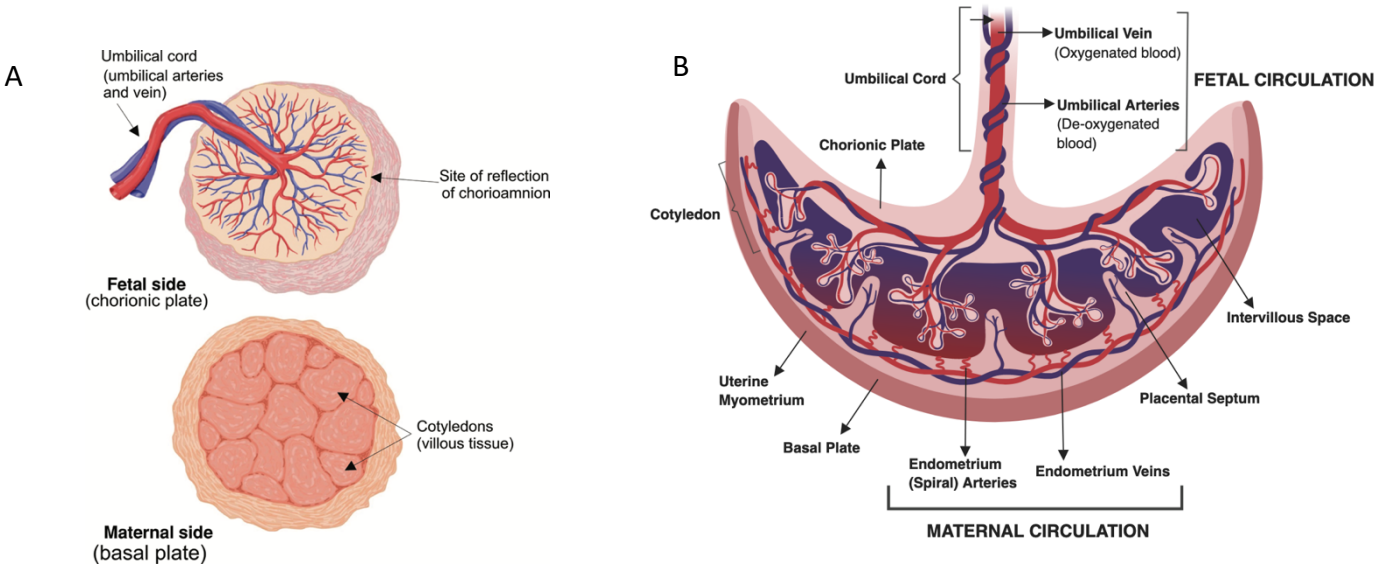

Figure 1. The human placenta. (A) Fetal and maternal placental surfaces. [Image taken from [29]; Chapter 3]. (B) Cross-section representation of the placenta. [Image created by Kelly Ann Hutchinson using ©BioRender 2019]

1.1.2.1 TROPHOBLASTIC INVASION, DIFFERENTIATION AND PROLIFERATION

As the fertilized egg travels through the fallopian tube to reach the uterus – its final destination – it undergoes many cycles of cell division until it becomes known as the blastocyst [30]. Once

the blastocyst arrives in the uterus, it is enveloped with a single layer of cells called trophoblasts and is ready for implantation within the uterine endometrium – a process heavily dependent on hormonal concentrations [30][31]. Following implantation, trophoblastic invasion and differentiation occur simultaneously to form the placenta. The trophoblast membrane differentiates into two specialized cell types: the outer syncytiotrophoblasts (ST) and the inner cytotrophoblasts (CT). The outer layer, composed of STs, fuses to form a multinucleated syncytium [31]. Both STs and CTs form the chorionic villi of the placenta, key structures that act as barriers between maternal-fetal circulation and that house fetal blood vessels.

Further differentiation and proliferation occur to form extravillous trophoblasts (EVT) – highly migratory cells that detach from placental villi [31]. There are two types of EVTs: interstitial EVTs and endovascular EVTs [31]. Interstitial EVTs can travel up to one-third of the uterine myometrium thickness, while endovascular EVTs line and cover spiral arteries [31]. EVT proliferation is crucial in ensuring proper invasion and thus the survival of the embryo.

1.1.2.2 Establishment of the Maternal-Fetal Circulation

Placental circulation is unique as it houses both maternal (uteroplacental circulation) and fetal (fetoplacental circulation) blood while keeping both separate (Figure 1B). Maternal blood originates from the spiral arteries and perfuses the intervillous spaces of the placenta where nutrient and gas exchange occur via the trophoblasts located on placental villi. Fetal arteries and veins, housed within the villi, are responsible for transport of materials to and from the fetus. For the fetus to grow and thrive within the uterine cavity, nutrients must continuously be provided to the placenta, via the maternal circulation, by crossing the chorionic villi barrier.

1.1.3 PLACENTAL MACRONUTRIENT TRANSPORT

The placenta acquires nutrients from maternal circulation through both active and passive transport mechanisms [32]. Nutrients must successfully travel across the outer most layer of the placenta – the ST membrane to subsequently reach fetal circulation [32]. The ST layer has two membranes: the microvillous plasma membrane (MVM) faces the maternal side while the basal plasma membrane (BM) is situated in closer proximity to the fetus [32]. Different classes of macronutrients (i.e., glucose, amino acids, and fatty acids) are mobilized through various transporters, embedded within both the MVM and BM of the ST layer, and are essential to support fetal development (Figure 2) [33][34]. Research suggests that, in addition to diet quality, PA may play a role in altering nutrient transporter expression and function in the placenta, thereby affecting the growth and development of the fetus [34][35].

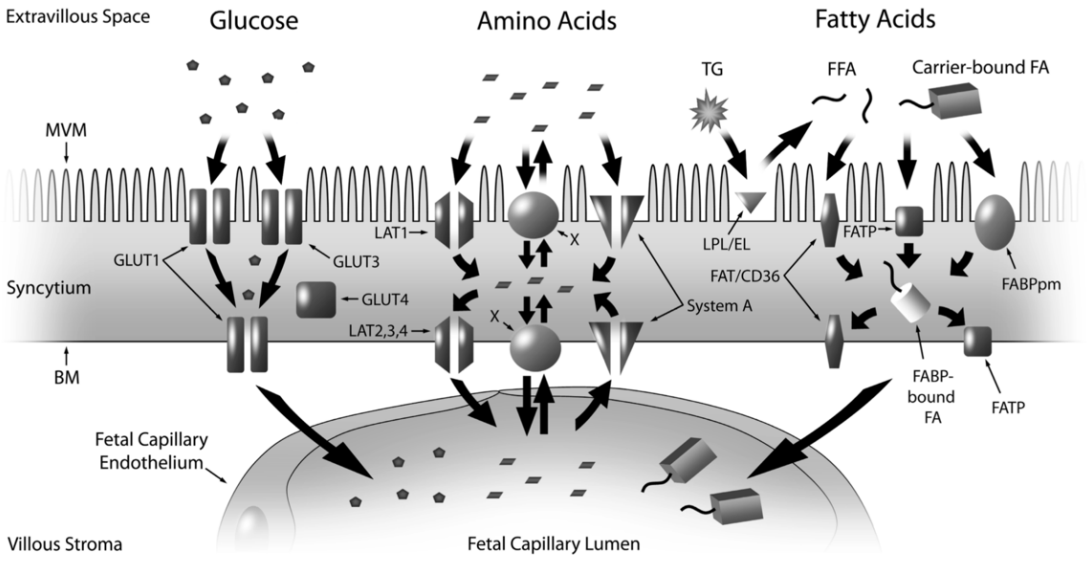

Figure 2. Representation of the syncytiotrophoblast layer and placental macronutrient transporters. MVM: microvillous membrane; BM: basal plasma membrane; GLUT1, GLUT2, GLUT3: glucose transporters; LAT1,LAT2,LAT3,LAT4: amino acid transporters; FATP: fatty acid transport protein; FABP: fatty acid binding protein; FAT/CD36: fatty acid translocase; $FABP_{pm}$: plasma membrane fatty acid binding protein; TG: triglycerides; FFA: free fatty acids; LPL: lipoprotein lipase; EL: endothelial lipase; X: amino acid exchangers. [Image taken from [36]].

1.1.3.1 GLUCOSE TRANSPORT

Glucose is the most important energy substrate for fetal and placental growth and development [32][36]. Fetal glucose is obtained through the maternal circulation and is transported by facilitated diffusion down its concentration gradient via specialized placental glucose transporters (GLUT) [36]. The GLUT family consists of fourteen isoforms, of which seven have been identified in the human placenta: GLUT1, GLUT3, GLUT4, GLUT8, GLUT9, GLUT10 and GLUT12 [37].

The GLUT-1 isoform is considered to be the primary glucose transporter in the placenta as it is highly expressed in the first and third trimester of pregnancy [36]. GLUT1 is present on both membranes of the ST layer; however, it is more prevalent on the MVM compared to the BM [36][38]. Both GLUT3 and GLUT4 are thought to be important transplacental glucose transporters in the first trimester of pregnancy as their expression is markedly reduced thereafter [36]. GLUT3 is mostly localized on the MVM membrane while GLUT4 is present in the cytosol of the ST layer [37]. Regulation of glucose transport is vital to ensure optimal maternal and fetal health outcomes. As such, over or under expression of various GLUT isoforms can lead to complications such as GDM and PE, respectively [32][37]. The previous results emphasize the importance of optimal glucose transport across and to the placenta.

1.1.3.2 AMINO ACID TRANSPORT

Amino acids are primarily necessary for protein synthesis but are also important precursors for specific molecules, such as neurotransmitters and nucleotides, in addition to being a source for carbon and nitrogen [39][33]. Unlike glucose, the concentration of amino acids is higher in fetal compared to maternal circulation [40]. Thus, the transport of amino acids to the fetus must be accomplished against a concentration gradient usually via secondary active transport [40].

Amino acid transporters can be divided into distinct categories based on substrate affinity (cationic, anionic and neutral) or sodium dependence (dependent and independent) [41]. Of the many identified classes of placental amino acid transporters, System A and L are the most well-characterized [36]. System A is comprised of three isoforms: SNAT1, SNAT2 and SNAT4, all of which are sodium-dependant and are responsible for the uptake of small, neutral amino acids including alanine and serine [42]. System A is expressed on both ST membranes although its expression is higher on the MVM compared to the BM [36].

In contrast, System L transporters function independently of sodium and transport large, neutral, branched and aromatic amino acids to the fetus [43]. The System L transporters act by exchanging non-essential amino acids, provided by System A, for essential amino acids [43]. Four isoforms of System L have been identified in the human placenta; LAT1 is found on the MVM membrane while LAT2, LAT3, and LAT4 are expressed on the BM [36].

Much like glucose transport, changes in function or expression of placental amino acid transporters leads to various health outcomes. Research shows that women with pregnancies complicated with IUGR, compared to healthy counterparts, have decreased function of System A transport [32]. The same trend has been reported for pregnant women with obesity [44]. In parallel, the placentas of fetuses with reduced growth have decreased activity of System L transporters [32]. Maternal PA is also a factor that could potentially alter amino acid transport to the fetus. Our group [34] provided preliminary evidence illustrating a 1.68-fold increase in the mRNA expression of *SNAT2* in active versus non-active pregnant women.

1.1.3.3 FATTY ACID TRANSPORT

Free fatty acids (FFA) must be metabolized from triglycerides (TG), which are present in maternal circulation, before being transported across the ST layer to the fetus [36]. Circulating

TGs are hydrolyzed into FFA by lipoprotein and endothelial lipases, both of which are expressed on the MVM [36]. Of particular importance for fetal development are essential fatty acids (EFA) [33]. There are two types of EFAs: linoleic acid (LA) and alpha-linolenic acid (ALA) [45]. ALA and LA are essential for the growth and development of the fetus as they are vital structural and functional components of cellular membranes [46]. EFAs must be processed into long-chain poly-unsaturated fatty acids (LCPUFA) such as arachidonic acid (ARA) and docosahexaenoic acid (DHA), to exhibit biological action in the fetus [46]. Both ARA and DHA play a pertinent role in the development of the fetal brain and nervous system [46].

Transport of fatty acids across the placenta to the fetus occurs primarily via four types of specialized transporters. Of the four, three are membrane-bound: those belonging to the fatty acid transport protein (FATP) family, plasma membrane fatty acid-binding protein ($FABP_{pm}$), and fatty acid translocase (FAT/CD36). The abovementioned transporters are all located on the MVM while FATP and FAT/CD36 are also expressed on the BM [36]. Amongst the FATP family, five isoforms are expressed in the placenta: FATP1, FATP2, FATP3, FATP4 and FATP6 [32]. The fourth fatty acid transporter class, fatty acid-binding protein (FABP), has five isoforms expressed in the cytosol of the ST layer; FABP1, FABP3, FABP4, FABP5 and FABP7 [32]. As with glucose and amino acid transport, changes in placental fatty acid transport has important implications for the fetus. For example, expression of the placental FABP1 isoform has been shown to be increased by 64% in pregnant women with GDM compared to controls [47]. Also, a study done in our lab shows a 2-fold decrease in the expression of the *FATP4* gene in physically active compared to non-active pregnant women [34]. Thus, these results suggest that PA decreases fatty acid transport to the fetus, possibly leading to a lower neonatal fat mass [34].

The literature consistently demonstrates that maternal behaviour and disease can alter nutrient transport to the fetus. Maternal PA is an adoptable behaviour known to optimize fetal health outcomes. Thus, it is reasonable to hypothesize that as a result of PA, mediators are being released into circulation, thereby altering and optimizing nutrient transport.

1.1.4 MYOKINES IN THE CONTEXT OF PREGNANCY

Myokines are a group of specialized cytokines and peptides released by skeletal muscle fibres most often as a result of contraction [48]. Considerable evidence from the literature has established that myokines exert beneficial effects on various tissues and organs in the human body via endocrine, autocrine and paracrine activity acting as mediators to PA (Figure 3) [48]. However, the role of myokines in human pregnancy, specifically on placental nutrient transport, has yet to be investigated. Research from our lab has proposed three ways through which myokines may potentially optimize fetal health: maternal physiology improvement, placenta development, and placental nutrient transport [49]. Identifying the role of exercise-induced myokines on placenta function is a logical next step in elucidating the benefits of PA during pregnancy. While the list of identified myokines is extensive, the next section will highlight some of the key myokines that have been established throughout the literature.

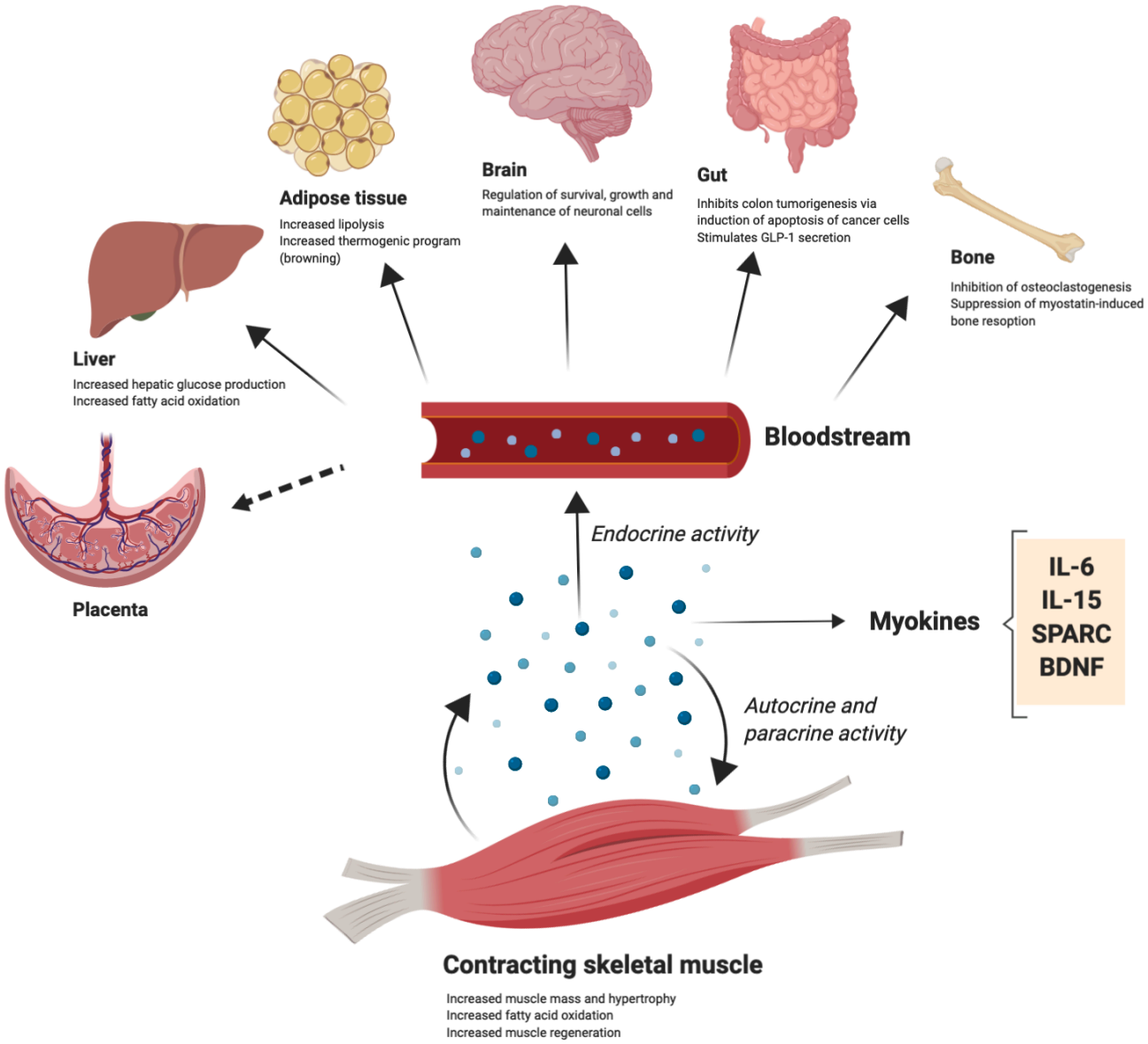

Figure 3. Representation of the synthesis and release of myokines from skeletal muscle upon contraction. Myokines may act in an autocrine, paracrine or endocrine fashion. Myokines reaching the bloodstream can act on various tissues and organs in the human body such as the liver, bones, brain, gut and adipose tissue [50][51][52][53][54][55][56][57]. The impact of myokines on placenta function, development and growth is not well understood. [Image created by Kelly Ann Hutchinson using ©BioRender 2019].

1.1.4.1 IL-6

Interleukin-6 (IL-6) is arguably the most well-characterized myokine. Before its discovery as an anti-inflammatory molecule synthesized and secreted by skeletal muscle, IL-6 was associated with obesity and found to have pro-inflammatory properties [58]. These findings lead researchers

to coin IL-6's mechanisms of action as paradoxical [58]. Since its classification as a myokine, IL-6 has been consistently shown to increase as a response to various types of exercise including resistance training and running [59][58][60][61]. Exercise-induced IL-6 is associated with an increase in fatty acid oxidation, insulin sensitivity, translocation of GLUT4 receptors to the plasma membrane and thereby increase in glucose uptake [62]. Papatheodorou and colleagues [63] have shown that in the active phase of human labour, IL-6 secretion is positively correlated with uterine contractions. In parallel, Dubé and colleagues suggest that the reduced inflammation and improved glycemic control seen in the non-pregnant population as a result of IL-6 secretion from PA, are likely to be observed in pregnant women [49]. However, very limited work investigates the role of IL-6 in human pregnancy. Specifically, the effect of exercise-induced IL-6 on placenta biology has yet to be explored.

1.1.4.2 IL-15

Interleukin-15 (IL-15) was originally identified as a mediator of the immune system as it was involved in natural killer T lymphocyte proliferation [64]. Eventually, IL-15 was also classified as a myokine due to its ability to be released by skeletal muscle as a result of PA [64]. In addition to its role with the immune system, IL-15 is involved in muscle-fat crosstalk and muscle growth [64]. While a study by Furmanczyk and Quinn [65] recognizes the potential anabolic properties of IL-15 by increasing myosin, a skeletal protein involved in stimulating muscle growth, current evidence disproves this hypothesis [66]. Rather, researchers are hypothesizing a role for IL-15 in the regulation of oxidation and fatigue in skeletal muscle [66]. In contrast, IL-15 reduces adipose tissue fat mass as it can act on the liver to decrease lipogenesis and increase fatty acid oxidation [67]. Interestingly, IL-15 mRNA is expressed in many tissues and organs in the body including the heart, lungs, and liver. However, its expression is highest in skeletal muscle

and placenta [52]. In the context of placental development, IL-15 has been shown to regulate trophoblast invasion and migration [68]. Sub-optimal invasion of placental cells can hinder implantation of the growing placenta within the uterine wall which subsequently can lead to miscarriage [69]. Additionally, insufficient invasion can lead to improper spiral artery remodelling which consequently has been associated with PE, fetal growth restriction and preterm birth [69].

1.1.4.3 SPARC

A newly discovered myokine, secreted protein acidic and rich in cysteine (SPARC) also known as osteonectin, has been shown to play a role in the suppression of colon tumorigenesis via apoptotic pathways [70]. Moreover, SPARC has been identified as a mediator in skeletal muscle regeneration, myogenesis and remodeling [50][71]. To our knowledge, our lab is the only to examine SPARC in the context of placental function or development. Serum samples obtained from active and non-active pregnant women at rest showed that of eleven myokines examined including IL-1ra, IL-6, IL-7, IL-8, IL-10, IL-13, IL-15, ANGPTL-4, MCP-1/CCL2, and Fractalkine/CX3CL1, SPARC was the only one higher in the active group (Dubé – unpublished). Additionally, we found that treating first-trimester placenta explants with SPARC *in vitro* was associated with improved placental invasion, suggesting its role in placental development. However, to date, SPARC's implication in placental function is unknown.

1.1.4.4 BDNF

In advance of brain-derived neurotropic factor (BDNF) being identified as a myokine, it had been shown to increase in brain tissue as a result of exercise and deemed a protective factor against neurodegenerative diseases [64]. Since then, studies have also identified skeletal muscle as a source for BDNF synthesis and release [72][73]. BDNF primarily ensures neuronal

maintenance and survival [74]. Matthews and colleagues [73] also found that BDNF increases fat oxidation via up-regulation of the AMPK pathway. Circulating levels of BDNF are lower in individuals with Alzheimer's disease, depression, type 2 diabetes and obesity [72]. Sakuma and colleagues [75] explain that BDNF optimizes the development, differentiation, and repair of muscle cells [64]. Moreover, exercise modality, intensity, and duration are all factors that can influence myokine synthesis and thereby release into the circulation. Short bouts of high-intensity interval training have been shown to induce a more substantial release of BNDF from skeletal muscle compared to more prolonged bouts of endurance training [76]. In animal models, research shows that offspring born from mothers that exercised throughout their pregnancy, compared to those born to sedentary mothers, report higher circulating BDNF in addition to an increase in neuronal and non-neuronal hippocampal cells [77]. With regard to the role of BDNF in the placenta, its release has been shown to promote trophoblast growth and invasion, *in vitro* [78]. Although BDNF's role in placental development has been investigated, its effect on function remains to be elucidated.

1.2 Study Rationale

The study of myokines in pregnancy is a novel research area. These exercise-induced molecules have been shown to act beneficially on various organs and tissues, from regulating neuronal growth in the brain to increasing hepatic glucose production in the liver [79]. Therefore, the procession of these beneficial effects may extend to yet another organ – the placenta. However, before examining the role of myokines on placental function and development, the myokine profile of pregnant women must first be characterized. This thesis aimed to examine the myokine response to a bout of generalizable moderate-intensity walking to identify which, if any, myokines are released in the circulation of pregnant women following exercise.

As previously discussed, engaging in PA during pregnancy confers numerous health benefits to both the mother and the fetus. Yet, the precise molecular mechanisms by which habitual maternal PA generates health benefits, specifically for the fetus, are not well understood. This thesis aimed to elucidate whether mediators present in post-exercise serum may be involved in the cross-talk between maternal PA and the placenta. Since the fetus relies entirely on the placenta for survival, growth, and development, understanding how PA impacts the growth, function, and development of the organ of pregnancy may be central in preventing pregnancy-related complications and diseases such as IUGR and PE and in improving fetal health. Nutrient transport to the fetus is arguably one of the most vital tasks of the placenta consequently gaining a better understanding of factors, such as PA, that may influence nutrient transporter expression, localization, and function is critical.

1.3 THESIS OBJECTIVES

Objective 1: Characterize the circulating myokine profile, pre- and post-exercise, of pregnant and non-pregnant women following a generalizable bout of acute moderate-intensity treadmill walking. Moderate-intensity is defined as 40-60% heart rate reserve.

Objective 2:

2.1 Assess the role of acute exercise on metrics of placental growth and function *in vitro*, including cellular proliferation as well as nutrient transporter (GLUT1, SNAT1 and FATP4) expression and localization. These experiments will be performed by treating BeWo choriocarcinoma and HTR-8/SVneo cell lines with pre- and post-exercise serum (collected in objective 1) from pregnant and non-pregnant women.

2.2 Evaluate the effect of chronic exercise on placental nutrient transporter (GLUT1, SNAT1, FATP4) expression and localization in term placenta tissue from active and non-active women.

2.1 PRESENTATION OF THESIS

The following Master of Science thesis is presented in manuscript format. **Chapter 1** provides an overview of the literature and introduces the relevant information needed to situate the existing gap in knowledge that this thesis attempts to address. **Chapter 2** is a manuscript corresponding to the first objective entitled "Examination of the myokine response in pregnant and non-pregnant women following an acute bout of moderate-intensity walking". This manuscript has been published in the journal *Frontiers in Physiology*. **Chapter 3** presents the manuscript entitled: "Physical activity engagement during pregnancy is associated to increased placental FATP4 protein expression", corresponding to the second objective of this thesis. This manuscript has been prepared for submission to the journal *Placenta*. Lastly, **Chapter 4** summarizes and discusses the collective findings of the thesis.

CHAPTER 2

PREAMBLE TO MANUSCRIPT 1

The manuscript titled: "Examination of the myokine response in pregnant and non-pregnant women following an acute bout of moderate-intensity walking" was submitted to the journal *Frontiers in Physiology – Exercise Physiology* as part of the research topic "The Role of the Muscle Secretome in Health and Disease" on April 16th, 2019. The submission ID number is 466465. The manuscript was submitted in accordance with the journal's specifications.

As of October 10th 2019, the manuscript has been *Published*.

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Examination of the Myokine Response in Pregnant and Non-pregnant Women Following an Acute Bout of Moderate-Intensity Walking

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Background: It is recommended that women accumulate 150-min of weekly moderate-intensity physical activity (MPA) when pregnant. Engaging in regular physical activity (PA) confers many health benefits to both the mother and the fetus. However, the molecular mechanisms by which these health benefits are bestowed are not well understood. One potential factor that may be contributing to the observed benefits is myokines, which are small peptides secreted by skeletal muscles. In the non-pregnant population, myokines are believed to be involved in the molecular mechanisms resulting from PA. The objective of this study was to characterize and compare the myokine profile of pregnant and non-pregnant women, after an acute bout of MPA.

Methods: Pregnant (n = 13) and non-pregnant (n = 17) women were recruited from the Ottawa region to undergo a treadmill walking session at moderate-intensity (40–60% heart rate reserve). Pre- and post-exercise serum samples were taken, and a set of 15 myokines were analyzed although only 10 were detected. IL-6 was analyzed using a high-sensitivity assay, while FGF21, EPO, BDNF, Fractalkine, IL-15, SPARC, FABP-3, FSTL-1, and oncostatin were analyzed using various multiplex assays.

Results: The pregnant and non-pregnant groups did not differ in terms of age, height, non/pre-pregnancy weight, BMI, and resting heart rate. Baseline levels of EPO and oncostatin were higher in the pregnant group while FGF21 was higher in the non-pregnant group. Circulating levels of three myokines, FGF21, EPO, and IL-15 significantly increased in response to the acute exercise in the pregnant group. Non-pregnant women exhibited an increase in three myokines, FABP-3, FSTL-1, and oncostatin, while one myokine, EPO, decreased post-exercise. SPARC, fractalkine and BDNF were shown to increase post-exercise regardless of pregnancy status while the response for BDNF was more pronounced in the non-pregnant group.

Conclusion: This is the first study examining myokine response following an acute bout of PA in pregnancy. Moderate intensity PA, which is recommended during pregnancy, elicited an increase in four myokines post-compared to pre-exercise in the pregnant group. Further research is warranted to understand the role of myokines in pregnancy.

Keywords: pregnancy, myokines, physical activity, exercise, gestational weight gain

INTRODUCTION

Contrary to outdated beliefs, evidence shows that engaging in regular physical activity (PA) during pregnancy is associated with a plethora of health benefits for both the mother and the fetus (Nascimento et al., 2012; Mudd et al., 2013). The 2019 Canadian guidelines for PA throughout pregnancy recommend that pregnant women without contraindications engage in at least 150-min moderate-intensity PA per week (Mottola et al., 2018). A combination of resistance and aerobic exercises has been deemed safe and is highly recommended to achieve greater benefits (Mottola et al., 2018). Systematic review evidence illustrates that pregnant women who are physically active compared to those that are not experience less musculoskeletal pain, gestational weight gain (GWG), postpartum weight retention, urinary incontinence as well as gestational diabetes and insulin resistance (Nascimento et al., 2012; Davenport et al., 2018a,b; Ruchat et al., 2018). Exercise interventions during pregnancy have been shown to decrease the odds of developing gestational hypertension and pre-eclampsia (Davenport et al., 2018c). Additionally, habitual PA while pregnant can decrease depressive symptoms during pregnancy and the postpartum period (Robledo-Colonia et al., 2012; Vargas-Terrones et al., 2018) and improve quality of life (Victoria et al., 2010). Alternatively, PA engagement during pregnancy may have positive downstream effects for the health of the infant (Ferraro et al., 2012). These findings are in line with the developmental origins of health and disease (DOHaD) hypothesis, suggesting that the intrauterine environment plays a critical role in determining health outcomes later in life (Wadhwa et al., 2009). Being physically active during pregnancy could contribute to optimizing the development of the fetus' immune and central nervous systems, thereby decreasing the risk of developing neurodevelopmental and psychiatric disorders (Marques et al., 2015). It is possible that maternal-fetal health benefits linked to PA may be accorded through changes in development and function of the placenta. Regular participation in PA throughout pregnancy is associated with improved placental function by way of optimizing nutrient transport to the fetus due to an increase in intervillous space blood volume (Jackson et al., 1995; Clapp, 2003).

Although it is well established that habitual PA is beneficial during pregnancy, the molecular mechanisms by which PA acts on different organs and body systems in pregnant women remain to be fully understood. It has been suggested that myokines, peptides that are synthesized by skeletal muscles and most often released in the body as a result of contraction (Pedersen et al., 2007), are responsible, in part, for the crosstalk between muscles and various organs and tissues in the body (Pedersen, 2013). The investigation of myokine response as a result of PA has been mainly conducted using male subjects. To date, the circulating myokine profile resulting from PA has yet to be examined in the context of human pregnancy. Dubé et al. (2017) postulate that myokines released during PA may play a role in the optimization of fetal and placental growth outcomes. Hundreds of these peptides have been identified, including, IL-6, IL-15, and fibroblast growth factor 21 (FGF21) (Leal et al., 2018). Myokines are involved in paracrine and endocrine signaling pathways (Pedersen, 2013), while some myokines, such as myostatin and leukemia inhibitory factor (LIF) exert their actions in an autocrine fashion upon the same muscle that synthesizes them (Pedersen, 2013; Carson, 2017). Most myokines are not exclusively produced and secreted by muscle fibers but can also be expressed or derived from other tissues or organs in the human body, such as bone, liver, adipose tissue and macrophages (Ishimi et al., 1990; García et al., 1999; Kakoti and Goswami, 2013; Salminen et al., 2017). Collectively, the myokine secretome has various functions attributable to the unique activity of each myokine. For instance, IL-6, the most well-characterized myokine, has been recognized as a key mediator of glucose metabolism as it increases insulin sensitivity (Steensberg et al., 2000). Although the function of each myokine is specific, it is suggested that there is one commonality in their roles, that of being mediators to the benefits and protective effects observed as a result of engaging in PA (Benatti and Pedersen, 2015; Whitham and Febbraio, 2016). However, the characterization of the myokine profile and thus its potential effects, in pregnant women, has yet to be investigated. This study aimed to compare the myokine response to moderate-intensity exercise between non-pregnant and pregnant women.

Abbreviations: BDNF, brain-derived neurotophic factor; BMI, body mass index; EPO, erythropoietin; EV, extracellular vesicles; FABP-3, fatty acid binding protein 3; FGF21, fibroblast growth factor 21; FSTL-1, follistatin-like 1; GDM, gestational diabetes mellitus; GLT, godin leisure time; GWG, gestational weight gain; HR_{max}, heart rate maximum; HR, heart rate; HRR, heart rate reserve; IOM, Institute of Medicine; LIF, leukemia inhibitory factor; MPA, moderate-intensity physical activity; PA, physical activity; RHR, resting heart rate; SPARC, secreted protein acidic and rich in cysteine.

MATERIALS AND METHODS

Ethics Approval and Informed Consent

This study was approved by the Research Ethics Board at the University of Ottawa (file number: H-06-18-634), and all aspects conform to the Declaration of Helsinki. Written

informed consent was obtained from each participant willing and eligible to participate.

Participants

Pregnant and non-pregnant women were recruited from the Ottawa region (ON, Canada) via recruitment flyers posted at the University of Ottawa and on social media platforms. Eligibility was confirmed via telephone by the researchers. Inclusion criteria were as follows: between 18 and 40 years of age, having a self-reported non/pre-pregnancy body mass index (BMI) classified as normal or overweight (18.5–29.9 kg/m^2) with no contraindication to exercise. Those with hypertension, diabetes, or untreated thyroid disease were excluded. Pregnant women in their second trimester (13–28 weeks gestation) were included. Participants were excluded if they were not able to complete the exercise session or if a blood sample was not obtained either pre- or post-exercise. Anthropometric measurements such as height and body weight were recorded at the time of the visit using a Tanita HR-200 wall-mounted stadiometer (Lachine, QC) and a Tanita BWB-800 scale, respectively. In the pregnant group, GWG was calculated by subtracting the weight measured at the study visit by the self-reported pre-pregnancy weight. Based on the Institute of Medicine (IOM) recommendations for GWG, women should gain a maximum of 9.7 kg in their first trimester, regardless of BMI (American College of Obstetricians and Gynecologists, 2013). Thereafter, a maximum of 2.2 and 1.5 kg of weight gain per week is recommended for women with a BMI classified as normal and overweight, respectively (American College of Obstetricians and Gynecologists, 2013). The GWG at the time of the visit in addition to gestational age of participants were used to calculate the percentage of upper-limit of weight gained in accordance to the IOM guidelines.

Exercise Protocol

Participants were asked to fast for 8 h and refrain from any PA for 12 h before the study visit. Upon arrival, participants were provided with, and asked to consume a standardized snack of approximately 340 kcal. The snack consisted of a fruit juice (orange or cranberry), a granola bar and a small fruit (apple or pear). However, we could not force a participant to eat if they chose not to. Following the snack, a 10-min seated resting phase began during which heart rate (HR) was monitored continuously and was recorded at 1-min intervals using a Polar V800 (Lachine, QC) heart rate monitor. Resting HR (RHR) was determined from the average of the last 5-min of measurements. The acute bout of exercise was conducted using a Woodway Pro XL 27 treadmill (Woodway USA, Waukesha, WI, United States) following the resting phase. Initially, participants underwent the acclimation phase starting with a warm-up for 3-min at 2.0 mph followed by an increase in the speed of 0.2 mph every minute, until the calculated moderate intensity, or 40–60% heart rate reserve (HRR), was achieved. The incline was set at 6% throughout both the acclimation and the acute bout of exercise. HRR was calculated using the Karvonen formula (Eqs. 1 and 2) (She et al., 2014).

$$\%HRR = [[HR_{max} - RHR] * \%intensity] + RHR \quad (1)$$

$$HR_{max} = 220 - age \quad (2)$$

Once the target HR intensity was met, the speed was kept constant for 30-min. HR was monitored throughout to ensure the target HR intensity zone was maintained. If HR was either below or above the desired range, the speed was adjusted by 0.2 mph accordingly. The rate of perceived exertion (RPE) was measured every 1-min during the acclimation phase and every 5-min during the acute exercise bout using the Borg Scale (Borg, 1982).

Blood Collection and Processing

A blood sample was taken pre- and post-exercise from the medial cubital vein and collected in serum blood collection tubes (#367820; BD Biosciences, Franklin Lakes, NJ) immediately before and after the completion of the exercise protocol. Serum was left to clot at room temperature for 30-min after which it was centrifuged for 15-min at 4°C at a speed of $1000 \times g$ using an Eppendorf 5702R centrifuge (Thermo Fisher Scientific Inc., Mississauga, ON, Canada). Serum samples were stored at −80°C until further analysis.

Human Myokine Assays

Serum samples from the pregnant and non-pregnant participants were assayed in duplicate for 15 myokines: apelin, brain-derived neurotrophic factor (BDNF), erythropoietin (EPO), fatty acid binding protein 3 (FABP-3), follistatin-like 1 (FSTL-1), fibroblast growth factor 21 (FGF21), fractalkine, interleukin-6 (IL-6), interleukin-15 (IL-15), irisin, LIF, myostatin, oncostatin M, osteocrin and SPARC. Of the 15 myokines analyzed, 10 were detected in our samples. Serum samples were shipped overnight, on dry ice to Eve Technologies (Calgary, AB) for analysis using the MILLIPLEX MAP Human Myokine Magnetic Bead Panel (HMYOMAG-56K, Millipore Sigma, Oakville, ON, Canada). Only four of the fifteen myokines were within the range of detection of the Milliplex assay: oncostatin, FABP-3, FSTL-1, and SPARC. Apelin, BDNF, EPO, FGF21, fractalkine, IL-6, IL-15, irisin, IF, myostatin and osteocrin were undetected in our samples. A custom high sensitivity IL-6 assay (Millipore Sigma) was conducted by Eve Technologies. Additionally, a U-PLEX Assay from Meso Scale Discoveries (MSD, Rockville, MD, United States) was used to analyze five myokines: BDNF, EPO, FGF21, Fractalkine, IL-15 while SPARC was re-analyzed using an R-PLEX assay (MSD, Rockville, MD, United States).

Statistical Analysis

The Student's t-test and the Mann-Whitney U test were used, as appropriate, to compare demographic variables and exercise session indices between pregnant and non-pregnant women. Based on the distribution of the data, either a parametric or non-parametric test was chosen. The main analysis was performed using a 2-way mixed ANOVA to assess changes in myokine

concentrations in pregnant compared to non-pregnant women following an acute bout of moderate-intensity walking (**Figure 2**). Data shown to deviate from normality following a Shapiro-Wilk test for normality were transformed using the natural logarithm. A Bonferroni *post hoc* correction for multiple comparisons was performed. Myokine data were excluded if they were not in the assay's detectable range. Pearson correlations were performed between delta change in myokine levels and BMI and age, respectively (data not shown), in addition to the exercise session indices and baseline characteristics of participants (**Figure 3**). For all statistical analyses, $p \leq 0.05$ was considered significant. Data are presented as mean ± standard deviation (SD). Data presented in **Table 1** and **Figure 3** were analyzed using GraphPad Prism Software (version 8.0.0, San Diego, CA, United States) while SPSS Software (version 13, Armonk, NY, United States) was used to analyze data in **Table 2** and **Figures 1-3**.

RESULTS

Baseline Characteristics and Exercise Parameters

In total, 13 pregnant and 17 non-pregnant women met the inclusion criteria and were included in the analysis. Baseline characteristics, such as age, height, non/pre-pregnant BMI, weight, and HR, did not differ between groups (**Table 1**). The RPE scores were compared between the pregnant and non-pregnant groups and did not differ. Thus, the perceived intensity of the exercise session was viewed equally in both groups and corresponded to moderate-intensity (Norton et al., 2010; Berghella and Saccone, 2017). Based on the same relative moderate-intensity exercise session, non-pregnant women were able to reach a significantly higher maximal ($p = 0.006$) and average speed ($p = 0.009$) compared to their pregnant counterparts (**Table 1**). Additionally, the duration of the exercise session was longer for non-pregnant compared to pregnant women ($p = 0.006$) (**Table 1**).

Comparison of Baseline Serum Myokine Levels Between Pregnant and Non-pregnant Women

The 2-way mixed ANOVA revealed differences in baseline serum myokine concentrations between the pregnant and non-pregnant groups. Of the 10 myokines, EPO ($p \leq 0.001$) and oncostatin ($p = 0.02$) were significantly increased in the pregnant group while FGF21 ($p = 0.001$) was higher in the non-pregnant group (**Figure 1**).

Myokine Serum Levels Pre- versus Post-exercise in Pregnant and Non-pregnant Groups

The 2-way mixed ANOVA revealed a significant interaction between time (pre- and post-exercise) and pregnancy status (pregnant and non-pregnant) for FGF21 ($F = 11.25$, $p = 0.002$), EPO ($F = 18.45$, $p < 0.0001$), oncostatin ($F = 20.56$, $p < 0.0001$), FABP-3 ($F = 20.29$, $p < 0.0001$), FSTL-1 ($F = 20.29$, $p = 0.002$), IL-15 ($F = 10.18$, $p = 0.003$), and BDNF ($F = 35.89$, $p < 0.0001$) (**Figure 2**). Bonferroni corrections were applied to multiple comparisons. Briefly, for the pregnant group, FGF21 ($p \leq 0.001$), EPO ($p = 0.004$), IL-15 ($p = 0.018$), and BDNF ($p = 0.025$) increased post-exercise (**Table 3**). Whereas, oncostatin ($p \leq 0.001$), FABP-3 ($p \leq 0.001$), FSTL-1 ($p \leq 0.001$), and BDNF ($p \leq 0.001$) increased post-exercise in the non-pregnant group, while EPO ($p = 0.002$) decreased post-exercise (**Table 3**). The main effect of time on SPARC ($F = 9.60$, $p = 0.005$) and fractalkine ($F = 20.6$, $p < 0.0001$) was significant: women, regardless of pregnancy status, exhibited an increase in both myokines post-exercise (**Tables 2**, **3**).

Correlations Between Maximum Speed Reached, and Weight Gained

The following indices were examined to help understand the difference in maximal and average speed reached during the exercise session in the pregnant and non-pregnant groups: age,

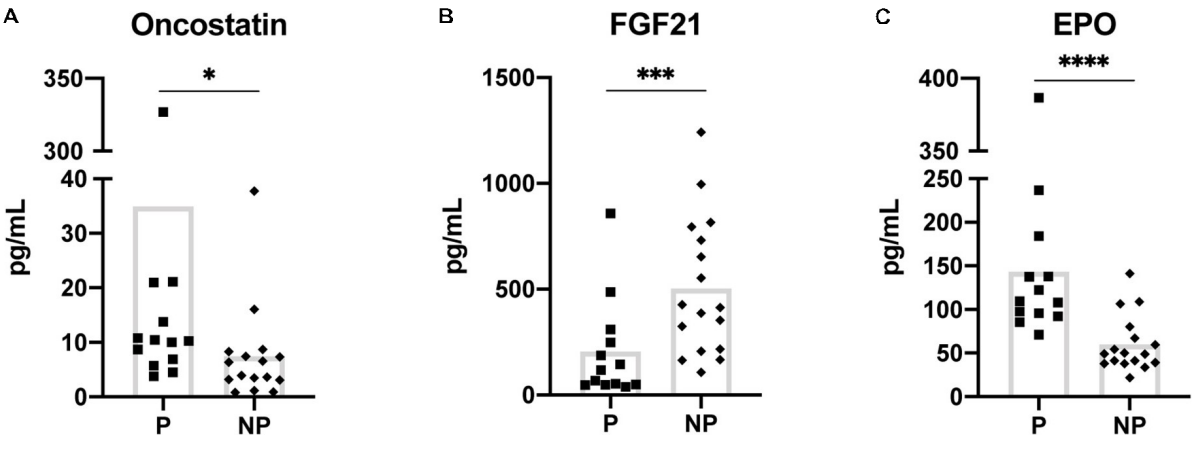

FIGURE 1 | Myokines showing a statistically significant difference in baseline (pre-exercise) serum concentration between pregnant and non-pregnant women (A–C). Means are shown with box on graphs. P, Pregnant; NP, Non-pregnant. * $p \leq 0.05$; *** $p \leq 0.001$; **** $p \leq 0.0001$.

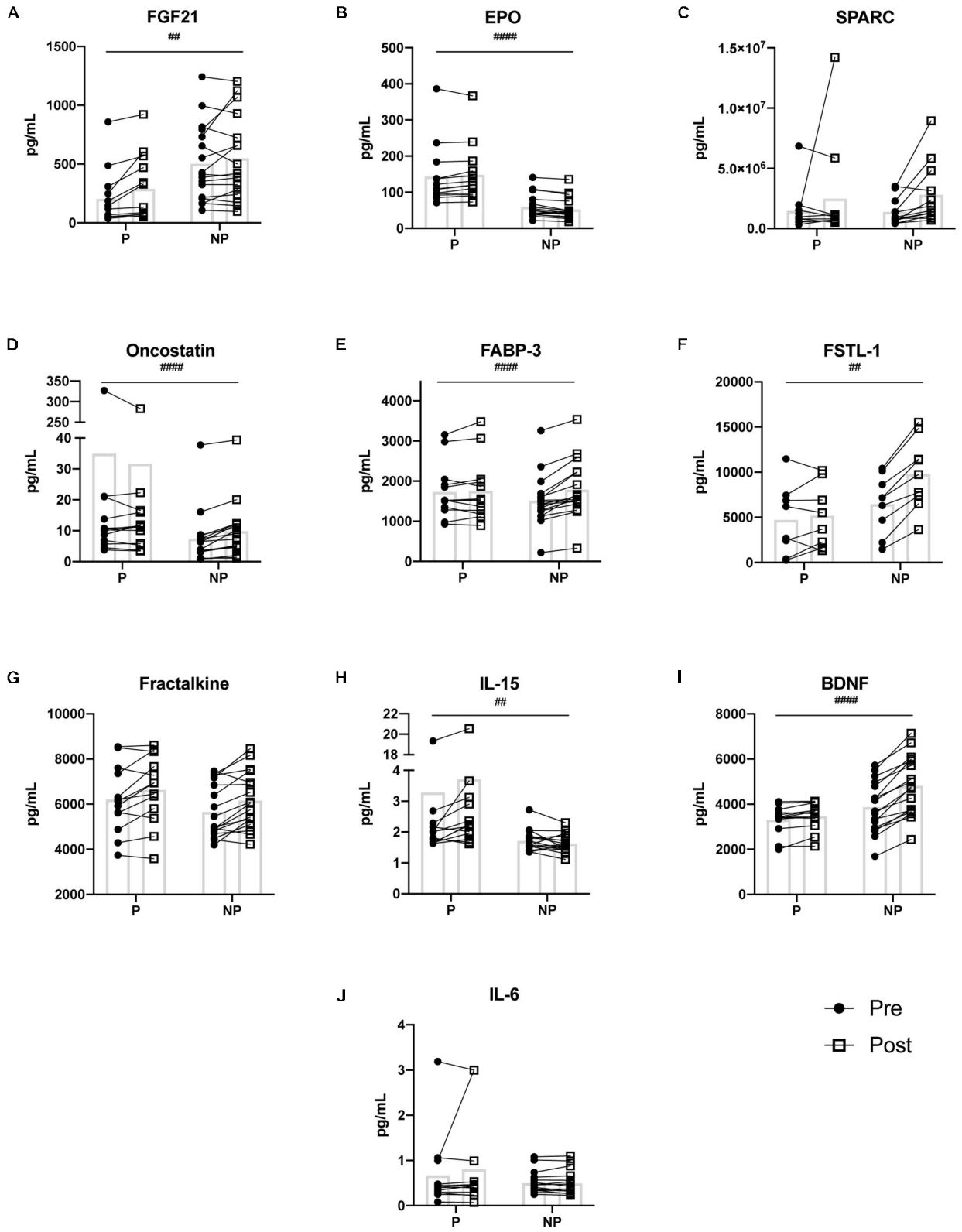

FIGURE 2 | Pre- and post-exercise serum concentrations of ten myokines (A–J) measured in serum of pregnant and non-pregnant women. Statistically significant pregnancy status by time interactions are depicted with '#' symbol. Means are shown with box on graphs. P, Pregnant; NP, Non-pregnant; Pre, Pre-exercise serum; Post, Post-exercise serum. ##$p \leq 0.01$; ###$p \leq 0.001$; ####$p \leq 0.0001$.

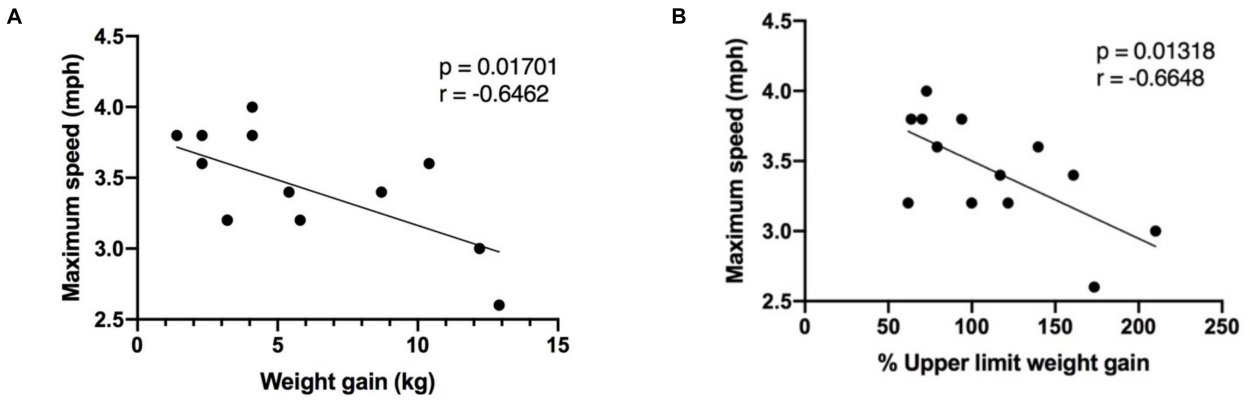

FIGURE 3 | Correlation between maximum speed reached during the exercise session, in pregnant women (n = 13), and (A) the weight gained at the time of the visit; (B) the percentage of upper limit weight gained in accordance to IOM gestational weight gain guidelines. Mph, miles per hour; kg, kilograms.

TABLE 1 | Study population demographics and exercise session indices.

	Pregnant N = 13	Non-pregnant N = 17	p-value
Age (years)	31.2 ± 3.5	30.2 ± 4.3	0.48
Gestational age (weeks)	20.1 ± 5.0	–	–
Gestational weight gain at time of session (kg)	5.8 ± 3.9	–	–
Height (cm)	166.7 ± 5.4	166.3 ± 6.3	0.87
Non/pre-pregnant BMI (kg/m^2)	23.7 ± 3.6	21.8 ± 2.3	0.09
Non/pre-pregnant body weight (kg)	63.7 ± 9.5	60.0 ± 8.4	0.27
Resting heart rate (bpm)	81.4 ± 14.6	74.3 ± 9.3	0.11
Rate of perceived exertion (Borg Scale)	12.7 ± 1.2	12.7 ± 1.3	0.90
Maximal speed reached (mph)	3.4 ± 0.4	3.8 ± 0.3	0.006*
Average speed (mph)	3.2 ±0.5	3.6 ±0.3	0.009*
Duration of exercise session (min)	40 ±2.1	42 ± 1.6	0.006*

*Values are presented as mean±standard deviation. BMI, body mass index; bpm, beats per minute; mph, miles per hour; min, minutes; * , indicates significance.*

RHR, pre-pregnancy BMI, gestational age and weight gained at the time of the visit in the pregnant group. There was an inverse correlation between weight gained (expressed both as absolute weight gained, and the percentage of upper limit weight gained according to IOM GWG guidelines) and maximal speed reached (**Figure 3**). Age, RHR, BMI, and gestational age were not significantly correlated with maximal speed reached or average speed achieved during the exercise session.

DISCUSSION

Following an acute bout of moderate-intensity walking, the concentration of three myokines; FGF21, EPO, and IL-15 significantly increased in pregnant women. The myokines FABP-3, FSTL-1, and oncostatin exhibited an increase in the non-pregnant group for the same relative intensity exercise. In contrast, EPO was the only myokine to decrease significantly post-exercise in the group of non-pregnant women. SPARC, fractalkine and BDNF were found to increase post-exercise in all women, regardless of pregnancy status. However, BDNF showed a stronger response in non-pregnant women. To our knowledge, this is the first study examining the myokine profile in pregnant women after an acute bout of exercise. Maternal, fetal, and placental health is optimized in women that engage in PA throughout their pregnancies, yet, how PA confers these observed benefits remains to be elucidated. Myokines may be one of the many mediators at play, hence, characterizing the myokine response post-exercise is an essential first step in understanding whether myokines may facilitate the health benefits resulting from PA engagement in pregnancy.

One of the myokines found to differ, FGF21, is a protein thought to regulate energy metabolism by increasing insulin sensitivity and glucose uptake in skeletal muscle and adipocytes

TABLE 2 | Myokines for which there was no statistically significant pregnancy by time interaction, but there was a statistically significant main effect of time.

			Pregnant		Non-pregnant	
Myokine	p-value	F-value	Pre-exercise mean ± SD	Post-exercise mean ± SD	Pre-exercise mean ± SD	Post-exercise mean ± SD
SPARC	0.005	9.6	1465435 ± 1846666	2491219 ± 4172003	1390862 ± 1083435	2809113 ± 2511117
Fractalkine	<0.0001	20.6	6210 ± 1481	6639 ± 1514	5658 ± 1165	6165 ± 1256

Pre- and post-exercise mean concentrations of myokines presented in pg/mL. SD, standard deviation.

TABLE 3 | Post-exercise response of myokines detected in serum of pregnant and non-pregnant women following the acute bout of treadmill walking.

Myokine	Pregnant women	Non-pregnant women	All women (regardless of pregnancy status)
FGF21	↑	–	n/a (i)
EPO	↑	↓	n/a (i)
SPARC	*n/a (n/i)*	*n/a (n/i)*	↑
Oncostatin	–	↑	n/a (i)
FABP-3	–	↑	n/a (i)
FSTL-1	–	↑	n/a (i)
Fractalkine	*n/a (n/i)*	*n/a (n/i)*	↑
IL-15	↑	–	n/a (i)
BDNF	↑	↑	n/a (i)

Post-exercise response of myokines with a statistically significant pregnancy status and time interaction with subsequent multiple comparison analysis (FGF21, EPO, Oncostatin, FABP-3, FSTL-1, IL-15, and BDNF) are depicted in bold font. Myokines without a significant interaction but with a statistically significant time effect, pre- versus post-exercise (SPARC and fractalkine), are depicted in italic font.↑, increase; ↓, decrease; –, no significant change; n/a (i), not applicable because of statistically significant interaction; n/a (n/i), not applicable because absence of statistically significant interaction.

(Ge et al., 2011; Mashili et al., 2011; Salminen et al., 2017). Based on our data illustrating an exercise-induced increase in circulating FGF21 levels in pregnant women, we hypothesize that this myokine may enhance glucose uptake and be an important mediator in decreasing the risk of developing gestational diabetes mellitus (GDM; glucose intolerance and insulin resistance in pregnancy). In support of this hypothesis, research shows that exercise interventions decrease the odds of developing GDM (Davenport et al., 2018c).

Increases in circulating EPO, a molecule known to stimulate erythropoiesis (Byts and Sirén, 2009), as a result of exercise in pregnancy, may be playing a role in blood volume adaptation. Blood volume can increase up to 50% during pregnancy compared to the pre-pregnancy period (Soma-Pillay et al., 2016). Thus, elevated EPO may be necessary to induce an increase in red blood cell production. On the contrary, BDNF is a molecule that plays an important role in the regulation of the nervous system as it is in part responsible for the maintenance, development, and survival of neuronal cells (Pedersen, 2009). Low circulating levels of BDNF have been associated with a plethora of diseases such as obesity, type 2 diabetes, depression, and cognitive impairments (Pedersen, 2009). In the context of pregnancy, low serum BDNF has been associated with antenatal depression (Fung et al., 2015) and increased risk of low birth weight (Christian et al., 2016). Increased levels of BNDF, made possible by exercise, may be important to promote the health of the nervous system.

Of the other myokines shown to increase in pregnant women post-exercise, IL-15 is known to increase trophoblast invasion and migration (Zygmunt et al., 1998). Moreover, IL-15 has been shown to play a role in muscle growth (Brandt and Pedersen, 2010) and the reduction of adipose tissue mass (Nielsen and Pedersen, 2008). Thus, increased IL-15 following exercise may help facilitate appropriate GWG corresponding to the IOM guidelines (American College of Obstetricians and Gynecologists, 2013). In summary, the increase in certain myokines following exercise may contribute to the optimization of maternal-fetal health and future work should aim to explore this hypothesis.

Brisk walking was chosen as the exercise modality for this study as it is a recommended moderate-intensity PA during pregnancy (Mottola et al., 2018). Since the walking session was of 'relative' intensity and our groups did not differ based on anthropometric and baseline characteristics (**Table 1**), it is particularly interesting that both groups demonstrated different myokine response profiles. A possible explanation as to why pregnant women are not exhibiting a change in certain circulating myokines compared to the non-pregnant group following a bout of MPA is the speed at which they were walking. Non-pregnant women were able to reach a higher maximal speed and averaged a higher speed than their non-pregnant counterparts, for the same relative intensity (**Table 1**). Furthermore, as illustrated in **Figure 3**, maximal speed reached in the pregnant group was inversely correlated to both GWG and percentage of upper-limit of recommended GWG, based on the IOM guidelines (Committee on Obstetric Practice, 2014). These results suggest that although the exercise session was of comparable relative intensity, and that the pregnant and non-pregnant groups did not differ in variables that would influence target HR zone intensity, such as age and RHR, the weight that pregnant women gain across pregnancy is likely hindering their ability to reach the higher speeds. Thus, it is possible that lower speed would translate to an insufficient muscle fiber recruitment or stimulation needed to elicit an observable change in a more substantial number of circulating myokines, in the pregnant participants. It is also conceivable that a higher intensity is necessary to stimulate myokine synthesis and release. For instance, circulating IL-6 is consistently shown to increase post-exercise, however, in this study, it remains unchanged in both the pregnant and non-pregnant groups following the walking session which may be attributed to the intensity of the exercise (Leal et al., 2018; Garneau and Aguer, 2019).

The myokine secretome is vast, complex, and the release of distinct myokines seem to be dependent on specific muscular stimulus. In light of this complexity, studies examining the same myokine report contradictory results. For instance, serum secreted protein acidic and rich in cysteine (SPARC) levels have been showed to increase following a 30-min aerobic bout of cycling in healthy young men (Aoi et al., 2013). In contrast, reports indicate no change in serum SPARC levels following a brief bout of supramaximal cycle sprint (Songsorn et al., 2017). These discrepancies are likely related to variations in experimental protocols. Exercise type or modality, intensity, and duration are all factors that could influence the rate of synthesis and thus, the release of detectable myokines in the bloodstream (Leal et al., 2018). Variables such as nutritional status, environmental conditions (Rai and Demontis, 2015) and disease states (Kurdiova et al., 2014) may also be other elements influencing myokine response. The duration of the exercise session in this study is one variable that may be contributing to the differing myokine response post-exercise between both groups. Pregnant women exercised for a significantly shorter

period (40-min vs. 42-min) compared to non-pregnant women (**Table 1**). Although this result was not by design, it indicates that the acclimation phase, during which the speed is increased every 1-min interval until the target heart rate range is met, was shorter for pregnant women who reached the desired intensity more rapidly. Thus, some myokines may require a longer duration rather than a higher intensity of exercise in order to be synthesized and released into the bloodstream.

While exercise session parameters may account for a proportion of the difference observed in the myokines released by the pregnant compared to the non-pregnant women, pregnancy-specific responses could also be at play. For instance, FGF21 increases exclusively in the pregnant group following the acute bout of exercise. Among the many physiological adaptions during pregnancy, cardiac hypertrophy occurs thereby accommodating the transient increase in blood volume (Li et al., 2012; Redondo-Angulo et al., 2017). Redondo-Angulo and colleagues explain that FGF21 is a key molecule responsible for cardiac remodeling during pregnancy. Thus, the increase of some and lack of change in other myokines in the pregnant compared to the non-pregnant women may have implications related to the physiological adaptations required as a result of pregnancy.

We also compared baseline myokine levels in the pregnant and the non-pregnant participants, as differences in baseline concentrations could potentially help clarify the main result. Of the 10 myokines studied, three demonstrated differences between pregnant and non-pregnant at baseline. Interestingly, pregnant participants had higher baseline levels of EPO and oncostatin, while FGF21 was lower. Regarding oncostatin, our results are consistent with research by Ogata et al. (2000) who demonstrated a higher level of serum oncostatin in pregnant versus non-pregnant women, attributed to production by decidual and chorionic tissues (Ogata et al., 2000). In line with these findings, oncostatin has been identified as a member of a cytokine family that plays a role in cellular differentiation (Rose and Bruce, 1991), a vital cellular process during pregnancy (Malassiné and Cronier, 2002) which could clarify the increased levels observed in pregnant individuals. Also, the known elevation in blood volume during pregnancy provides logical reasoning for higher levels of EPO in pregnant women. In brief, although myokines are synthesized and secreted by skeletal muscle; similar molecules are also regulated via other tissues and organs, regardless of PA engagement. Likewise, cardiovascular adaptions during pregnancy are particularly relevant when considering serum myokine levels. Blood volume increases disproportionately during pregnancy as plasma volume exhibits a more significant increase compared to red blood cell mass, creating a concept known as hemodilution (Costantine, 2014; Soma-Pillay et al., 2016). Thus, it is possible that blood volume adaptations and fluctuations in the serum proteome across gestation may influence observed myokine levels in pregnant women (Romero et al., 2017).

Pregnancy is undoubtedly a critical period for the health of both the fetus and the mother. While it is well established that maternal PA is essential for the short- and long- term health of mom and baby, the pathways and mediators involved in the crosstalk between skeletal muscle, the placenta and subsequently the fetus are mostly unknown. The results of this study propose four possible mediators: FGF21, EPO, BDNF, and IL-15. Recognizing the preliminary nature of this work focusing on a panel of well-characterized myokines, other myokines are likely being released during pregnancy that were not examined, and various exercise parameters may elicit differing circulatory responses in this particular population.

Limitations

Although our study had a similar sample size to other studies examining myokines post-exercise, such as six (Steensberg et al., 2002) and nine males (Aoi et al., 2013), given the inter-individual variability in our pregnant group, a larger sample size could potentially translate to differences in myokines that remained unchanged. However, the lack of information and published results on myokines in pregnant women did not allow us to perform a power calculation to determine an appropriate sample size. Additionally, a measure of fitness would have been helpful in providing insight as to the role of chronic exercise on myokine release. However, obtaining such a measure in our pregnant population would have required multiple visits and in turn render the study less feasible.

Future Directions

Our results warrant further investigation of the role of myokines in pregnancy. As this was an initial characterization of the myokine profile following a single bout of moderate-intensity walking, follow-up work is needed to understand whether myokine signaling is a vital part of placental and fetal health optimization. Future studies should aim to investigate whether different exercise modalities, such as cycling, swimming, or resistance training, which are all deemed safe during pregnancy, influence the myokine response in pregnant women. The exercise duration chosen for this study was based on current guidelines for PA during pregnancy (Mottola et al., 2018) and ranged from 36 to 43 min for the pregnant participants. Longer exercise durations or bouts of higher intensity may elicit different responses. Examination of the relationship between objectively measured PA level (volume) during pregnancy and the myokine response would also be valuable. Further exploration of these variables would allow us to identify if prior fitness level or chronic exposure to exercise influences circulating myokines following PA.

CONCLUSION

This novel study found that walking at moderate-intensity between 36 and 43 min elicited a change in four of the ten myokines measured in the pregnant participants and five of the ten myokines in non-pregnant controls, while two myokines increased post-exercise regardless of pregnancy status. Future studies should aim to explore whether the myokines shown to

be elevated post-exercise in the pregnant group of this study are involved in the molecular mechanism by which maternal, fetal and placental health is optimized as a result of PA engagement.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the manuscript/supplementary files.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of the Research Ethics Board at the University of Ottawa (file number: H-06-18-634) with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by Research Ethics Board at the University of Ottawa.

AUTHOR CONTRIBUTIONS

KH drafted the manuscript. KH and SM primarily performed data collection and designed the study. KM, LG, and CA secondarily performed the data collection. All authors contributed to the design of the study, revised and edited the manuscript, and read and approved the final version of the manuscript.

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REFERENCES

American College of Obstetricians and Gynecologists (2013). ACOG committee opinion no. 548: weight gain during pregnancy. *Obstet. Gynecol.* 121, 210–212. doi: 10.1097/01.aog.0000425668.87506.4c

Aoi, W., Naito, Y., Takagi, T., Tanimura, Y., Takanami, Y., Kawai, Y., et al. (2013). A novel myokine, secreted protein acidic and rich in cysteine (SPARC), suppresses colon tumorigenesis via regular exercise. *Gut* 62, 882–889. doi: 10.1136/gutjnl-2011-300776

Benatti, F. B., and Pedersen, B. K. (2015). Exercise as an anti-inflammatory therapy for rheumatic diseases - myokine regulation. *Nat. Rev. Rheumatol.* 11, 86–97. doi: 10.1038/nrrheum.2014.193

Berghella, V., and Saccone, G. (2017). Exercise in pregnancy. *Am. J. Obstet. Gynecol.* 216, 335–337. doi: 10.1016/j.csm.2017.05.005

Borg, G. A. (1982). Psychophysical bases of perceived exertion. *Med. Sci. Sports Exerc.* 14, 377–382.

Brandt, C., and Pedersen, B. K. (2010). The role of exercise-induced myokines in muscle homeostasis and the defense against chronic diseases. *J. Biomed. Biotechnol.* 2010:520258. doi: 10.1155/2010/520258

Byts, N., and Sirén, A.-L. (2009). Erythropoietin: a multimodal neuroprotective agent. *Exp. Transl. Stroke Med.* 1, 1–10. doi: 10.1186/2040-7378-1-4

Carson, B. P. (2017). The potential role of contraction-induced myokines in the regulation of metabolic function for the prevention and treatment of type 2 diabetes. *Front. Endocrinol.* 8:97. doi: 10.3389/fendo.2017.00097

Christian, L. M., Mitchell, A. M., Gillespie, S. L., and Palettas, M. (2016). Serum brain-derived neurotrophic factor (BDNF) across pregnancy and postpartum: associations with race, depressive symptoms, and low birth weight. *Psychoneuroendocrinology* 74, 69–76. doi: 10.1016/j.psyneuen.2016.08.025

Clapp, J. F. (2003). The effects of maternal exercise on fetal oxygenation and feto-placental growth. *Eur. J. Obstet. Gynecol. Reprod. Biol.* 110(Suppl.), 80–85. doi: 10.1016/S0301-2115(03)00176-3

Committee on Obstetric Practice (2014). Committee opinion. *Female Pelvic Med. Reconstr. Surg.* 20, 245–247. doi: 10.1097/SPV.0000000000000113

Costantine, M. M. (2014). Physiologic and pharmacokinetic changes in pregnancy. *Front. Pharmacol.* 5:65. doi: 10.3389/fphar.2014.00065

Davenport, M. H., Marchand, A., Mottola, M. F., Poitras, V. J., Gray, C. E., Garcia, A. J., et al. (2018a). Exercise for the prevention and treatment of low back, pelvic girdle and lumbopelvic pain during pregnancy: a systematic review and meta-analysis. *Br. J. Sports Med.* 53, 90–98. doi: 10.1136/bjsports-2018-099400

Davenport, M. H., Nagpal, T. S., Mottola, M. F., Skow, R. J., Riske, L., Poitras, V., et al. (2018b). Prenatal exercise (including but not limited to pelvic floor muscle training) and urinary incontinence during and following pregnancy: a systematic review and meta-analysis. *Br. J. Sports Med.* 52, 1397–1404. doi: 10.1136/bjsports-2018-099780

Davenport, M. H., Ruchat, S. M., Poitras, V. J., Jaramillo Garcia, A., Gray, C. E., and Barrowman, N. (2018c). Prenatal exercise for the prevention of gestational diabetes mellitus and hypertensive disorders of pregnancy: a systematic review and meta-analysis. *Br. J. Sports Med.* 52, 1367–1375. doi: 10.1136/bjsports-2018-099355

Dubé, C., Aguer, C., Adamo, K., and Bainbridge, S. (2017). A role for maternally derived myokines to optimize placental function and fetal growth across gestation. *Appl. Physiol. Nutr. Metab.* 42, 459–469. doi: 10.1139/apnm-2016-0446

Ferraro, Z. M., Gaudet, L., and Adamo, K. B. (2012). The potential impact of physical activity during pregnancy on maternal and neonatal outcomes. *Obstet. Gynecol. Surv.* 67, 99–110. doi: 10.1097/OGX.0b013e318242030e

Fung, J., Gelaye, B., Zhong, Q. Y., Rondon, M. B., Sanchez, S. E., Barrios, Y. V., et al. (2015). Association of decreased serum brain-derived neurotrophic factor (BDNF) concentrations in early pregnancy with antepartum depression. *BMC Psychiatry* 15:43. doi: 10.1186/s12888-015-0428-7

García, J. E. L., Rodríguez, F. M., de Cabo, M. R. M., Salgado, M. J. G., Losada, J. P., Villarón, L. G., et al. (1999). Evaluation of inflammatory cytokine secretion by human alveolar macrophages. *Mediators Inflamm.* 8, 43–51. doi: 10.1080/09629359990711

Garneau, L., and Aguer, C. (2019). Role of myokines in the development of skeletal muscle insulin resistance and related metabolic defects in type 2 diabetes. *Diabetes Metab.* doi: 10.1016/j.diabet.2019.02.006 [Epub ahead of print].

Ge, X., Chen, C., Hui, X., Wang, Y., Lam, K. S. L., and Xu, A. (2011). Fibroblast growth factor 21 induces glucose transporter-1 expression through activation of

the serum response factor/Ets-like protein-1 in adipocytes. *J. Biol. Chem.* 286, 34533–34541. doi: 10.1074/jbc.M111.248591

Ishimi, Y., Miyaura, C., Jin, C. H., Akatsu, T., Abe, E., Nakamura, Y., et al. (1990). IL-6 is produced by osteoblasts and induces bone resorption. *J. Immunol.* 145, 3297–3303.

Jackson, M. R., Gott, P., Lye, S. J., Ritchie, J. W., and Clapp, J. F. (1995). The effects of maternal aerobic exercise on human placental development: placental volumetric composition and surface areas. *Placenta* 16, 179–191. doi: 10.1016/0143-4004(95)90007-1

Kakoti, A., and Goswami, P. (2013). Heart type fatty acid binding protein: structure, function and biosensing applications for early detection of myocardial infarction. *Biosens. Bioelectron.* 43, 400–411. doi: 10.1016/j.bios.2012.12.057

Kurdiova, T., Balaz, M., Vician, M., Maderova, D., Vlcek, M., Valkovic, L., et al. (2014). Effects of obesity, diabetes and exercise on Fndc5 gene expression and irisin release in human skeletal muscle and adipose tissue: in vivo and in vitro studies. *J. Physiol.* 592, 1091–1107. doi: 10.1113/jphysiol.2013.264655

Leal, L. G., Lopes, M. A., and Batista, M. L. Jr. (2018). Physical exercise-induced myokines and muscle-adipose tissue crosstalk: a review of current knowledge and the implications for health and metabolic diseases. *Front. Physiol.* 9:1307. doi: 10.3389/fphys.2018.01307

Li, J., Umar, S., Amjedi, M., Iorga, A., Sharma, S., Nadadur, R. D., et al. (2012). New frontiers in heart hypertrophy during pregnancy. *Am. J. Cardiovasc. Dis.* 2, 192–207.

Malassiné, A., and Cronier, L. (2002). Hormones and human trophoblast differentiation: a review. *Endocrine* 19, 3–11.

Marques, A. H., Bjørke-Monsen, A.-L., Teixeira, A. L., and Silverman, M. N. (2015). Maternal stress, nutrition and physical activity: impact on immune function, CNS development and psychopathology. *Brain Res.* 1617, 28–46. doi: 10.1016/j.brainres.2014.10.051

Mashili, F. L., Austin, R. L., Deshmukh, A. S., Fritz, T., Caidahl, K., Bergdahl, K., et al. (2011). Direct effects of FGF21 on glucose uptake in human skeletal muscle: implications for type 2 diabetes and obesity. *Diabetes Metab. Res. Rev.* 27, 286–297. doi: 10.1002/dmrr

Mottola, M. F., Davenport, M. H., Ruchat, S. M., Davies, G. A., Poitras, V. J., Gray, C. E., et al. (2018). 2019 Canadian guideline for physical activity throughout pregnancy. *Br. J. Sports Med.* 52, 1339–1346. doi: 10.1136/bjsports-2018-100056

Mudd, L. M., Owe, K. M., Mottola, M. F., and Pivarnik, J. M. (2013). Health benefits of physical activity during pregnancy: an international perspective. *Med. Sci. Sports Exerc.* 45, 268–277. doi: 10.1249/MSS.0b013e31826cebcb

Nascimento, S. L., Surita, F. G., and Cecatti, J. G. (2012). Physical exercise during pregnancy: a systematic review. *Curr. Opin. Obstet. Gynecol.* 24, 387–394. doi: 10.1097/GCO.0b013e328359f131

Nielsen, A. R., and Pedersen, B. K. (2008). The biological roles of exercise-induced cytokines: IL-6, IL-8, and IL-15. *Appl. Physiol. Nutr. Metab.* 32, 833–839. doi: 10.1139/h07-054

Norton, K., Norton, L., and Sadgrove, D. (2010). Position statement on physical activity and exercise intensity terminology. *J. Sci. Med. Sport* 13, 496–502. doi: 10.1016/j.jsams.2009.09.008

Ogata, I., Shimoya, K., Moriyama, A., Shiki, Y., Matsumura, Y., Yamanaka, K., et al. (2000). Oncostatin M is produced during pregnancy by decidual cells and stimulates the release of HCG. *Mol. Hum. Reprod.* 6, 750–757. doi: 10.1093/molehr/6.8.750

Pedersen, B. K. (2009). The diseasome of physical inactivity - and the role of myokines in muscle-fat cross talk. *J. Physiol.* 587, 5559–5568. doi: 10.1113/jphysiol.2009.179515

Pedersen, B. K. (2013). Muscle as a secretory organ. *Compr. Physiol.* 3, 1337–1362. doi: 10.1002/cphy.c120033

Pedersen, B. K., Akerström, T. C. A., Nielsen, A. R., and Fischer, C. P. (2007). Role of myokines in exercise and metabolism. *J. Appl. Physiol.* 103, 1093–1098. doi: 10.1152/japplphysiol.00080.2007

Rai, M., and Demontis, F. (2015). Systemic nutrient and stress signaling via myokines and myometabolites. *Annu. Rev. Physiol.* 78, 85–107. doi: 10.1146/annurev-physiol-021115-105305

Redondo-Angulo, I., Mas-Stachurska, A., Sitges, M., Tinahones, F. J., Giralt, M., Villarroya, F., et al. (2017). Fgf21 is required for cardiac remodeling in pregnancy. *Cardiovasc. Res.* 113, 1574–1584. doi: 10.1093/cvr/cvx088

Robledo-Colonia, A. F., Sandoval-Restrepo, N., Mosquera-Valderrama, Y. F., Escobar-Hurtado, C., and Ramirez-Velez, R. (2012). Aerobic exercise training during pregnancy reduces depressive symptoms in nulliparous women: a randomised trial. *J. Physiother.* 58, 9–15. doi: 10.1016/S1836-9553(12)70067-X

Romero, R., Erez, O., Maymon, E., Chaemsaithong, P., Xu, Z., Pacora, P., et al. (2017). The maternal plasma proteome changes as a function of gestational age in normal pregnancy: a longitudinal study. *Am. J. Obstet. Gynecol.* 217, 67.e1–67.e21. doi: 10.1016/j.ajog.2017.02.037

Rose, T. M., and Bruce, G. (1991). Oncostatin M is a Member of a Cytokine Family that Includes Leukemia-Inhibitory Factor, Granulocyte Colony-Stimulating Factor, and Interleukin 6. *Proc. Natl. Acad. Sci. U.S.A.* 88, 8641–8645. doi: 10.1073/pnas.88.19.8641

Ruchat, S., Mottola, M. F., Skow, R. J., Nagpal, T. S., Meah, V. L., James, M., et al. (2018). Effectiveness of exercise interventions in the prevention of excessive gestational weight gain and postpartum weight retention: a systematic review and meta-analysis. *Br. J. Sports Med.* 52, 1347–1356. doi: 10.1136/bjsports-2018-099399

Salminen, A., Kauppinen, A., and Kaarniranta, K. (2017). FGF21 activates AMPK signaling: impact on metabolic regulation and the aging process. *J. Mol. Med.* 95, 123–131. doi: 10.1007/s00109-016-1477-1

She, J., Nakamura, H., Makino, K., Ohyama, Y., and Hashimoto, H. (2014). Selection of suitable maximum-heart-rate formulas for use with karvonen formula to calculate exercise intensity. *Int. J. Autom. Comput.* 12, 62–69. doi: 10.1007/s11633-014-0824-3

Soma-Pillay, P., Nelson-Piercy, C., Tolppanen, H., and Mebazaa, A. (2016). Physiological changes in pregnancy. *Cardiovasc. J. Africa* 27, 89–94.

Songsorn, P., Ruffino, J., and Vollaard, N. B. J. (2017). No effect of acute and chronic supramaximal exercise on circulating levels of the myokine SPARC. *Eur. J. Sport Sci.* 17, 447–452. doi: 10.1080/17461391.2016.1266392

Steensberg, A., Keller, C., Starkie, R. L., Osada, T., Febbraio, M. A., and Pedersen, B. K. (2002). IL-6 and TNF-α expression in, and release from, contracting human skeletal muscle. *Am. J. Physiol. Endocrinol. Metab.* 283, E1272–E1278. doi: 10.1152/ajpendo.00255.2002

Steensberg, A., Van Hall, G., Osada, T., Sacchetti, M., Saltin, B., and Pedersen, B. K. (2000). Production of interleukin-6 in contracting human skeletal muscles can account for the exercise-induced increase in plasma interleukin-6. *J. Physiol.* 529, 237–242. doi: 10.1111/j.1469-7793.2000.00237.x

Vargas-Terrones, M., Barakat, R., Santacruz, B., Fernandez-Buhigas, I., and Mottola, M. F. (2018). Physical exercise programme during pregnancy decreases perinatal depression risk: a randomised controlled trial. *Br. J. Sports Med.* 53, 348–353. doi: 10.1136/bjsports-2017-098926

Victoria, M. A. A., Orozco Buitrago, L., Aguilar de Plata, A. C., Mosquera Escudero, M., and Ramirez-Velez, R. (2010). Aerobic exercise during pregnancy improves health-related quality of life: a randomised trial. *J. Physiother.* 56, 253–258. doi: 10.1016/s1836-9553(10)70008-4

Wadhwa, P. D., Buss, C., Entringer, S., and Swanson, J. M. (2009). Developmental origins of health and disease: bried history of the approach and current focus on epigenetic mechanism. *Semin. Reprod. Med.* 27, 358–368. doi: 10.1055/s-0029-1237424.Developmental

Whitham, M., and Febbraio, M. A. (2016). The ever-expanding myokinome: discovery challenges and therapeutic implications. *Nat. Rev. Drug Discov.* 15, 719–729. doi: 10.1038/nrd.2016.153

Zygmunt, M., Hahn, D., Kiesenbauer, N., Münstedt, K., and Lang, U. (1998). Invasion of cytotrophoblastic (JEG-3) cells is up-regulated by interleukin-15 *in vitro*. *Am. J. Reprod. Immunol.* 40, 326–331. doi: 10.1111/j.1600-0897.1998.tb00061.x

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

CHAPTER 3

PREAMBLE TO MANUSCRIPT 2

The manuscript titled: “Physical activity engagement during pregnancy is associated to increased placental FATP4 protein expression” is prepared for submission to the journal *Placenta*. The manuscript is in accordance with the journal’s specifications.

Physical activity engagement during pregnancy is associated to increased placental FATP4 protein expression

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Abstract

Background: Placental function is of utmost importance to ensure proper fetal development *in utero*. Amongst the placenta's many roles includes ensuring the passage of sufficient macronutrients to the fetus. The fetus relies heavily on the maternal supply of glucose, amino acids, and fatty acids. These macronutrients are carried to the fetus across transporters embedded within the placenta. The objective of this study was to examine the impact of i) an acute bout of exercise and ii) chronic exercise, which is strongly recommended throughout pregnancy, on changes in placenta nutrient transporter expression, and localization *in vitro*.

Methods: Pre- and post-exercise serum was collected from pregnant (n=5) and non-pregnant (n=5) women who underwent a moderate-intensity exercise session. To investigate the effect of acute exercise, the collected serum was used to treat placental-derived cell lines (BeWo choriocarcinoma). Protein expression and localization for the transporters GLUT1, SNAT1, and FATP4 were then examined. To assess chronic physical activity exposure, we measured protein expression patterns in term placenta from women categorized as active (n=10) vs. inactive (n=10) during pregnancy based on accelerometry.

Results: GLUT1 expression in BeWo cells treated with serum from pregnant women was higher compared to treatment with serum from non-pregnant women, independently of exercise. FATP4 protein expression was elevated in term placenta of active women. These results were further supported by immunohistochemistry, which illustrated higher levels of FATP4 in placental tissue of active compared to non-active women.

Conclusion: These results show that engagement in chronic exercise during pregnancy increases the expression of the placental fatty acid transporter, FATP4. Future work should aim to investigate the role of chronic exercise on other key placental fatty acid transporters in addition

to fatty acid transport. Moreover, this study suggests that serum from pregnant women contains factors or molecules that increase GLUT1 protein expression.

Introduction

Placental growth and function are of utmost importance for the development and long-term health outcomes of the fetus. Nutrient transport and trophoblast proliferation are key markers of placental health and development. Research shows that increased or decreased nutrient transport can lead to excessive or restricted fetal growth, respectively [1][2]. Parallel to these findings, maternal pregnancy-related pathologies such as intra uterine growth restriction (IUGR), pre-eclampsia (PE) and gestational diabetes mellitus (GDM) are associated with altered placental nutrient transport [2][3]. The fetus relies greatly on the maternal supply of nutrients via the placenta. Although some nutrient transfer occurs through simple diffusion, the demands of the fetus far exceed the supply of nutrients obtained solely through diffusion [4][5]. Thus, nutrient transporters for glucose, amino acids, and fatty acids are crucial for facilitating the delivery of macronutrients needed to support fetal development [4].

Glucose is the most important macronutrient for fetal growth and development [6]. The three main placental glucose transporter isoforms are GLUT1, GLUT3, and GLUT4 [2]. Changes in GLUT transporter expression can lead to complications during pregnancy. As such, increased expression of GLUT3 has been associated with IUGR [7] while women with PE exhibit decreased placental GLUT1 expression [6]. Moreover, GLUT1 is generally considered the predominant placental glucose transporter, as it is significantly expressed in placental tissue early in pregnancy and at term [2].

The transport of amino acids is essential as these molecules are not only precursors for fetal proteins, but also neurotransmitters, polyamines, and nitric oxide [8]. System L and A are the best-characterized placental amino acid transport systems [2]. Large neutral and aromatic amino acids are transported through the placenta via LAT1 and LAT2, isoforms of the System L

sodium-independent transporter family [9]. System A transporters are sodium-dependent and are responsible for the transport of small, neutral amino acids such as alanine, serine and glutamine [10] and comprised of three isoforms: SNAT1, SNAT2 and SNAT3 [11]. System L acts as an exchanger, and its activity is dependent on the supply of amino acids from System A [2].

The fetus is unable to synthesize essential fatty acids (EFA) and thus relies on the maternal circulation for its supply [4]. EFAs are vital for the development of the fetal brain, lungs, and cardiovascular system [4]. The primary membrane-bound placental fatty acid (FA) transporters include the FA transporter protein family (FATP), plasma membrane FA binding protein ($FABP_{pm}$) and FA translocase (FAT/CD36), while the FA binding protein family (FABP) is the main cytoplasmic FA transporter in the placenta [1].

Although limited, evidence shows that physical activity (PA) during pregnancy can alter placental nutrient transport and function. Brett and colleagues [12] found that women who accumulated a minimum of 150 min of weekly moderate-to-vigorous intensity PA, compared to sedentary women, exhibited a 1.95-fold decrease in placental gene expression for *FATP4* and a 1.68-fold increase in the gene expression for *SNAT2*. Additionally, Clapp [13] suggests that placental function is improved in women who engage in PA throughout pregnancy in part due to increased intervillous blood volume and resting maternal plasma volume. It is well established throughout the literature that fetuses born to mothers that were physically active during their pregnancies have optimized health outcomes at birth and throughout their lifespan [14][15][16]. However, the mechanistic pathways and targets responsible for such outcomes are relatively unknown. We hypothesize that the health benefits bestowed upon the fetus as a result of habitual maternal PA may be achieved via the placenta through changes in nutrient transport.

The objective of this study was to examine whether exposure of trophoblast cell lines *in vitro* to serum obtained before and after an acute bout of exercise was associated with alterations in important metrics of placental growth and function including the expression and localization of three key nutrient transporters: GLUT1, SNAT1 and FATP4 and to cellular proliferation. Additionally, the role of chronic exercise on nutrient transporter expression and localization was assessed in term human placenta tissue of pregnant women categorized as physically active and non-active.

Methods

Ethical Approval

Participants gave informed written consent to participate in either the PhysicaL Activity and diEtary implicatioNs Throughout pregnAncy (PLACENTA) study (file number: H11-15-29) or the Acute Exercise study (file number: H-06-18-634), at the University of Ottawa. For this article, the PLACENTA study will be referred to as the chronic exercise study vs. the Acute Exercise study. Both studies were approved by the Research Ethics Board (REB) of the University of Ottawa and conformed with all aspects of the Declaration of Helsinki.

Participants and exercise parameters

Acute exercise study

Pregnant and non-pregnant women were recruited from the Ottawa region for participation in the Acute Exercise study. Women aged between 18-40 years, having a pre-pregnancy body mass index (BMI) considered normal or overweight (18.5 – 29.9 kg/m^2), with no contraindication to exercise were included. Those with hypertension, diabetes, or untreated thyroid disease were excluded. Additionally, only women in the second trimester (between 13-27 weeks gestation) of

pregnancy were included. Non-pregnant controls were also recruited using the same inclusion and exclusion criteria. All women in this study reported to our laboratory for one session of moderate-intensity treadmill walking.

Acute exercise session

Participants were asked to refrain from eating and exercising before their morning visit at our laboratory. Upon arrival, height and weight of participants were recorded by researchers. A standardized snack of approximately 340 calories was provided to each participant, and they were asked to consume the snack, but not forced to do so. Following the snack, a 10 min seated resting phase preceded the walking session during which resting heart rate (RHR) was determined using a Polar V800 heart rate monitor (Lachine, QC), to determine the target heart rate required for moderate-intensity exercise – equivalent to 40-60% heart rate reserve (HRR). The walking session was conducted using a Woodway Pro XL 27 treadmill (Woodway USA, Waukesha, WI) as previously described [17]. Briefly, the participants walked for 30min at a 6% incline while the speed was adjusted by 0.2 mph until the target HR was met.

Blood collection and processing

A pre- and post-exercise blood draw was taken from the medial cubital vein and collected in serum tubes (BD Biosciences, Franklin Lakes, NJ). The blood was allowed to clot at room temperature for 30 min before it was centrifuged for 15 min at 4°C at a speed of 1000 x g. Serum was stored at -80°C until further experimentation.

Chronic exercise study

Pregnant women were recruited from the Ottawa region for participation in the PLACENTA study. Inclusion and exclusion criteria for pregnant women were the same as those presented for the Acute Exercise study. Free-living PA measurements were obtained prospectively from

participants for 7 consecutive days, during the second trimester (between 24-28 weeks gestation) using an Actical accelerometer (Phillips Respironics, Montreal, QC). Data analyses were performed as previously described using SAS version 9.4 (SAS Institute, Cary, NC) [18]. Briefly, classification as either 'active' or 'non-active' was based on the 2019 Canadian PA Guideline Throughout Pregnancy [16], which recommends 150 min of weekly moderate-intensity PA (MPA). Participants that did not have 7 valid days of wear were classified as 'active' if they accumulated 21.4 min of daily MPA (equivalent to 150 min/7 days). For any participants to be included in the analysis, a minimum of 4 valid days of wear per week and 10 hours of wear per day were necessary. Furthermore, the height and weight of participants were recorded at the laboratory by researchers the day before the Actical measurement period. Upon delivery, placenta samples were collected from each participant (see section 2.3). The most physically active (n=10) and least active (n=10), herein referred to as non-active, participants were selected for this study based on accelerometry. Infant birthweight was measured 24-48 hr after delivery to the nearest gram.

Cell culture

The choriocarcinoma cytotrophoblast BeWo and transformed extravillous trophoblast HTR-8/SVneo cell lines were purchased from ATCC (Manassas, VA). Nutrient transporter expression and localization were examined using BeWo cells as this cell line has been recommended to study transplacental transport [19][20]. In contrast, HTR-8/SVneo cells were used to analyze cellular proliferation as this first trimester immortalized cell line has been shown to have similar characteristics to first-trimester placental trophoblasts [21][22]. Cells were cultured in Gibco RPMI 1640 Media (Thermo Fischer Scientific, Nepean, ON) supplemented with 10% fetal bovine serum (FBS) and incubated at 37°C and 5% CO_2. Before any experiment, cells were

seeded and allowed to adhere overnight. Following attachment, cells were starved in serum-free media for 24 hr. Treatment of cells with experimental conditions began 48 hr post-seeding.

Placenta tissue sampling and preparation

Term placenta of participants from the chronic exercise study (n=10) was weighed, and samples were collected within 30 min of delivery. Placental tissue was randomly sampled on ice from the central (minimum 2 cm away from the umbilical cord insertion) and peripheral (minimum 2 cm away from the edge of the placental disc) areas of the placenta (approximately 2.5 cm^3 each), avoiding areas of necrosis or calcification. Tissue from the basal (decidua) and chorionic plates were also avoided. In total, three central and two peripheral samples were further dissected into smaller pieces (0.25 cm^3) and washed twice with ice-cold phosphate buffer saline (PBS) and distributed into cryovials in a 2:1 ratio, respectively. Tissues were flash-frozen in liquid nitrogen and stored at -80°C until further analysis. The placenta tissue was powdered on dry ice and homogenized in radioimmunoprecipitation assay (RIPA) buffer with an electric homogenizer. Lysate protein concentration was assessed using a Bradford Protein Assay (Bio Rad, Mississauga, ON). Additionally, a 1.5 cm by 1 cm full-thickness biopsy was obtained from the central area of the placenta for immunohistochemical studies. The samples were rinsed twice with PBS for fixation in 10% neutral-buffered formalin for 48 hr at room temperature. After fixation, samples were rinsed with PBS and prepared for processing and paraffin embedding using standard methodologies.

Protein analysis

BeWo cells were plated (1.5×10^6) in 60 mm dishes. At 48 hr post-seeding, cells were treated for 1 hr and 8 hr, with 1640 RPMI media supplemented with 10% pre- or post-exercise serum collected from pregnant (n=5) and non-pregnant (n=5) women. Following the treatment, cells

were lysed, and protein was extracted and quantified for western blot analysis. Briefly, cells were washed twice with PBS followed by a 5 min incubation at room temperature (RT) with Mammalian Protein Extraction Reagent (M-PER) (Thermo Fisher Scientific, Nepean, ON, cat. #78501) supplemented with 1% protein inhibitor cocktail (Millipore Sigma, Oakville, ON, cat. #P8340). The lysate was collected and spun using a Sorvall ST 16R centrifuge (Thermo Fisher Scientific, Nepean, ON) at 14,000 x g for 5 min. The supernatant was collected, and protein was quantified using the Bradford Protein Assay (Bio Rad, Mississauga, ON) according to the manufacturer's instructions.

Western blot analysis

Total protein was loaded (10-30 µg) in Bio-Rad TGX Stain-Free pre-cast gels (Bio Rad, Mississauga, ON). Samples analyzed for SNAT1 and FATP4 expression were boiled for 5 min at 95°C. Total cellular protein was separated by gel electrophoresis and transferred onto 0.22-micron polyvinylidene difluoride (PVDF) membrane. Membranes for SNAT1 and FATP4 were blocked in TBST containing 5% milk for 1 hr at RT, while the membranes for GLUT1 were blocked in TBST with 10% milk for 2 hr at RT. Membranes were probed overnight at 4°C for their respective primary antibodies: GLUT1 (0.001µg/mL; Abcam, Cambridge, MA, cat. #ab115730) SNAT1 (2µg/mL; Millipore Sigma, Oakville, ON, cat. #MABN502), FATP4 (50µg/mL; Santa Cruz Biotechnology Inc, Santa Cruz, CA, cat. #sc101271). Membranes were then incubated with horseradish-peroxidase conjugated secondary antibodies for 1 hr at RT as follows: SNAT1 and FATP4 (1:2000; Goat Anti-Mouse IgG, Bio Rad, Mississauga, ON, cat. #1706516), and GLUT1 (1:5000; Goat Anti-Rabbit IgG, Bio Rad, Mississauga, ON, cat. #1721019). Clarity Western ECL Blotting Substrate was used for visualization (Bio Rad, Mississauga, ON, cat. #1705061). Membranes were imaged for protein bands using the

ChemiDoc XRS+ Molecular Imager (Bio Rad, Mississauga, ON). Following imaging, membranes were stripped and permanently stained with Amido Black for whole protein lane quantification.

Immunofluorescence staining

To examine changes in transporter localization, immunofluorescence staining was conducted using BeWo cells. Cells (5.3×10^4) were plated in 8 well chamber slides (ibidi USA, Fitchburg, WI, cat. #80826) and treated with 10% pre- or post-exercise human serum in 1640 RPMI media from pregnant (n=5) and non-pregnant (n=5) women and incubated at 37°C and 5% CO_2 for 1 hr. Cells were subsequently fixed with cold 4% paraformaldehyde in PBS for 10 min at RT, permeabilized with 0.2% Triton X-100 in PBS for 10 min at RT and blocked for 1 hr at RT in 5% goat serum in PBS. Subsequently, cells were incubated with GLUT1 primary antibody at 4°C overnight (1:100; Abcam, Cambridge, MA, Cat. #ab15309). The following day, cells were incubated with an Alexa-fluor 488 conjugated secondary antibody for 1 hr at RT (1:1000 for Goat anti-Rabbit IgG, Thermo Fischer Scientific, Nepean, ON, cat. #A27034). NucBlue™ Fixed Cell ReadyProbes™ Reagent was added (Thermo Fischer Scientific, Nepean, ON, cat. #R37606) to stain for nuclei while actin was stained using ActinRed™ 555 Ready Probes™ Reagent (Thermo Fischer Scientific, Nepean, ON, cat. R37112). Mounting media (ibidi USA, Fitchburg, WI, cat. #50001) was added to each well prior to imaging using a Zeiss AxioObserver D1 microscope (Carl Zeiss Canada Ltd, Toronto, ON) equipped with Zen Blue software (version, info) at 40x magnification. Four representative fields-of-view (FOV) were analyzed for differences between experimental treatments. Blinded semi-quantitative visual assessment of membrane intensity staining using a four-point ordinal scale was conducted as follows:

0=negative, 1=weak, 2=moderate and 3=strong. Negative controls were treated with 10% pooled pre- or post-exercise serum from pregnant participants without primary antibody.

Immunohistochemistry

Formalin-fixed paraffin-embedded tissues were sectioned at a thickness of 4 μm, and deparaffinized and rehydrated. Epitopes were unmasked using heat-induced epitope retrieval with 10mM sodium citrate buffer (pH 6.0). Tissue sections were blocked for endogenous peroxidase activity using 3% H_2O_2, and sera were blocked for 1 hr at room temperature using 10% normal goat serum. Tissue sections were incubated overnight at 4°C with anti-SLC27A4 (probing for FATP4; 1:50, Sigma, cat. #HPA007293). Sections were then incubated with biotin-conjugated AffiniPure goat anti-rabbit IgG (1:1000, JacksonImmunoResearch Laboratories, Inc., West Grove, PA) for 1 hr at RT followed by incubation with ExtrAvidin®-Peroxidase (1:100, Sigma, cat. # E2886) room temperature for 30 minutes. Staining was visualized with DAB chromogen (Abcam, cat. #ab64238) and counterstained using Harris' hematoxylin. Slides were rehydrated, cleared, and coverslipped in preparation for imaging using a Zeiss AxioImager M2 microscope (Zeiss) with Zen Blue (version 2.3, Zeiss). Images were taken using 40x magnification for comparisons. Sections incubated without primary antibody (diluent only) served as negative controls.

Cellular proliferation

Cellular proliferation was assessed using immunofluorescence staining for the nuclear proliferative marker Ki67. HTR-8/SVneo cells were seeded (1.4×10^4) in 8 well chamber slides (ibidi USA, Fitchburg, WI, cat. #80826). Cells were then treated with 10% pre- or post-exercise serum from pregnant (n=3) and non-pregnant (n=3) in 1640 RPMI media for 24 hr. Following incubation with serum, the immunofluorescence technique was performed as described above.

Cells were incubated with primary antibody Ki67 (1:100; Abcam, Cambridge, MA, cat. #ab16667) overnight at 4°C. Following probing, cells were treated with an Alexa-fluor 488 conjugated secondary antibody, Goat Anti-Rabbit IgG (1:1000, Thermo Fischer Scientific, Nepean, ON, cat. #A27034) for 1 hr at RT. Nuclei of cells were stained using NucBlue™ Fixed Cell ReadyProbes™ Reagent (Thermo Fischer Scientific, Nepean, ON, cat. #R37606). Mounting media (ibidi USA, Fitchburg, WI, cat. #50001) was added to each well prior to imaging with a Zeiss AxioObserver D1 microscope at 20x magnification. Image J software (National Institutes of Health, Bethesda, MD) was used to count cells in six representative FOV for each experimental treatment. The primary antibody was omitted from cells acting as negative controls. Negative control cells were treated with 10% pooled pre- or post-exercise serum from pregnant participants.

Statistical Analyses

All data are presented as mean ± standard deviation (SD) and were analysed using GraphPad Prism Software (version 8.0.0, San Diego, CA). Participant characteristics (Table 1 and Table 2), physical activity parameters of participants (Figure 3), and expression levels for nutrient transporters in the chronic exercise study (Figure 4) were compared using either the unpaired t-test or the Mann-Whitney U test. Parametric or non-parametric tests were used according to the distribution of the data determined via the Shapiro-Wilk test for normality. The 2-way mixed ANOVA was used to assess changes in nutrient transporter expression (Figure 1C, 1F, 1I), GLUT1 localization (Figure 2G), and cellular proliferation (Figure 6G) in the acute exercise study in pregnant compared to non-pregnant women. No test for multiple comparisons was utilized as no significant interaction for pregnancy status (pregnant and non-pregnant) and time

(pre-exercise and post-exercise) was detected. For all statistical analyses, $p<0.05$ was considered significant.

Results

Pregnant serum leads to differences in GLUT1 expression in BeWo cells

The 2-way mixed ANOVA revealed a significant effect of pregnancy status (pregnant versus non-pregnant) for GLUT1 expression ($F = 21.0, p = 0.002$) (Figure 1C). GLUT1 expression was significantly higher in cells treated with serum from pregnant women compared to serum from non-pregnant women (Figure 1C). No significant interaction effect for pregnancy status and exercise was uncovered for GLUT, SNAT1, and FATP4 for the 8hr serum treatment in BeWo cells (Figure 1C, 1F, 1I). Moreover, cells treated for 1 hr with serum from pregnant and non-pregnant women showed no significant interaction between exercise and pregnancy status in the expression of GLUT1, SNAT1 or FATP4 (data not shown).

GLUT1 membrane localization

Since cells treated with serum from pregnant participants compared to those treated with non-pregnant serum demonstrated higher cellular expression of GLUT1, we wanted to explore whether cellular membrane localization between groups followed the same pattern. Thus, the same cell line (BeWo) was used to perform localization staining using an immunofluorescence technique. We observed no difference in cellular localization of GLUT1 in cells treated with pre- or post-exercise serum from pregnant and non-pregnant women (Figure 2).

Participant characteristics and PA levels for women in the chronic exercise study

Participant characteristics such as age, pre-pregnancy BMI and weight for the active and non-active women participating in the chronic exercise study are presented in Table 2. Gestational

age at delivery was the only characteristic that differed between both groups (Table 2B). Active pregnant women delivered earlier (39.16 ± 0.7 weeks of gestation), albeit full term, compared to their non-active counterparts (41.0 ± 0.4 weeks of gestation). As designed, the active group accumulated more steps as well as daily minutes of MPA and MVPA in their second trimester of pregnancy compared to the non-active group (Figure 3).

FATP4 expression in term placenta of active compared to non-active women

The expression of FATP4 was higher in term placenta of active versus non-active women (Figure 4G-4H). Immunohistochemical staining of tissue sections confirmed this observation (Figure 5). Conversely, western blot analysis showed no difference in the expression of the glucose transporter GLUT1 and the amino acid transporter SNAT1 in term placental tissue of the active compared to the non-active group (Figure 4A-4F).

Cell proliferation

HTR-8/SVneo cells treated with pre- or post- exercise serum from pregnant and non-pregnant women (Table 1B) demonstrated no differences in proliferation between or within groups (Figure 6). Regardless of treatment, 96% of the HTR-8/SVneo cells were in the proliferation phase.

Discussion

The relationship between PA engagement throughout pregnancy and beneficial health outcomes for both mother and fetus is increasingly substantiated by research [16][23][24]. Identifying the molecules and mechanisms through which PA confers these observed benefits could potentially assist obstetric health care providers in rationalizing and prescribing appropriate exercise regimens to patients. This study aimed to elucidate potential cellular targets of maternal PA. The main findings of this study are two-fold. First, we showed that expression of the fatty-acid

transport protein, FATP4, is higher in term placenta of active compared to non-active women. Second, we found that cells treated for 8hr with serum from pregnant women demonstrated a higher GLUT1 expression than those treated with serum from non-pregnant women. Our second finding pursued a novel question by using a unique methodology – the treatment of placental cell lines with serum from pregnant women following exercise – that, to our knowledge, has not previously been done in the literature.

Increased protein expression of placental FATP4 in women who are active versus non-active during pregnancy challenges previous findings from our lab. A study performed by Brett and colleagues [12] shows that in pregnant women classified as active, defined as those that met PA guidelines (150min of weekly moderate-to-vigorous PA), mRNA expression levels for the *FATP4* gene were 1.95-fold lower than in non-active women. Assessing mRNA levels for specific genes is an important first step in understanding cellular mechanisms. Although mRNA expression levels for a particular gene may be an indication of protein abundance, this correlation does not always hold true. Post-transcriptional and translational modifications along with protein degradation, are some of the factors that may create a discrepancy between mRNA levels and protein concentrations [25]. While mRNA expression for *FATP4* was shown in a similar study to be lower, our higher protein expression in women categorized as active suggests post-transcriptional modifications may be at play. There exists a multitude of post-transcriptional mechanisms through which mRNAs may be modified which can thereby alter protein abundance. For instance, Fu and colleagues [26] explain that mRNAs with shorter 3' untranslated regions (UTRs), which can often be a result of alternative polyadenylation (APA), have greater translational efficiency. In line with these findings, researchers have been able to increase protein translation by 30-150% in four human and mice genes by introducing modified

antisense oligonucleotides (ASOs) that bind to specific areas of mRNA [27]. Moreover, a study conducted by Dubé and colleagues reports that mRNA and protein expression for FATP4 is higher in pregnant women with a BMI classified as normal compared to obese [28].

Further investigation regarding the results obtained in this study relating to FATP4 is warranted. Although protein abundance is arguably more functionally relevant than mRNA expression levels, it has its limitations. The observed increased placental FATP4 protein expression in women who are active in pregnancy compared to non-active does not give insight into the functional capacity of this transporter. Moreover, placental FA uptake is complex and orchestrated by various different types of membrane embedded and cytoplasmic transporters. Protein expression quantification is crucial in gaining an understanding as to whether PA plays a role in altering FA transport. Future investigations should aim to explore the effect of PA on FA uptake in placenta.

Our second finding was that GLUT1 protein expression was higher in BeWo cells treated with serum from pregnant compared to non-pregnant women. These results could suggest that certain mediators released by the placenta and present in the serum of pregnant women, regardless of PA, elicit an increase in GLUT1 production. As glucose is the primary substrate for fetal growth, higher expression of GLUT1 stimulated by extrinsic factors present in serum from pregnant women, compared to serum from non-pregnant women, is possible, but had yet to be shown in the literature.

The observed differences in GLUT1 protein expression related to pregnancy did not translate to changes in membrane localization, as we found no difference between cells treated with serum from the pregnant and non-pregnant women. However, a difference in the experimental time frame must be noted. BeWo cells evaluated for protein expression were

treated with serum for 8hr while those examined for membrane localization were treated for 1hr. Furthermore, extrapolating results from our cell culture model to placental tissue presents limitations. In creating a cell monolayer, we are unable to represent the actual complexities of the syncytiotrophoblasts double-membrane present in the placenta. Additionally, as Kaur and Dufour [29] explain, cell lines are studied in the absence of their local environment and are genetically modified which could possibly alter their response to various stimuli. Moreover, changes in protein expression do not give insight into the function of GLUT1. Thus, in order to gain a better understanding of the effect of acute exercise and its impact on GLUT1, glucose uptake should be explored.

In addition to our main findings, while we found that active women in the chronic exercise study had a shorter gestation period compared to the non-active women, the gestational age at the time of delivery for both groups is considered full term [30]. These results may initially be misleading as historically unsubstantiated claims posited that exercise during pregnancy was associated to adverse health outcomes including premature delivery. Yet, research shows that engaging in regular exercise during pregnancy is not associated to pre-term birth [31].

The results obtained from the cellular proliferation immunofluorescence experiments are also worth discussing. We found no difference in proliferation between HTR-8/SVneo cells treated with pre- or post-exercise serum from either pregnant or non-pregnant women. As HTR-8/SVneo cells are a good model for first trimester trophoblasts, they are expected to be highly proliferative and post-exercise serum does not alter this capacity. These results further support the literature in highlighting the safety of PA during pregnancy.

Although our two main findings are novel and add to the growing body of literature regarding the effect of PA on placental function, certain limitations inherent to human studies must be considered. In the chronic exercise study, term placenta tissue was used to evaluate protein transporter expression. Since the placenta is a transient organ, examining its functional capacity at term may not be representative of its function throughout the course of pregnancy. Examining first trimester placental explants may offer additional understating of nutrient transport throughout pregnancy. Moreover, the acute exercise study also has its limitations. This study was designed based on the assumption that the placenta is altered by acute exercise through metabolites present in the circulation. However, it is possible that other mechanisms, independently of circulation, impact placental transport as a result of acute PA. Additionally, cell lines were used as a first step in the exploration of the role of PA on placental function, but future research should aim to expand on this work and treat placental explants with maternal serum.

Conflict of interest statement

The authors declare no conflict of interest.

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Abbreviations

BMI	Body mass index
EFA	Essential fatty-acids
FA	Fatty acid
FABP	Fatty acid binding protein
$FABP_{pm}$	Plasma membrane fatty acid binding protein
FAT	Fatty acid translocase
FATP	Fatty acid transporter protein
FBS	Fetal bovine serum
FOV	Field of view
GDM	Gestational diabetes mellitus
HR	Heart rate
HRR	Heart rate reserve
IUGR	Intra-uterine growth restriction
M-PER	Mammalian protein extraction reagent
MPA	Moderate-intensity physical activity

MVPA	Moderate-to-vigorous intensity physical activity
PA	Physical activity
PBS	Phosphate buffer saline
PE	Pre-eclampsia
PVDF	Polyvinylidene difluoride
REB	Research Ethics Board
RHR	Resting heart rate
RIPA	Radioimmunoprecipitation assay
RT	Room temperature
SD	Standard deviation

Table 1. Acute exercise study participant characteristics.

A

	Pregnant	Non-pregnant	p-value
N	5	5	-
Age (years)	32.4 ± 2.9	32.2 ± 3.3	0.9
Gestational age (weeks)	19.8 ± 5.5	-	-
Non/pre-pregnant BMI (kg/m²)	24.2 ± 3.2	22.8 ± 2.1	0.4
Non/pre-pregnant weight (kg)	65.8 ± 5.4	62.0 ± 5.0	0.3
Height (cm)	167.8 ± 4.0	165.4 ± 8.8	0.6
Resting Heart Rate (RHR) (bpm)	71.3 ± 11.0	71.8 ± 5.3	0.9

B

	Pregnant	Non-pregnant	p-value
N	3	3	-
Age (years)	30.7 ± 2.1	30.7 ± 2.5	0.9
Gestational age (weeks)	20.2 ± 6.7	-	-
Non/pre-pregnant BMI (kg/m²)	21.8 ± 1.8	23.1 ± 0.3	0.3
Non/pre-pregnant weight (kg)	62.9 ± 4.4	61.8 ± 7.5	0.8
Height (cm)	168.0 ± 1.0	163.2 ± 8.7	0.4
Resting Heart Rate (RHR) (bpm)	72.7 ± 19.5	66.9 ± 5.5	0.6

(A) Participants included for analysis of nutrient transporter (GLUT1, SNAT1, FATP4) protein expression by western blot analysis and GLUT1 localization by immunofluorescence. (B) Participants included for examination of cellular proliferation by immunofluorescence. Values are presented as mean ± SD. BMI= body mass index; bpm= beats per minute; SD= standard deviation.

Table 2. Chronic exercise study pregnant participant characteristics.

A

	Active	Non-active	p-value
N	10	10	-
Age (years)	32.7 ± 3.9	31.1 ± 4.2	0.4
Pre-pregnancy BMI (kg/m²)	22.9 ± 2.6	24.1 ± 4.6	0.5
Pre-pregnancy weight (kg)	63.8 ± 8.7	66.9 ± 14.0	0.6
Height (cm)	166.9 ± 4.1	165.7 ± 7.5	0.6
Gestational age at delivery (weeks)	40.0 ± 1.2	40.6 ± 1.1	0.3
Mode of delivery	**CS:** 2 **V:** 8	**CS:** 1 **V:** 9	-
Infant weight (g)	3373.0 ± 374.6	3551.0 ± 376.7	0.3
Placenta weight (g)	571.4 ± 117.1	660.6 ± 97.8	0.08

B

	Active	Non-active	p-value
N	5	5	-
Age (years)	32.4 ± 4.2	31.6 ± 4.0	0.8
Pre-pregnancy BMI (kg/m²)	23.7 ± 2.8	24.9 ± 5.8	0.7
Pre-pregnancy weight (kg)	64.2 ± 9.7	70.0 ± 15.0	0.5
Height (cm)	165.5 ± 3.8	166.6 ± 8.5	0.8
Gestational age at delivery (weeks)	39.16 ± 0.7	41.0 ± 0.4	0.0009*
Mode of delivery	**CS:** 1 **V:** 4	**CS:** 0 **V:** 5	-
Infant weight (g)	3194.0 ± 360.3	3668.0 ± 394.6	0.08
Placenta weight (g)	511.2 ± 91.7	653.2 ± 138.8	0.09

(A) Participants included for analysis of nutrient transporter (GLUT1, SNAT1, FATP4) protein expression by western blot analysis. (B) Participants included for examination of FATP4 localization by immunohistochemistry. Values are presented as mean ± SD. BMI= body mass index; CS= caesarian section; V= vaginal; AV= assisted vaginal; SD= standard deviation; (*)= indicates significance.

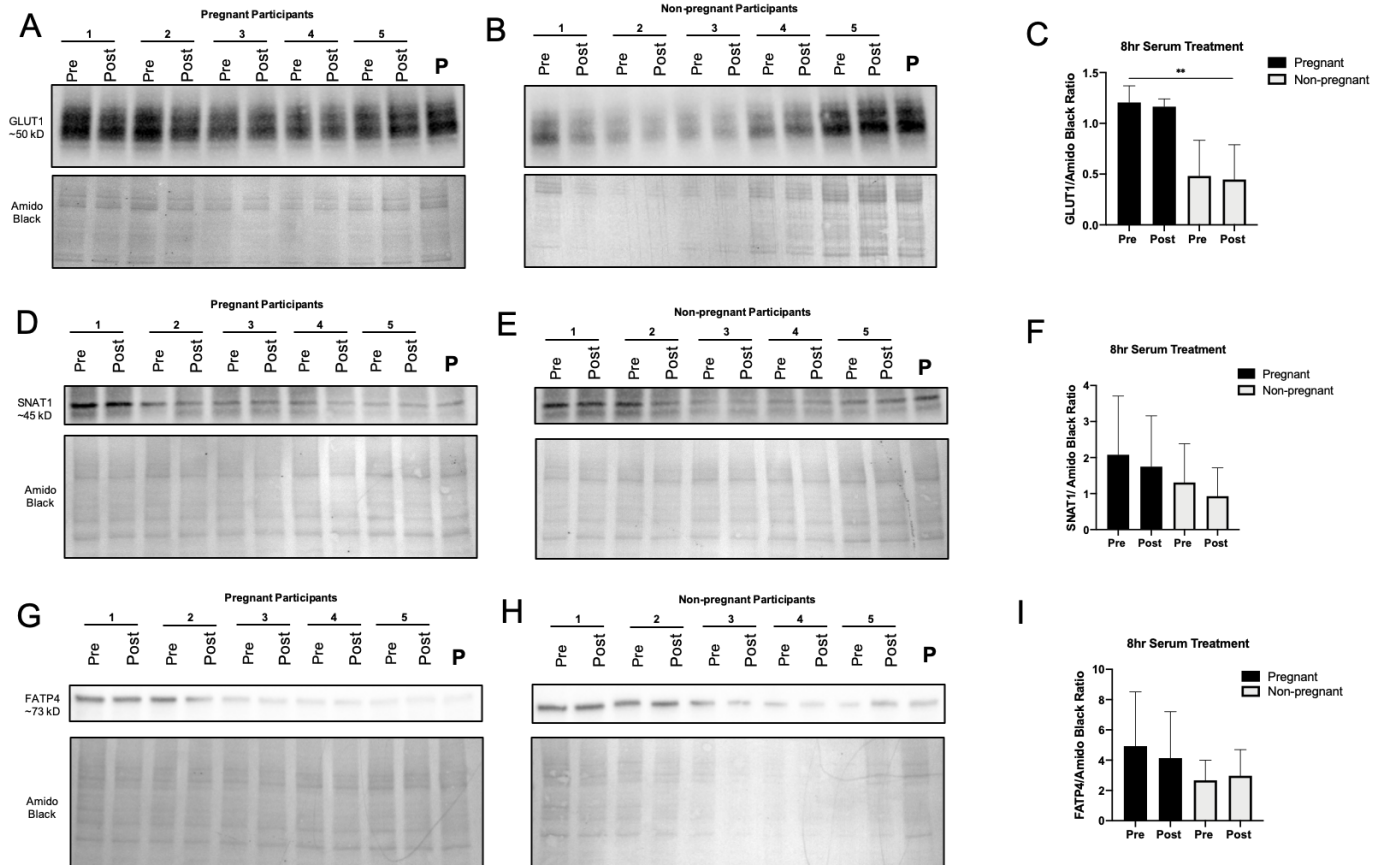

Figure 1. The effect of pre- or post-exercise serum treatment on GLUT1 (A-C), SNAT1 (D-F) and FATP4 (G-I) protein expression in pregnant (n=5) and non-pregnant (n=5) women from the acute exercise study. Changes in expression were assessed in cell lysates collected from BeWo cells following treatment with 10% pre- or post-exercise serum respectively in 1640 RPMI media for 8hr. Band density was normalized to total lane protein density determined by Amido Black staining. Each blot was further normalized to its respective pooled (P) lane for inter-blot comparisons. Values are presented as mean ± standard deviation. Data (C, F, I) was analyzed using a 2-way mixed ANOVA. Statistically significant effect of pregnancy status (pregnant compared to non-pregnant) depicted with '*' symbol. Pre= Pre-exercise serum treatment; Post= Post-exercise serum treatment; P= pooled lysate (n=5 pregnant and n=5 non-pregnant); (**)= $p \leq 0.01$.

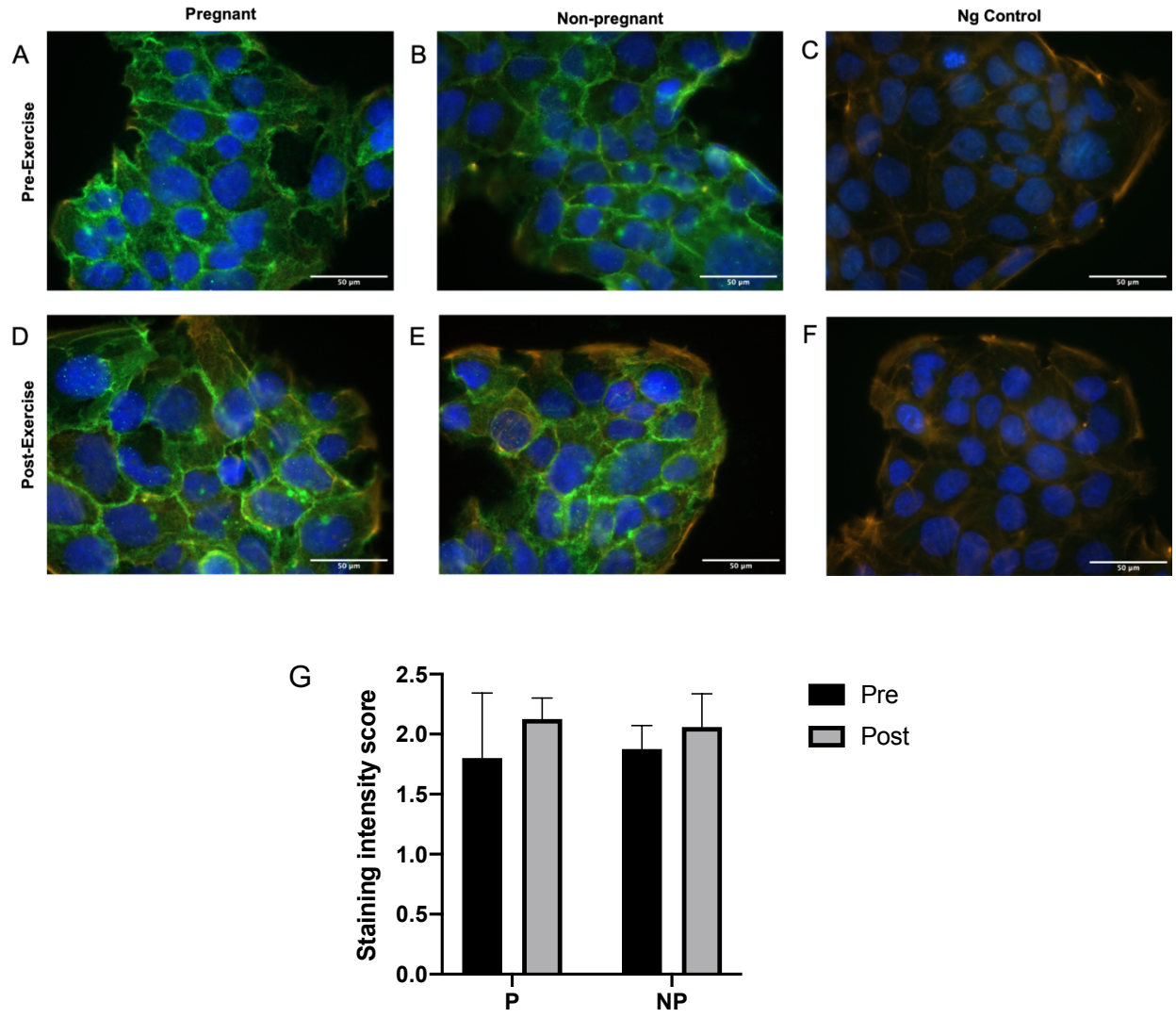

Figure 2. Effect of pre- or post-exercise serum from pregnant and non-pregnant women on cellular distribution of GLUT1. BeWo cells were treated with pre- or post-exercise serum (10% in 1640 RPMI media) from pregnant (A, D) and non-pregnant (B, E) women for 1hr. Cells were probed with anti-GLUT1 antibody (depicted in green) and imaged using an immunofluorescent microscope. Blue staining is DAPI, representing nuclei while Actin is depicted with the orange stain. Images shown are representative of five biological replicates for each experimental condition. Negative (Ng) control images (C, F) were taken of cells treated with pooled pre- and post-exercise serum from the pregnant participants while omitting primary GLUT1 antibody. Blinded scoring of GLUT1 membrane intensity was performed by two different researchers independently and results are depicted in (G). Values are presented as mean ± standard deviation and were analyses using a 2-way ANOVA. Pre= Pre-exercise serum treatment; Post= Post-exercise serum treatment; P= Pregnant; NP= Non-pregnant.

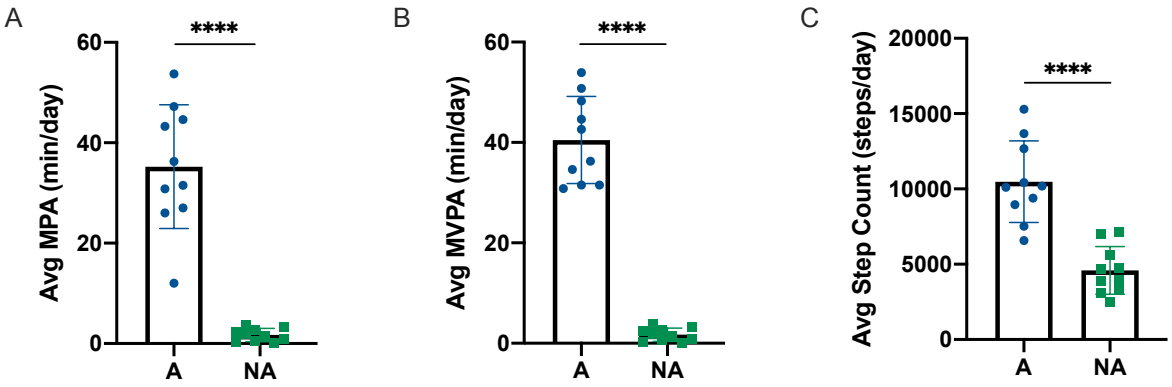

Figure 3. Physical activity parameters of participants in the chronic exercise study. Representative of second trimester activity levels in physically active (n=10) and non-active (n=10) participants. A= active; NA= non-active; Avg= Average; MPA= moderate-intensity physical activity; MVPA=moderate to vigorous-intensity physical activity; (****)= $p \leq 0.0001$.

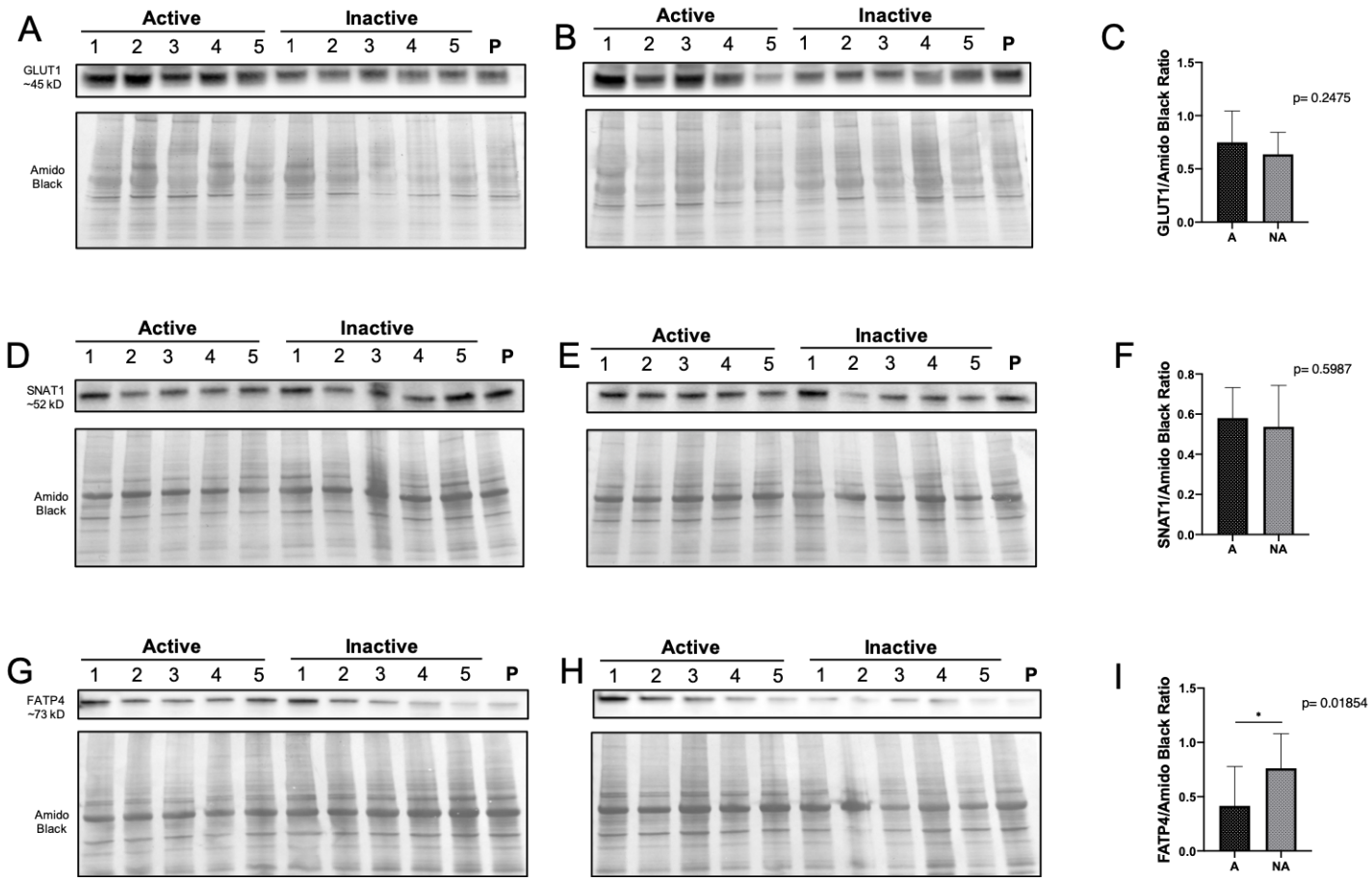

Figure 4. Protein expression of GLUT1 (A-C), SNAT1 (D-F) and FATP4 (G-I) in active (n=10) and non-active (n=10) pregnant women from the chronic exercise study. Protein lysate was prepared from term placenta tissue. Protein band density was normalized to total lane protein density determined by Amido Black staining. Each blot was further normalized to its respective pooled (P) lane for inter-blot comparisons. Values are presented as mean ± standard deviation. Data (D-F) was analyzed using the unpaired t-test or the Mann-Whitney U test depending on normality of the data. A= active participants; NA= non-active participants; P= pooled lysate (n=10 active and n=10 non-active); (*)= p <0.05.

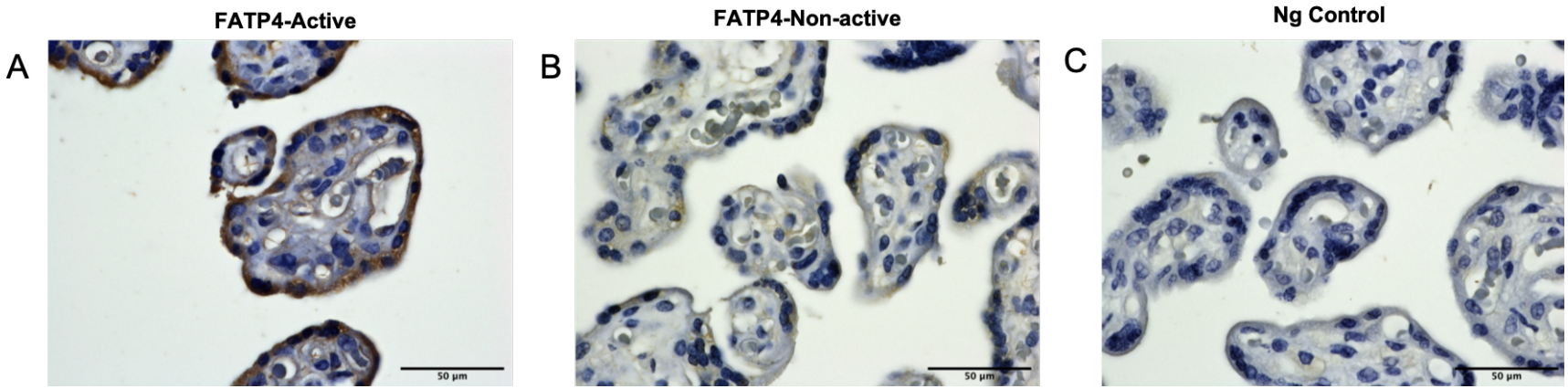

Figure 5. FATP4 localization in term placenta of physically active versus non-active women. Representative photomicrographs of five biological replicates at 40X magnification show higher positive FATP4 staining (brown) in the syncytiotrophoblasts of the chorionic villi of active (A) compared to non-active (B) women. Representative negative control image is shown in panel (C).

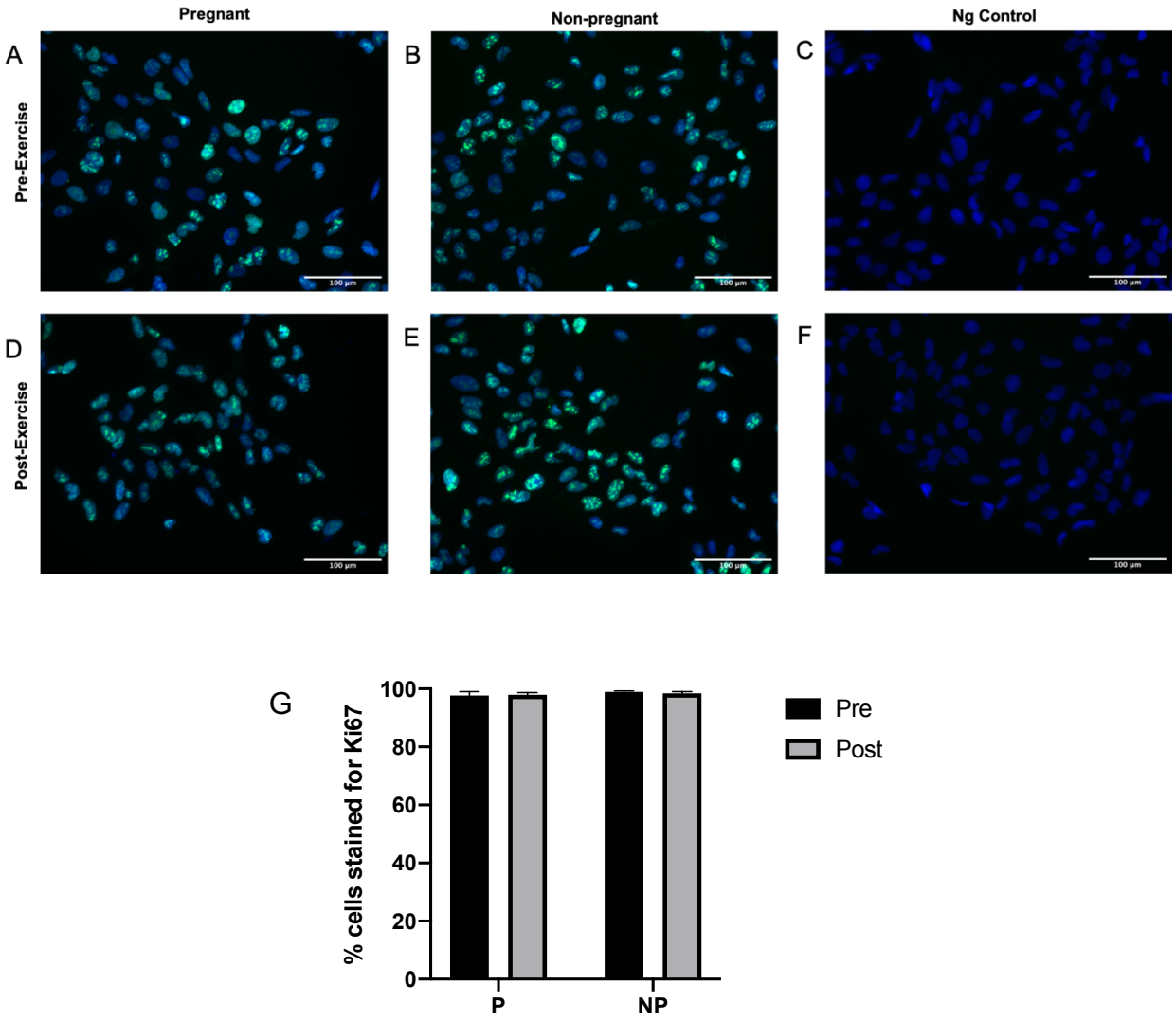

Figure 6. Effect of pre- or post-exercise serum from pregnant (A, D) and non-pregnant (B, E) women on HTR-8/SVneo proliferation. Changes in cellular proliferation were assessed using the nuclear proliferative marker Ki67 following treatment with 10% human serum in 1640 RPMI media for 24hr. Images shown are representative of three biological replicates for each experimental condition and were taken using an immunofluorescent microscope. Negative (Ng) control images (C, F) were taken from cells treated with pooled serum from pregnant participants without treatment with primary antibody. The number of positive cells (stained in green for Ki67) were manually counted and expressed as a percentage of total cell number (stained in blue for DAPI). Values are presented as mean ± standard deviation. Data shown in (G) were analyzed using a two-way ANOVA. Pre= Pre-exercise serum treatment; Post= Post-exercise serum treatment; P= Pregnant; NP= Non-pregnant.

References

[1] S. Lager and T. L. Powell, "Regulation of Nutrient Transport across the Placenta," *J. Pregnancy*, pp. 1–14, 2012.

[2] K. E. Brett, Z. M. Ferraro, J. Yockell-Lelievre, A. Gruslin, and K. B. Adamo, "Maternal–Fetal nutrient transport in pregnancy pathologies: The role of the placenta," *Int. J. Mol. Sci.*, vol. 15, no. 9, pp. 16153–16185, 2014.

[3] Y. Aiko *et al.*, "Differential levels of amino acid transporters System L and ASCT2, and the mTOR protein in placenta of preeclampsia and IUGR," *BMC Pregnancy Childbirth*, vol. 14, no. 181, pp. 1–12, 2014.

[4] G. T. Knipp, K. L. Audus, and M. J. Soares, "Nutrient transport across the placenta," *Adv. Drug Deliv. Rev.*, vol. 38, pp. 41–58, 1999.

[5] E. Larqué, M. Ruiz-Palacios, and B. Koletzko, "Placental regulation of fetal nutrient supply," *Curr. Opin. Clin. Nutr. Metab. Care*, vol. 16, no. 3, pp. 292–297, 2013.

[6] B. P. Lüscher *et al.*, "Placental glucose transporter (GLUT)-1 regulation in pre-eclampsia," *Placenta*, vol. 55, pp. 94–99, 2017.

[7] C. Janzen, M. Y. Y. Lei, P. Sullivan, B.-C. Shin, and S. U. Devaskar, "Placental glucose transporter 3 (GLUT3) is up-regulated in human pregnancies complicated by late-onset intrauterine growth restriction," *Placenta*, vol. 34, no. 11, pp. 1072–1078, 2013.

[8] M. A. Grillo, A. Lanza, and S. Colombatto, "Transport of amino acids through the placenta and their role," *Amino Acids*, vol. 34, no. 4, pp. 517–523, 2008.

[9] F. Verrey, "System L: Heteromeric exchangers of large, neutral amino acids involved in directional transport," *Eur. J. Physiol.*, vol. 445, no. 5, pp. 529–533, 2003.

[10] M. Desforges *et al.*, "SNAT4 isoform of system A amino acid transporter is expressed in human placenta," *Am. J. Physiol. Physiol.*, vol. 290, no. 1, pp. C305–C312, 2005.

[11] I. L. M. H. Aye, T. Jansson, and T. L. Powell, "TNF-α stimulates system a amino acid transport in primary human trophoblast cells mediated by p38 MAPK signaling," *Physiol. Rep.*, vol. 3, no. 10, pp. 1–11, 2015.

[12] K. E. Brett, Z. M. Ferraro, M. Holcik, and K. B. Adamo, "Prenatal physical activity and diet composition affect the expression of nutrient transporters and mTOR signaling molecules in the human placenta," *Placenta*, vol. 36, no. 2, pp. 204–212, 2015.

[13] J. F. Clapp, "The effects of maternal exercise on fetal oxygenation and feto-placental growth," *Eur. J. Obstet. Gynecol. Reprod. Biol.*, vol. 110, no. SUPPL., pp. 80–85, 2003.

[14] K. B. Adamo, Z. M. Ferraro, and K. E. Brett, "Can we modify the intrauterine environment to halt the intergenerational cycle of obesity?," *Int. J. Environ. Res. Public Health*, vol. 9, no. 4, pp. 1263–1307, 2012.

[15] Z. M. Ferraro, L. Gaudet, and K. B. Adamo, "The Potential Impact of Physical Activity During Pregnancy on Maternal and Neonatal Outcomes," *Obstet. Gynecol. Surv.*, vol. 67, no. 2, pp. 99–110, 2012.

[16] M. F. Mottola *et al.*, "2019 Canadian guideline for physical activity throughout pregnancy," *Br. J. Sports Med.*, vol. 52, no. 21, pp. 1339–1346, 2018.

[17] K. A. Hutchinson, S. Mohammad, L. Garneau, K. Mcinnis, C. Aguer, and K. B. Adamo, "Examination of the Myokine Response in Pregnant and Non-pregnant Women Following an Acute Bout of Moderate-Intensity Walking," *Front. Physiol.*, vol. 10, no. 1188, pp. 1–10, 2019.

[18] K. E. Brett, S. Wilson, Z. M. Ferraro, and K. B. Adamo, "Self-report Pregnancy Physical

Activity Questionnaire overestimates physical activity," *Can. J. Public Heal.*, vol. 106, no. 5, pp. e297–e302, 2015.
[19] F. Liu, M. J. Soares, and K. L. Audis, "Permeability properties of monolayers of teh human trophoblast cell line BeWo," vol. 273, no. 5, pp. 1596–1604, 1997.
[20] C. J. Bode, H. Jin, E. Rytting, P. S. Silverstein, A. M. Young, and K. L. Audis, "In Vitro Models for Studying Trophoblast Transcellular Transport," *Methods Mol Med.*, vol. 122, no. 2, pp. 225–239, 2006.
[21] C. H. Graham *et al.*, "Establishment and Characterization of First Trimester Human Trophoblast Cells with Extended Lifespan," *Exp. Cell Res.*, vol. 206, pp. 204–211, 1993.
[22] M. Weber, I. Knoefler, E. Schleussner, U. R. Markert, and J. S. Fitzgerald, "HTR8/SVneo cells display trophoblast progenitor cell-like characteristics indicative of self-renewal, repopulation activity, and expression of 'stemness-' associated transcription factors," *Biomed Res. Int.*, vol. 2013, pp. 1–10, 2013.
[23] L. M. Mudd, K. M. Owe, M. F. Mottola, and J. M. Pivarnik, "Health benefits of physical activity during pregnancy: An international perspective," *Med. Sci. Sports Exerc.*, vol. 45, no. 2, pp. 268–277, 2013.
[24] S. Ruchat *et al.*, "Effectiveness of exercise interventions in the prevention of excessive gestational weight gain and postpartum weight retention : a systematic review and meta-analysis," *Br. J. Sports Med.*, vol. 52, pp. 1347–1356, 2018.
[25] C. Vogel and E. M. Marcotte, "Insights into regulation of protein abundance from proteomics and transcriptomis analyses.," *Nat. Rev. Genet.*, vol. 13, no. 4, pp. 227–232, 2013.
[26] Y. Fu *et al.*, "Crosstalk between alternative polyadenylation and miRNAs in the regulation of protein translational efficiency," *Genome Res.*, vol. 28, no. 11, pp. 1656–1663, 2018.
[27] X. H. Liang, W. Shen, H. Sun, M. T. Migawa, T. A. Vickers, and S. T. Crooke, "Translation efficiency of mRNAs is increased by antisense oligonucleotides targeting upstream open reading frames," *Nat. Biotechnol.*, vol. 34, no. 8, pp. 875–880, 2016.
[28] E. Dubé *et al.*, "Modulation of Fatty Acid Transport and Metabolism by Maternal Obesity in the Human Full-Term Placenta1," *Biol. Reprod.*, vol. 87, no. 1, pp. 1–11, 2012.
[29] G. Kaur and J. M. Dufour, "Cell lines: Valuable tools or useless artifacts.," *Spermatogenesis*, vol. 2, no. 1, pp. 1–5, 2012.
[30] A. R. Fleischman, M. Oinuma, and S. L. Clark, "Rethinking the definition of 'term pregnancy,'" *Obstet. Anesth. Dig.*, vol. 31, no. 3, pp. 144–145, 2011.
[31] R. Barakat, J. R. Stirling, and A. Lucia, "Does exercise training during pregnancy affect gestational age? A randomised controlled trial," *Br. J. Sports Med.*, vol. 42, no. 8, pp. 674–678, 2008.

CHAPTER 4 – DISCUSSION AND GENERAL CONCLUSIONS OF THE THESIS

This thesis presents the first characterization of the circulating myokine profile of pregnant women following an acute bout of generalizable moderate-intensity PA. Myokines have been studied extensively in various healthy and diseased populations however, as with most fields of research, especially relating to exercise and PA, healthy pregnant women are often overlooked. Contrary to outdated suppositions, current evidence nearly unanimously shows not only the safety but also the benefits tied to engaging in PA during pregnancy. Nonetheless, current knowledge and understanding of the role of PA on maternal, fetal and placental physiology during pregnancy is limited.

The first objective of this thesis yielded salient results. Following a 30-min bout of moderate-intensity walking, the serum levels for FGF21, EPO, BDNF, and IL-15 were elevated in pregnant women (Figure 4). Additionally, pregnant women had a higher baseline level of EPO and oncostatin compared to non-pregnant women – highlighting the potential role for these molecules in pregnancy (Figure 4). Although these findings are significant and will add to the growing body of literature regarding pregnancy and PA, much remains to be explored. A panel of only ten myokines was evaluated in this study, despite hundreds of these molecules being known. Their specific roles in pregnancy remain unknown. The myokines chosen for evaluation in this study are well-characterized in the literature, nevertheless it is possible that myokines connected to pregnancy were not evaluated. As stated in the first manuscript of this thesis, the exercise modality and intensity for this study were chosen based on the Canadian Guideline [3] to be generalizable to the pregnant population. Although we did find differences in pre- and post-exercise levels for four myokines in pregnant women, different intensities of exercise (e.g., light or vigorous), in addition to different modalities (e.g., cycling or swimming), should be

investigated in the context of myokines. Furthermore, factors such as chronic exercise warrant future consideration when examining the myokine response in pregnant women as its effect is currently unknown. Lastly, there are measurement limitations regarding serum myokine levels. Most of these molecules are known to be secreted by various other tissues and organs in the human body, in addition to skeletal muscle. Thus, the design of this study does not allow confirmation that circulating serum cytokines are solely produced by skeletal muscle as a result of PA.

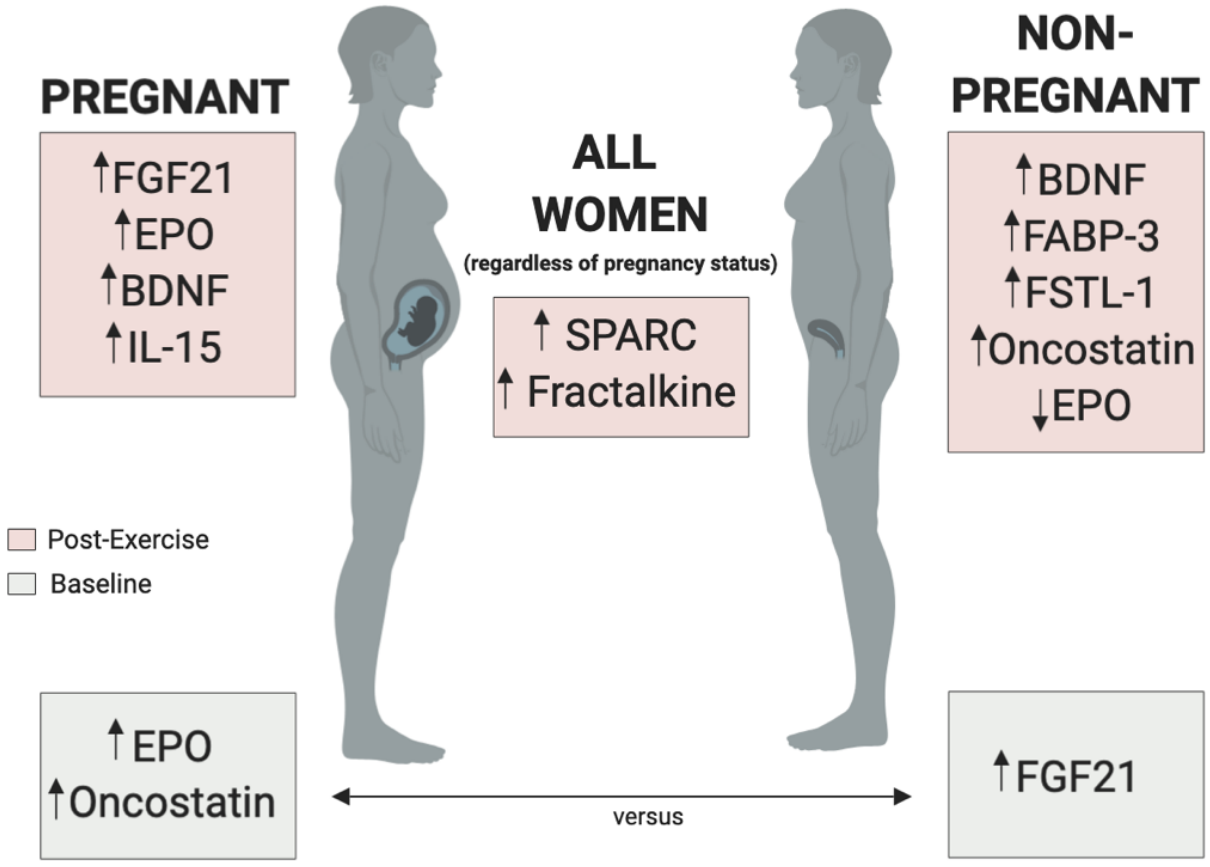

Figure 4. Summary of the findings from the first objective of the thesis. Pregnant women exhibited an increase in four myokines post-exercise: FGF21, EPO, BDNF and IL-15. Four myokines were increased post-exercise in the non-pregnant group: BDNF, FABP-3, FSTL-1 and oncostatin while EPO decreased following the exercise session. SPARC and fractalkine were elevated in all women, regardless of pregnancy status. Pregnant women had a higher baseline level of EPO and oncostatin compared to non-pregnant women, while non-pregnant women exhibited higher baseline FGF21. [Image created by Kelly Ann Hutchinson using ©BioRender 2019].

The second objective of this thesis was to examine whether acute and/or chronic exercise instigates changes in metrics of placental growth and function, namely cellular proliferation as well as nutrient transporter expression and localization. The rationale behind this exploration lies in knowing that PA brings about benefits to the fetus and the placenta, but that the mechanisms and molecules responsible for such outcomes remain unknown. To assess the effect of acute exercise on the above-mentioned metrics, pre- and post-exercise serum collected from the first objective was used to treat placental cell lines (BeWo and HTR-8/SVneo). Second, to assess the role of chronic exercise, term placenta collected from active and non-active pregnant women (PA levels determined by objective accelerometry data) were analyzed for nutrient transporter expression and localization.

We found that BeWo cells treated for 8hr with either pre- or post-exercise serum from pregnant compared to non-pregnant women, exhibited a higher protein expression for GLUT1. Glucose provided via maternal circulation is the primary substrate for fetal growth. As such, GLUT1, highly expressed throughout pregnancy, is considered the most prominent placental glucose transporter. Thus, it is possible that factors or molecules in the serum of pregnant women are responsible for the observed higher expression of GLUT1. Also, we observed no difference in the expression of GLUT1 in cells treated for 1hr with pre- versus post-exercise serum from pregnant. This result further validates existing literature supporting the safety of PA engagement during pregnancy as we show that exercise does not decrease the expression of a prominent placental glucose transporter.

Secondly, the expression for FATP4 was found to be greater in highly active pregnant women compared to those categorized as non-active. As discussed in the second manuscript of this thesis, these findings are unexpected given the work previously conducted in our lab

showing higher levels of mRNA expression for the *FATP4* gene in pregnant women who are active compared to non-active women. However, Vogel and Marcotte [80] explain that regulatory processes, such as post-transcriptional and translational modifications as well as protein degradation, are often overlooked when associating mRNA expression to protein abundance. In fact, they state that nearly all mRNA transcripts that have been studied in the literature, originating from various organisms, can only "partially predict protein abundance" [80]. Furthermore, work conducted in our lab has illustrated that many non-coding RNAs, generally associated with inhibiting translation, are downregulated in the placenta of highly active pregnant women. In light of this knowledge, future research should aim to assess whether FA uptake is also altered as a result of exercise.

The results obtained in the second objective of this thesis enhance our knowledge as to the role of PA in pregnancy. However, much research remains to be undertaken for a more thorough understanding of the processes involved in optimizing maternal, fetal and placental health as a result of PA. In terms of nutrient transport, there exist many placental transporters that were not examined in this thesis but may be specific targets of PA. Although challenging, the role of PA on other vital placental transporters should be investigated. In addition to expression and localization of transporters, other metrics of placental growth and function, namely invasion and migration in addition to macronutrient transport should also be explored. Lastly, the cellular model we used to examine the effect of acute exercise on placental nutrient transport has its limitations. Although placental cancer cell lines are beneficial in that they allow for timely and efficient experimentation, they are not true models of healthy trophoblasts.

The importance of this thesis lies in identifying potential mechanistic and molecular targets as well as mediators involved in optimizing maternal, fetal and placental health as a result

of a physically active pregnancy. Future research should also aim to explore different exercise modalities, duration and intensities as they may benefit the placenta via unique mechanistic pathways. Identifying molecules and understanding pathways through which placental health is improved as a result of PA is crucial in potentially developing therapeutic agents and treatments as well as prescribing appropriate exercise regimens for women experiencing complicated pregnancies.

CHAPTER 5 – REFERENCES

[1] D. E. R. Warburton, C. W. Nicol, and S. S. D. Bredin, "Health benefits of physical activtity: the evidence," *CMAJ*, vol. 174, no. 6, pp. 801–809, 2006.

[2] M. Reiner, C. Niermann, D. Jekauc, and A. Woll, "Long-term health benefits of physical activity- a systematic review of longitudinal studies," *BMC Public Health*, vol. 13, no. 813, pp. 1–9, 2013.

[3] M. F. Mottola *et al*., "2019 Canadian guideline for physical activity throughout pregnancy," *Br. J. Sports Med*., vol. 52, no. 21, pp. 1339–1346, 2018.

[4] R. Lindsay, "What is gestational diabetes?," *Gestation. Diabetes Orig. Complicat. Treat*., vol. 30, pp. 33–48, 2014.

[5] L. Bellamy, J.-P. Casas, A. D. Hingorani, and D. Williams, "Type 2 diabetes mellitus after gestational diabetes: a systematic review and meta-analysis," *Lancet*, vol. 373, pp. 1773–1779, 2009.

[6] R. Kessous, I. Shoham-Vardi, G. Pariente, M. Sherf, and E. Sheiner, "An association between gestational diabetes mellitus and long-term maternal cardiovascular morbidity," *Heart*, vol. 99, no. 15, pp. 1118–1121, 2013.

[7] M. H. Davenport *et al*., "Prenatal exercise for the prevention of gestational diabetes mellitus and hypertensive disorders of pregnancy: A systematic review and meta-analysis," *Br. J. Sports Med*., vol. 52, no. 21, pp. 1367–1375, 2018.

[8] B. W. J. Mol, C. T. Roberts, S. Thangaratinam, L. A. Magee, C. J. M. De Groot, and G. J. Hofmeyr, "Pre-eclampsia," *Lancet*, vol. 387, pp. 999–1011, 2016.

[9] L. Bellamy, J. P. Casas, A. D. Hingorani, and D. J. Williams, "Pre-eclampsia and risk of cardiovascular disease and cancer in later life: Systematic review and meta-analysis," *Br. Med. J*., vol. 335, no. 974, pp. 1–12, 2007.

[10] E. R. Magro-Malosso, G. Saccone, M. Di Tommaso, A. Roman, and V. Berghella, "Exercise during pregnancy and risk of gestational hypertensive disorders : a systematic review and meta-analysis," *AOGS*, vol. 96, no. 1, pp. 921–931, 2017.

[11] Z. M. Ferraro, L. Gaudet, and K. B. Adamo, "The Potential Impact of Physical Activity During Pregnancy on Maternal and Neonatal Outcomes," *Obstet. Gynecol. Surv*., vol. 67, no. 2, pp. 99–110, 2012.

[12] M. H. Davenport *et al*., "Exercise for the prevention and treatment of low back , pelvic girdle and lumbopelvic pain during pregnancy : a systematic review and meta-analysis," *Br. J. Sports Med*., vol. 53, pp. 90–98, 2018.

[13] B. Muktabhant, L. Theresa, L. Pisake, and L. Malinee, "Diet or exercise, or both, for preventing excessive weight gain in pregnancy (Review)," *Cochrane Database Syst. Rev*., no. 6, 2015.

[14] C. M. Olson and M. S. Strawderman, "Modifiable behavioral factors in a biopsychosocial model predict inadequate and excessive gestational weight gain," *J. Am. Diet. Assoc*., vol. 103, no. 1, pp. 48–54, 2003.

[15] I. of M. (U. S. . C. to R. I. P. W. G. Rasmussen, KM, Yaktine, AL, *Weight gain during pregnancy: Reexamining the guidelines*. The National Academic Press, 2009.

[16] N. H. Morken, K. Klungsøyr, P. Magnus, and R. Skjærven, "Pre-pregnant body mass index, gestational weight gain and the risk of operative delivery," *Acta Obstet. Gynecol. Scand*., vol. 92, no. 7, pp. 809–815, 2013.

[17] I. Thorsdottir, J. E. Torfadottir, B. E. Birgisdottir, and R. T. Geirsson, "Weight Gain in

Women of Normal Weight Before Pregnancy: Complicatiosn in Pregnancy or Delivery and Birth Outcome," *Obstet. Gynecol.*, vol. 99, no. 5, pp. 799–806, 2002.
[18] J. Crane, P. Murphy, L. Burrage, J. White, and D. Hutchens, "The effect of gestational weight gain by body mass index on maternal and neonatal outcomes," *Int. J. Gynecol. Obstet.*, vol. 107, pp. S437–S437, 2009.
[19] J. Robitaille, "Excessive gestational weight gain and gestational diabetes: importance of the first weeks of pregnancy," *Diabetologia*, vol. 58, no. 10, pp. 2203–2205, 2015.
[20] J. Johnson *et al.*, "Pregnancy Outcomes With Weight Gain Above or Below the 2009 Institute of Medicine Guidelines," *Obs. Gynecol*, vol. 121, no. 5, pp. 969–975, 2013.
[21] L. Schack-Nielsen, K. F. Michaelsen, M. Gamborg, E. L. Mortensen, and T. I. A. Sørensen, "Gestational weight gain in relation to offspring body mass index and obesity from infancy through adulthood," *Int. J. Obes.*, vol. 34, no. 1, pp. 67–74, 2010.
[22] Z. M. Ferraro *et al.*, "Excessive gestational weight gain predicts large for gestational age neonates independent of maternal body mass index," *J. Matern. Neonatal Med.*, vol. 25, no. 5, pp. 538–542, 2012.
[23] K. B. Adamo, Z. M. Ferraro, and K. E. Brett, "Can we modify the intrauterine environment to halt the intergenerational cycle of obesity?," *Int. J. Environ. Res. Public Health*, vol. 9, no. 4, pp. 1263–1307, 2012.
[24] M. Persson, D. Pasupathy, U. Hanson, and M. Norman, "Disproportionate body composition and perinatal outcome in large-for-gestational-age infants to mothers with type 1 diabetes," *BJOG An Int. J. Obstet. Gynaecol.*, vol. 119, no. 5, pp. 565–572, 2012.
[25] A. Weissmann-Brenner *et al.*, "Maternal and neonatal outcomes of large for gestational age pregnancies," *Acta Obstet. Gynecol. Scand.*, vol. 91, no. 7, pp. 844–849, 2012.
[26] A. H. Marques, A.-L. Bjørke-Monsen, A. L. Teixeira, and M. N. Silverman, "Maternal stress, nutrition and physical activity: Impact on immune function, CNS development and psychopathology," *Brain Res.*, vol. 1617, pp. 28–46, 2015.
[27] L. E. May, S. A. Scholtz, R. Suminski, and K. M. Gustafson, "Aerobic exercise during pregnancy in fl uences infant heart rate variability at one month of age," *Early Hum. Dev.*, vol. 90, no. 1, pp. 33–38, 2014.
[28] K. Benirschke, "Remarkable placenta," *Clin. Anat.*, vol. 11, no. 3, pp. 194–205, 1998.
[29] H. H. Kay, M. D. Nelson, and Y. Wang, Eds., "The Placenta: from Development to Disease," First., Blackwell Publishing Ltd., 2011.
[30] K. Red-Horse *et al.*, "Trophoblast differentiation during embryo implantation and formation of the maternal-fetal interface," *J. Clin. Invest.*, vol. 114, no. 6, pp. 744–754, 2004.
[31] S. R. Anin, G. Vince, and S. Quenby, "Trophoblast invasion," *Hum. Fertil.*, vol. 7, no. 3, pp. 169–174, 2004.
[32] S. Lager and T. L. Powell, "Regulation of Nutrient Transport across the Placenta," *J. Pregnancy*, pp. 1–14, 2012.
[33] G. T. Knipp, K. L. Audus, and M. J. Soares, "Nutrient transport across the placenta," *Adv. Drug Deliv. Rev.*, vol. 38, pp. 41–58, 1999.
[34] K. E. Brett, Z. M. Ferraro, M. Holcik, and K. B. Adamo, "Prenatal physical activity and diet composition affect the expression of nutrient transporters and mTOR signaling molecules in the human placenta," *Placenta*, vol. 36, no. 2, pp. 204–212, 2015.
[35] H. N. Jones, L. A. Woollett, N. Barbour, P. D. Prasad, T. L. Powell, and T. Jansson, "High-fat diet before and during pregnancy causes marked up-regulation of placental

nutrient transport and fetal overgrowth in C57/BL6 mice," *FASEB J.*, vol. 23, no. 1, pp. 271–278, 2009.
[36] K. E. Brett, Z. M. Ferraro, J. Yockell-Lelievre, A. Gruslin, and K. B. Adamo, "Maternal–Fetal nutrient transport in pregnancy pathologies: The role of the placenta," *Int. J. Mol. Sci.*, vol. 15, no. 9, pp. 16153–16185, 2014.
[37] N. P. Illsley and M. U. Baumann, "Human placental glucose transport in fetoplacental growth and," *BBA-Molecular Basus Dis.*, no. December, 2018.
[38] N. P. Illsley, "Glucose Transporters in the Human Placenta," *Placenta*, vol. 21, no. 1, pp. 14–22, 2000.
[39] M. A. Grillo, A. Lanza, and S. Colombatto, "Transport of amino acids through the placenta and their role," *Amino Acids*, vol. 34, no. 4, pp. 517–523, 2008.
[40] H. N. Jones, T. L. Powell, and T. Jansson, "Regulation of Placental Nutrient Transport - A Review," *Placenta*, vol. 28, no. 8–9, pp. 763–774, 2007.
[41] T. Jansson, "Amino acid transporters in the human placenta," *Pediatr. Res.*, vol. 49, no. 2, pp. 141–147, 2001.
[42] I. L. M. H. Aye, T. Jansson, and T. L. Powell, "TNF-α stimulates system a amino acid transport in primary human trophoblast cells mediated by p38 MAPK signaling," *Physiol. Rep.*, vol. 3, no. 10, pp. 1–11, 2015.
[43] F. Verrey, "System L: Heteromeric exchangers of large, neutral amino acids involved in directional transport," *Eur. J. Physiol.*, vol. 445, no. 5, pp. 529–533, 2003.
[44] D. M. Farley *et al.*, "Placental amino acid transport and placental leptin resistance in pregnancies complicated by maternal obesity," *Placenta*, vol. 31, no. 8, pp. 718–724, 2010.
[45] A. K. Dutta-Roy, "Transport mechanisms for long-chain polyunsaturated fatty acids in the human placenta," *Am. J. Clin. Nutr.*, vol. 71, pp. 315S-322S, 2000.
[46] A. K. Duttaroy, "Transport of fatty acids across the human placenta: A review," *Prog. Lipid Res.*, vol. 48, no. 1, pp. 52–61, 2009.
[47] A. L. Magnusson, I. J. Waterman, M. Wennergren, T. Jansson, and T. L. Powell, "Triglyceride hydrolase activities and expression of fatty acid binding proteins in the human placenta in pregnancies complicated by intrauterine growth restriction and diabetes," *J. Clin. Endocrinol. Metab.*, vol. 89, no. 9, pp. 4607–4614, 2004.
[48] B. K. Pedersen, T. C. A. Akerström, A. R. Nielsen, and C. P. Fischer, "Role of myokines in exercise and metabolism.," *J. Appl. Physiol.*, vol. 103, no. 3, pp. 1093–8, 2007.
[49] C. Dubé, C. Aguer, K. Adamo, and S. Bainbridge, "A role for maternally derived myokines to optimize placental function and fetal growth across gestation," *Appl. Physiol. Nutr. Metab.*, vol. 42, no. 5, pp. 459–469, 2017.
[50] L. H. Jørgensen *et al.*, "Secreted protein acidic and rich in cysteine (SPARC) in human skeletal muscle," *J. Histochem. Cytochem.*, vol. 57, no. 1, pp. 29–39, 2009.
[51] A. Rodríguez, S. Becerril, S. Ezquerro, L. Méndez-Giménez, and G. Frühbeck, "Crosstalk between adipokines and myokines in fat browning," *Acta Physiol.*, vol. 219, no. 2, pp. 362–381, 2017.
[52] S. Schnyder and C. Handschin, "Skeletal muscle as an endocrine organ: The role of myokines in exercise adaptations," *Bone*, vol. 7, no. 11, pp. 115–125, 2015.
[53] B. K. Pedersen, "The diseasome of physical inactivity - and the role of myokines in muscle-fat cross talk," *J. Physiol.*, vol. 587, no. 23, pp. 5559–5568, 2009.
[54] J. H. Lee and H. S. Jun, "Role of myokines in regulating skeletal muscle mass and

function," *Front. Physiol.*, vol. 10, no. JAN, pp. 1–9, 2019.
[55] G. Colaianni, T. Mongelli, S. Colucci, S. Cinti, and M. Grano, "Crosstalk Between Muscle and Bone Via the Muscle-Myokine Irisin," *Curr. Osteoporos. Rep.*, vol. 14, no. 4, pp. 132–137, 2016.
[56] H. Ellingsgaard *et al.*, "Interleukin-6 enhances insulin secretion by increasing glucagon-like peptide-1 secretion from L cells and alpha cells," *Nat. Med.*, vol. 17, no. 11, pp. 1481–1489, 2011.
[57] T. Bettis, B. J. Kim, and M. W. Hamrick, "Impact of muscle atrophy on bone metabolism and bone strength: implications for muscle-bone crosstalk with aging and disuse," *Osteoporos. Int.*, vol. 29, no. 8, pp. 1713–1720, 2018.
[58] M. Pal, M. A. Febbraio, and M. Whitham, "From cytokine to myokine: The emerging role of interleukin-6 in metabolic regulation," *Immunol. Cell Biol.*, vol. 92, no. 4, pp. 331–339, 2014.
[59] A. Steensberg, C. Keller, R. L. Starkie, T. Osada, M. A. Febbraio, and B. K. Pedersen, "IL-6 and TNF-α expression in, and release from, contracting human skeletal muscle," *Am. J. Physiol. - Endocrinol. Metab.*, vol. 283, no. 6, pp. E1272–E1278, 2002.
[60] L. Gough, R. S. Penfold, R. J. Godfrey, and L. Castell, "The immune response to short-duration exercise in trained, eumenorrhoeic women," *J. Sports Sci.*, vol. 33, no. 13, pp. 1396–1402, 2015.
[61] B. K. Pedersen, T. C. A. Akerström, A. R. Nielsen, and C. P. Fischer, "Role of myokines in exercise and metabolism.," *J. Appl. Physiol.*, vol. 103, no. 3, pp. 1093–8, 2007.
[62] A. L. Carey *et al.*, "Interleukin-6 increases insulin-stimulated glucose disposal in humans and glucose uptake and fatty acid oxidation in vitro via AMP-activated protein kinase," *Diabetes*, vol. 55, no. 10, pp. 2688–2697, 2006.
[63] D. C. Papatheodorou *et al.*, "Pulsatile interleukin-6 leads CRH secretion and is associated with myometrial contractility during the active phase of term human labor," *J. Clin. Endocrinol. Metab.*, vol. 98, no. 10, pp. 4105–4112, 2013.
[64] B. K. Pedersen, "Muscle as a secretory organ," *Compr. Physiol.*, vol. 3, no. 3, pp. 1337–1362, 2013.
[65] P. S. Furmanczyk and L. S. Quinn, "Interleukin-15 increases myosin accretion in human skeletal myogenic cultures.," *Cell Biol. Int.*, vol. 27, no. 10, pp. 845–51, 2003.
[66] E. E. Pistilli and L. S. Quinn, "From anabolic to oxidative: Reconsidering the roles of IL-15 and IL-15Rα in skeletal muscle," *Exerc. Sport Sci. Rev.*, vol. 41, no. 2, pp. 100–106, 2013.
[67] A. R. Nielsen *et al.*, "Association between interleukin-15 and obesity: Interleukin-15 as a potential regulator of fat mass," *J. Clin. Endocrinol. Metab.*, vol. 93, no. 11, pp. 4486–4493, 2008.
[68] P. Bischof, A. Meisser, and A. Campana, "Paracrine and autocrine regulators of trophoblast invasion - A review," *Placenta*, vol. 21, no. 14, pp. 55–60, 2000.
[69] C. L. Lee, J. H. W. Veerbeek, T. K. Rana, B. B. van Rijn, G. J. Burton, and H. W. Yung, "Role of Endoplasmic Reticulum Stress in Proinflammatory Cytokine–Mediated Inhibition of Trophoblast Invasion in Placenta-Related Complications of Pregnancy," *Am. J. Pathol.*, vol. 189, no. 2, pp. 467–478, 2019.
[70] W. Aoi *et al.*, "A novel myokine, secreted protein acidic and rich in cysteine (SPARC), suppresses colon tumorigenesis via regular exercise," *Gut*, vol. 62, no. 6, pp. 882–889, 2013.

[71] L. H. Jørgensen *et al*., "SPARC Interacts with Actin in Skeletal Muscle in Vitro and in Vivo," *Am. J. Pathol*., vol. 187, no. 2, pp. 457–474, 2017.
[72] B. K. Pedersen, "Exercise-induced myokines and their role in chronic diseases," *Brain. Behav. Immun*., vol. 25, no. 5, pp. 811–816, 2011.
[73] V. B. Matthews *et al*., "Brain-derived neurotrophic factor is produced by skeletal muscle cells in response to contraction and enhances fat oxidation via activation of AMP-activated protein kinase," *Diabetologia*, vol. 52, no. 7, pp. 1409–1418, 2009.
[74] B. K. Pedersen, "Muscle as a secretory organ," *Compr. Physiol*., vol. 3, no. 3, pp. 1337–1362, 2013.
[75] K. Sakuma and A. Yamaguchi, "The Recent Understanding of the Neurotrophin's Role in Skeletal Muscle Adaptation," *J. Biomed. Biotechnol*., vol. 2011, pp. 1–12, 2011.
[76] C. M. Saucedo Marquez, B. Vanaudenaerde, T. Troosters, and N. Wenderoth, "High-intensity interval training evokes larger serum BDNF levels compared with intense continuous exercise," *J. Appl. Physiol*., vol. 119, no. 12, pp. 1363–1373, 2015.
[77] S. Gomes Da Silva *et al*., "Maternal exercise during pregnancy increases BDNF levels and cell numbers in the hippocampal formation but not in the cerebral cortex of adult rat offspring," *PLoS One*, vol. 11, no. 1, pp. 1–15, 2016.
[78] K. Kawamura, N. Kawamura, W. Sato, J. Fukuda, J. Kumagai, and T. Tanaka, "Brain-derived neurotrophic factor promotes implantation and subsequent placental development by stimulating trophoblast cell growth and survival," *Endocrinology*, vol. 150, no. 8, pp. 3774–3782, 2009.
[79] L. Pedersen and P. Hojman, "Muscle-to-organ cross talk mediated by myokines," *Adipocyte*, vol. 1, no. 3, pp. 164–167, 2012.
[80] C. Vogel and E. M. Marcotte, "Insights into regulation of protein abundance from proteomics and transcriptomis analyses.," *Nat. Rev. Genet*., vol. 13, no. 4, pp. 227–232, 2013.

APPENDIX A: RESEARCH ETHICS BOARD (REB) APPROVAL

A.1 UNIVERSITY OF OTTAWA CERTIFICATE OF ETHICS APPROVAL

27/07/2018

Université d'Ottawa
Bureau d'éthique et d'intégrité de la recherche

University of Ottawa
Office of Research Ethics and Integrity

CERTIFICAT D'APPROBATION ÉTHIQUE I CERTIFICATE OF ETHICS APPROVAL

Numéro du dossier / Ethics File Number	H-06-18-634
Titre du projet / Project Title	The potential role for exercise-induced myokines in the optimization of placental growth and function
Type de projet / Project Type	Thèse de maîtrise / Master's thesis
Statut du projet / Project Status	Approuvé / Approved
Date d'approbation (jj/mm/aaaa) / Approval Date (dd/mm/yyyy)	27/07/2018
Date d'expiration (jj/mm/aaaa) / Expiry Date (dd/mm/yyyy)	26/07/2019

Équipe de recherche / Research Team

Chercheur / Researcher	Affiliation	Role
Kelly Ann HUTCHINSON	École des sciences de l'activité physique / School of Human Kinetics	Chercheur Principal / Principal Investigator
Kristi ADAMO	École des sciences de l'activité physique / School of Human Kinetics	Superviseur / Supervisor
Nhung VUONG	École des sciences de l'activité physique / School of Human Kinetics	Coordonnateur de recherche / Research Coordinator
Shuhiba MOHAMMAD	École des sciences de l'activité physique / School of Human Kinetics	Étudiant-chercheur / Student-researcher

Conditions spéciales ou commentaires / Special conditions or comments

550, rue Cumberland, pièce 154
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www.recherche.uottawa.ca/deontologie I www.recherche.uottawa.ca/ethics

APPENDIX B: MYOKINE STUDY DOCUMENTATION

B.1 PARTICIPANT INFORMED CONSENT

PARTICIPANT INFORMED CONSENT

Faculté des sciences
de la santé
Pavillon Roger-Guindon

Faculty of Health Sciences
Roger Guindon Hall

613-562-5800 (5603)
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health.uOttawa.ca

451 Smyth
Ottawa ON K1H 8M5
Canada

Title of Study: The potential role for exercise-induced myokines in the optimization of placenta growth and function

Researchers:

Kristi Adamo Ph. D.	Associate Professor, University of Ottawa
Nhung Vuong Ph. D.	Research Coordinator, Adamo Lab
Kelly Ann Hutchinson	Graduate Student, Adamo Lab

The Principal Investigator of this study is Kelly Ann Hutchinson, under supervision of Dr. Kristi Adamo. This research project is being conducted as part of Ms. Hutchinson's Master's thesis.

Funding Agency: Natural Sciences and Engineering Research Council (NSERC).

Participation in this study is voluntary. Please read this Participant Informed Consent Form carefully before you decide if you would like to participate. Please ask the Principal Investigator and the study team as many questions as you would like. We encourage you to discuss your options with family, friends or your health care provider.

Invitation to Participate:
You are being asked to participate in this research study because you are either a pregnant or a non-pregnant woman, are between 18 to 40 years old and have indicated that you are interested in learning more about our research study.

Purpose of the Study:
This study is designed to look at how physical activity during pregnancy can affect how nutrients move to the growing fetus. The placenta is an organ that forms during pregnancy to support the baby. The placenta controls the transfer of nutrients (sugar, protein and fat) and oxygen to the fetus, and removes waste. If the placenta is not working properly, the baby may receive too few or too many nutrients. It is not well known how physical activity affects the transfer of nutrients to the fetus.

The goal of this study is to observe whether myokines, which are peptide and cytokines, released from skeletal muscle when engaging in physical activity, affects certain measurements of placental growth and function. More specifically, we want to test whether myokines affect the ability of placental cells to survive and replicate in vitro (test-tube experiment), as well as whether they play a role in optimizing the

transfer of nutrients from the mother to the fetus. We hope that the information we learn in this study will be helpful in designing preventive health strategies for pregnancy.

We will be analyzing the pre- and post-exercise myokines in the blood samples collected. Further, we will be treating cultured placental cells with a synthetic mixture of myokines mimicking the profile detected in the blood samples. Placental metrics including cell proliferation, cell viability and nutrient transporter expression as well as function will be analyzed.

Participation:
If you consent to participate in this study, you will be asked to come the University of Ottawa-Lees Campus for one exercise session.

Exercise session details:
The visit will last 3 hours maximum

- We ask that you refrain from exercise 12 hours before the session.
- A small fasting (no food for 8 hours before) blood sample (15mL, ~1 tablespoon) will be taken. Since this blood sample requires being fasted, we recommend that we book the visit in the morning.
- After the blood sample, you be given a small snack, for example a granola bar and some juice.
- You will be asked to complete i) the International Physical Activity Questionnaire (IPAQ), ii) the Godin Leisure Time questionnaire so that we can get a better idea of how active you are on a regular basis and, iii) a Sociodemographic questionnaire.
- We will measure your height and weight.
- We will then ask you to put on a heart rate monitor and a face mask for indirect calorimetry measures. We will ask you to rest seated for 30 minutes to allow relaxation of your muscles and digestion of snack.
- After the relaxation phase, you will undergo a moderate-intensity steady-state treadmill walking session (60 minutes maximum duration).
- If your heart rate reaches 85% of your predicted heart rate maximum, the test will be stopped.
- Immediately following the cool-down, we will take another blood draw (15mL).
- Following the blood draw, we will ask you to remain seated for a second post-exercise resting phase of a 30-minute duration.

Risks:

1. **Pregnant participants**

There is little risk to you or your baby by participating in this study.

Blood drawing causes some pain and may cause bruising, bleeding or infections at the site of the needle stick. A nurse or certified phlebotomist has been trained in safely drawing blood. Care will be taken to avoid these complications.

The walking sesssion will occur in a safe environment and will incorporate the most recent evidence for exercise guidelines during pregnancy. CPR and first aid trained personnel, specially

trained to perform exercise testing, will coordinate and monitor the testing. Heart rate will be continually monitored to ensure that you do not reach an unsafe heart rate. Research staff will make sure the treadmill is adjusted properly and will conduct a proper warm-up and cool-down to prevent injuries.

The risk of an adverse event is minimized through the supervision of testing by qualified personnel. In the unlikely event that you experience an injury, medical or psychological crisis (i.e. chest pains, heart attack, panic attack, etc.) during the fitness test, a safety protocol is in place and the university emergency response team will be contacted immediately.

2. Non-pregnant participants

There is little risk to you by participating in this study.

Blood drawing causes some pain and may cause bruising, bleeding or infections at the site of the needle stick. A nurse or certified phlebotomist has been trained in safely drawing blood. Care will be taken to avoid theses complications.

The walking session will occur in a safe environment. CPR and first aid trained personnel, specially trained to perform exercise testing, will coordinate and monitor the testing. Heart rate will be continually monitored to ensure that you do not reach an unsafe heart rate. Research staff will make sure the treadmill is adjusted properly and will conduct a proper warm-up and cool-down to prevent injuries.

The risk of an adverse event is minimized through the supervision of testing by qualified personnel. In the unlikely event that you experience an injury, medical or psychological crisis (i.e. chest pains, heart attack, panic attack, etc.) during the fitness test, a safety protocol is in place and the university emergency response team will be contacted immediately.

Benefits:

1. Pregnant Participants

The results of these tests may not be directly beneficial to you and your baby, but the results will help define the potential role of physical activity in pregnancy. The knowledge gained from this study may benefit other pregnant women in the future. The group results from this study will be shared with health care professionals including general practitioners, obstetricians and gynecologists, exercise and nutrition professionals as well as policy makers and health care planners.

2. Non-pregnant participants

The results of these tests may not be directly beneficial to you. However, this study will help determine whether physical activity results in the release of different myokines by skeletal muscle in pregnant compared to non-pregnant women and how these potential differences may affect the development of the placenta and thereby the fetus. The group results from this study will be shared with health care professionals including general practitioners, obstetricians and gynecologists, exercise and nutrition professionals as well as policy makers and health care planners.

Anonymity, Confidentiality and Data Storage:

- All information and samples collected during your participation in this study will be identified with a unique study number, and will not contain information that identifies you, such as your name, address, etc.
- The link between your unique study number and your name and contact information will be stored securely, password protected and separate from your study records, at The University of Ottawa. The link will not leave the University of Ottawa.
- Any documents or samples leaving the University of Ottawa will contain only your unique study number. This includes publications or presentations resulting from this study.
- Research records will be kept for 10 years.
- At the end of the storage time, all paper records will be shredded, and all electronic records will be securely deleted.

Voluntary Participation:
Your participation in this study is voluntary. You may decide not to participate in this study, or to participate in the study now, and then change your mind later without affecting the medical care, education, or other services to which you are entitled or are presently receiving at this institution. You may withdraw from the study at any time. Information and samples collected for the study before you withdraw this consent will be destroyed unless you request otherwise.

What compensation will I receive if I am injured or become ill in this study?
In the event of a study-related injury or illness, you will be provided with appropriate medical treatment and care. Financial compensation for lost wages, disability or discomfort due to an injury or illness is not available. You are not waiving any of your legal rights by agreeing to participate in this study.

Will I be paid for my participation or will there be any additional costs to me?
You will not be paid to be a participant in the study; however, a parking voucher will be provided to you to cover bus or parking costs for all visits attended with the study team at the University of Ottawa-Lees Campus.

At the end of the study you will also be given a thank you card with a $10 gift certificate to either a grocery store, book store, or coffee shop (based on your preference) as a token of appreciation for your time to participate in the study visit.

Conflict of Interest:
The investigators have no conflicts of interest to declare related to this study.

Consent to participate in study:

- I understand that I am being asked to participate in a research study about physical activity during pregnancy.
- This study was explained to me by _____.
- I have read, or someone has read to me, each page of this Participant Informed Consent Form.
- All of my questions have been answered to my satisfaction.

- If I decide later that I would like to withdraw my participation and/or consent from the study, I can do so at any time.
- I voluntarily agree to participate in this study.
- I will be given a copy of this signed Participant Informed Consent Form.

Please initial:

___ Yes __ No I realize that my participation is voluntary, and I am free to withdraw from the study at any time.

___ Yes __ No Having my blood samples stored and used for future ethics approved research on health behaviours (i.e. nutrition & physical activity), pregnancy outcomes and weight regulation.

___ Yes __ No To be contacted in the future for follow up studies

If I have any questions about the study, I may contact the researcher or her supervisor.

If I have any questions regarding the ethical conduct of this study, I may contact the Protocol Officer for Ethics in Research, University of Ottawa, Tabaret Hall, 550 Cumberland Street, Room 154, Ottawa, ON K1N 6N5
Tel.: (613) 562-5387
Email: ethics@uottawa.ca

There are two copies of the consent form, one of which is mine to keep.

Participant full name (Print):

Participant's signature: **Date:**

Investigator or Delegate Statement
I have carefully explained the study to the study participant. To the best of my knowledge, the participant understands the nature, demands, risks and benefits involved in taking part in this study.

Researcher's signature: **Date:**

B.2 EXERCISE SESSION DOCUMENTATION

PARTICIPANT ID #: MYO-___- ___ ___ DATE: _____

uOttawa

Faculty of Health Sciences
School of Human Kinetics

MYOKINE STUDY

Exercise Session

Heart Rate Maximum: _____

85% Heart Rate Maximum: _____

1. **RESTING PHASE PRE-EXERCISE [10 minutes]**

A. Time of day (start): _____

Time of Test (min)	Heart Rate (bpm)	Time of Test (min)	Heart Rate (bpm)
1:00		6:00	
2:00		7:00	
3:00		8:00	
4:00		9:00	
5:00		10:00	

B. Time of day (end): _____
C. Time of day blood draw (pre-exercise) taken: _____
D. Average Resting Heart Rate: _____
E. Heart Rate Maximum (220-age): _____
F. Heart Rate Reserve [=%intensity (HR_{max} - HR_{rest}) + HR_{rest}]

40% HRR	50% HRR	59% HRR

Evaluators: _____ (Version: June 2018) Page 1 of 3

2. ACCLIMATION PHASE

TOTAL TIME (minutes)	STAGE TIME (minutes)	SPEED (mph)	INCLINE (%)	HEART RATE (bpm)	Borg Scale (6-20)
3	3 (warm up)	2.0	2.0		
4	1	2.2	6.0		
5	1	2.4	6.0		
6	1	2.6	6.0		
7	1	2.8	6.0		
8	1	3.0	6.0		
9	1	3.2	6.0		
10	1	3.4	6.0		
11	1	3.6	6.0		
12	1	3.8	6.0		
13	1	4.0	6.0		
14	1	4.2	6.0		
15	1	4.4	6.0		

C. **TESTING PHASE [30 minutes]** IDEAL HRR ZONE: _____

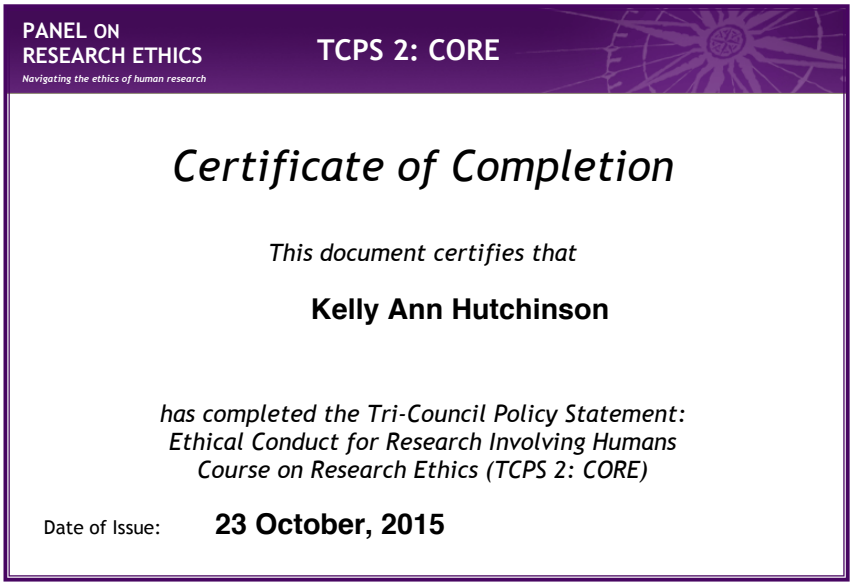
TOTAL TIME (minutes)	STAGE TIME (minutes)	TESTING PHASE TIME (min.)	SPEED (mph)	INCLINE (%)	HEART RATE (bpm)	Borg Scale (6-20)
	1	1		6.0		[redacted]
	1	2		6.0		
	1	3		6.0		
	1	4		6.0		
	1	5		6.0		
	1	6		6.0		[redacted]
	1	7		6.0		
	1	8		6.0		
	1	9		6.0		
	1	10		6.0		
	1	11		6.0		[redacted]
	1	12		6.0		
	1	13		6.0		
	1	14		6.0		
	1	15		6.0		
	1	16		6.0		[redacted]
	1	17		6.0		
	1	18		6.0		
	1	19		6.0		
	1	20		6.0		
	1	21		6.0		[redacted]
	1	22		6.0		
	1	23		6.0		
	1	24		6.0		
	1	25		6.0		
	1	26		6.0		[redacted]
	1	27		6.0		
	1	28		6.0		
	1	29		6.0		
	1	30		6.0		

G. Time of day (end): _____
H. Was the test stopped at any point? _____
   a. Reason (if answer above is 'yes'): _____

I. Time of day blood draw (post-exercise) taken: _____

APPENDIX C: TRAINING CERTIFICATES

C.1 TRI-COUNCIL POLICY STATEMENT: ETHICAL CONDUCT FOR RESEARCH INVOLVING HUMANS COURSE ON RESEARCH ETHICS (TCPS 2: CORE)



PANEL ON RESEARCH ETHICS
Navigating the ethics of human research

TCPS 2: CORE

Certificate of Completion

This document certifies that

Kelly Ann Hutchinson

has completed the Tri-Council Policy Statement:
Ethical Conduct for Research Involving Humans
Course on Research Ethics (TCPS 2: CORE)

Date of Issue: **23 October, 2015**

C.2 UNIVERSITY OF OTTAWA LAB SAFETY TRAINING

Université d'Ottawa

Ce certificat atteste que

Kelly Ann Hutchinson

a suivi l'atelier

Lab Safety Training

Bureau de la gestion du risque

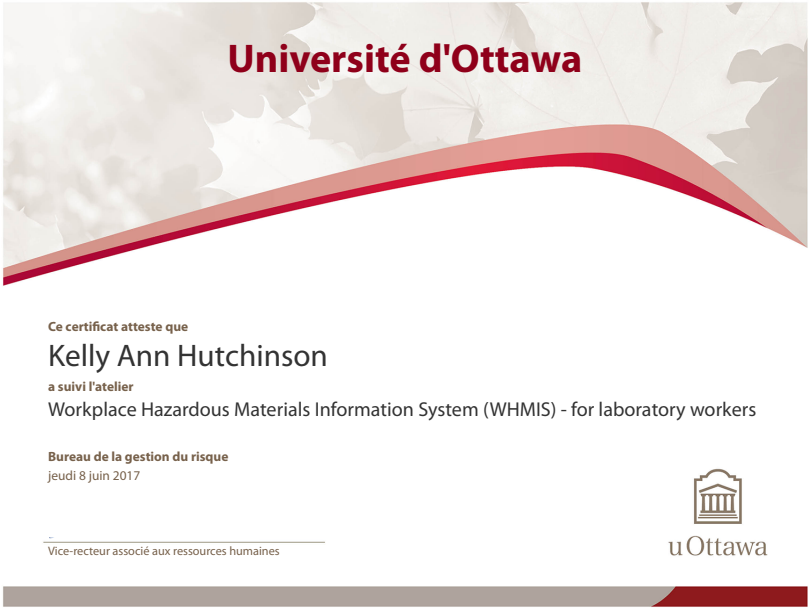
jeudi 8 juin 2017

Vice-recteur associé aux ressources humaines

uOttawa

C.3 University of Ottawa Principles of Biosafety

C.4 Workplace Hazardous Materials Information System (WHMIS) - for laboratory workers



C.5 University of Ottawa Radiation Safety Training

University of Ottawa

This certifies that

Kelly Ann Hutchinson

has completed the following workshop

Radiation Safety Training - For Users

ORM, Radiation and Biosafety

Wednesday May 29, 2019

Associate Vice-President, Human Resources

uOttawa

APPENDIX D: SCHOLARLY ACHIEVEMENTS

D.1 SCHOLARSHIPS

2018 - 2019	Canadian Graduate Scholarship Master's –Natural Sciences and Engineering Research Council (NSERC)
2018 - 2019	Ontario Graduate Scholarship [DECLINED]
2017 - 2018	Ontario Graduate Scholarship
2017 - 2019	University of Ottawa Excellence Scholarship
2017 - 2019	University of Ottawa Entrance Scholarship

D.2 PRESENTATIONS

February 2019	**Hutchinson, K.A.**, Mohammad, S., McInnis, K., Adamo, K.B. Characterizing the myokine response in pregnant and non-pregnant women after an acute bout of moderate-intensity exercise. Abstract accepted for 6th Annual Canadian National Perinatal Research Meeting (CNPRM); Mont-Tremblant, QC.

D.3 PUBLICATIONS

Hutchinson, K. A., Mohammad, S., Garneau, L., McInnis, K., Aguer, C., & Adamo, K. B. (2019). Examination of the Myokine Response in Pregnant and Non-pregnant Women Following an Acute Bout of Moderate-Intensity Walking. *Frontiers in Physiology*, *10*(1188), 1–10. https://doi.org/10.3389/fphys.2019.01188

Fernandes da Silva, D., Mohammad, S., **Hutchinson, K. A.** & Adamo, K. B. The measurement period for resting heart rate variability can be reduced in pregnant and non-pregnant women. *Submitted to Applied Physiology, Nutrition, and Metabolism – Accepted for publication September 2019*.

Halili, L., Liu, R., **Hutchinson K. A.**, Semeniuk, K. & Adamo, K.B. (2018). Development and pilot evaluation of a pregnancy-specific mobile health tool: a qualitative investigation of *SmartsMoms Canada*. *BMC Medical Informatics and Decision Making, 18*(9).

Fernandes da Silva, D., Mohammad, S., **Hutchinson, KA.** & Adamo, K. B. (2019). Cross-validation of ratings of perceived exertion derived from heart rate target ranges recommended for pregnant women. *Submitted to The Journal of Physical Activity and Health – October 1st, 2019; currently in review.*