THE EFFECT OF PHYSICAL ACTIVITY AND GESTATIONAL WEIGHT GAIN ON LIPID MARKERS THROUGHOUT PREGNANCY: DOES ONE OUTWEIGH THE OTHER?

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PREFACE TO THIS THESIS

Amid the growing cases of the COVID-19 pandemic in Canada following the 2019 worldwide outbreak, the province of Ontario declared a state of emergency in March 2020 closing all schools, recreation programs, restaurants/bars, and non-essential retail stores, to name a few. The Prime Minister of Canada advised all Canadians to stay at home whenever possible, in addition to practicing social and physical distancing to minimize the spread of the virus.

In accordance with the state of emergency declared by the Premiere of Ontario, the University of Ottawa halted all non-essential research and in-person data collection. As the bulk of my research consisted of using previously collected participant information and tissue samples, I was able to complete the majority of my thesis objectives. Unfortunately, the main experiment in my thesis, radioactively labelled fatty acid uptake in fresh placental explants, had to be removed due to the lack of in person data collection. In preparation for my experiment, I had completed the University of Ottawa radiation safety training, prepared an experimental protocol, in addition to reaching out to the University of Ottawa Office of Risk Management to discuss any logistical requirements for using radioactive material at Lees Campus. My supervisor (Dr. Kristi Adamo) and committee members Dr. Shannon Bainbridge and Dr. François Haman supported the modifications necessary to my thesis due to these unforeseen circumstances.

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To my committee members, Dr. Shannon Bainbridge and Dr. Francois Haman, the expertise you possess in your respective fields is invaluable. I would like to thank you for being a part of my thesis committee and supporting my research questions.

To my best friend Emily, you've been a constant in my life for over 10 years. You introduced me to the Adamo lab, you've been my anchor in hard times, and my sail in good ones. You have always supported me in my dreams and having someone like you to celebrate my achievements makes them even more special. You'll always be family to me.

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look forward to the time I can come visit you and Danilo in Brazil. Taniya, you are the reason our group is so successful. Had it not been for your team building spirit, and your constant encouragement in my work, my career as a masters student would not have been as rewarding. Rhea, your excitement for research is unquestionable, and I don't doubt you will do great things in your career; thank you for being a part of my thesis work.

To my parents, grandparents, and sister, I love you with all my heart. I know you listened but never understood a word I said, yet you always encouraged me in my schooling. Thank you for supporting me in my undergraduate career, which allowed me the opportunity to continue into graduate school. I would not be here without you.

Lastly, to my love Antoine, thank you for being there for me. From picking me up after school, to listening to me talk about my research, you are my biggest supporter and my rock. I couldn't have done it without you. Thank you for everything you've done, in addition to adding joy to my life with our deux ti loups, Lola et Kygo.

ABSTRACT

Background: In the pregnant population, being physical active and meeting gestational weight gain (GWG) guidelines have numerous health benefits for both mother and infant. Markers of lipid metabolism are known to be influenced by these two variables in the non-pregnant population. However, the relationship between physical activity (PA) and GWG on lipid markers has yet to be assessed during pregnancy. My thesis aims to address this gap in the literature.

Methods: The first objective of my thesis was to examine the relationship between maternal PA and GWG on gross measurements of fetal and placental development (n=40). Specifically, three markers of placental efficiency (Pl-E) were examined (birthweight [BW], BW-to-placenta weight ratio, and residual BW). The second objective of my thesis was to analyze maternal serum lipid and glucose markers (n=40), in mid (24-28 weeks) and late (34-38 weeks) gestation as well as from the umbilical cord (UC) as they relate to both PA and GWG. The third objective of my thesis was to explore how PA level and GWG status affect markers of lipid metabolism in term placenta (n=31). Markers of placental lipid transport (FATP1, FABP4, FAT/CD36) were assessed at the protein level, and enzymatic activity of placental lipoprotein lipase was also measured. Lastly, placental lipid storage was assessed by examining triglyceride content, paired with lipid droplet staining.

Results: There was no relationship between PA independently or in combination with GWG on any Pl-E markers. A significant association was found between GWG and BW in women who gained weight excessively compared to insufficiently. Neither PA nor GWG categorization was associated with maternal lipid and glucose markers. Total cholesterol levels measured in UC serum were significantly lower in women categorized as active throughout pregnancy ($p<0.0001$) or whose activity dropped in late gestation ($p<0.0001$) compared to those who were inactive

throughout gestation. Glucose levels were lower in UC blood of women who gained weight appropriately in mid-gestation compared to those who gained insufficient ($p=0.040$) or excessive ($p=0.021$) weight. In terms of placental fatty acid transport, there was a significant interaction between PA status and GWG categorization and placental FATP1 protein expression ($F=14.62$, $p<0.0001$). Finally, while no differences were found in placental lipid droplet staining, the droplets were more likely to be clustered within the syncytiotrophoblast border.

Conclusion: In conclusion, maternal PA had no association with Pl-E, while GWG was only associated with BW. My thesis work found that while maternal serum lipid markers were not associated with PA and GWG, both maternal PA and GWG status were related to changes in UC and placental lipid markers throughout pregnancy. In combination with previous research from our lab, it is suggested that women who are physically active during pregnancy, and gain weight appropriately may be transporting fewer nutrients (i.e. fatty acid, glucose, cholesterol) to the placenta than those who are inactive, yet simultaneously increasing metabolization. Future research should further investigate these findings by performing functional experiments.

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ABBREVIATIONS

AGA	Appropriate-for-gestational-age
ATP	Adenosine triphosphate
BMI	Body mass index
BW	Birth weight
DNA	Deoxyribonuclease acid
EFA	Esterified fatty acids
FABP	Fatty acid binding protein
FATP	Fatty acid transport protein
FAT	Fatty acid translocase
GDM	Gestational diabetes mellitus
GWG	Gestational weight gain
HDL-c	High density lipoprotein cholesterol
IOM	Institute of medicine
LCPUFA	Long chain poly unsaturated fatty acid
LDL-c	Low density lipoprotein cholesterol
LGA	Large-for-gestational-age
MPA	Moderate physical activity
MVPA	Moderate-to-vigorous physical activity
NEFA	Non-esterified fatty acid
PA	Physical activity
Pl-E	Placental efficiency
PW	Placental weight
SGA	Small-for-gestational-age
VPA	Vigorous physical activity
W/A	Weight-for-age
W/L	Weight-for-length

CHAPTER 1: THESIS OVERVIEW

1.1 PRESENTATION OF THESIS

The following Master of Science thesis will be presented in manuscript format. **Chapter 1** will provide an overview of the topics covered in this thesis and assess the current state of literature and gaps in knowledge. **Chapter 2** presents the first objective of the thesis entitled "*The effect of maternal physical activity and gestational weight gain on placental efficiency*". This manuscript was published in *Medicine & Science in Sports & Exercise,* April 2021. **Chapter 3** presents the second objective of the thesis entitled "*Physical activity throughout pregnancy is associated with umbilical cord cholesterol".* **Chapter 4** presents the third objective entitled "*Placental FATP1 is differentially expressed in physically active women"*. **Chapter 5** presents the overall findings and discussion of this thesis.

1.2 INTRODUCTION AND LITERATURE OVERVIEW

1.2.1 THE PLACENTA

The placenta is a specialized organ found only in mammals and marsupials. Functionally, it is a highly complex organ. The placenta acts as a surrogate lung for the transport of respiratory gases, functions as a kidney to remove waste, behaves like the immune barrier to protect the fetus from pathogens, and serves as the digestive system transferring all necessary nutrients from the mother for fetal development. In humans, egg fertilization is followed by cell division where the fertilized egg, or zygote, begins to exit the ovary, and by the fifth day post-fertilization, the zygote undergoes increased cell division, becoming a blastocyst. Upon reaching the uterus, the blastocyst is enveloped by a single layer of rapidly proliferating trophoblast cells [1]. The trophoblast cells differentiate into two main placental cell types: the outer syncytiotrophoblast, and the inner cytotrophoblast, the former being the primary cell type responsible for nutrient transport [2]. In

combination with maternal spiral arteries, the placenta can uptake nutrients for transport to the fetus through passive diffusion and active transport, reaching maturity around 34 weeks gestation and discarded post-birth [3].

In 1986, Dr. D.J. Barker published an epidemiological report in *The Lancet* that would later be credited for the origin of the "DOHaD" concept, or the Developmental Origins of Health and Disease [4]. This study suggested that the intrauterine environment is impacted by various exposures (environmental, maternal, etc) over gestation, creating predispositions for later in life health and disease by influencing the expression of genes *in utero* [5]. In recent times, the placenta is being recognized as the programming agent, whose phenotype alters in response to changes in maternal diet or metabolic status [6]. As the placenta is the conduit for maternal to fetal communication, alterations in placental phenotype can influence fetal development [6]. Due to this important relationship, research investigating pregnancy related complications or outcomes, in both mother and newborn, are beginning to recognize the critical role of the placenta and include its physiological and metabolomic makeup in their investigation.

1.2.2 PHYSICAL ACTIVITY THROUGHOUT PREGNANCY

1.2.2.1 PHYSICAL ACTIVITY GUIDELINES AND MATERNAL HEALTH

A healthy, active lifestyle is beneficial at any stage of life, and pregnancy is no exception. The Society of Obstetricians and Gynaecologists of Canada and the Canadian Society for Exercise Physiology have collaboratively published evidence-based recommendations for physical activity (PA) during pregnancy, which states that pregnant women should engage in at least 150 min of moderate PA (MPA) per week, accumulated over 3 or more days [7]. The guidelines state that women with contraindications to PA or who have been told by their health care provider to withhold from PA during pregnancy should modify these recommendations as needed. For this

thesis, PA will be defined as any bodily movement, while exercise will be defined as planned bodily movement for the purpose of fitness or body training. PA, such as aerobic exercise (e.g., walking, jogging, stationary biking), during pregnancy, has a protective effect on maternal health by reducing the risk for gestational diabetes mellitus (GDM), pre-eclampsia, urinary incontinence, excessive gestational weight gain (GWG), and postpartum weight retention [8–11]. A greater proportion of women who meet the recommendations for an active pregnancy stay within their recommended GWG guidelines than their non-active counterparts [12]. However, it is common for women who engage in PA to reduce their activity levels as pregnancy progresses due to the inevitable changes of the maternal body that accompanies the growing fetus [13]. Additional factors are also believed to create barriers for PA during pregnancy including: fatigue, lack of sleep, low motivation, lack of knowledge about PA, lack of social support, and inclement weather [14].

1.2.2.2 IMPACT OF MATERNAL PHYSICAL ACTIVITY ON FETAL HEALTH

Historically, PA during pregnancy was not supported by the medical community for fear of the potential negative impact on fetal health. Currently, evidence-based research consistently illustrates that moderate PA is not only safe for the mother and fetus, but beneficial [7]. One widely held belief was that an exercise-induced increase in maternal core temperature could cause overheating in the fetus [15]. A recent systematic review assessed maternal thermoregulatory capacity during passive heat exposure (20°C-25°C air temperature and 30°C- 40°C water temperature) and wide ranges of exercise, such as cycling, running, and water aerobics, performed between 50%-90% of maximum heart rate and did not find any reports of maternal core temperature exceeding the teratogenic threshold (i.e., 39°C) across pregnancy timepoints [16]. While neonates born to physically active women have been reported to be slightly smaller with lower body fat, they are nonetheless within the healthy size limit and develop at rates comparable to

babies born to non-active women [17–19]. Unfortunately, limited studies follow the offspring born to women who were physically active during pregnancy. Those who do have long-term follow-up assessments show that at the 1- and 5- year checkups, children of women who were physically active in pregnancy were lighter and leaner than their non-exercise counterparts, yet still fell within the normal population growth range [20].

1.2.2.3 IMPACT OF MATERNAL PHYSICAL ACTIVITY ON PLACENTAL OUTCOMES

The placenta is a transient organ responsible for the exchange of nutrients, gas, and signaling molecules from the mother to the fetus [21]**.** Acute maternal PA is believed to temporarily modify the gas and nutrient availability to the placenta through reduced blood flow; however, physiological adaptations have resulted in an intrauterine environment that compensates for these intermediate disruptions through increased maternal blood flow at rest [22]. Optimal placental growth and function are crucial for supplying nutrients and removing waste through the movement of blood, thereby providing a favourable environment for appropriate fetal growth (Figure 1) [23]. Studies have shown that women who perform aerobic exercise during pregnancy have higher placental parenchymal volume, villous surface area, and vascular volume, which has the potential to increase the transfer of nutrients to the fetus - resulting in a more efficient placenta [24].

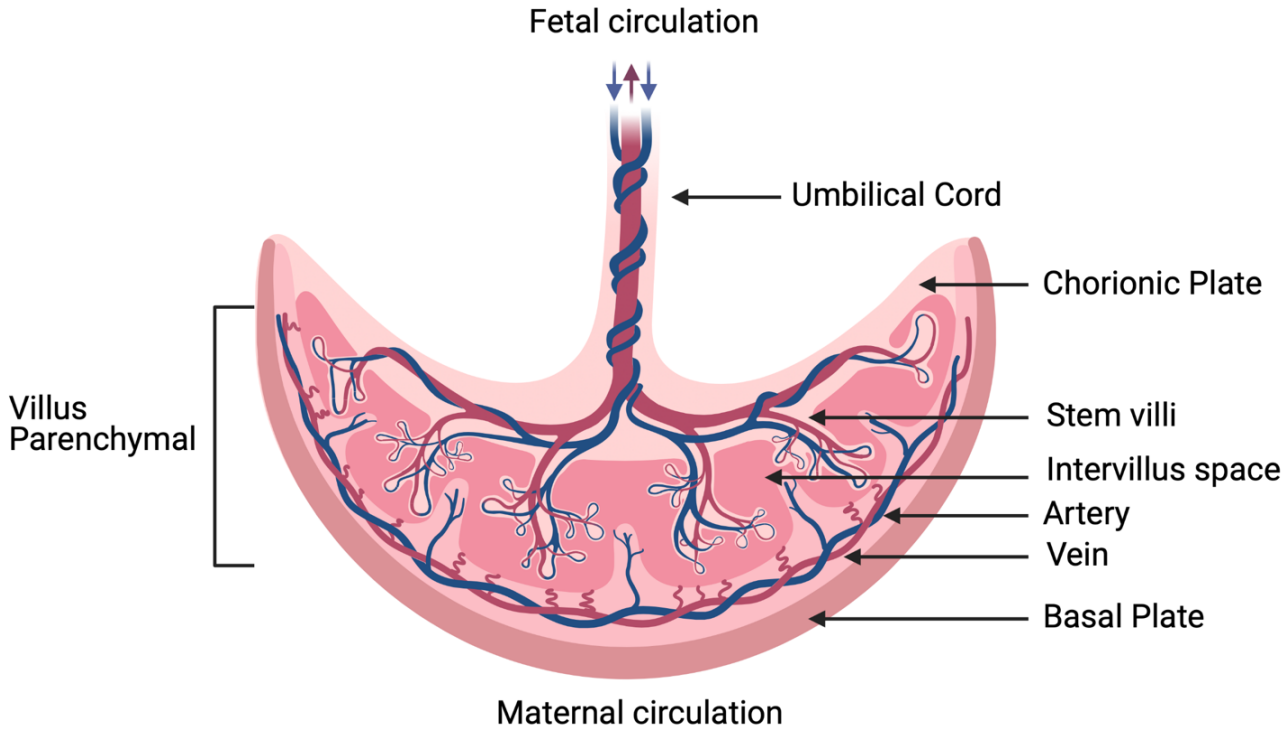

Figure 1. Representative Image of Placental Structure. Placental vasculature structures are responsible for the diffusion and passive or active transport of nutrients and gas molecules. Molecules and nutrients from the maternal system pass through the basal plate where the placental vein carries nutrient-rich oxygenated blood to the fetus, and the placental arteries carry deoxygenated blood to the be expelled by the maternal system. Created with Biorender.com.

A surrogate marker often used to interpret the placenta's ability to exchange nutrients and gas based on the available surface area is placental efficiency (Pl-E), or the measurement of grams of fetus produced per grams of placenta [25]. Placental development has been implicated in fetal growth patterns, one major outcome being birth weight (BW) [26]. The positive linear relationship between BW and placental weight (PW) has been the foundation of the traditional methodology of measuring Pl-E: birth weight to placental weight (BW:PW) ratio. A greater ratio is believed to represent a more efficient placenta because less placenta is required to grow a larger fetus. The majority of research to date has investigated Pl-E in disease states and linking measures to future health outcomes. Yet, the literature is scarce on the relationship between Pl-E and positive behaviours such as maternal PA [27]. Although widely used, the methodology of applying BW:PW

ratio as a measurement of Pl-E has been criticized because it has a non-zero intercept, presenting a challenge for biological statistics [28]. Christians *et al.* suggests the use of residuals using a linear regression model, which would be the difference between the predicted BW based on PW, and the measured BW [28]. Placental efficiency indicators other than BW:PW ratio have not been investigated in a healthy (non-diseased) population and represent a novel area of research that will be explored in this thesis.

1.2.3 GESTATIONAL WEIGHT GAIN

During pregnancy, a woman's weight gain does not solely comprise the growing fetus; it also accounts for increases in bodily fluid, blood volume, breast tissue, uterus tissue, amniotic fluid, fat, protein, nutrient storage, and the growth of the placenta. In 2009, the US Institute of Medicine (IOM) released guidelines for GWG [29], recommending specific weight gain ranges for women, based on their pre-pregnancy body mass index (BMI), that were those *'associated with the lowest prevalence of the outcomes of greatest interest'*, essentially balancing the benefits and risks. While each woman is unique and has different gestational needs, the IOM guidelines are the most up-to-date research-based evidence available to women and their health care providers. Many factors can contribute to inappropriate weight gain during pregnancy, including inadequate advice given about the importance of nutrition and PA by their health care provider [30].

1.2.3.1 MATERNAL NUTRITION OVER GESTATION

Maternal nutrient intake is a key factor in the growth and development of the placenta and fetus, as associations have been found between a nutrient-rich diet before and during pregnancy, and improved fetal health, optimized BW, and increased rates of maternal and fetal survival [31]. Research has shown that preconception and the first trimester of pregnancy are key periods requiring optimized nutrition, as organogenesis and other critical processes of fetal development

occur during these initial few months [32,33]. Women in their first trimester of pregnancy have similar caloric requirements as non-pregnant women. These requirements increase as pregnancy progresses into the second and third trimester, when pregnant women are advised to add approximately 340 kcal, and 452 kcal per day, respectively. These changes equate to approximately 300 kcal/day over the entire pregnancy [34]. In addition to an increase in caloric intake, pregnant women require greater amounts of vitamins and minerals such as; folate, vitamin A, vitamin D, riboflavin, and vitamin B6/12, generally consumed in a prenatal multivitamin [34]. Dietary modifications are needed to ensure the maternal body can provide the necessary building blocks to the growing fetus while simultaneously supplying her own body with the daily required energy. These factors are important for fetal development; however, consuming calories in excess is a growing concern, with the focus of this thesis being excess maternal lipid intake, and subsequent placental uptake. Research has shown that increases in triglycerides and cholesterol in early and late pregnancy are associated with an increased risk for a large for gestational age (LGA) newborn [35–37]. The majority of women require a positive energy balance for a healthy pregnancy (i.e., energy intake > energy output), but excessive energy intake may lead to excessive GWG. Habitual PA, a marker for energy output during pregnancy, can be used as a mediation tool to moderate excessive energy intake and any associated complications [38].

1.2.3.2 IMPACT OF EXCESSIVE GESTATIONAL WEIGHT GAIN ON PLACENTAL AND FETAL OUTCOMES

Considerable attention has been paid to the increased risk of birthing an LGA neonate when women i) have GDM, ii) have a BMI ≥ 25 kg/m^2, or iii) gain weight excessively during pregnancy [39,40]. In addition, a fetus born LGA may require an extended hospital stay and is more likely to be admitted to the neonatal intensive care unit due to a compromised immune system [41,42]. It has been proposed that high levels of maternal fatty acids may contribute to LGA newborns, especially in

women living with obesity [43]. Increased adiposity during pregnancy, often found in cases of women living with obesity, may also negatively affect placental vascularization, impeding blood flow to the placenta and affecting nutrient transfer due to an increase in non-branching placental angiogenesis [44,45]. While women who gain weight excessively during pregnancy may grow a functional placenta and birth an infant of appropriate weight, altered intrauterine conditions during gestation may result in programming for later-life obesity and metabolic-related disorders to the infant [46]. In contrast, limited research investigates insufficient GWG and its effect on fetal or placenta outcomes, especially in an otherwise healthy population. One study investigating pregnant women living with GDM found that the infants of those who gained weight insufficiently had an increase in umbilical cord glucose levels compared to those who gained weight appropriately [47]. Placental and fetal outcomes are rarely investigated in association with GWG in uncomplicated pregnancies, and therefore represent a novel area of research.

1.2.4 PLACENTAL LIPID METABOLISM

1.2.4.1 PLACENTAL LIPID TRANSPORT

There are multiple different families of fatty acid transport proteins that support placental lipid transport, though the location, role, and existence of specific isoforms of fatty acid transporter proteins in the placenta are still under investigation (Figure 2). Certain species and isoforms have been identified in the placenta, such as fatty acid-binding proteins (FABP) 3-5 and 7, fatty acid translocase (FAT/CD36), and fatty acid transporter proteins (FATP) 1-6 [48]. Evidence suggests a positive association between the mother's triglyceride levels and the BW of the fetus, as pregnant women living with obesity have higher triglyceride content than their normal-weight counterparts and have a higher chance of birthing an LGA newborn [49]. One study indicated that placental lipoprotein lipase (LPL) activity, a potential major hydrolyzer of free fatty acids (FFA) from

triglycerides at the placental barrier, positively correlates with newborn BW and body fat percentage [50]. In contrast, research conducted by Calabuig-Navarro *et al.* determined that in isolated placental trophoblast cells from women living with obesity and their normal weight counterparts, there was no difference in fatty acid oxidation as measured by radioactive transport of [^{3}H]-palmitate, a long-chain fatty acid [51]. Another study suggests that there is a decrease in placental FATP1/4 in the placentas of women with high pre-pregnancy BMI and GDM, with no difference in fatty acid composition [52]. Finally, pregnant women classified as inactive had a significantly lower protein expression of placental FATP4 than those deemed physically active [53]. There is much debate in the literature about the effect of fatty acid metabolism in the placentas of women living with obesity and little is known about the impact of PA and GWG in a non-complicated population on lipid transport from mother to fetus via the placenta.

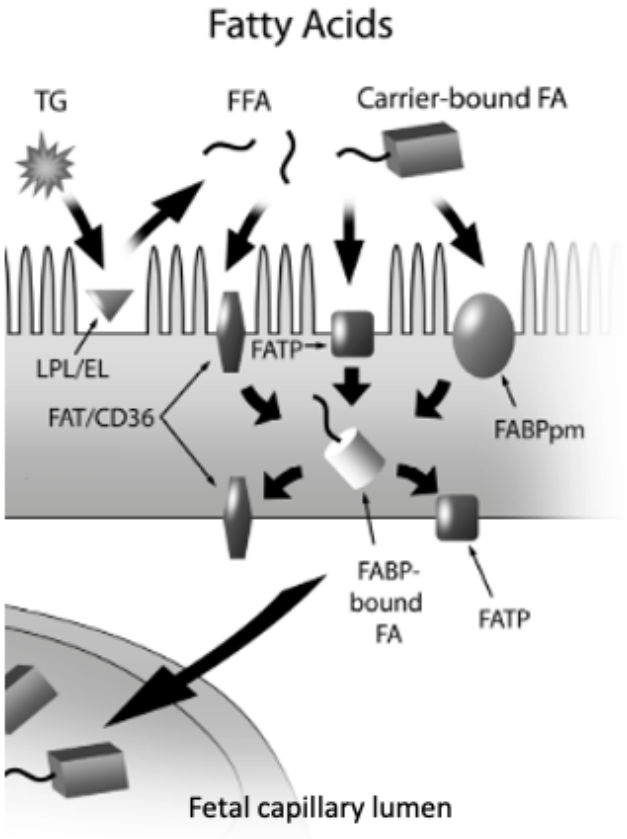

Figure 2. Representation of fatty acid transport across the placental syncytiotrophoblast membrane. TG: triglycerides; FFA: free fatty acids; FA: fatty acids; LDL: lipoprotein lipase; EL: endothelial lipase; FATP: fatty acid transport protein; FABP: fatty acid binding protein; FABPpm: plasma membrane fatty acid binding protein; FAT/CD36: fatty acid translocase. [Image modified from Brett et al. (2014) [54]]

1.2.4.2 PLACENTAL LIPID STORAGE

The fetus receives nutrients through the mother and must therefore store lipids and fatty acids as reserves in the placenta [55]. Neutral lipids such as triglycerides and cholesterol are stored in organelles known as lipid droplets, which are found in almost every cell type [56]. It has been observed that the placentas of mothers living with obesity contain higher amounts of lipids such as triglycerides, free cholesterol, and cholesterol esters, resulting in a lipotoxic environment [51,57]. Triglycerides are the most common form of stored lipids and are the result of a positive energy balance. As more mothers enter pregnancy with high levels of adiposity, fetal macrosomia is more prevalent, and the risk further increases with GDM [58,59]. In addition, high maternal lipid levels can negatively influence placental physiology (e.g., mitochondrial function) through increased lipid storage [60]. Pregnant women living with obesity are more susceptible to placental mitochondrial dysfunction, marked by abnormal mitochondrial morphology and increased mitochondrial DNA copy number, presumed to be a compensation mechanism to regulate the metabolism of the increased lipid load [51,61]. There is currently little to no research on the relationship of maternal PA and placental mitochondrial function. As placental lipid metabolism and mitochondrial function are highly interconnected, there is a significant knowledge gap of the potential beneficial effect PA may have on this relationship [62].

1.2.4.3 PLACENTAL LIPID METBAOLISM

Lipids are primary macronutrients for the growing fetus because they are key components of cell membranes. Glucose and fatty acids are both used as primary sources of energy in the placenta

for ATP production and play an interconnected role in energy metabolism [63]. Regardless, glucose is the main energy source for the placenta, whereas fatty acids serve a primary role as essential building blocks in the development of the fetus [54]. In cell culture models, supraphysiological glucose levels, similar to those found in GDM, affected lipid metabolism but not uptake or transport [64]. The fetus is believed to be able to synthesize some fatty acids from glucose; however, the majority are received from maternal circulation [55]. Previous research from our lab did not find any difference between maternal PA and placental glucose transport 1 (GLUT1) protein expression [53], and was therefore not investigated in this thesis. In the last three months of gestation, lipids are essential for developing the fetal nervous system and brain [65]. Arachidonic acid (20:4w-6) and docosahexaenoic acid (22:6w-3) are two such lipids, also known as non-esterified fatty acids (NEFA), crucial to the developing brain and retina [66]. To cross from mom to fetus through the placenta, NEFA and esterified fatty acids (EFA) must first be hydrolyzed by LPLs into FFA [67]. The placenta syncytiotrophoblast is the regulator of nutrient transport, recruiting the activity of lipoproteins to engage transport proteins for uptake of FFAs [50]. Lipids must first be metabolized in the maternal circulation into FFAs (e.g., long-chain polyunsaturated fatty acid [LCPUFA] and medium-length fatty acids), to be transported within the placenta because the placenta does not have the necessary enzymes to perform this conversion [48,68]. Human placental perfusion experiments found that LCPUFA (e.g., C-12 and C-16) accumulated at a higher rate in trophoblast cells, while medium-length fatty acids (e.g. C-5) accumulated at a higher rate in fetal capillaries [69]. After transport into the placenta, the LCPUFA were found to be more likely esterified and stored in lipid droplets, whereas medium length fatty acids were transported against a concentration gradient, suggesting being more actively transferred for fetal use [69].

1.2.4.4 MATERNAL INFLUENCE ON PLACENTAL LIPID METABOLISM

Maternal lifestyle patterns, such as dietary intake and PA, in addition to pregnancy outcomes like GWG, have considerable influence on fetal development. Maternal hyperlipidemia (i.e., increase in circulating plasma triacylglycerols, NEFA, phospholipids, and cholesterol) is often present during the third trimester of pregnancy due to an increased breakdown of maternal fat depots primarily found in adipose tissue [66]. Positive associations between maternal aerobic exercise and maternal lipid levels have been correlated with an increase in infant head circumference, BW, and birth length [35–37,67]. Whereas infant "fatness", measured by ultrasound, is linked with GWG [36]. The literature is scarce on the relationship between PA and GWG and placental lipid metabolism in a lean pre-pregnancy, uncomplicated pregnant population. Research by our lab on PA during pregnancy found opposing results for protein and mRNA expression of placental FATP4, where protein levels were increased in the placentas of healthy physically active women and the potential to produce proteins (mRNA expression) decreased [53,70]. In PA intervention studies with pregnant women living with obesity, a decrease has been noted in the placental lipid droplet quantity [71]. As discussed, the maternal environment plays a major role in determining placental function, which subsequently regulates fetal development. This cascade of interactions ultimately controls pregnancy and future infant outcomes, with lipids playing an important role in driving a healthy pregnancy.

1.3 STUDY RATIONALE

The study of lipid transport and storage over gestation is salient because lipids are a crucial factor in fetal growth, especially brain development [65]. To date, little research has been conducted on the relationship between PA during pregnancy independently or in combination with GWG on markers of lipid transport and storage. Some studies have investigated the before (maternal) and end (fetal) result of PA and GWG on lipids throughout pregnancy, yet few, if any, explore the two

variables in combination or consider the role of the placenta. It is well known that PA during pregnancy and appropriate GWG offers health benefits to the mother and infant. Yet, there is a considerable knowledge gap in how those two variables affect the nutrient transporting function of the placenta in healthy mothers. The overall aim of this thesis is to investigate the effect of maternal PA and GWG, individually and in combination, on lipid markers over gestation and their subsequent relationship with placental and fetal development.

1.4 THESIS OBJECTIVES

Objective 1: To examine the relationship between maternal physical activity and gestational weight gain on gross markers of placental health and newborn outcomes.

Objective 2: To investigate the relationship between trimester-specific and habitual maternal physical activity and gestational weight gain on maternal and umbilical cord serum lipid and glucose profile.

Objective 3: To explore if placental lipid transport and storage at term differ based on maternal physical activity over gestation and according to gestational weight gain.

CHAPTER 2: THE EFFECT OF MATERNAL PHYSICAL ACTIVITY AND GESTATIONAL WEIGHT GAIN ON PLACENTAL EFFICIENCY

PREAMBLE TO MANUSCRIPT 1

The manuscript titled: "*The effect of maternal physical activity and gestational weight gain on placental efficiency*" was submitted to the journal of *Medicine & Science in Sports & Exercise* on the 19th of May 2020 in accordance with the journal's specifications. The manuscript identified as MSSE-D-20-00488R2, was accepted for publication on the 19th of September 2020; doi: 10.1249/MSS.0000000000002524 and published in the April 2021 issue. This chapter represents

the first objective of this thesis, as it aims to evaluate gross measurements of placental and fetal health and their association with PA and GWG.

The Effect of Maternal Physical Activity and Gestational Weight Gain on Placental Efficiency

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ABSTRACT

EVEREST, C., T. S. NAGPAL, S. C. S. SOUZA, D. F. DA SILVA, L. GAUDET, S. MOHAMMAD, J. BHATTACHARJEE, and K. B. ADAMO. The Effect of Maternal Physical Activity and Gestational Weight Gain on Placental Efficiency. *Med. Sci. Sports Exerc.*, Vol. 53, No. 4, pp. 756–762, 2021. **Introduction:** Adherence to physical activity (PA) and gestational weight gain (GWG) recommendations during pregnancy has been shown to improve maternal and fetal health outcomes, including reducing the risk for chronic diseases. Limited research has evaluated the effect of meeting PA in combination with GWG recommendations on placental efficiency (Pl-E), a surrogate marker of the placenta's ability to exchange nutrients and gas based on surface area. The purpose of this study was to measure and compare Pl-E based on meeting PA and GWG recommendations. **Method:** Healthy pregnant women ($n = 61$) wore accelerometers in their second and third trimesters to objectively measure PA. Women were classified as active or inactive at each time point based on meeting the 2019 Canadian prenatal PA guidelines. Total GWG was calculated as weight measured in the third trimester minus self-reported prepregnancy weight, and were categorized as insufficient ($n = 19$), adequate ($n = 22$), and excessive ($n = 20$) according to the 2009 Institute of Medicine guidelines. Placental weight (PW) and birth weight (BW) were measured within 30 min of delivery and 24–48 h postdelivery, respectively. Pl-E was determined in three ways: BW:PW ratio, residual BW, and measured BW, with a higher value indicating better Pl-E. Pl-E was compared by PA and GWG status using a two-way ANOVA. **Results:** No differences were found in the BW:PW ratio or residual BW corresponding to PA and GWG status. Measured BW was significantly higher in newborns of women who gained weight excessively compared with those who gained insufficient weight ($P < 0.05$). **Conclusion:** These findings suggest that prenatal PA does not compromise Pl-E; however, further research is required to evaluate the potential mechanistic benefits of meeting PA and GWG guidelines on the placenta. **Key Words:** PREGNANCY, EXERCISE, PRENATAL CARE, NEWBORN, MATERNAL-FETAL EXCHANGE, PLACENTA

The placenta is the most vital organ for fetal growth, and its development directly affects neonatal health outcomes (1). Research illustrates that placental parenchymal volume, vascular volume, and villous surface area increase when aerobic exercise is performed during pregnancy, and this can improve placental function by increasing the flow of nutrients to the fetus (2). In addition, previous research has shown that excessive gestational weight gain (GWG) may alter gene expression involved in regulating nutrient transport and can increase the risk of delivering a large for gestational age infant (3). Placental efficiency (Pl-E) is a surrogate marker of the placenta's ability to exchange nutrients and gas to support fetal growth (4). Placental morphometry has been closely linked to fetal growth, as it is the mediator of fetal development (5). This relationship has been shown through multiple methods, such as decreases in placental weight (PW) measured by ultrasound corresponding to low birth weight (BW), uteroplacental blood flow and BW closely relating to PW, and finally placental growth and shape being indicators in abnormalities of abdominal circumference and head-to-abdominal circumference ratio, both measurements of fetal growth restrictions (6). Pl-E as a biological marker represents a case-by-case interpretation relative to placenta (g) and fetal (g) size, with values ranging, on average, from 5 to 6 $g \cdot g^{-1}$ (4,7). To date, researchers have not examined the effect of meeting prenatal physical activity (PA) and GWG guidelines on Pl-E.

Women without contraindications to exercise are recommended to be active throughout gestation, supported by several international guidelines. The *2019 Canadian Guideline for Physical Activity throughout Pregnancy* (8) recommends that women without contraindications to exercise should aim for 150 min of moderate-intensity PA every week throughout gestation, with at least 30 min of activity attained on most days

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of the week. Meeting PA guidelines during pregnancy has protective effects on maternal health, including reducing the risk for gestational diabetes mellitus, preeclampsia, excessive GWG, urinary incontinence, and postpartum weight retention (9,10). Furthermore, the health benefits of PA during pregnancy extend to neonatal outcomes, including prevention of macrosomia, without increasing the risk of adverse outcomes such as preterm delivery or intrauterine growth restriction (11). Engaging in PA during pregnancy has preventive downstream effects, namely, reducing the risk of future chronic health conditions such as obesity and diabetes, for both mother and infant (12). In addition to PA, adherence to GWG recommendations is associated with better maternal and neonatal health outcomes, reduced risk for postpartum weight retention, and macrosomia, which reduces risk for early-onset diseases such as cardiovascular diseases, diabetes, and obesity (13). Taken together, currently available evidence suggests that meeting prenatal PA and GWG guidelines may effectively prevent pregnancy complications and future chronic health conditions. However, limited research has examined the combination of meeting prenatal PA and GWG recommendations on placenta growth and development.

The ratio of grams of BW produced per grams of placenta (BW:PW) is the most common measurement of Pl-E (the lower the ratio, the less efficient the placenta) (14). The use of BW:PW ratio as a measurement of Pl-E has been criticized for two major reasons: (i) the intercept (i.e., the predicted BW when PW is zero) is not zero, and (ii) an artifact of ratios is observed (15), where less mature placentas at term are deemed as "efficient" (16). These two critiques prompted Christians et al. (16) to conduct an investigation into Pl-E through birth records provided by the National Collaborative Perinatal Project. Analysis of that data set suggested an advantage to the use of residual BW (i.e., the difference between predicted and measured values) as a marker of Pl-E. Residual BW requires using measured BW as the dependent variable and measured PW as the independent variable in a linear regression model. This concept generates an equation based on the linear regression to calculate the predicted BW according to measured PW. Higher residual BW values would thus be interpreted as greater Pl-E. The authors also suggest using measured BW as a surrogate marker of Pl-E, given that residual BW and measured BW should be highly and positively correlated. As the methodologies put forth by Christians et al. are considered acceptable measures of Pl-E, all three proposed methodologies were investigated as part of this study.

Women who meet prenatal PA recommendations are more likely to stay within recommended GWG guidelines than their nonactive counterparts (13). Despite the known health benefits of PA, only 15% of pregnant women meet PA guidelines, and adherence to recommendations significantly decreases as gestation progresses (17). In addition, 57.7% of women exceed GWG guidelines based on prepregnancy body mass index (BMI) (18). Research suggests that maternal PA level and GWG status are predictors of optimal placenta development (19,20), and therefore women who adhere to both guidelines (independently and combined) would likely have higher Pl-E than women who do not meet one or both of the guidelines. The purpose of this study was to evaluate the effects of meeting PA (active or inactive) and GWG guidelines (insufficient, appropriate, or excessive), individually and combined, on three different measures of Pl-E (BW:PW, residual BW, and measured BW).

METHODS

Participant recruitment. An *a priori* sample size calculation was performed to determine the minimum number of participants needed to identify a group difference between physically active and inactive women, based on a 1-unit difference for BW:PW ratio and standard deviation (SD) values of 1.5 for the inactive group and 1.0 for the active group from previous literature (21). The analysis suggested a minimum of 54 participants (27 active and 27 inactive). The lack of data in the literature regarding the effects of meeting GWG guidelines on Pl-E did not allow us to incorporate expected differences in our sample size calculation. Pregnant women were recruited from the Ottawa region (ON, Canada) to participate in the Physical Activity and Dietary Implications throughout Pregnancy (PLACENTA) study. This study, approved by the University of Ottawa Research Ethics board (file no. H11-15-29), followed all ethical standards outlined in the Declaration of Helsinki. All participants gave informed written consent before inclusion in the study. Eligibility criteria included pregnant women between the ages of 18 and 40 yr, in their second trimester (24–28 wk gestation), carrying a single fetus, self-reported prepregnancy BMI of normal to overweight (18.5–29.9 $kg \cdot m^{-2}$), and weight stable for 6 months (±2 kg) before conception. Women were excluded if they had prepregnancy diabetes, hypertension, or contraindications to exercise during gestation as deemed by their health care provider. Participants were not asked if they developed pregnancy-related complications throughout gestation.

All participants visited the laboratory once in their second trimester between 24 and 28 wk of gestation, and again in their third trimester between 34 and 38 wk. In both trimesters, participants were provided with an omniaxial Actical® accelerometer (Philips Respironics, QC, Canada) and were instructed to wear it for the following 7 d. Actical® accelerometers have shown to provide valid and reliable measurements of PA, particularly moderate to vigorous PA (MVPA), in nonpregnant adults according to intraclass reliability, test–retest reliability analyses, and Bland–Altman mean error score (22,23). Although Actical® accelerometers have not been specifically validated in the pregnant population, they have been used in studies involving pregnant women at different time points throughout gestation (24,25), and results have shown agreement with the trends of decreased PA levels expected during pregnancy (17).

Actical® data were downloaded and analyzed using SAS version 9.4 (SAS Institute, Cary, NC) following the Canadian Health Measures Survey to determine MVPA (26). In both pregnancy trimesters, women were considered physically active if they reached ≥ 21.4 $min \cdot d^{-1}$ of MVPA (8) over a period of a minimum of 4 d for at least 10 h of wear time during each of these days (27). Total GWG was calculated by subtracting

weight at the third trimester visit (34–36 wk gestation) from their self-reported prepregnancy weight. Women were classified into GWG groups (i.e., insufficient, appropriate, and excessive GWG) based on the Institute of Medicine 2009 recommendations (28), according to their self-reported prepregnancy BMI.

Outcome measurements. Placentas delivered after 37 wk of gestation (i.e., term placentas) were collected 30 min after vaginal or cesarean section delivery by the researchers, and PW was recorded after the removal of the umbilical cord and fetal membranes, in addition to any coagulated blood. Neonatal weight was measured 24–48 h after delivery by the researchers to ensure standardized methodology. Neonatal weight and PW were measured using an Ultrascale MBSC-55 (MyWeigh, China) to the nearest gram. Neonatal skinfold measurements were taken using a Harpenden Skinfold Caliper (Baty International, Burgess Hill, UK), at the biceps, triceps, subscapular, and suprailiac. As per standardized protocols, measurements were taken twice to the nearest 0.4 mm, and if outside 0.2 mm, a third measurement was taken. Body fat percentages were estimated as outlined by Schmezle and Fusch (29).

Pl-E was determined according to three different approaches:

1. BW:PW ratio—the division between measured BW and measured PW (16);
2. Residual BW—the subtraction between measured BW and predicted BW.
   Measured PW was used to calculate the predicted BW by using the following equation (16):

$$\text{predicted BW} = 2190.62 + 2.30 \times \text{measured PW}$$

3. BW—measured BW was considered a marker of Pl-E as suggested by Christians et al. (16) because this variable should be highly correlated with residual BW.

Statistical analysis. Data are presented as mean ± SD. Normality was assessed by the Shapiro–Wilk test, and homogeneity was assessed according to the Levene test. Participant characteristics were compared between different categories of GWG (i.e., insufficient, appropriate, and excessive) and PA levels (active and inactive) using one-way ANOVA and independent *t*-test. For categorical variables, the Pearson chi-square test and the Fisher exact test were used. Residual BW were determined and correlated with BW using Spearman's coefficient of correlation. A two-way ANOVA was used to assess the effects of both PA levels (in both second and third trimesters) and GWG classification, as well as their interaction on Pl-E. Adjustments for multiple comparisons were performed according to the Bonferroni correction, and the magnitude of potential observed differences was computed as effect sizes (eta squared, η^2). The significance level was set at $P < 0.05$. All statistical analyses were completed using SPSS software version 13 (IBM Corp, Armonk, NY).

RESULTS

There was a total of $n = 71$ pregnant women in the PLACENTA study; however, only 61 participants were deemed eligible for the current analysis in the second trimester and 49 participants in the third trimester. Reasons for exclusion included incomplete fetal outcomes, incomplete maternal outcomes, or invalid accelerometer wear time. Participant characteristics are presented according to GWG classification (Table 1) and PA levels (Table 2) in the second and third trimesters. Residual BW was found to be strongly correlated with BW in our sample ($\rho = 0.858$, $P < 0.001$).

Second trimester outcome data. ANOVA including PA data revealed no PA categorization effect ($F = 0.024$, $P = 0.878$, $\eta^2 = 0.0004$), no GWG classification effect ($F = 1.215$, $P = 0.305$, $\eta^2 = 0.042$), and no interaction between PA category and GWG classification ($F = 0.301$, $P = 0.741$, $\eta^2 = 0.011$) for the residual BW (Table 3).

Similar results were observed for BW:PW ratio, with no PA levels effect ($F = 1.082$, $P = 0.303$, $\eta^2 = 0.019$), no GWG classification effect ($F = 0.866$, $P = 0.426$, $\eta^2 = 0.031$), and no interaction between these two factors ($F = 0.541$, $P = 0.586$, $\eta^2 = 0.019$) (Table 3).

The third index related to Pl-E was BW, and there was a significant GWG classification effect ($F = 3.838$, $P = 0.028$, $\eta^2 = 0.122$). The multiple correction analysis based on GWG classification demonstrated that neonate BW was lower for women whose weight gain was insufficient compared with excessive (mean difference = −353.0 [−671.7 to −34.2] g,

TABLE 1. Participant characteristics by GWG classification ($n = 61$).

	Insufficient ($n = 19$)	Appropriate ($n = 22$)	Excessive ($n = 20$)	*P*
Maternal demographics				
Age (yr), mean ± SD	32.5 ± 3.3	31.8 ± 3	32.0 ± 3.1	0.771
Ethnicity, *n*				
White	18	17	19	0.954
Other	1	5	1	
Marital status, *n*				
Married	15	19	19	0.098
Common law	3	3	1	
Single	1	0	0	
Level of education, *n*				
Graduate	10	13	14	0.195
Bachelor	7	5	6	
Nonuniversity certification	1	2	0	
Other	1	2	0	
Maternal anthropometrics and birth outcomes				
Pregravid BMI, *n*				
Normal	17	19	7	<0.001
Overweight	2	3	13	
GWG (kg), mean ± SD	8.7 ± 2.8	13.5 ± 1.6*	16.5 ± 3.4*,**	<0.001
Gestational age at delivery (wk), mean ± SD	39.8 ± 0.9	40.4 ± 1.0	40.0 ± 1.2	0.122
Neonatal body fat (%), mean ± SD	15.9 ± 2.3	18.0 ± 2.5*	17.4 ± 3.0	0.036
Placenta weight (g), mean ± SD	448.2 ± 77.9	507.0 ± 97.8	521.8 ± 89.6*	0.031
Maternal PA outcomes—trimester 2				
MVPA (min·d^{-1}), mean ± SD	29.3 ± 24.5	26.6 ± 17.1	24.1 ± 16.0	0.706
Total steps per day, mean ± SD	7128 ± 3286	7579 ± 2426	6926 ± 2632	0.745
Maternal PA outcomes—trimester 3[a]				
MVPA (min·d^{-1}), mean ± SD	21.3 ± 15.8	17.3 ± 16.5	12.9 ± 8.4	0.237
Total steps per day, mean ± SD	6132 ± 2306	6384 ± 2228	5389 ± 1425	0.343

*Significantly different from insufficient GWG.
**Significantly different from appropriate GWG.
[a]Insufficient GWG ($n = 16$), appropriate GWG ($n = 16$), excessive ($n = 17$).

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TABLE 2. Participant characteristics by maternal PA status in the second (n = 61) and third trimesters (n = 49).

	Second Trimester			Third Trimester		
	Inactive (n = 30)	Active (n = 31)	P	Inactive (n = 35)	Active (n = 14)	P
Maternal Demographics						
Age (yr), mean ± SD	32.2 ± 3.4	32.0 ± 3.0	0.870	32.6 ± 3.2	31.3 ± 2.7	0.179
Ethnicity, n						
White	25	29	0.255	30	14	0.303
Other	5	2		5	0	
Marital status, n						
Married	27	26	0.363	33	12	0.568
Common law	3	4		2	2	
Single	0	1		0	0	
Level of education, n						
Graduate	14	23	0.032	18	13	0.026
Bachelor	12	6		13	1	
Nonuniversity certification	1	2		3	0	
Other	3	0		1	0	
Maternal anthropometrics and birth outcomes						
Pregravid BMI, n						
Normal	23	20	0.298	24	10	0.845
Overweight	7	11		11	4	
GWG (kg), mean ± SD	12.9 ± 4.0	13.0 ± 4.3	0.964	13.3 ± 4.0	12.2 ± 5.1	0.441
Gestational age at delivery (wk), mean ± SD	40.1 ± 1.2	40.1 ± 0.9	0.868	40.0 ± 1.0	40.3 ± 0.9	0.276
Neonatal body fat (%), mean ± SD	16.8 ± 2.5	17.4 ± 2.9	0.368	17.0 ± 2.5	16.5 ± 2.6	0.585
Placenta weight (g), mean ± SD	508.1 ± 100.1	479.3 ± 84.3	0.231	494.8 ± 88.1	446.4 ± 88.1	0.089
Maternal PA outcomes						
MVPA (min·d^{-1}), mean ± SD	11.0 ± 6.2	41.7 ± 14.8	<0.001	9.8 ± 6.2	35.4 ± 11.3	<0.001
Total steps per day, mean ± SD	5217 ± 1355	9220 ± 2309	<0.001	5259 ± 1658	7700 ± 1810	<0.001

P = 0.025) (Table 3). However, no PA level effect (F = 0.290, P = 0.593, η^2 = 0.005) nor interaction between the two factors (F = 0.446, P = 0.643, η^2 = 0.016) was observed (Table 3).

There was no PA level effect in estimated body fat percentage between active (17.4% ± 2.9%) and inactive (16.8% ± 2.5%) neonates (F = 0.603, P = 0.441, η^2 = 0.011) (Table 2). There was a significant GWG classification effect (F = 3.408, P = 0.040, η^2 = 0.110), the multiple correction analysis demonstrating that neonates born to women who gained weight insufficiently (15.9% ± 2.3%) had lower body fat percentage than those who gained weight appropriately (18.0% ± 2.5%). There were no differences in measured BW of neonates born to women who gained weight excessively (17.4% ± 3.0%) (Table 1). There was no interaction between the two factors (F = 0.378, P = 0.687, η^2 = 0.014).

Third trimester outcome data. ANOVA including PA data demonstrated no effect of PA levels (F = 0.213, P = 0.647, η^2 = 0.005), no GWG classification effect (F = 0.760, P = 0.474, η^2 = 0.034), and no interaction between PA category and GWG classification (F = 0.235, P = 0.792, η^2 = 0.011) for the residual BW (Table 3).

BW:PW ratio showed similar results, with no PA levels effect (F = 0.261, P = 0.612, η^2 = 0.006), no GWG classification effect (F = 0.523, P = 0.597, η^2 = 0.024), and no interaction between these two factors (F = 0.207, P = 0.814, η^2 = 0.010) (Table 3).

The analysis for BW revealed no significant GWG classification effect (F = 2.667, P = 0.081, η^2 = 0.110). In addition, no PA level effect (F = 1.000, P = 0.323, η^2 = 0.023) nor interaction between the two factors (F = 0.676, P = 0.514, η^2 = 0.030) was observed (Table 3).

There was no PA level effect in estimated body fat percentage between active (16.5% ± 2.6%) and inactive (17.0% ± 2.5%) neonates (F = 0.256, P = 0.615, η^2 = 0.006) (Table 2). There was a significant GWG classification effect (F = 3.820, P = 0.030, η^2 = 0.151), the multiple correction analysis demonstrating that

TABLE 3. Effects of PA status and GWG classification on PI-E markers.

		Second Trimester (n = 61)			Third Trimester (n = 49)		
		PA Status					
	GWG Classification	Active	Inactive	Total	Active	Inactive	Total
Residual BW	Insufficient	−129.8 ± 175.8	−52.5 ± 335.6	−89.1 ± 267.7	−148.8 ± 198.9	8.2 ± 290.1	−50.7 ± 264.3
	Appropriate	38.1 ± 298.4	−60.6 ± 539.6	−2.3 ± 405.3	38.7 ± 375.7	83.7 ± 304.3	66.8 ± 321.1
	Excessive	99.1 ± 198.7	77.4 ± 460.9	87.2 ± 358.6	98.6 ± 43.8	58.1 ± 403.8	62.9 ± 378.1
	Total	7.0 ± 251.0	−7.3 ± 439.2		−33.1 ± 284.4	51.1 ± 338.8	
BW:PW ratio (g·g^{-1})	Insufficient	7.3 ± 1.0	6.9 ± 1.0	7.1 ± 1.0	7.6 ± 1.2	7.1 ± 0.9	7.3 ± 1.0
	Appropriate	7.0 ± 1.0	6.4 ± 1.2	6.7 ± 1.1	7.2 ± 1.1	6.9 ± 1.0	7.0 ± 1.0
	Excessive	6.8 ± 0.8	6.9 ± 1.4	6.8 ± 1.1	6.8 ± 0.4	6.9 ± 1.3	6.9 ± 1.2
	Total	7.0 ± 0.9	6.7 ± 1.2		7.3 ± 1.0	7.0 ± 1.1	
BW (g)	Insufficient	3032 ± 223	3225 ± 396	3134 ± 332	2970 ± 228	3276 ± 371	3161 ± 351
	Appropriate	3349 ± 307	3365 ± 680	3356 ± 480	3303 ± 459	3450 ± 387	3395 ± 407
	Excessive	3502 ± 281	3461 ± 400	3480 ± 343*	3480 ± 184	3406 ± 346	3415 ± 328
	Total	3302 ± 327	3354 ± 491		3186 ± 381	3381 ± 361	

Data are presented as mean ± SD.
* P < 0.05 in comparison with insufficient GWG group (total).

neonates born to women who gained weight insufficiently (15.6% ± 2.2%) had lower body fat percentage than those who gained weight appropriately (18.0% ± 2.4%). There were no differences in measured BW of neonates born to women who gained weight excessively (17.0% ± 2.4%) (Table 1). There was no interaction between the two factors ($F = 0.015$, $P = 0.985$, $\eta^2 = 0.001$).

DISCUSSION

The purpose of this study was to compare Pl-E markers measured by three different methods (BW:PW, residual BW, and measured BW), based on adherence to prenatal PA (active vs inactive) and GWG recommendations (insufficient vs appropriate vs excessive). In the current analysis of a sample of healthy pregnant women without any preexisting complications, we did confirm that Pl-E was not compromised by engaging in PA. Measured BW was significantly higher among women in our cohort who gained weight excessively, in line with previous investigations (18). Finally, there was no interaction found for PA and GWG.

Previous research has shown that women who engage in PA during pregnancy are more likely to stay within GWG guidelines than those who do not (13). Although our results showed no difference in GWG based on activity status, this may be due to our limited sample size. In line with the existing literature (30), we found a significant difference between the measured BW of neonates born to women who gained weight excessively compared with those who gained weight insufficiently. The use of measured BW as a surrogate marker of Pl-E may be misleading, as higher values may not be indicative of greater Pl-E, especially in cases of macrosomia and large for gestational age newborns. Fetal adiposity and fat mass may be more relevant birth outcomes as they are predictors of childhood obesity, especially in pregnancies complicated by gestational diabetes mellitus and maternal obesity (31,32). For example, neonates born to women who exercised a minimum of 30 min, three or more times per week before conception had a reduction in skinfold percentile, calculated body fat percentage, and calculated fat mass compared with their control counterparts (33). Interestingly, in our population, infants of women who were active during pregnancy, regardless of the pregnancy trimester, were not different in measured BW or neonatal adiposity as measured by skinfold thickness.

Measured BW and PW, used to calculate Pl-E, are gross measurements that give limited insight into the underlying functionality of the placenta. Unlike other organs that can send signals to the body through the nervous system, the placenta is not innervated, and therefore interorgan communication must take place by other means, such as through signaling molecules (34). Biomolecules that may function in an endocrine or paracrine fashion may be more valuable predictors of Pl-E. A logical candidate biological marker of placental function is placental growth factor (PlGF), a key protein involved in placental development and angiogenesis (35). Low levels of serum PlGF have been linked to pregnancy complications such as preeclampsia or fetal growth restriction and increased maternal cardiovascular risk (36–38). Because PA and GWG protect against pregnancy complications, biological measurement approaches may be more useful in interpreting Pl-E.

Previous studies have shown that both gaining within GWG guidelines and meeting recommended PA levels may have a positive effect on placental development (2,19). The use of Pl-E as a marker of placental function has been debated, as it is a relative measurement used to compare a population or sample; however, it is still widely used in a clinical setting. A recent review questioned its clinical merit in certain populations, such as instances of uncomplicated pregnancies (39). A healthy and homogeneous population, as we examined, might be expected to have normal values for Pl-E. The placenta is an adaptive organ and is often described as highly resilient; therefore, drastic variations in Pl-E may be difficult to observe in a low risk group (39). This concept has been illustrated in a randomized clinical exercise trial, where no difference in placenta weight was reported after 85 exercise sessions with high adherence (40). Because Pl-E values are likely consistent across an uncomplicated pregnant population (where placentas without pathological complications will adapt according to fetal size), conventional methods of measuring Pl-E may therefore be considered clinically irrelevant. As a result, although meeting PA and GWG recommendations are clearly healthy pregnancy behaviors, we were unable to demonstrate that they positively influenced Pl-E. Although the results are inconclusive, PA and GWG have been nonetheless shown to influence other factors of pregnancy, justifying the relevance of investigating its effect on Pl-E. As previously mentioned, an inactive lifestyle and/or gaining weight outside the recommended amount increases the risk for pregnancy complications, and as our population of pregnant women have no contraindications otherwise, it is important to investigate how PA and GWG behaviors can affect the three markers of Pl-E.

The strengths of this study include the objective measurement of maternal PA level by accelerometry. In addition, we were able to include PA data from two different pregnancy time points representing two different trimesters, and the effects of PA levels during trimesters 2 and 3 on Pl-E markers were very similar. Another strength is that we objectively calculated Pl-E by measuring placenta and fetal weight immediately (placenta) and 24–48 h postbirth (neonate). Limitations of our study include the sample size of $n = 49$ for trimester 3 data, which is below the sample size calculation. Moreover, PA was only assessed once per trimester using a single device. Another limitation of this study is the homogeneity of our population, as it is composed of educated, healthy pregnant women, mostly Caucasian, and with a BMI <29.9 $kg \cdot m^{-2}$. Moreover, participant dietary intake has the possibility to influence maternal GWG and fetal BW, regardless of PA levels, but this was not addressed in the present analysis.

Future studies should consider including a larger, more diverse population of pregnant women to increase the generalizability and overall statistical power needed to detect potential differences, using our results (i.e., effect sizes) as guidance

for sample size calculation. Because research has shown nutrient transport to be perturbed in complicated pregnancies (39), future studies should evaluate the potential protective effects of meeting PA and GWG recommendations in pregnancies with a high prevalence of reduced Pl-E (e.g., women living with obesity, high blood pressure, or preeclampsia) using a biomarker such as PlGF.

Although no significant differences in Pl-E were found based on PA and GWG, we have shown that PA does not negatively affect markers of Pl-E. This finding further supports guideline recommendations that PA is safe among pregnant women who do not have any contraindications to exercise. Given the known growth and developmental benefits of meeting PA and GWG recommendations during pregnancy, a closer examination into the functional response of the placenta would be beneficial. Understanding the positive impact of meeting PA and GWG recommendations during pregnancy will help to further advocate for the promotion of guideline adherence within primary care to achieve healthy pregnancy outcomes.

The results of this study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation. The results of this study do not constitute endorsement by the American College of Sports Medicine. The PLACENTA study is funded by a CIHR Operating Grant. T. S. N. is funded by a Mitacs Postdoctoral Fellowship in partnership with The Society of Obstetricians and Gynecologists of Canada. SM is funded by an Ontario Graduate Scholarship.

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CHAPTER 3: PHYSICAL ACTIVITY THROUGHOUT PREGNANCY IS ASSOCIATED WITH UMBILICAL CORD CHOLESTEROL

PREAMBLE TO MANUSCRIPT 2

The manuscript titled: "*Physical activity throughout pregnancy is associated with umbilical cord cholesterol*" is the third chapter of this thesis and covers the second objective, which aims to investigate the impact of PA (i.e. in mid and late gestation and PA patterns over gestation) and GWG on maternal (mid and late gestation) and umbilical cord serum lipid and glucose profile. This article is in preparation to be submitted to the *Journal of Obstetrics and Gynaecology Canada.* As gross measurements of the placenta were inconclusive on the relationship between maternal PA and GWG on birth outcomes, this objective is the next step in exploring the relationship between maternal outcomes and lipid markers.

PHYSICAL ACTIVITY THROUGHOUT PREGNANCY IS ASSOCIATED WITH UMBILICAL CORD CHOLESTEROL

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Abstract

Maternal serum and umbilical cord (UC) lipid and glucose levels are known to be influenced by a variety of maternal factors over the course of pregnancy. Yet, their relationship with physical activity (PA) and gestational weight gain (GWG) are rarely investigated in combination, and in terms of gestational patterns. The purpose of this study was to investigate mid (24-28 weeks) and late (34-38 weeks) gestation maternal and UC serum lipid and glucose profile in relationship to maternal PA status and GWG categorization. Pregnant women (n=40) were categorized into PA groups based on the 2019 Canadian Guidelines for PA throughout pregnancy, and GWG categorization based on 2009 Institute of Medicine recommendations. Fasted maternal serum was taken in mid and late gestation, in addition to venous UC serum at birth. No relationship was found between maternal serum and PA or GWG. Infants born to women who were physically active across pregnancy, or whose activity status dropped in late gestation, had lower UC total cholesterol than those who were inactive throughout pregnancy ($p<0.0001$ for both). Women who gained weight appropriately in mid gestation had significantly lower UC glucose than those who gained weight insufficiently ($p=0.040$) or excessively ($p=0.021$). While PA and GWG may not have affected maternal serum, positive pregnancy behaviors and outcomes may provide prophylactic effects on UC serum, potentially providing long-term health benefits to the newborn.

Introduction

Maternal lifestyle behaviours such as physical activity (PA) and pregnancy outcomes such as gestational weight gain (GWG) have considerable influence on maternal and fetal health. Evidence-based international guidelines for PA during pregnancy are available to assist pregnant women with maintaining an active lifestyle, including the *2019 Canadian Guideline for Physical Activity Throughout Pregnancy* [1]. PA during pregnancy is an integral component of optimizing maternal and fetal health, including supporting women to meet their recommended GWG range target [2]. Despite known health benefits, most women reduce their PA levels as gestation progresses, which may increase the risk of excessive GWG [3]. There is a dearth of studies, either observational or interventional, which have investigated the relationship between habitual PA in combination with GWG on serum lipid outcomes in healthy pregnant women experiencing uncomplicated pregnancies. Certain PA studies have suggested a link between increased PA status and high HDL-c [4] or low triglycerides (TG) [5]. Regarding GWG, gaining in excess of recommendations compared to appropriate has been associated with increased maternal TG levels [6]. There is a substantial amount of research investigating maternal serum cholesterol, TGs, and glucose levels at varying stages of pregnancy, as well as the relationship with newborn outcomes [7–9]. A multitude of factors have been found to influence umbilical cord (UC) lipid and glucose levels, including maternal pre-pregnancy BMI status, maternal obesity, pre-eclampsia, and gestational diabetes mellitus (GDM) [7,10–14]. Despite this, there are little to no studies that examine habitual PA patterns together with GWG and their subsequent relationship with maternal and UC serum lipid and glucose markers.

The physiological benefits of PA and GWG within recommendations can extend to the growing fetus, reducing the likelihood of preterm birth, miscarriage, and increasing the likelihood of the neonate being born at an appropriate birth weight [15–17]. Exposures such as maternal PA and

appropriate GWG are believed to modify the lipids available to the fetus, impacting fetal growth, fetal development, and predisposing the fetus for positive health outcomes later in life [18]. As the placenta is the intermediate between maternal and fetal nutrient transport, venous UC serum may act as a proxy measurement for fetal lipid and glucose levels at birth. A prospective cohort study by Collings *et al.* (2020) investigated the relationship between self-reported PA status in mid-gestation and UC serum lipid profile at birth, finding a higher concentration of HDL-c in those deemed somewhat active and moderately active compared to inactive [19]. Umbilical cord lipid and glucose levels have been positively correlated with newborn outcomes such as birth weight and adiposity [20,21]. High levels consistently predict an increased risk of obesity and cardiovascular diseases later in life [22]. As UC lipid and glucose markers are known to predict future health outcomes, positive pregnancy behaviours such as habitual PA and outcomes like appropriate GWG may have beneficial effects on the developing fetus through optimized UC lipid and glucose levels.

As gestation progresses there are barriers that cause changes in PA activity and GWG patterns, which have the potential to produce downstream effects on maternal and infant health [23]. Investigating multiple time points during gestation provides a more comprehension understanding of a women's pregnancy outcomes; however, there is a lack of research that attempt to understand the effect of PA and GWG patterns over gestation. Maternal and UC serum lipid and glucose markers have been shown to be influenced by both PA and GWG in a pregnant population [5,13], nonetheless rarely in combination. Thus, this study aims to explore the relationship between maternal PA and GWG in mid and late gestation, and gestational patterns, on maternal and UC lipid and glucose serum profile.

Materials and Methods

Participant recruitment

Pregnant women from the Ottawa, ON region were recruited to participate in the PhysicaL ACtivity and diEtary implicatioNs Throughout pregnAncy (PLACENTA) study. This study, approved by the University of Ottawa Research Ethics Board (REB: H11-15-29), followed all ethical standards outlined in the Declaration of Helsinki, and participants gave their written, informed consent prior to being included in the study. Participant eligibility criteria were: pregnant women between the ages of 18-40 years, in mid-pregnancy (24-28 weeks gestation), carrying a singleton fetus, weight stable for 6 months (± 2 kg) prior to conception, and self-reported pre-pregnancy body mass index (BMI) of normal to overweight (18.5-29.9 kg/m^2). Exclusion criteria included: pre-pregnancy diabetes, hypertension, or contraindications to PA as deemed by their health care provider.

Study factors: maternal physical activity and gestational weight gain

Participants were recruited during mid-gestation (24-28 weeks) and visited the University of Ottawa campus at that time, and once again in late pregnancy (34-38 weeks gestation). Serum samples were taken at both visits after an 8 or more hour fast. To objectively measure PA status at both pregnancy time points, participants were provided with an omniaxial Actical® accelerometer (Philips Respironics, QC, Canada). The Actical® accelerometer was to be worn for seven days following each visit, for a minimum of 10 hours of wear time per day [24]. At least three valid days per pregnancy time point were necessary to include the participant in the analysis [25]. We used the software SAS 9.4 (SAS Institute, NC, USA) to analyze the Actical® accelerometer data, in accordance with the Canadian Health Measures Survey [24]. Light-, moderate-, and vigorous-intensity PA were analyzed in minutes/day using ≥100 to <1,535 counts per minute (cpm), ≥1,535 to <2,962 cpm, and ≥2,962 cpm as the cut-offs for these intensities, respectively [24]. Women were considered active in each period of gestation if they engaged in ≥ 21.4 min/day of moderate-to-

vigorous PA (MVPA), following the 2019 Canadian Guideline for Physical Activity Throughout Pregnancy [1]. Women were classified into gestational PA categories based on their PA patterns; i) active throughout gestation were considered active in mid and late gestation, ii) activity dropped over gestation were active in mid gestation but not late gestation, and iii) inactive throughout gestation were inactive at both pregnancy time points.

To calculate GWG, participant self-reported pre-pregnancy weight was subtracted from their measured weight (Tanita BWB-800 scale, Lachine, QC) at each visit. Participants were further classified into GWG categories (insufficient, appropriate, and excessive) for each pregnancy assessment according to IOM 2009 recommendations [26], based on their pre-pregnancy BMI. For example, if a woman had gained 5.3 kg by 28 weeks' gestation, and the lower and upper limit of weight gain for that week of pregnancy is 7.3 and 9.6 kg respectively, she would be classified as insufficient weight gain based on the GWG guideline.

Covariates: dietary intake

Participants dietary intake was tracked through an Automated Self-Administered 24-hour (ASA24) Dietary Assessment Tool, version ASA24-Canada- 2018 (National Cancer Institute, Bethesda, MD). ASA24 is a cost-saving, online assessment tool that was found to report 80% of the items truly consumed [27]. Participants total food intake was recorded for three days (2 weekdays and 1 weekend day) during a 1-week period in each pregnancy timepoint. Unsaturated fat and total sugar were taken from the dietary assessment and used as covariates in our analysis [28–30]. Dietary intake data was excluded if participants had less than three days completed.

Study outcomes: serum analysis

Mid and late gestation maternal and term venous umbilical cord serum samples were taken using serum blood collection tubes (#367820; BD Biosciences, Franklin Lakes, NJ) and used for

measurement of total cholesterol, HDL-c, LDL-c, remnant cholesterol, TG, and glucose. Maternal and UC serum samples were analyzed using Cholestech LDX™ Lipid Profile•Glu Cassette (Abbott, IL, USA). Maternal LDL-c and remnant cholesterol concentration were calculated by the Cholestech LDX™ analyzer. UC serum triglyceride and total cholesterol levels were below the sensitivity range of the Cholestech LDX™ analyzer and could not be used to calculate LDL-c and remnant cholesterol concentration. UC serum TG and total cholesterol were analyzed using Triglyceride Colorimetric Assay Kit (cat.10010303, Cayman Chemical, MI, USA) and Cholesterol/Cholesteryl Ester Colorimetric Quantification Assay Kit (ab65359, Abcam, Cambridge, UK), respectively.

Statistical Analysis

Data were presented as mean (standard deviation [SD]). Normality was assessed by the Shapiro-Wilk test and homogeneity according to Levene's test. Participant demographics were compared between different categories of PA (i.e., active throughout gestation, activity dropped over gestation, inactive over gestation) and GWG categories (i.e., insufficient, appropriate, and excessive) using one-way ANOVA. Adjustments for multiple comparisons were performed according to Bonferroni correction. A two-way ANCOVA was used to assess the effects of both PA status (i.e., active/ inactive in mid and late gestation, in addition to long-term PA status), GWG classification (i.e., mid gestation and total GWG), as well as their interaction on maternal and UC serum lipid markers and glucose profile. Maternal unsaturated fat and sugar during each pregnancy time point were used as covariates in the analysis [28,30]. Adjustments for multiple comparisons were made using the Bonferroni correction. If an ANCOVA effect or interaction were found and the Bonferroni correction revealed no significant difference, we applied the LSD post-hoc to minimize the risk of type II statistical errors. The significance level was set at $p<0.05$.

Results

Forty participants (n=40) were included in this study, of which 10 were considered active throughout gestation, 11 fell into the activity dropped over gestation group, and 19 were considered inactive throughout gestation. In addition, 10 participants late gestation weight gain was classified as insufficient, 15 as appropriate, and 15 as excessive. Participant demographics can be found in Table 1 according to gestational PA pattern (i.e., active throughout gestation, activity dropped over gestation, and inactive throughout gestation), and late GWG categories (i.e., insufficient, appropriate, and excessive).

Table 1. Maternal and newborn characteristics according to gestational pattern PA status and total GWG classification (n=40).

Maternal demographics	**Active Throughout Gestation (n=10)**	**Activity dropped over gestation (n=11)**	**Inactive throughout gestation (n=19)**	***p-value***
Age (years)	31.70±2.95	32.45±3.62	31.63±3.17	0.785
Ethnicity, *n*				
White	10	10	16	0.421
Other	0	1	3	
Pre-pregnancy BMI (kg/m^2)	22.56±2.56	23.67±3.00	22.73±2.85	0.605
Gestational weight gain (kg)				
Mid gestation	9.21±3.14	8.24±2.02	8.56±2.75	0.700
Late gestation	13.05±5.35	13.98±3.22	13.54±4.09	0.881
Gestational weight gain classification, *n*				
Mid gestation				
Insufficient	2	1	3	
Appropriate	2	2	5	0.920
Excessive	6	8	11	
Late gestation				
Insufficient	4	1	6	
Appropriate	4	5	5	0.410
Excessive	2	5	7	
Gestational age at delivery (weeks)	40.45±0.92	39.67±1.01	39.88±1.23	0.258
Mid gestation MVPA (min/day)	43.30±7.52	40.66±10.62	12.49±5.89*,†	<0.001
Late gestation MVPA (min/day)	34.29±9.34	10.05±5.95*	9.49±6.22*	<0.001
Mid gestation dietary intake (g)				
Total calories	2606.02±693.54	2166.80±344.58	2154.66±410.05	0.050
Unsaturated fat	58.79±17.51	50.29±11.96	47.75 ±11.16	0.111
Sugar	142.78±47.30	119.27±31.01	120.90±37.41	0.289
Late gestation dietary intake (g)				
Total calories	2684.34±500.33	2612.92±529.49	2509.82±553.22	0.715
Unsaturated fat	62.80±12.63	61.78±16.18	66.13±12.99	0.672
Sugar	150.20±35.15	138.77±35.90	138.40±35.23	0.668
Maternal demographics	**Insufficient (n=10)**	**Appropriate (n=15)**	**Excessive (n=15)**	***p-value***
Age (years)	33.00±3.40	31.60±3.29	31.40±2.95	0.439
Ethnicity, *n*				
White	10	12	14	0.241
Other	0	3	1	

Pre-pregnancy BMI (kg/m^2)	22.11±1.67	21.88±2.35	24.57±3.14$^{\#,\ddagger}$	0.013
Gestational weight gain (kg)				
Mid gestation	5.87±1.93	8.88±1.21$^{\#}$	10.23±2.69$^{\#}$	<0.001
Late gestation	8.71±2.12	13.81±1.72$^{\#}$	17.02±3.08$^{\#,\ddagger}$	<0.001
Mid gestation weight gain classification, *n*				
Insufficient	6	0	0	
Appropriate	4	4	1	<0.001
Excessive	0	11	14	
Gestational age at delivery (weeks)	39.60±0.88	40.35±1.07	39.83±1.25	0.223
Mid gestation MVPA (min/day)	25.01±15.58	31.99±17.87	25.85±16.66	0.505
Late gestation MVPA (min/day)	17.09±10.22	16.97±17.68	13.89±8.30	0.765
Mid gestation dietary intake (g)				
Total calories	2131.00±354.00	2144.00±507.60	2491.00±544.2	0.103
Unsaturated fat	45.53±10.57	51.63±16.94	54.57±11.10	0.270
Sugar	120.40±22.35	111.20±40.84	144.40±39.83	0.052
Late gestation dietary intake (g)				
Total calories	2347.00±342.30	2489.00±468.00	2831.00±665.50	0.068
Unsaturated fat	64.71±13.34	64.05±12.52	63.74±15.69	0.986
Sugar	130.7±18.10	127.3±25.62	162.8±41.6$^{\#,\ddagger}$	0.008

Data presented as mean±SD. BMI: body mass index; Mid gestation: 24-28 weeks gestation; Late gestation: 34-38 weeks gestation. * Significantly different from active throughout gestation; †Significant different from activity dropped over gestation; #Significantly different from the insufficient group; ‡Significantly different from the appropriate group.

Association of mid and late gestation PA and GWG on maternal and umbilical cord blood lipid and glucose profile

No GWG classification, PA status, or interaction was found when investigating mid and late gestation maternal serum lipid and glucose markers (supplementary Table 1). We found an effect of GWG classification ($F = 3.460$; $p = 0.044$; $\eta^2 = 0.178$) at mid pregnancy on UC glucose; *posthoc* analysis revealed a significantly higher level of UC serum glucose for women who gained insufficiently ($p = 0.040$) and excessively ($p = 0.021$) compared to women who gained appropriately in mid pregnancy. Active pregnant women in mid ($F = 33.875$; $p < 0.001$; $\eta^2 = 0.514$) and late gestation ($F = 5.462$; $p = 0.026$; $\eta^2 = 0.146$) presented lower UC blood total cholesterol than their inactive counterparts (Figure 1, data presented in Appendix Table 1C).

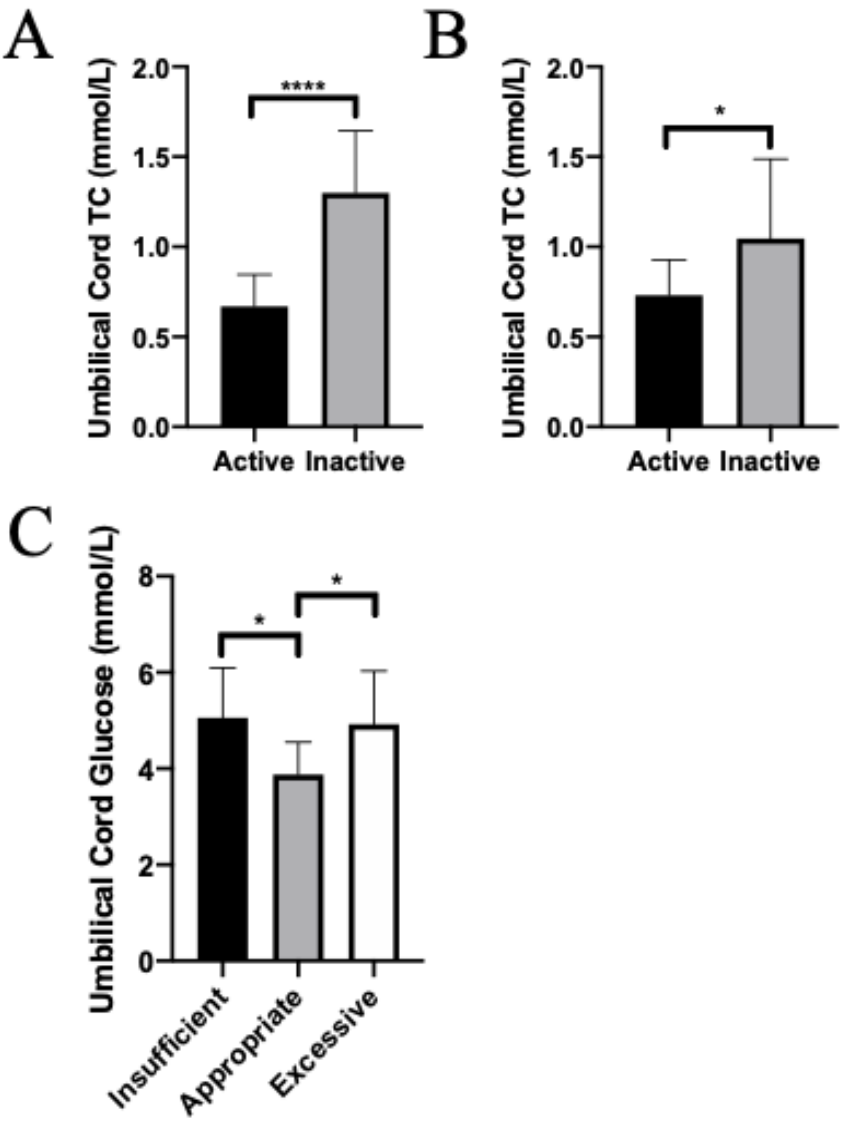

Figure 1. Mid gestation gestational weight gain and mid and late gestation physical activity on umbilical cord total cholesterol and glucose (mmol/L) markers (n=40). A: Mid gestation physical activity status; B: late gestation physical activity status; C: mid gestational weight gain classification. TC: total cholesterol. * $p < 0.05$, **** $p < 0.0001$.

Association of gestational PA patterns and total GWG with umbilical cord serum and the delta change of maternal serum lipid and glucose profile

Maternal PA patterns and late gestation weight gain were analyzed individually and in combination on the relationship with the delta change ([late gestation – mid gestation]/ mid gestation * 100) of maternal serum lipid and glucose profiles. No effect of gestational PA patterns, GWG categorization, or interaction was observed (supplementary Table 2). In terms of the relationship between gestational PA patterns and UC serum, there was a gestational PA pattern effect ($F = 19.232$; $p < 0.001$; $\eta^2 = 0.570$) for total cholesterol where the active throughout gestation group ($p < 0.0001$) and the group whose activity dropped over gestation ($p < 0.0001$) presented lower UC serum total cholesterol than the inactive throughout pregnancy group (Figure 2 & 3, data presented in Appendix Table 2C).

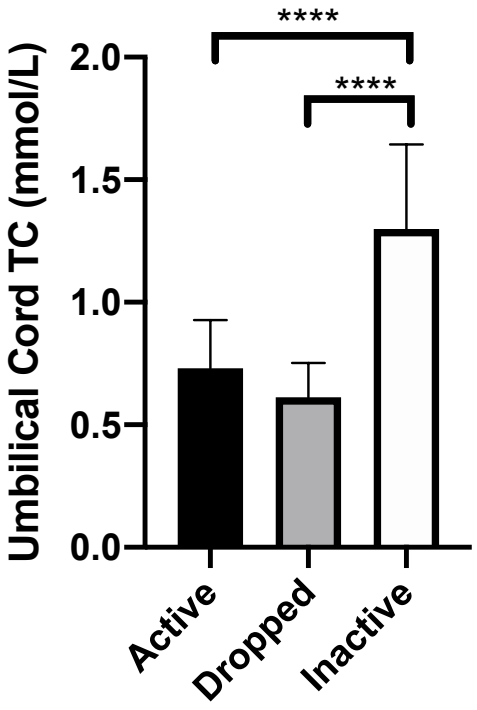

Figure 2. Gestational physical activity patterns on umbilical cord serum total cholesterol (mmol/L, n=40). Active: active throughout gestation; Dropped: activity dropped over gestation; Inactive: inactive throughout gestation. TC: total cholesterol. **** $p < 0.0001$

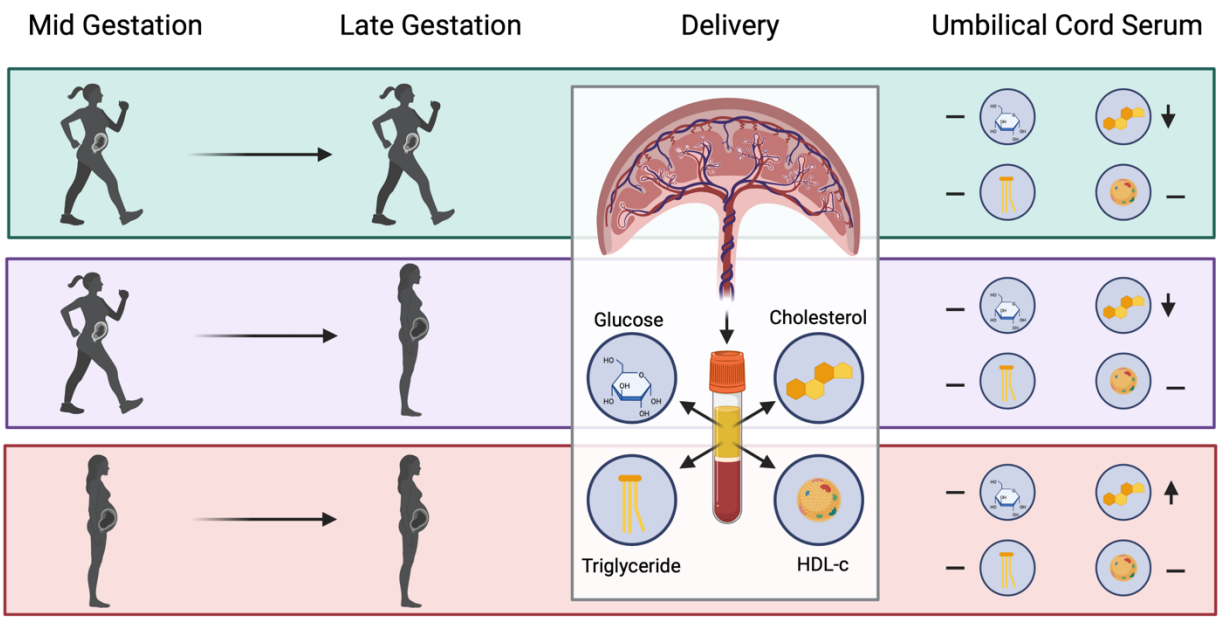

Figure 3. Relationship between habitual physical activity patterns in mid and late gestation on umbilical cord serum lipid and glucose concentrations. HDL-c: high density lipoprotein cholesterol; ↓: decrease; ↑: increase; —: no change.

Discussion

Serum lipid levels in a non-pregnant population are known to be influenced by PA and GWG outcomes; however, their objective measurement and subsequent interaction are rarely investigated in a non-complicated pregnant population. The lipid markers examined in this study are seldom researched in both maternal and UC serum − a proxy measurement for infant serum. Venous UC glucose levels were found to be significantly lower in those who gained weight appropriately by mid gestation compared to those who gained excessively or insufficiently. Total cholesterol concentration was significantly lower in UC serum of women deemed active throughout gestation, and in those whose activity dropped over gestation, compared to those who were inactive throughout gestation.

Despite data illustrating that PA and GWG can independently impact serum lipid and glucose markers, our study did not confirm this relationship. When examining serum lipids, studies rarely control for diet, even though it has considerable influence on serum lipid and glucose levels,

and may be the reason we do not see any differences [28–30]. Maternal GWG did not influence maternal lipid outcomes, which may be due to our population being relatively healthy, with a BMI <29.9 kg/m^2, and non-complicated pregnancies. The majority of studies where weight related outcomes impact lipid levels are those in cases of obesity, GDM, or a combination of these complications [11,13,31]. In addition, although PA is a positive behaviour that can protect a pregnant person against many pregnancy-related complications [32], our population experienced non-complicated pregnancies, are of a higher socio-economic status, and are well-educated; thus MVPA intensity may not be powerful enough to influence maternal serum lipid and glucose levels.

The timing of maternal exposures during fetal development (e.g., mid and/or late pregnancy) is known to influence pregnancy outcomes [33], with habitual PA and GWG being no exception. Physical activity has not only been shown to benefit newborn outcomes, but also the placenta; increasing villous surface area, vasculature volume, and promoting increased expression of vascular endothelial growth factor [34,35]. As the placenta is the intermediate for maternal to fetal communication, it acts as a gatekeeper shuttling oxygenated, nutrient-rich blood for fetal use. The placenta stores lipids in the form of triglycerides and neutral esters, yet those destined for fetal use are transported by the venous UC blood [36]. Maternal lipid and glucose levels are much higher than those of the UC, suggesting that the placenta is limiting excessive nutrient transport to possibly protect the developing fetus. Maternal PA and GWG may therefore influence the physiology of the placenta to dictate lipid and glucose transport to the fetus.

Glucose is the main substrate for normal development *in utero*, yet fetal production of glucose is minimal [37]. The fetus is therefore dependent on maternal glucose production, and the subsequent transport across the placenta. Mid gestation weight gain was associated with UC glucose levels, with findings indicating that the UC serum of women who gained weight

insufficiently or excessively had higher levels of glucose than those who gained appropriately. One explanation for this outcome is that the timing of weight gain during gestation is highly linked to fetal birthweight [38]. Given mid gestation is the period where most fetal organ development occurs, the fetus would require increased energy supply. One mouse-based model has suggested that in response to maternal undernutrition, the fetus may signal to the placenta to increase nutrient uptake to support fetal growth [39], thereby potentially shuttling excessive glucose. Few studies investigate the impact of GWG in a healthy population on newborn glucose levels, yet one study whose data was extracted from medical records examined the relationship of GWG from pregnant women living with GDM on newborn glucose levels [31]. The authors reported that the newborns of those who gain weight appropriately were less likely to have high glucose levels or be hypoglycemic within the first 48 hours after delivery than those who gained weight insufficiently or excessively [31]. As most research investigating UC glucose involves cases of GDM, it should be noted that GWG has a greater impact on overall pregnancy outcomes than GDM [40], and future research should be dedicated to understanding the mechanism by which GWG in uncomplicated pregnancies is involved in gestational glucose metabolism.

An active PA status, especially in mid gestation, was significantly associated with a lower concentration of UC total cholesterol when compared to their inactive counterparts. An unanticipated finding of this study is that even if a women's activity dropped over gestation (i.e., in late gestation), venous UC serum total cholesterol levels had similar amounts to those who were chronically active over gestation. This finding may be because fetal liver development, which produces a significant portion of the fetus' own cholesterol supply, begins in early pregnancy, and is completed by mid-gestation [41]. PA may be influencing fetal derived cholesterol producing hepatocyte cells to optimize its production, signaling to the placenta that it does not require

excessive cholesterol transport. Cholesterol is an important part of a healthy pregnancy for both mother and fetus, acting as the precursor to various hormones required for growth; however, in excess, it has been associated with metabolic conditions such as cardiovascular disease and obesity later in life [42]. The mechanisms by which this occurs may not be fully understood, but PA may influence mechanisms of placental cholesterol transport, providing prophylactic effects on UC serum total cholesterol levels and reducing the possibility for future health complications for the fetus.

The strengths of this study include the objective measurements of PA and standardized weight measurement at multiple time points throughout pregnancy. While our population is of similar ethnic background (mostly Caucasian) and non-complicated pregnancies, we followed the same participants throughout pregnancy and acquired multiple indices of maternal and fetal lipid markers. Our population is limited in size and not adequately powered; yet this study could be helpful in informing future investigations.

In conclusion, while PA status and GWG categorization in an observational setting may not have been powerful enough to influence maternal lipid and glucose profile, yet an active PA status over gestation, specifical mid gestation, was associated with a significantly lower UC total cholesterol concentration. In addition, appropriate mid gestation weight gain resulted in a lower UC glucose concentration. These positive pregnancy behaviours (PA) and outcomes (GWG) may offer a physiological benefit to the fetus by means of optimized lipid and glucose transport from the placenta, potentially improving future newborn health outcomes.

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CHAPTER 4: PLACENTAL FATP1 IS DIFFERENTIALLY EXPRESSED IN PHSYICALLY ACTIVE WOMEN

PREAMBLE TO MANUSCRIPT 3

The manuscript titled: "*Placental FATP1 is differentially expressed in physically active women*" represents the third objective of this thesis, which aims to investigate the impact of PA over pregnancy and GWG categorization on markers of placental lipid transport and storage at term. As maternal PA and GWG were shown to influence umbilical cord lipids, the final investigation of this thesis is to determine whether the placenta acts as an intermediate to mediate these associations.

PLACENTAL FATP1 IS DIFFERENTIALLY EXPRESSED IN PHYSICALLY ACTIVE WOMEN

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Abstract

Physical activity (PA) and weight gain influence fatty acid transport and storage in muscle and adipose tissue, yet their relationship has rarely been investigated in the placenta. The purpose of this study is to examine the relationship between maternal PA across gestation, in addition to gestational weight gain (GWG) on markers of placental lipid storage and transport at term. Pregnant women (n=31) were recruited before 28 weeks gestation, where objective PA and weight gain measures were taken at late gestation (34-38 weeks). At birth, placental tissue samples were collected for protein expression, immunohistochemistry analysis, triglyceride content, and lipoprotein lipase enzymatic activity. Physically active participants who gained insufficient weight had lower expression of placental FATP1 protein than their inactive, insufficient weight gain comparator (p=0.0008). In contrast, active women who gained excessive weight had higher placental FATP1 protein expression compared to their inactive, excessive weight gain counterparts (p=0.0052). Finally, gene expression of placental FABP4 was 2.194 fold higher in women who gained weight excessively compared to appropriately. Overall, this study in women with uncomplicated pregnancies suggests that PA and GWG in combination may alter the transport of fatty acids to the placenta. Further research is required to investigate whether this relationship is similarly associated with metabolic functions.

Introduction

The placenta is a specialized organ that develops throughout gestation to support the growing fetus by facilitating nutrient transport from the maternal circulation. In combination with maternal arteries, the placenta transfers nutrients such as lipids from mother to fetus through passive diffusion and active transport [1]. Maternal fat depots that accumulate in adipose tissue over gestation undergo increased rates of lipolysis in late gestation, becoming readily available for rapid fetal growth in preparation for delivery [2]. This process is key in the last three months of gestation, as essential and non-essential fatty acid (EFA and NEFA, respectively), which are key building blocks for nervous system and brain development, are transported to the fetus [3]. For the fetus to achieve a maximal growth rate, proteins associated with placental fatty acid metabolism must be functioning at optimal levels to deliver the appropriate amount of lipids [2]. These proteins include placental lipoprotein lipase ([LPL], which hydrolyzes EFA and NEFA into free fatty acids) [4], scavenger proteins (fatty acid translocase, [FAT/CD36]) [5], transport proteins such as fatty acid transport protein (FATP) 1 and 4, and fatty acid binding protein (FABP) 4 [5]. These proteins work together to transport free fatty acids across the placental barrier for either storage in lipid droplets, fatty acid oxidation, or transport to the fetus via the feto-placental circulation. Associations between pregnancy- related complications (i.e., obesity, gestational diabetes mellitus, excessive GWG) have been found with markers of fatty acid transport and storage such as decreases in placental FATP1/4 protein expression and increase in placental triglyceride composition [6]. Further, as the fetus is the recipient of nutrients passed through the placenta, it is important to consider the effects of placental lipid transport and storage on newborn anthropometric outcomes (i.e., weight-for-age [W/A] percentile and weight-for-length [W/L] z-score). Women living with obesity have been found to have elevated maternal to fetal transfer of free fatty acids [7], while complications such as intrauterine growth restriction may limit lipid transport to the fetus and cause fetal growth

complications [4]. Hence, it is important to investigate placental lipids and associated fetal developmental outcomes that may be linked to maternal behaviours.

The majority of studies investigating placental lipid transport and storage focus on cases of severe pregnancy- related complications, with few investigating the relationship with pregnancy behaviours such as physical activity (PA) and appropriate gestational weight gain (GWG). In the non-pregnant population, habitual exercise can reduce tissue mass and fatty acid uptake and improve metabolic functions in adipose tissue [8]. Furthermore, aerobic exercise training increases fatty acid uptake and transport in skeletal muscles in response to heightened mitochondrial activity [9]. There is a known relationship between high pre-pregnancy body mass index and mRNA expression of placental proteins involved in lipid transport and storage, such as FATP1, FATP4, and FAT/CD36 [10]; however, there is a knowledge gap in placental lipid transport and storage in relation to PA status. As weight gain patterns and PA habits fluctuate over gestation [11,12], it is unknown whether PA across gestation and weight gain influence placental lipid transport or storage at term. This study investigates the relationship between PA and GWG in mid and late gestation and markers of placental lipid transport and storage. It is hypothesized that those who are physically active throughout gestation, coupled with gaining appropriate weight, will have lower expression of fatty acid transport and lipid storage markers.

Methods

Participant recruitment and newborn outcomes

Pregnant women from the Ottawa region were recruited through the Physical Activity and diEtary implicatioNs Throughout pregnAncy (PLACENTA) study (Canadian Institute of Health Research MOP: 142298). Participants had given written informed consent before participation and ethics approval for this study was obtained from the University of Ottawa Research Ethics board

(file number:H11-15-29), in adherence to the ethical guidelines of the Declaration of Helsinki. Pregnant women were recruited in mid pregnancy (24- 28 weeks) between the ages of 18-40 years, carrying a singleton fetus, having a pre-pregnancy body mass index (BMI) considered normal or overweight (18.5 – 29.9 kg/m^2), weight stable for 6 months (±2 kg) prior to conception, and no contraindication to PA during pregnancy. Exclusion criteria included those with hypertension, pre-pregnancy diabetes, or untreated thyroid disease. The most and least physically active participants throughout pregnancy were chosen based on PA status throughout pregnancy (measured in mid gestation; 24-28 weeks, and late gestation; 34-38 weeks); Participants wore an omniaxial Actical® accelerometer (Philips Respironics, Bend, OR, USA) to measure PA status in mid and late pregnancy, and were instructed to wear it for seven days, for a minimum of 10 hours of wear time per day [13]. Participants required at least three valid days per pregnancy time point to be included in the analysis [14]. The software SAS 9.4 (SAS Institute, NC, USA) was used to analyze the Actical® accelerometer data, in accordance with the Canadian Health Measures Survey methods[13]. Participants were deemed physically active if they engaged in a minimum of 150 minutes of moderate-to-vigorous PA (MVPA) per week, or >21.4 minutes MVPA/day, as recommended by the 2019 Canadian pregnancy guidelines [15]. Women were considered inactive if they engaged 2 standard deviations or more below the minimum weekly MVPA. Participants visited the University of Ottawa Campus at each pregnancy time point, where maternal weight was taken (Tanita BWB-800 scale, Lachine, QC) and GWG calculated based on self-reported pre-pregnancy weight. Participants were classified into GWG categories (i.e., insufficient, appropriate, excessive) according to 2009 Institute of Medicine (IOM) guidelines based on calculated pre-pregnancy BMI [16].

Neonate weight and length were taken 24-48 hours after delivery by a trained research team member, following the WHO-recommended assessment methodology. Birth weight was measured using an Ultrascale MBSC-55 (MyWeigh, China) to the nearest gram. Weight-for-age percentile and W/L z-score were calculated based on WHO (2006) child growth standards [17]. A Harpenden Skinfold Caliper (Baty International, UK) was used for neonatal skinfold estimation, taking two measurements from the right side of the infant, at the biceps, triceps, subscapular, and suprailiac to the nearest 0.4 mm, and averaged. If measurements differed by greater than 0.4 mm, a third measurement was taken. Fetal body fat percentage was estimated as described by Schmezle and Fusch [18].

Placental tissue sampling

Term placentas (i.e., after 37 weeks gestation) were processed on ice within 60 minutes of delivery. According to standardized lab protocol, placental tissue samples (washed of excess blood) were biopsied at three central and two peripheral cotyledons to acquire appropriate representation of the placenta. Samples were flash frozen in liquid N_2 for protein analysis. Frozen placental tissues were weighed and homogenized on ice using a Powergen 125 homogenizer (Fisherbrand, PA, USA) in radioimmunoprecipitation assay buffer (Alfa Aesar, MA, USA) for protein extraction. The lysates were centrifuged at 1000 x g for 10 minutes at 4°C and stored at -80°C for Western Blot analysis and LPL activity assay. For histological purposes, a full thickness placental biopsy was dissected approximately 2 cm from the insertion of the umbilical cord and flash frozen in optical cutting temperature compound (Fisher HealthCare, MA, USA) and stored at -80°C until sectioned.

Western blotting

Total protein (20-40 µg) from each tissue sample was loaded onto Mini-PROTEAN® TGX gel (Bio-Rad, CA, USA). The resolved proteins were wet-transferred onto a polyvinylidene di-fluoride membranes (Bio-Rad) at 100 V for 1.5 hours. Following the transfer, the membrane was blocked with 5% powdered milk in TBST for 1 hr at room temperature (RT). Membranes were incubated overnight at 4^{o}C with the following primary antibodies: ADFP (1:500, ab181452, abcam, Cambridge, MA), FAT/CD36 (1:500, ab133625, abcam), FATP1 (1:1000, ab69458, abcam), and FABP4 (1:1000, ab66682, abcam). Following day, the membranes were washed with TBST and incubated with horseradish-peroxidase conjugated secondary antibodies for 1 hr at RT as follows: ADFP and CD36 (1:2500; Goat Anti-Rabbit IgG, Bio Rad), FATP1 and FABP4 (1:5000; Goat Anti-Mouse IgG, Bio Rad). Blots were developed using Clarity ECL Western Substrate (Bio-Rad) and imaged using ChemiDoc™ XRS+ Imaging System (Bio-Rad). Blots were standardized to a pooled total protein lysate sample. Following imaging, membranes were stripped and permanently stained with 1% Amido Black for whole protein quantification. Densitometry analysis of the bands was performed using ImageJ (Bio-Rad).

qPCR analysis

Total placental RNA was isolated from flash frozen tissue samples using an Illustra RNAspin Mini Kit (GE Healthcare Life Sciences, ON, Canada) following manufacturer's instructions. After isolation, 1 ug of total RNA was reverse transcribed into cDNA using 5X iScript Reverse Transcription Supermix (Bio-Rad). One-ten ug of cDNA was amplified by one-step fast real-time quantitative polymerase chain reaction (qPCR) using Roto-Gene RG-3000 (Corbett Research, Australia). The gene expression of FATP1 (Hs.PT.58.25764893, Integrated DNA Technologies [IDT] New Jersey, USA), FABP4 (Hs.PT.58.20106818, IDT), and FAT/CD36

(Hs.PT.56a.3615957, IDT) were analyzed in triplicates with by prime-time qPCR probe, using YWHAZ (Hs.PT.39a.22214858 IDT) as an endogenous control. The threshold cycle (CT) value was used to calculate relative gene expression of each gene according to the equation $2^{-\Delta\Delta Ct}$. Placental gene expression of physically active women was calculated relative to that of physically inactive women.

Immunohistochemistry

Unfixed, frozen placental biopsies were sectioned to a thickness of 4 μm and fixed in a 10% formalin buffer for 10 min. Samples were stained with Oil Red O solution (cat. ab150678, abcam) following manufacturer recommendation with the following modification: incubation in Oil Red O Solution for 15 min and counterstained using ¼ diluted Harris hematoxylin for 1 min. Non-consecutive sections (4 μm apart) were stained in duplicate, and those omitted from Oil Red O staining served as negative controls. Slides were imaged at a 40X magnification and tiled to represent a full-thickness image of the placenta using the Thermo Scientific™ Invitrogen™ EVOS™ FL Auto 2 Imaging System. Lipid droplets were quantified using ImageJ software based on previously described methodology [19]. Images were converted to a 16-bit grayscale format. The threshold feature was used to distinguish droplets from background tissue, and subsequently, the particles were analyzed to quantify the number of droplets in the image. Droplet number was normalized to tissue area by converting the image to binary colour, with tissue and background space denoted as white and black pixels, respectively. Tissue pixels were divided by the total number of pixels to obtain a percentage of tissue coverage. The number of oil droplets was then divided by this proportion to calculate the number of droplets per tissue area for each slide. The average number of droplets per participants were calculated between the two slides using the following formula:

Lipid droplet count = Pixel count / [Tissue area / (Tissue area + negative space)]

In the case of compromised slide quality (i.e., smearing of tissue, improper mounting, etc), slides were excluded from analysis, and a single image was analyzed for the respective participant.

Lipase Activity

The activity of placental LPL was determined by Lipase Activity Assay Kit (cat. 700640, Cayman Chemical, MI, USA) according to manufacturer's recommendations using a 1/10 diluted protein lysate. Samples were assayed in triplicate.

Placental triglyceride quantification

Triglyceride concentration was assessed using a Triglyceride Colorimetric Assay Kit (cat.10010303, Cayman Chemical) with the following modification: approximately 200 mg of frozen placental tissue was homogenized in 1 mL of acetone on ice using Powergen 125 homogenizer (Fisherbrand, PA, USA). Samples were left to gently rock overnight at 4°C followed by at 15,000 x g for 15 min at 4°C. Samples were analyzed in triplicate and normalized to tissue weight (g).

Statistical analysis

Data are presented as mean ± standard deviation. Normality was assessed by the Shapiro-Wilks test, and parametric or non-parametric tests were applied where appropriate. Participant characteristics and Oil Red O visualization were analyzed by students t-test or Mann-Whitney U test according to normality. Placental protein and gene expression, placental LPL enzyme activity, and triglyceride quantification were analyzed by two-way analysis of variance (ANOVA) controlling for PA status and GWG categorization. Multiple comparison tests were applied according to Bonferroni correction where applicable. All statistical analyses were performed using

GraphPad version 8.3.0. (San Diego, CA). For all statistical analyses, $p<0.05$ was deemed significant.

Results

Participant characteristics

Maternal demographic and newborn outcomes are described in Table 1 according to PA status throughout gestation. By study design, the physically active group had significantly higher MVPA (min/day) than their inactive counterparts ($p<0.0001$). There were no differences in GWG between active and inactive women. As seen in Table 1 an active PA status was associated with a lower newborn W/A percentile ($p=0.010$).

Table 1. Maternal demographic according to physical activity status throughout gestation (in both mid (24-28 weeks) and late (34-38 weeks) gestation (n=31). Newborn outcomes were recorded from term infants born after 37 weeks gestation.

Maternal demographics	**Active** (n=12)	**Inactive** (n=19)	p-value
Age (yrs)	31.4 ±2.8	32.1 ±3.1	0.534
Pre-pregnancy weight (kg)	64.2 ±9.9	65.5 ±11.0	0.796
Pre-pregnancy BMI (kg/m^2)	23.4 ±2.5	23.2 ±3.0	0.746
Gestational age at birth (weeks)	40.2 (39.5, 41.4)	40.3 (39.0, 41.3)	0.382
MVPA (min/day)			
Mid Gestation	42.7 (39.4, 56.5)	6.6 (4.0, 11.6)	<0.0001
Late Gestation	32.6 (28.5, 42.9)	4.0 (2.0, 10.4)	<0.0001
GWG (kg)	12.4 ±5.4	12.7 ±3.8	0.850
GWG classification, *n*			
Insufficient	5	6	
Appropriate	5	7	0.400
Excessive	2	6	
Newborn outcomes			
Birth weight (kg)	3.2 (2.9, 3.6)	3.6 (3.2, 3.8)	0.108
Birth length (cm)	50.0 ±2.5	51.0 ±1.8	0.221
Body fat (%)	16.3 ±2.8	17.2 ±2.3	0.339
Sex, *n*			
Male	7	11	0.982
Female	5	8	
W/A percentile	12.7 (10.9, 42.5)	52.9 (26.2, 74.4)	0.010
W/L z-score	-0.6 ±1.2	-0.7 ±1.2	0.779
Placental weight (g)	451 (417, 525)	480 (460, 546)	0.143

Values are presented as mean ± standard deviation or mean (interquartile range) according to normality. BMI: body mass index; GWG: gestational weight gain; MVPA: moderate to vigorous physical activity; W/A: weight-for-age; W/L: weight-for-length. Mid gestation: 24-28 weeks gestation; Late gestation: 34-38 weeks gestation.

Placental FATP1 protein expression is lower in active mothers than inactive mothers.

The protein expression of placental lipid transporters FATP1, FABP4, and FAT/CD36 were analyzed by western blot (Figure 1), and the activity of placental LPL was assessed using an enzymatic assay (Figure 1). Two-way ANOVA, including PA and GWG, indicated there was no PA effect, GWG effect, or interaction for proteins FABP4 and FAT/CD36, or for placental LPL enzymatic activity. Gestational weight gain status was significantly associated FATP1 protein expression ($F=6.22$, $p=0.0064$), and interaction between PA status and GWG was noted ($F=14.62$, $p<0.0001$). Bonferroni post-hoc analysis revealed a significantly lower FATP1 protein expression in the placenta from active women who gained insufficient weight compared to their inactive insufficient weight gain counterparts ($p=0.0008$), while active women who gained weight excessively had higher placental FATP1 protein expression than inactive women who gained weight excessively ($p=0.0052$). The relative fold difference in gene expression of physically active women compared physically inactive women were 0.915 for FATP1, 0.842 for FAPB4, and 1.067 for FAT/CD36. When comparing women who gained weight excessively and insufficiently compared to appropriately, 0.787 and 1.355 for FATP1, 2.194 and 1.656 for FABP4, and 1.494 and 1.219 for FAT/CD36, respectively. Two-way ANOVA, including PA and GWG, indicated there was no PA effect, GWG effect, or interaction for any of the target genes.

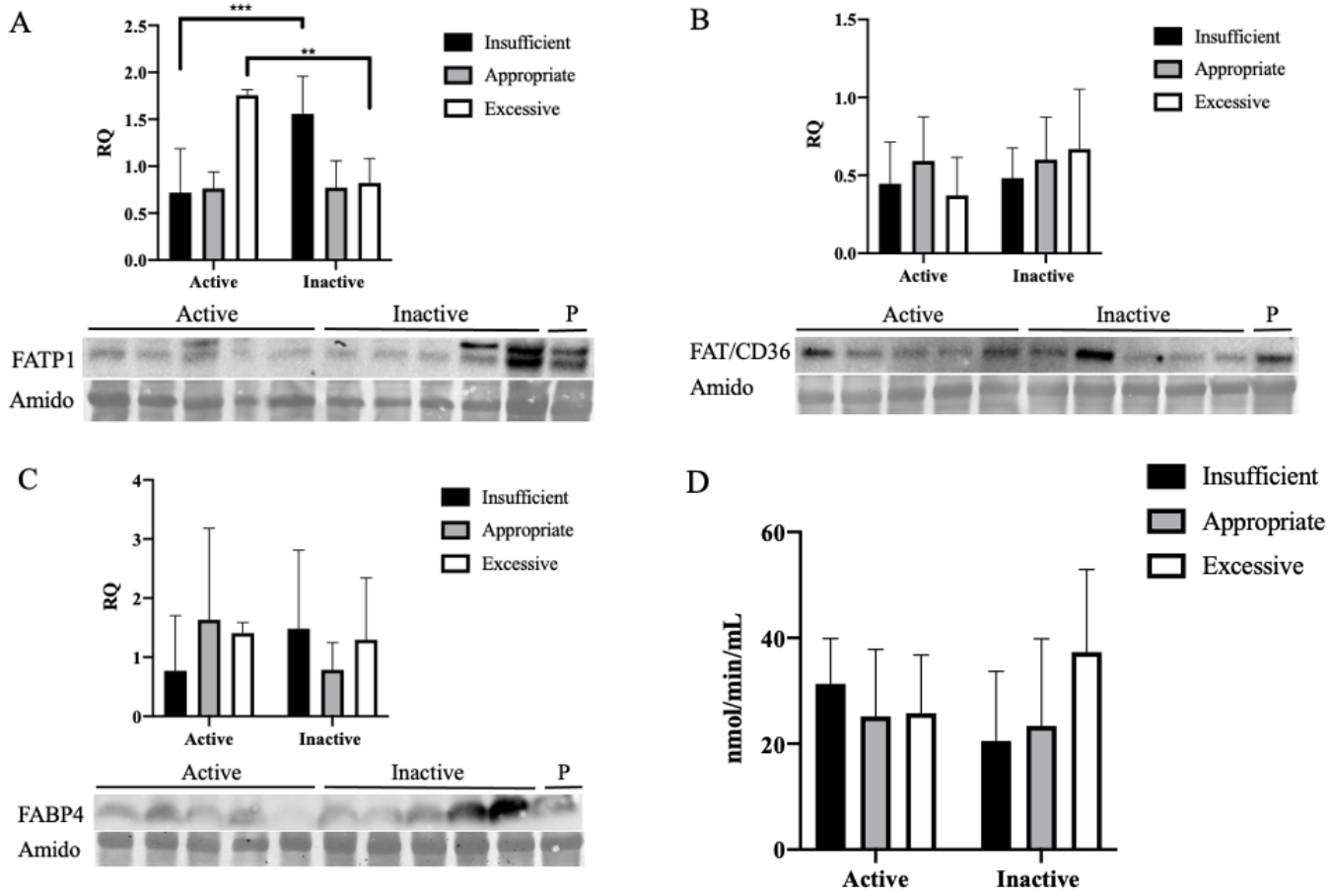

Figure 1. Placental protein expression of FATP1 (A), FAT/CD36 (B), FABP4 (C) and placental lipoprotein enzymatic activity (D) in physically active and inactive participants who gained weight insufficiently, appropriately, or excessively (n=31). Representative blots are shown. Blots were normalized to total participant pooled (P) sample for inter-blot comparison. Placental lipoprotein activity was normalized to total protein volume (mL). Values are presented as mean ± standard deviation. Protein densitometry was analyzed by two-way ANOVA for physical activity status and total gestational weight gain. RQ: relative quantity; P: total participant pooled sample. ** $p < 0.01$, *** $p < 0.001$.

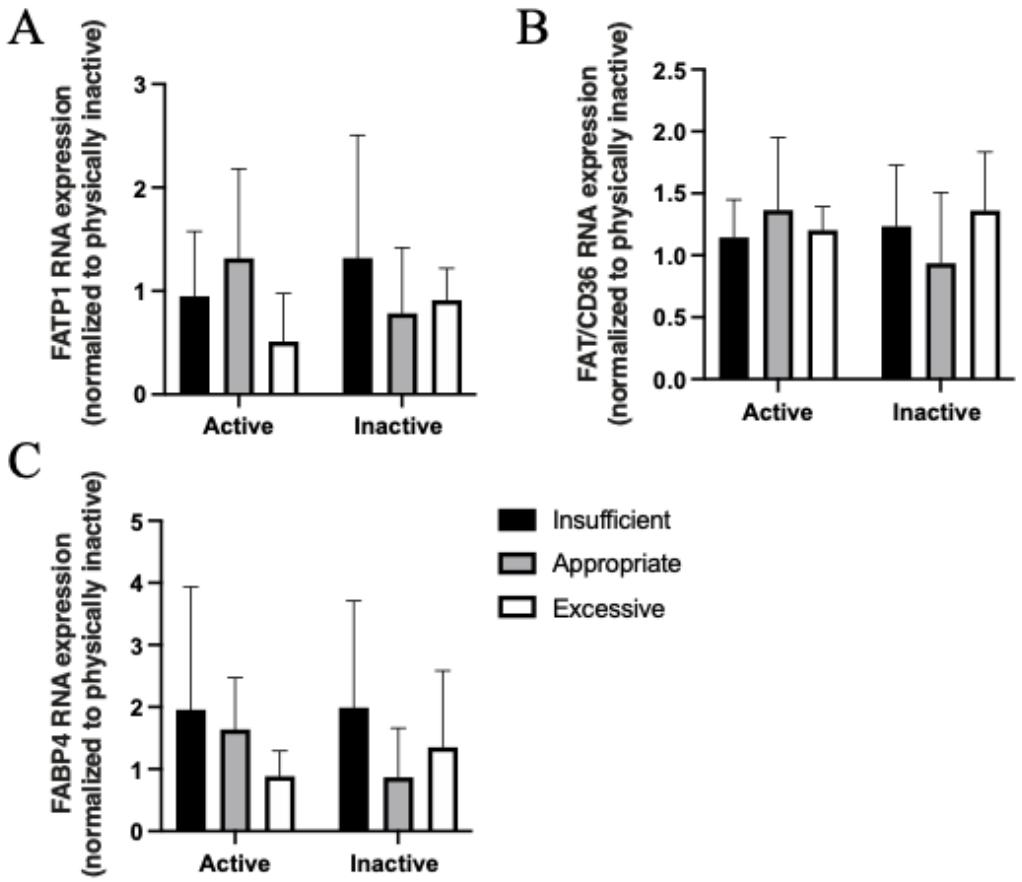

Figure 2. Relative quantification of placental RNA expression of FATP1 (A), FAT/CD36 (B), FABP4 (C) in physically active and inactive participants who gained weight insufficiently, appropriately, or excessively (n=31). Target gene expression was normalized to the expression of YWHAZ in all samples. All data presented as mean ± standard deviation.

Lipid droplets cluster near chorionic villi

To examine how PA and GWG affect the expression of the ADFP protein (a lipid droplet marker) we used two-way ANOVA. No PA effect, GWG effect, or interaction between the two variables and ADFP was found (Figure 3 A, B). The triglyceride content of placental tissue was assessed using a colorimetric assay, and no significant differences were found (Figure 3 C). Due to the limited sample, the visualization and pixel intensity of Oil Red O staining in placenta tissue were analyzed according to PA status or GWG only (Figure 4). A one-tailed student t-test was performed according to PA status, and no significant differences were found in lipid droplet quantification between active and inactive participants ($p=0.129$). Clusters of placental lipid droplets were most frequently localized near the chorionic villi and maternal/fetal interactions sites along the syncytiotrophoblast border.

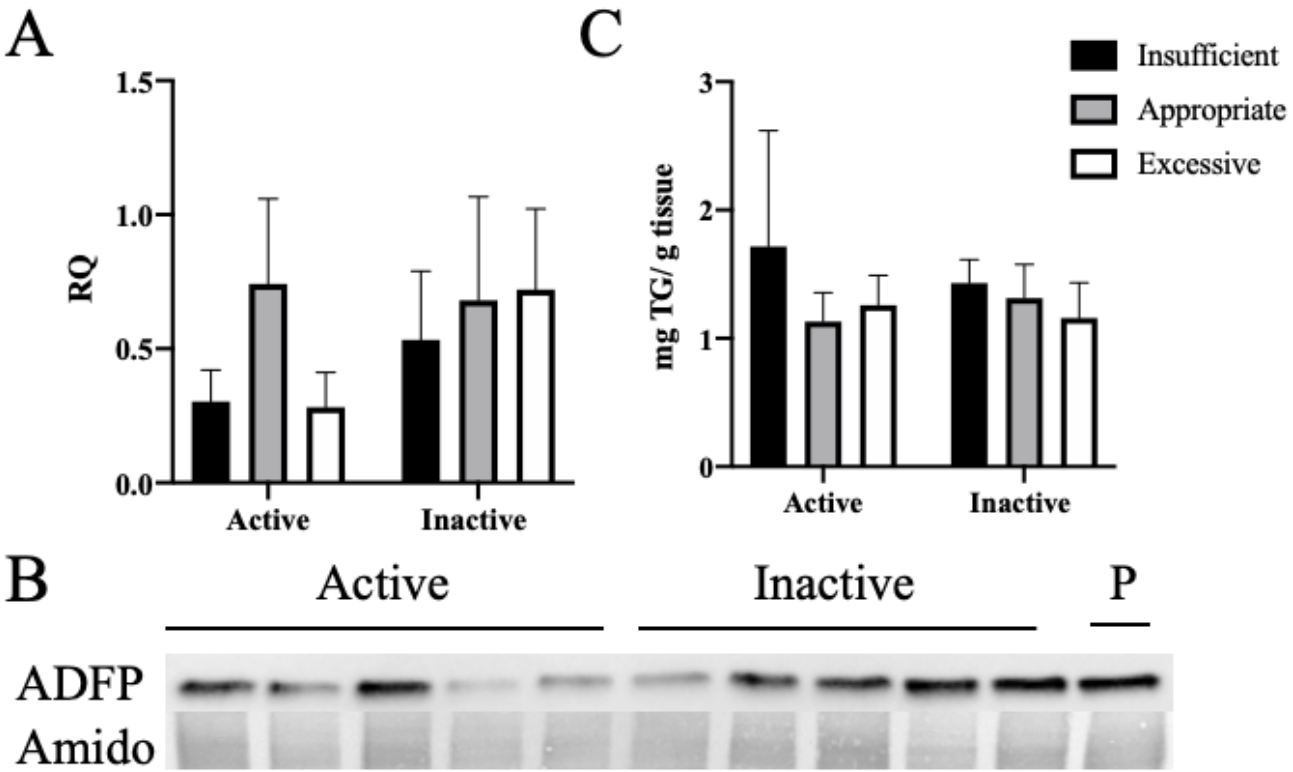

Figure 3. Placental protein expression of ADFP (A-B, n=31) and placental triglyceride content (C, n=28) of active and inactive participants who gained weight insufficiently, appropriately, or excessively. Representative blot is shown. Blots were normalized to total participant pooled (P) sample for inter-blot comparison. Placental triglyceride (mg) was extracted from whole tissue and normalized to tissue weight (g). Values are presented as mean ± standard deviation. Protein densitometry and triglyceride (mg) was analyzed by two-way ANOVA for physical activity status and gestational weight gain. TG: triglyceride; RQ: relative quantity; P: total participant pooled sample.

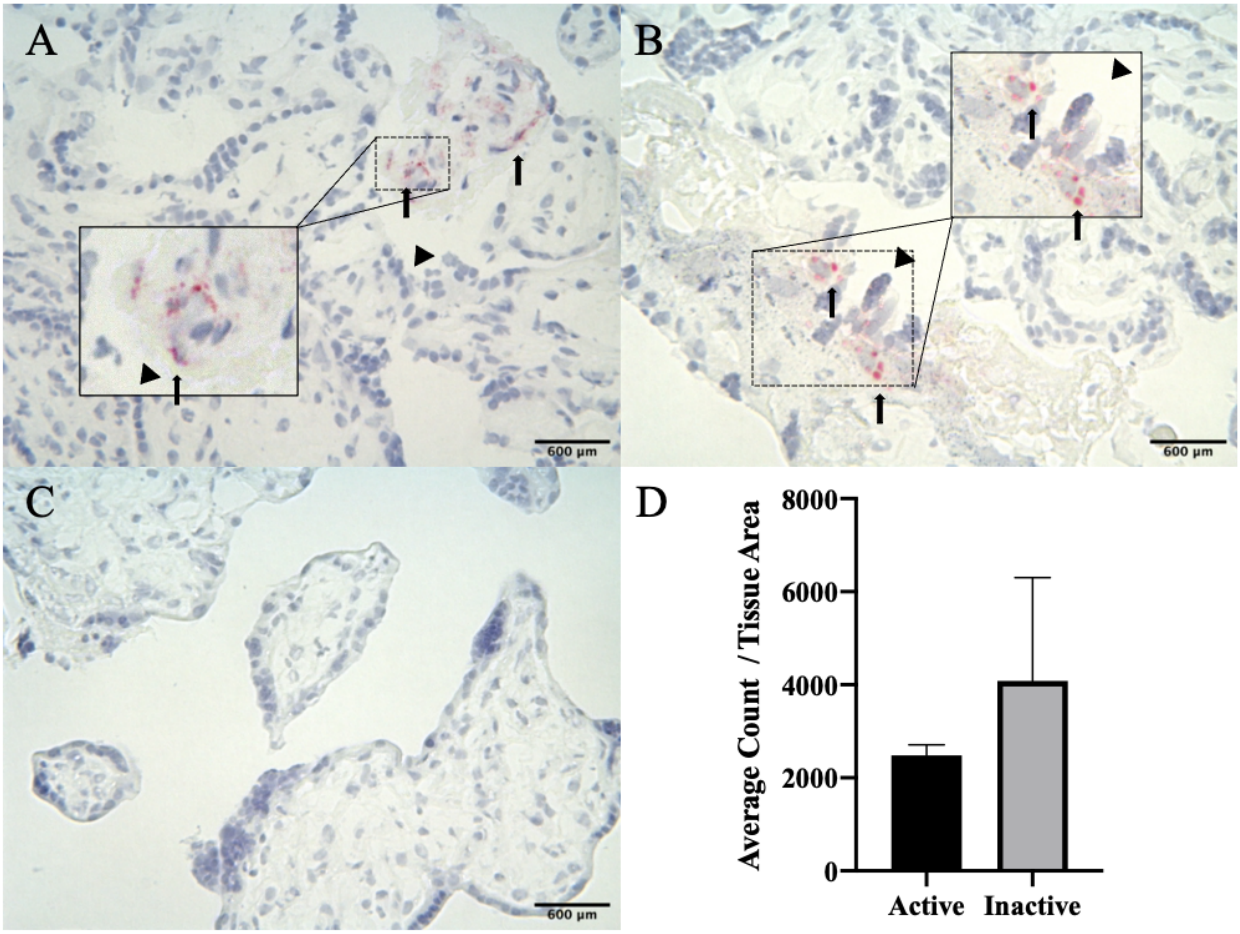

Figure 4. Staining intensity and localization of lipid droplet in term placenta tissue of active (A, n=3) and inactive (B, n=8) participants according to PA status. Arrows indicate lipid droplets (red), found to be clustered near sites of maternal/fetal interaction (arrow heads). Lipid droplet pixel density was normalized to total tissue coverage. Images are representative of two biological replicates per participant. Slides that did not undergo Oil Red O staining served as negative control (C). Values presented as mean ± standard deviation (D).

Discussion

Research investigating placental lipid markers is abundant in cases of pregnancy complications such as gestational diabetes mellitus and mothers living with obesity. While these studies are important to understand the possible complications that could impact fetal development and future health outcomes, research is minimal to non-existent on the effect of healthy behaviours, such as PA, and outcomes like appropriate GWG on placental lipid markers. It is known that in other tissue types, PA and GWG influence lipid transport and storage, yet this is the first study to investigate these outcomes in combination on the placenta. In this study, PA status and GWG categorization were jointly found to be associated with placental FATP1 protein expression. An active PA status coupled with gaining insufficient weight resulted in lower placental FATP1 protein expression compared to inactive women weight gain counterparts. In comparison, those gaining excessive weight were found to have higher placental FAPT1 protein expression if they were physically active.

The placenta is a complex organ that is tightly regulated for the transport of nutrients and waste removal to support fetal growth. As the placenta is non-innervated, communication between mother and fetus (by the placenta) cannot occur through neurotransmitters and must therefore take place through maternal blood. Hormones and nutrients transported by maternal blood may act as conduits of communication, where the placenta metabolises various biological molecules to recirculate for either maternal or fetal use [20]. Previous research from our lab found that the protein expression of placental FATP4 was higher in the placentas of physically active women compared to inactive [21]. FATP4 is a plasma membrane protein also found in the endoplasmic reticulum, serving as an activator of long-chain polyunsaturated fatty acids (LCPUFA) into acyl-CoA for cellular metabolic uses [22,23]. While an increase in markers of metabolic function is generally

considered a positive outcome, it does not depict a full view of how fatty acids are transported to the placenta, nor does it provide insight regarding implications of storage for later use. FATP1 acts as a plasma membrane transporter of LCPUFA, forming a complex with very-long-chain acyl Co-A synthases at the membrane to facilitate transportation and activation of fatty acids for metabolic purposes [24]. In the current study, FATP1 protein expression was found to be differentially expressed based on both PA status, and GWG categorization. Comparable findings were published in skeletal muscle tissue of young men after an 8-week aerobic training program, where FATP1 protein expression was found to be decreased [25]. There are no studies in the published literature that have investigated placental FATP1 protein expression in terms of PA, though women living with obesity were found to have lower placental FATP1 protein expression than those with a normal pre-pregnancy BMI [26]. As FATP1 is an insulin-sensitive receptor, this may explain the relationship with both variables as insulin has been shown to be regulated by both PA [27] and weight gain [28]. While no statistically significant differences were found, FABP4 gene expression was 2.194-fold higher in women who gained weight excessively compared to appropriately. As FABP4 is an intercellular fatty acid transport, the placenta may be increasing FABP4 gene expression in an effort to potentially shuttle fatty acids for metabolic use or storage. Our lab's FATP1/4 protein and FABP4 gene expression data suggest that PA and weight gain may interact during pregnancy to regulate fatty acid transport across the placental membrane by FATP1, while potentially enhancing the conversion of fatty acids into acyl-CoA for mitochondrial use through intracellular trafficking of FATP4 [28].

When investigating uncomplicated pregnancies, placental triglyceride storage was found to be five-times greater near the chorionic plate than the anchoring villi[29]. These findings suggest that triglycerides are stored in closer proximity to the umbilical cord than elsewhere in the placenta,

thereby providing readily available access to the fetus if signaled. In skeletal muscle tissue, there is a greater capacity to store triglycerides than glycogen, ensuring that triglycerides are accessible in times of high energy demand when glycogen is depleted [30]. Our study showed no differences in protein markers of lipid droplet storage between those classified as physically active and inactive, or GWG categories. While no differences were found, lipid droplet clusters were more likely to be found near capillaries and sites of maternal-fetal interaction, believed to be for rapid transport or metabolization of nutrients when signaled by the fetus.

As gestation progresses, it is more common for women to decrease their PA habits [11], while simultaneously gaining weight to meet the demands of the growing fetus. While the whole of gestation is critical for fetal and placental development, the placenta reaches maturity at approximately 34 weeks gestation. As such, it is essential for women to follow through with healthy behaviours throughout pregnancy, as PA and GWG patterns may create predispositions that are carried forward to term. When considering neonatal anthropometrics, no associations were found between W/A percentile or W/L z-score of the newborns and markers of placental lipid transport and storage (data not shown). However, the W/A percentile of newborns of women who were physically active during pregnancy was significantly lower, albeit within the appropriate growth range, than their inactive counterparts. This trend is known to carry through to the 1- and 5- year checkups for children of active mothers, who were leaner than inactive mothers, yet they nonetheless fell within a normal population growth range [31,32]. While this study found that placental lipid transport and storage may not be a major factor influencing fetal development, other physiological processes associated with maternal PA may be responsible for these findings.

The present study is the first of its kind to examine the relationship between maternal PA and GWG and multiple markers of fatty acid transport and storage in term placenta of

uncomplicated pregnant individuals. The strengths of this study include validated objective measurements of PA by accelerometer and weight taken at multiple time points throughout pregnancy, in addition to standardized placenta tissue collection and use of a pooled sample from multiple placental locations. A limitation of this study includes the lack of functional data (i.e., fatty acid uptake), the relatively homogenous sample of women, and the small sample size for placental lipid droplet staining. Overall, the findings of this study suggests that PA and GWG in combination may be involved in regulating placental fatty acid transport, and future research should investigate the uptake of fatty acids from the placenta to the fetus to elucidate any physiological effect on the growing fetus.

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CHAPTER 5: DISCUSSION AND GENERAL CONCLUSIONS OF THE THESIS

The key findings of this thesis include a novel approach to investigating placental and fetal health markers in an uncomplicated population. Pl-E is a widely used clinical marker of placental

health that was not affected by maternal PA in either mid or late gestation, although BW (an accepted measurement of placental health) was significantly increased in women who gained weight excessively compared to insufficiently or appropriately. It is widely known in the literature that BW is highly and positively correlated with GWG; however, no other markers of Pl-E (BW:PW ratio, residual BW) were found to be associated with PA or GWG.

The lipid and glucose profile of maternal and venous UC serum in relation to both PA and GWG were investigated in Chapter 3. No associations were found between PA and GWG in mid or late gestation on maternal outcomes. Yet, appropriate weight gain in mid gestation was significantly associated with lower venous UC glucose levels compared to gaining weight excessively or insufficiently. Finally, lower total cholesterol levels were detected in venous UC serum from women who were active throughout pregnancy, or whose activity dropped in late gestation compared to those who were inactive throughout pregnancy.

Chapter 4 of this thesis demonstrated the relationship between PA and GWG on markers of lipid transport and storage in term placenta. Women who were physically active and gained weight below the recommended weight range had lower placental protein expression of FATP1 compared to those that were inactive. Women who were physically active but gained excessive weight had higher levels of FATP1 protein expression than those who were inactive and gained weight excessively.

Linking placental health, serum lipid and glucose, and markers of placental lipid transport and storage

One's physical activity behaviours, and magnitude of GWG during pregnancy are variables that may cause physiological changes to the mother, the placenta, and developing fetus. The

placenta acts as a conduit relaying important messages and transporting essential building blocks from mother to fetus. We hypothesized that PA and guideline concordant GWG could impact gross markers of placental health and improve Pl-E. As reported in Chapter 2, we found that women with excessive GWG birthed significantly larger infants than women with insufficient or appropriate weight gain; however, no relationship was found between gross measurements of placental health and PA. Therefore, Pl-E might not be indicative of the benefits that PA may have on the placenta. Previous research has shown that women who performed aerobic exercises during pregnancy displayed differences in placental morphology, including increased parenchymal surface area and vasculature [24], while simultaneously having a negative relationship with absolute placental weight [72]. The relationship between PA and placental structure could indicate that PA influences internal placenta physiology (i.e., vasculature), therefore potentially increasing the flow of nutrients and metabolites required for an optimal *in utero* environment.

Given our data on gross measurements of placental health (BW:PW ratio, BW, residual BW), we next examined whether maternal serum lipid and glucose that potentially will be shuttled to the placenta and venous UC serum (surrogate marker of fetal serum), are associated with maternal PA and GWG. This study was performed as other maternal pregnancy outcomes (i.e., GDM, obesity, hypertension) have been shown to influence metabolites (more specifically lipids) found in maternal and UC serum [73–75]. We hypothesized that PA and GWG would behave similarly and be significantly associated with serum lipid and glucose, providing further understanding of the relationship between pregnancy behaviour, health outcomes, and placental/fetal development. Glucose levels were lower in UC serum of women who gained weight appropriately in mid gestation compared to women who gained weight insufficiently and excessively, which may be the result of several different outcomes; i) decreased transport of glucose to the placenta [76], or ii)

the fetus is not signalling to the placenta to transport glucose [77]. The second outcome regarding venous UC serum is that women who were physically active in pregnancy, especially mid pregnancy, had lower UC total cholesterol than their inactive counterparts. While UC serum total cholesterol were not examined to determine their contents, cholesterol molecules are known to be rich in triglycerides and LCPUFA [78]. As fetal nutrient lipid metabolism transitions to a state of greater *de-novo* synthesis closer to delivery [79,80], newborns of physically active mothers could possibly have more highly optimized nutrient production than those of inactive mothers, requiring less cholesterol to be transported by the UC. In cases of intrauterine growth restriction and small-for-gestational- age (SGA), UC serum cholesterol is significantly lower than in non-complicated pregnancies [81], believed to be due to a downregulation in hepatic activity [82]. As only three of the 40 participants in this study birthed SGA newborns (two from women considered physically inactive throughout pregnancy), we believe that the reduced UC serum total cholesterol is unlikely to be due to downregulated hepatic activity. Rather, it is possible that newborns of women who were physically active during pregnancy have upregulated hepatic or cholesterol synthesis activity. In brief, the synthesis of cholesterol during development is mainly via two pathways: the Kandutsch-Russel and Bloch pathways. The mevalonate pathway produces lanosterol as a final product, which enters the enzyme-mediated Kandutsch-Russel cholesterol producing pathway [81]. Another possible cholesterol-producing pathway highly activated in the fetal brain is the Bloch pathway, which begins with desmosterol [83]. Both pathways have the possibility of influencing fetal cholesterol production, and by analyzing the lanosterol or desmosterol to total cholesterol ratios, it is possible to determine whether maternal PA is associated with upregulation of either cholesterol-producing pathway. The investigation of fetal or newborn human cholesterol synthesis

in association with maternal PA is virtually non-existent in the current literature and would represent a novel area of research.

To further investigate the work highlighted above, the final study of this thesis was to investigate biological markers of lipid transport and storage in the placenta. Placental FATP1 protein expression was found to be significantly associated with both PA and GWG, unlike FATP4 placental protein expression, which was found to be higher in active women than inactive (although the interaction between PA and GWG was not reported) [53]. The two transport proteins play very different cellular roles; FATP1 transports LCPUFA at the plasma membrane into the placenta, while FATP4 traffics the lipids intracellularly, to be re-esterified into lipid droplets or oxidized by the mitochondria [84,85]. Mitochondria is one of the major sources for fatty acid oxidation, and previous literature has found a significant relationship between women living with obesity, placental lipids, and mitochondria function; women living with obesity were found to have significantly higher amounts of placental mtDNA copy number [61], abnormal mitochondrial morphology [61], and increase in fatty acid esterification/ decrease in fatty acid oxidation [61]. The placentas of active women were found to have a significantly lower mtDNA copy number compared to inactive women (Everest *et al*., unpublished results, appendix Figure 1C). This unpublished finding together with the association between PA, GWG, and FATP1/4 protein expression, may suggest a more efficient mitochondrial function.

Chapter 3 of this thesis found that venous UC serum of women whose GWG was appropriate had lower glucose concentration than their excessive and insufficient weight gain counterparts, which may influence fatty acid transport and storage as glucose and fatty acids have a complex, intertwined metabolic relationship [86]. Glucose is the primary cellular energy source for most cell types in humans, and as such, an increase in glucose oxidation inhibits fatty acid

oxidation [86]. In the placenta, glucose and fatty acids are connected, as demonstrated by data from women with GDM, whose fatty acid oxidation was lower and glucose uptake higher than in uncomplicated pregnancies [87]. Markers of glucose metabolism were not tested in this study, although previous research from our lab did not find any differences between the expression of placental glucose transporter (GLUT) 1 protein expression between physically active and inactive women (PA was assessed in mid gestation only), nor was the relationship between GWG and placental GLUT1 protein expression investigated [53]. Similar to FAPT1, GLUT4 is an insulin-dependent transport protein and could be comparably regulated, potentially explaining the differences in UC glucose levels [88,89]. In conclusion, PA and GWG were associated with multiple markers of lipid metabolism throughout pregnancy, such as placental FATP1 protein expression, venous UC serum cholesterol, and venous UC glucose levels. We, therefore, believe that PA and GWG in combination may be affecting placental glucose metabolism and fatty acid oxidation, potentially through increased mitochondrial function.

Future directions

The findings of this thesis support the current literature that PA and appropriate GWG during pregnancy could result in beneficial pregnancy outcomes through optimized placental function. Furthermore, we are left with two major unanswered questions;

i) is maternal PA during pregnancy associated with upregulation of fetal cholesterol synthesis?, and

ii) does PA during pregnancy coupled with appropriate GWG have a significant relationship with mitochondrial function via glucose and fatty acid metabolism?

Maternal serum lipid and glucose levels are generally higher than those found in the UC, suggesting the placenta acts as a gatekeeper restricting transport. In combination with the relationship between PA and UC total cholesterol, the placenta might be limiting the uptake of

cholesterol through its various cholesterol transport proteins. To further investigate whether this relationship exists, analysis of placental cholesterol transport proteins would be required. Major cholesterol transport proteins worth examining include LDL receptors (LDLR), LDLR-related proteins, ABCA1, ABCG1, scavenger receptors A, and HDL binding scavenger receptors B1 [90]. In addition, work in progress in our lab has illustrated that regardless of PA or GWG status, there are significant associations between maternal cholesterol and newborn anthropometrical measurements (Everest *et al*., *in prep*). For example, mid gestation maternal serum total cholesterol and remnant cholesterol are associated with newborn weight-for-age (W/A) percentile, and maternal HDL-c and weight-for-length (W/L) z-score (Appendix Table 3C). Likewise, in late gestation, maternal serum total cholesterol and LDL-c were associated with newborn W/L z-score (Appendix Table 4C). These sets of results suggest that cholesterol has a significant relationship with fetal development, and the placenta acting as a conduit moderating maternal cholesterol and impacting fetal growth. To further understand this relationship, placental cholesterol transporters, placental cholesterol content, and the lanosterol/ desmosterol to total cholesterol ratio (as previously discussed) of UC serum cholesterol molecules should be investigated. Lastly, the differences found in placental FATP1 protein expression and venous UC glucose levels, combined with previous research on FATP4 and placental mtDNA copy number, suggest that LCPUFA placental uptake, fatty acid oxidation, and subsequent glucose metabolism may have a significant relationship with PA and GWG during pregnancy. The differences found in FATP1/4 protein expression might potentially hint towards a mechanistic explanation. Still, to answer these questions, an examination of fatty acid uptake would need to be conducted to determine if placental tissue of physically active women, or those who gain weight appropriately, have lower uptake of LCPUFA than their counterparts. In addition, glucose uptake experiments in placental explants,

and analysis of GLUT4 expression in the placenta would be required to investigate the differences seen in UC serum glucose levels. Finally, high-resolution respirometry using fresh placental explants would be performed to investigate whether the differences identified in placental FATP4 protein expression and mtDNA copy number lower are due to higher rates of mitochondrial function, as FATP4 shuttles fatty acid towards the mitochondria.

The findings from this thesis contribute to the growing body of literature on the relationship between PA and GWG and pregnancy outcomes in uncomplicated pregnancies. Recognizing that behaviours and outcomes during pregnancy, even in a non-complicated pregnant population, have a significant association with placental and fetal health will help further support clinical pregnancy guidelines. This thesis can help inform future studies, either observation or interventional, on targets (placental cholesterol transport, placental mitochondria, fatty acid oxidation) or covariates (maternal cholesterol, placental glucose transport) that could influence their findings. By doing so, this thesis would serve to guide researchers on the effects and the interplay between PA and GWG on lipid markers throughout pregnancy.

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APPENDICES
APPENDIX A: TRAINING CERTIFICATION

A.1. UNIVERSITY OF OTTAWA BIOSAFETY TRAINING


University of Ottawa

This certifies that
Catherine Starr Everest
has completed the following workshop
Biosafety Training - For Users

Office of Risk Management
Friday September 28, 2018

Associate Vice-President, Human Resources

uOttawa

A.2. UNIVERSITY OF OTTAWA LAB SAFETY TRAINING


University of Ottawa

This certifies that
Catherine Everest
has completed the following workshop
Lab Safety Training

Office of Risk Management
Wednesday September 5, 2018

Associate Vice-President, Human Resources

uOttawa

A.3. UNIVERSITY OF OTTAWA RADIATION SAFETY TRAINING

APPENDIX B: SCHOLARLY ACHIEVEMENTS

B.1. PUBLICATIONS.

Goudreau A.D., **Everest C.**, Tzaneva V., Tanara L., Adamo K.B. Does physical activity the influence polarization state of placenta-resident macrophages? (*To be submitted Jan 2022*)

Everest C., da Silva D.F., Puranda J.L., Souza S.C.S., Goudreau A.D., Nagpal T.S., Edwards C.M., Gupta R., Adamo K.B. Physical activity and weight gain throughout pregnancy are associated with umibilcal cord markers. *Journal of Obstetrics and Gynaecology Canada*. (*to be submitted Dec 2021*).

Everest C., Puranda J.L., da Silva D.F., Souza S.C.S., Goudreau A.D., Nagpal T.S., Edwards C.M., Tzaneva V., Adamo K.B. Maternal Serum Cholesterol is Associated with Newborn Anthropometrics (*in preparation*).

Goudreau A.D., **Everest C,** Nagpal T.S., Puranda, J.L., Bhattacherjee J, Vasanthan T., Adamo K.B. Elucidating the interaction between maternal physical activity and circulating myokines throughout gestation: A scoping review. *American Journal of Reproductive Immunology. (online ahead of print August 2021).* doi:10.1111/aji.13488

Nagpal T.S, **Everest C**, Goudreau A.D., Mannicks M, Adamo K.B. To HIIT or not to HIIT? The question pregnant women may be searching for online- An exploratory study. *Perspectives In Public Health.* (2021);141(2):81-88. doi:10.1177/1757913920985898.

Everest C, Nagpal T.S, Souza S, F. da Silva D, Gaudet L, Mohammad S, Bhattacharjee J, Adamo K.B. The effect of maternal physical activity and gestational weight gain on placental efficiency. *Medicine & Science in Sports & Exercise.* (2021); 53(4): 756- 762. doi: 10.1249/MSS.0000000000002524.

Nagpal T.S, **Everest C**, Souza S, F. da Silva D, Mohammad S, Bhattacherjee J, Adamo K.B. Does " Sitting " Stand Alone ? A Brief Report Evaluating the Effects of Prenatal Sedentary Time on Maternal and Newborn Anthropometric Outcomes. *Journal of Physical Activity and Health.* 2020;17(9);915-919. doi:10.1123/jpah.2020-0175.

Hutchinson K.A., Vuong N.H., Mohammad S, **Everest C**, Leung M, Bhattacharjee J, Adamo KB. Physical Activity During Pregnancy Is Associated with Increased Placental FATP4 Protein Expression. *Reproductive Sciences*. 2020;27(10);1909-1919. doi:10.1007/s43032-020-00210-w.

B.2. CONFERENCE ABSTRACTS AND PRESENTATIONS.

Society for Reproductive Investigation, Boston, Massachusetts
July 2021, poster presentation

Everest C., Puranda J.L., da Silva D.F., Souza S.C.S., Goudreau A.D., Tzaneva V., Adamo K.B. "Association between Maternal and Cord Serum Lipid Profile Markers with Anthropometrical Newborn Outcomes".

Obesity Canada, online conference
May 2021, virtual presentation

Everest C., da Silva D.F., Goudreau A.D., Puranda J.L., Adamo K.B. "Does maternal Physical Activity Impact Cord Serum Lipid Levels?"

Canadian National Perinatal Meeting Research, Banff, Alberta
March 2020, Oral Presentation

Everest C., Vuong N., Leung M., Rankin J., Mohammad S., Adamo K.B. "Characterization of placental mitochondria in response to chronic maternal exercise".

BPS, BCH, and CHM honors students poster presentation, University of Ottawa, Ontario
April 2019**,** Poster Presentation

Everest, C., Hutchinson, K.A., Vuong, N., Adamo, K.B. "The effects of maternal serum on GLUT1 *in vitro* after an acute sub maximal walking exercise".

Scinapse Undergraduate Science Case Competition: Cannabis, harm or health? Ottawa, Ontario
March 2019

Everest C., Goudreau A., Valko E. Prenatal cannabidiol administration on mice growth and development: A preliminary study for use during human labour. In: Scinapse 2018-2019 Undergraduate Science Case Competition: Cannabis, harm or health? March 2019; Ottawa, ON. Abstract 34.

B.3. COMPETITIONS AND COMMUNITY INVOLVEMENT.

2021 SPARC Virtual Research Competition Mentor, [Finalist]

2019-2021 Let's Talk Science Student Mentor

2019 Scinapse Undergraduate Science Case Competition: Cannabis, harm or health? [Top 20%]

APPENDIX C: SUPPLEMENTARY DATA.

Appendix Table 1C. Effects of PA status and GWG classification at mid and late gestation on maternal and umbilical cord serum lipid and glucose profile (mmol/L).

		Mid Gestation			Late Gestation		
		PA status					
	M. Total Cholesterol	Active	Inactive	Total	Active	Inactive	Total
GWG classification	Insufficient	6.42 ±1.52	6.02 ±1.41	6.22 ± 1.33	7.82 ±1.41)	5.76 ±1.05)	6.38 ±1.47)
	Appropriate	6.04 ±0.41	5.31 ±1.19	5.63 ±0.96	6.86 ±1.43	6.48 ±1.30	6.61 ±1.31
	Excessive	6.17 ±1.03	6.08 ±1.03	6.13 ±1.01	6.88 ±2.94	6.29 ±0.99	6.37 ±1.23
	Total	6.18 ±0.98	5.87 ±1.12		7.15 ±1.59	6.23 ±1.11	
Main effects and interaction	PA status	F = 1.507; p = 0.229; η^2 = 0.045			F = 2.210; p = 0.147; η^2 = 0.065		
	GWG classification	F = 0.426; p = 0.657; η^2 = 0.026			F = 0.442; p = 0.647; η^2 = 0.027		
	Interaction	F = 0.700; p = 0.504; η^2 = 0.042			F = 1.317; p = 0.282; η^2 = 0.076		
		PA status					
	M. HDL-c	Active	Inactive	Total	Active	Inactive	Total
GWG classification	Insufficient	1.93 ±0.37	2.10 ±0.12	2.02 ±0.27	1.71 ±0.60	1.73 ±0.33	1.73 ±0.39
	Appropriate	2.37 ±0.25	2.13 ±0.23	2.24 ±0.25	1.86 ±0.52	1.93 ±0.35	1.91 ±0.40
	Excessive	2.22 ±0.29	2.27 ±0.18	2.25 ±0.25	2.16 ±0.16	1.98 ±0.34	2.00 ±0.33
	Total	2.21 ±0.31	2.21 ±0.19		1.88 ±0.48	1.91 ±0.35	
Main effects and interaction	PA status	F < 0.001; p = 0.994; η^2 < 0.001			F = 0.023; p = 0.882; η^2 = 0.001		
	GWG classification	F = 2.988; p = 0.065; η^2 = 0.157			F = 1.244; p = 0.302; η^2 = 0.072		
	Interaction	F = 1.639; p = 0.210; η^2 = 0.093			F = 0.034; p = 0.967; η^2 = 0.002		
		PA status					
	M. LDL-c	Active	Inactive	Total	Active	Inactive	Total
GWG classification	Insufficient	3.70 ±1.66	3.27 ±1.29	3.48 ±1.35	4.91 ±1.45	3.05 ±0.89	3.61 ±1.34
	Appropriate	3.15 ±0.42	2.55 ±1.01	2.82 ±0.82	3.98 ±0.85	3.49 ±0.94	3.65 ±0.91
	Excessive	2.92 ±0.61	3.07 ±0.86	2.99 ±0.72	3.55 ±2.76	3.14 ±0.77	3.20 ±1.04
	Total	3.07 ±0.78	2.96 ±0.94		4.18 ±1.39	3.24 ±0.85	
Main effects and interaction	PA status	F = 1.397; p = 0.246; η^2 = 0.042			F = 3.026; p = 0.092; η^2 = 0.086		
	GWG classification	F = 0.311; p = 0.735; η^2 = 0.019			F = 1.465; p = 0.246; η^2 = 0.084		
	Interaction	F = 1.485; p = 0.242; η^2 = 0.085			F = 1.725; p = 0.194; η^2 = 0.097		

		PA status					
	M. Remnant Cholesterol	Active	Inactive	Total	Active	Inactive	Total
GWG classification	Insufficient	0.80 ±0.27	0.65 ±0.34	0.72 ±0.28	1.20 ±0.56	0.98 ±0.41	1.04 ±0.44
	Appropriate	0.52 ±0.24	0.64 ±0.13	0.59 ±0.19	1.01 ±0.32	1.06 ±0.37	1.05 ±0.34
	Excessive	0.75 ±0.17	0.73 ±0.20	0.74 ±0.18	1.17 ±0.03	0.98 ±0.26	1.01 ±0.25
	Total	0.71 ±0.21	0.69 ±0.20		1.10 ±0.35	1.01 ±0.33	
Main effects and interaction	PA status	F = 0.166; p = 0.687; η^2 = 0.005			F = 1.438; p = 0.239; η^2 = 0.043		
	GWG classification	F = 2.037; p = 0.147; η^2 = 0.113			F = 0.324; p = 0.725; η^2 = 0.020		
	Interaction	F = 0.726; p = 0.491; η^2 = 0.043			F = 0,492; p = 0.616; η^2 = 0.030		
		PA status					
	M. Triglyceride	Active	Inactive	Total	Active	Inactive	Total
GWG classification	Insufficient	1.75 ±0.57	1.42 ±0.74	1.58 ±0.62	1.91 ±0.92	2.14 ±0.90	2.07 ±0.86
	Appropriate	1.18 ±0.47	1.36 ±0.28	1.28 ±0.36	2.24 ±0.63	2.32 ±0.81	2.29 ±0.73
	Excessive	1.59 ±0.40	1.59 ±0.44	1.59 ±0.41	2.57 ±0.06	2.18 ±0.52	2.23 ±0.50
	Total	1.54 ±0.45	1.50 ±0.44		2.21 ±0.65	2.22 ±0.70	
Main effects and interaction	PA status	F = 0.269; p = 0.608; η^2 = 0.008			F = 0.212; p = 0.648; η^2 = 0.007		
	GWG classification	F = 1.794; p = 0.183; η^2 = 0.101			F = 0.877; p = 0.426; η^2 = 0.052		
	Interaction	F = 0.591; p = 0.560; η^2 = 0.036			F = 0.454; p = 0.639; η^2 = 0.028		
		PA status					
	M. Glucose	Active	Inactive	Total	Active	Inactive	Total
GWG classification	Insufficient	4.32 ±0.50	4.33 ±0.20	4.33 ±0.34	3.80 ±0.26	3.90 ±0.32	3.87 ±0.29
	Appropriate	4.37 ±0.58	4.51 ±0.34	4.45 ±0.43	4.06 ±0.25	4.02 ±0.57	4.04 ±0.48
	Excessive	4.49 ±0.50	4.66 ±0.50	4.57 ±0.50	3.92 ±0.08	4.10 ±0.46	4.08 ±0.43
	Total	4.44 ±0.49	4.57 ±0.43		3.95 ±0.24	4.03 ±0.47	
Main effects and interaction	PA status	F = 0.632; p = 0.432; η^2 = 0.019			F = 0.525; p = 0.474; η^2 = 0.016		
	GWG classification	F = 0.488; p = 0.619; η^2 = 0.030			F = 0.550; p = 0.582; η^2 = 0.033		
	Interaction	F = 0.119; p = 0.888; η^2 = 0.007			F = 0.214; p = 0.809; η^2 = 0.013		
		PA status					
	CB. Total Cholesterol	Active	Inactive	Total	Active	Inactive	Total
GWG classification	Insufficient	0.62 ±0.09	1.30 ±0.17	0.96 ±0.39	0.74 ±0.22	1.21 ±0.30	1.07 ±0.35
	Appropriate	0.76 ±0.23	1.33 ±0.15	1.08 ±0.35	0.72 ±0.25	0.93 ±0.43	0.86 ±0.38
	Excessive	0.65 ±0.18	1.28 ±0.45	0.93 ±0.45	0.74 ±0.01	1.05 ±0.51	1.01 ±0.49

	Total	**0.67 ±0.18**	**1.30 ±0.35***		**0.73 ±0.20**	**1.05 ±0.44***	
Main effects and interaction	PA status	$F = 33.875$; $p < 0.001$; $\eta^2 = 0.514$			$F = 5.462$; $p = 0.026$; $\eta^2 = 0.146$		
	GWG classification	$F = 0.384$; $p = 0.684$; $\eta^2 = 0.023$			$F = 0.427$; $p = 0.656$; $\eta^2 = 0.026$		
	Interaction	$F = 0.153$; $p = 0.859$; $\eta^2 = 0.009$			$F = 0.898$; $p = 0.417$; $\eta^2 = 0.053$		
				PA status			
	CB. Triglyceride	Active	Inactive	Total	Active	Inactive	Total
GWG classification	Insufficient	0.25 ±0.04	0.43 ±0.21	0.34 ±0.16	0.30 ±0.07	0.49 ±0.24	0.43 ±0.22
	Appropriate	0.35 ±0.04	0.47 ±0.28	0.42 ±0.21	0.40 ±0.07	0.46 ±0.18	0.44 ±0.15
	Excessive	0.54 ±0.24	0.56 ±0.37	0.55 ±0.30	0.74 ±0.45	0.55 ±0.37	0.57 ±0.37
	Total	0.46 ±0.23	0.52 ±0.32		0.44 ±0.23	0.50 ±0.28	
Main effects and interaction	PA status	$F = 1.300$; $p = 0.263$; $\eta^2 = 0.039$			$F = 0.367$; $p = 0.549$; $\eta^2 = 0.011$		
	GWG classification	$F = 1.649$; $p = 0.208$; $\eta^2 = 0.093$			$F = 0.427$; $p = 0.656$; $\eta^2 = 0.026$		
	Interaction	$F = 0.338$; $p = 0.716$; $\eta^2 = 0.021$			$F = 0.470$; $p = 0.629$; $\eta^2 = 0.029$		
				PA status			
	CB. HDL-c	Active	Inactive	Total	Active	Inactive	Total
GWG classification	Insufficient	0.60 ±0.15	0.77 ±0.13	0.69 ±0.16	0.62 ±0.16	0.61 ±0.18	0.61 ±0.17
	Appropriate	0.67 ±0.16	0.55 ±0.25	0.60 ±0.21	0.61 ±0.22	0.63 ±0.16	0.62 ±0.17
	Excessive	0.57 ±0.13	0.67 ±0.16	0.61 ±0.15	0.64 ±0.14	0.62 ±0.17	0.62 ±0.17
	Total	0.59 ±0.14	0.65 ±0.19		0.62 ±0.17	0.62 ±0.16	
Main effects and interaction	PA status	$F = 0.655$; $p = 0.424$; $\eta^2 = 0.020$			$F = 0.033$; $p = 0.857$; $\eta^2 = 0.001$		
	GWG classification	$F = 0.378$; $p = 0.688$; $\eta^2 = 0.023$			$F = 0.087$; $p = 0.917$; $\eta^2 = 0.005$		
	Interaction	$F = 1.891$; $p = 0.167$; $\eta^2 = 0.106$			$F = 0.099$; $p = 0.906$; $\eta^2 = 0.006$		
				PA status			
	CB. Glucose	Active	Inactive	Total	Active	Inactive	Total
GWG classification	Insufficient	5.70 ±1.21	4.42 ±0.04	**5.06 ±1.03**‡	5.31 ±1.42	4.54 ±0.63	4.77 ±0.92
	Appropriate	3.78 ±0.46	3.96 ±0.85	**3.88 ±0.67**	4.50 ±0.55	4.33 ±1.03	4.39 ±0.88
	Excessive	5.02 ±0.92	4.80 ±1.35	**4.92 ±1.11**‡	5.92 ±0.16	4.84 ±1.39	4.99 ±1.34
	Total	4.88 ±1.04	4.52 ±1.14		5.03 ±0.97	4.60 ±1.12	
Main effects and interaction	PA status	$F = 0.782$; $p = 0.383$; $\eta^2 = 0.024$			$F = 2.456$; $p = 0.127$; $\eta^2 = 0.071$		
	GWG classification	$F = 3.460$; $p = 0.044$; $\eta^2 = 0.178$			$F = 1.760$; $p = 0.188$; $\eta^2 = 0.099$		
	Interaction	$F = 0.739$; $p = 0.486$; $\eta^2 = 0.044$			$F = 0.627$; $p = 0.541$; $\eta^2 = 0.038$		

Data presented as mean ±SD. PA = physical activity; GWG = gestational weight gain; M. = maternal; CB. = cord blood. Total = total grouping (i.e. active PA status, insufficient GWG). *Significantly different compared to the active group. ‡Significantly different compared to the appropriate group.

Appendix Table 2C. Effects of gestational PA pattern and GWG classification on the delta change of late and mid gestation maternal serum lipid and glucose concentrations, in addition to umbilical cord serum lipid and glucose profile (mmol/L).

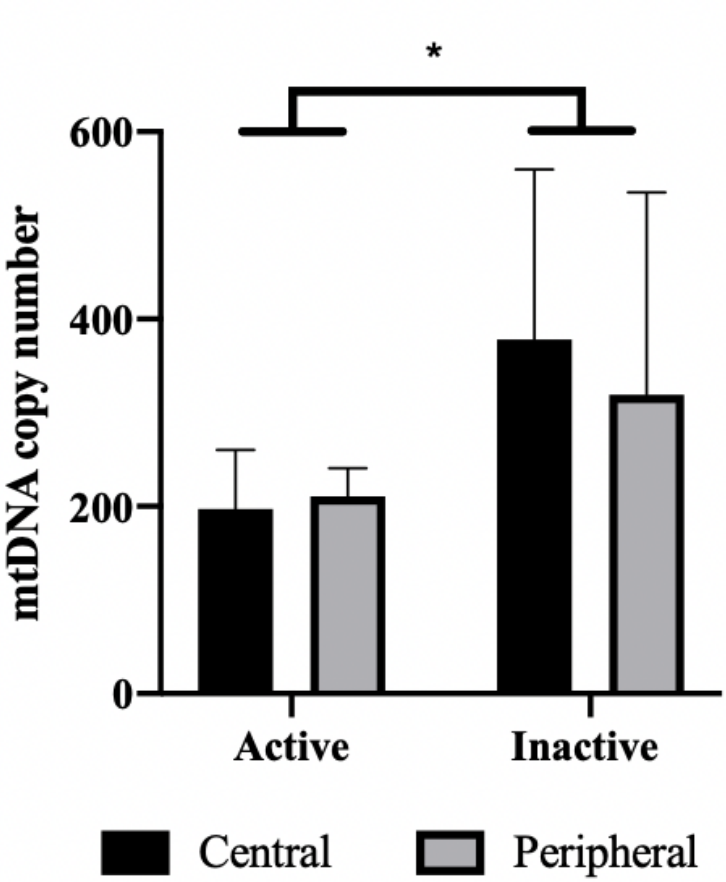
		PA status			
	M. Delta Change Total Cholesterol	Active throughout gestation	Activity dropped over gestation	Inactive throughout gestation	Total
GWG classification	Insufficient	24.34 ±17.24	3.88 (-)	7.48 ±11.14	12.18 ±14.38
	Appropriate	6.04 ±18.34	10.62 ±13.44	-1.45 ±15.09	5.07 ±15.47
	Excessive	1.76 ±6.77	1.43 ±8.15	6.71 ±9.98	4.29 ±8.90
	Total	10.68 ±17.67	5.83 ±10.97	4.81 ±11.77	
Main effects and interaction	PA status		$F = 0.618$; $p = 0.546$; $\eta^2 = 0.041$		
	GWG status		$F = 1.561$; $p = 0.227$; $\eta^2 = 0.097$		
	Interaction		$F = 1.510$; $p = 0.225$; $\eta^2 = 0.172$		
		PA status			
	M. Delta Change HDL-c	Active throughout gestation	Activity dropped over gestation	Inactive throughout gestation	Total
GWG classification	Insufficient	-18.78 ±30.01	-19.67 (-)	-14.06 ±13.48	-16.04 ±17.54
	Appropriate	-16.93 ±9.08	-14.79 ±15.20	-15.91 ±20.04	-15.87 ±14.32
	Excessive	-11.74 ±4.61	-14.18 ±17.68	-13.38 ±6.74	-13.43 ±10.68
	Total	-16.45 ±15.68	-14.96 ±14.83	-14.26 ±12.59	
Main effects and interaction	PA status		$F = 0.036$; $p = 0.964$; $\eta^2 = 0.002$		
	GWG status		$F = 0.050$; $p = 0.951$; $\eta^2 = 0.003$		
	Interaction		$F = 0.077$; $p = 0.989$ $\eta^2 = 0.011$		
		PA status			
	M. Delta Change LDL-c	Active throughout gestation	Activity dropped over gestation	Inactive throughout gestation	Total
GWG classification	Insufficient	45.14 ±37.72	-1.37 (-)	21.99 ±29.39	26.60 ±31.81
	Appropriate	23.59 ±29.20	21.24 ±24.06	9.06 ±13.66	17.96 ±22.49

	Excessive	-2.60 ±27.37	0.55 ±11.98	17.19 ±21.81	9.00 ±20.38
	Total	24.82 ±32.92	9.78 ±20.24	16.57 ±22.18	
Main effects and interaction	PA status		F = 0.335; p = 0.718; η^2 = 0.023		
	GWG status		F = 1.623; p = 0.215; η^2 = 0.101		
	Interaction		F =1.415; p = 0.254; η^2 = 0.163		
			PA status		
	M. Delta Change Remnant Cholesterol	Active throughout gestation	Activity dropped over gestation	Inactive throughout gestation	Total
	Insufficient	171.27 ±244.56	69.47 (-)	40.49 ±31.66	82.62 ±132.93
GWG classification	Appropriate	41.18 ±28.94	57.70 ±23.10	33.97 ±30.48	44.28 ±27.62
	Excessive	64.78 ±13.38	44.93 ±17.71	46.03 ±37.25	48.16 ±29.02
	Total	84.93 ±131.61	52.97 ±20.24	41.11 ±32.40	
Main effects and interaction	PA status		F = 1.398; p = 0.263; η^2 = 0.088		
	GWG status		F = 1.147; p = 0.332; η^2 = 0.073		
	Interaction		F = 1.051; p = 0.398; η^2 = 0.127		
			PA status		
	M. Delta Change Triglyceride	Active throughout gestation	Activity dropped over gestation	Inactive throughout gestation	Total
	Insufficient	45.29 ±32.86	70.05 (-)	40.72 ±32.23	45.33 ±29.99
GWG classification	Appropriate	51.81 ±30.78	57.76 ±21.74	30.93 ±28.43	46.83 ±27.90
	Excessive	60.23 ±19.18	44.36 ±17.82	46.07 ±36.72	47.39 ±28.62
	Total	51.84 ±26.98	52.79 ±19.84	40.40 ±32.12	
Main effects and interaction	PA status		F = 1.593; p = 0.221; η^2 = 0.099		
	GWG status		F = 0.166; p = 0.848; η^2 = 0.011		
	Interaction		F = 0.564; p = 0.690; η^2 = 0.072		
			PA status		
	M. Delta Change Glucose	Active throughout gestation	Activity dropped over gestation	Inactive throughout gestation	Total
	Insufficient	-2.93 ±8.96	-17.67 (-)	-10.97 ±3.19	-9.22 ±6.83
GWG classification	Appropriate	-13.38 ±9.94	-7.05 ±7.96	-18.11 ±14.71	-12.84 ±11.41
	Excessive	-11.08 ±0.83	-12.17 ±7.98	-6.47 ±8.43	-8.98 ±7.85
	Total	-9.78 ±9.22	-10.34 ±7.96	-10.95 ±10.09	

Main effects and interaction	PA status		F = 0.239; p = 0.789 η^2 = 0.016		
	GWG status		F = 0.192; p = 0.827 η^2 = 0.013		
	Interaction		F = 1.766; p = 0.163; η^2 = 0.196		
		PA status			
	CB. Total Cholesterol	Active throughout gestation	Activity dropped over gestation	Inactive throughout gestation	Total
GWG classification	Insufficient	0.74 ±0.23	0.63 (-)	1.31 ±0.17	1.07 ±0.35
	Appropriate	0.72 ±0.25	0.54 ±0.15	1.31 ±0.11	0.86 ±0.38
	Excessive	0.74 ±0.01	0.68 ±0.12	1.29 ±0.53	1.01 ±0.49
	Total	**0.73 ±0.20***	**0.61 ±0.14***	1.30 ±0.35	
Main effects and interaction	PA status		F = 19.232; p < 0.001; η^2 = 0.570		
	GWG status		F = 0.296; p = 0.746; η^2 = 0.020		
	Interaction		F = 0.469; p = 0.758; η^2 = 0.061		
		PA status			
	CB. Triglyceride	Active throughout gestation	Activity dropped over gestation	Inactive throughout gestation	Total
GWG classification	Insufficient	0.30 ±0.07	0.22 (-)	0.53 ±0.23	0.43 ±0.22
	Appropriate	0.40 ±0.07	0.50 ±0.20	0.41 ±0.17	0.44 ±0.15
	Excessive	0.74 ±0.45	0.51 ±0.28	0.57 ±0.44	0.57 ±0.37
	Total	0.44 ±0.23	0.48 ±0.23	0.52 ±0.32	
Main effects and interaction	PA status		F = 0.280; p = 0.758; η^2 = 0.019		
	GWG status		F = 0.562; p = 0.576; η^2 = 0.037		
	Interaction		F = 0.467; p = 0.760; η^2 = 0.060		
		PA status			
	CB. HDL-c	Active throughout gestation	Activity dropped over gestation	Inactive throughout gestation	Total
GWG classification	Insufficient	0.62 ±0.16	0.62 (-)	0.61 ±0.20	0.61 ±0.17
	Appropriate	0.61 ±0.22	0.57 ±0.09	0.70 ±0.19	0.62 ±0.17
	Excessive	0.64 ±0.14	0.56 ±0.11	0.66 ±0.20	0.62 ±0.17
	Total	0.62 ±0.17	0.57 ±0.09	0.65 ±0.19	
Main effects and interaction	PA status		F = 0.400; p = 0.674; η^2 = 0.027		
	GWG status		F = 0.032; p = 0.969; η^2 = 0.002		

	Interaction		F = 0.190; p = 0.942; η^2 = 0.026		
			PA status		
	CB. Glucose	Active throughout gestation	Activity dropped over gestation	Inactive throughout gestation	Total
GWG classification	Insufficient	5.31 ±1.42	5.60 (-)	4.37 ±0.47	4.77 ±0.92
	Appropriate	4.50 ±0.55	4.67 ±1.37	3.99 ±0.45	4.39 ±0.88
	Excessive	5.92 ±0.16	4.65 ±1.05	4.96 ±1.63	4.99 ±1.34
	Total	5.03 ±0.97	4.75 ±1.13	4.52 ±1.14	
Main effects and interaction	PA status		F = 1.650; p = 0.210; η^2 = 0.102		
	GWG status		F = 1.785; p = 0.186; η^2 = 0.110		
	Interaction		F = 0.712; p = 0.590; η^2 = 0.089		

Data presented as mean ±SD. PA = physical activity; GWG = gestational weight gain; M. = maternal; CB. = cord blood. Total = total grouping (i.e. active throughout gestation, insufficient GWG). *Significantly different compared to the inactive throughout gestation group. Delta change is calculated by: [(late gestation – mid gestation)/ mid gestation] *100.



Appendix Figure 1C (unpublished results): Placental Mitochondrial DNA (mtDNA) copy number of active (n=5) and inactive (n=5) participants. Eight samples of roughly two grams of placenta tissue each were taken from each participant; four samples located near the centre of the placenta (central), four samples located on the outer edge (peripheral). mtDNA copy number was determined using NovaQUANT™ Human Mitochondrial to Nuclear DNA Ratio Kit (Sigma-Aldrich,MO, USA) according to manufactures recommendation. The ΔCt of ND1/BECN1 pair and the ND6/NEB pair were used to calculate copy number using the equation $2^{\Delta Ct}$.

Appendix Table 3C (manuscript *in prep*).
Associations between mid-gestation maternal serum lipid and glucose profile with neonate outcomes (n=40).

	Birthweight (kg)		Body fat (%)		Weight-for-age percentile		Weight-for-length z-score	
	β (95% CI)	p-value	β (95% CI)	p-value	β (95% CI)	p-value	β (95% CI)	p-value
Total cholesterol (mmol/L)								
Model 1	**0.12 (0.01; 0.22)**	**0.036**	-0.01 (-0.89; 0.87)	0.981	**0.29 (0.06; 0.52)**	**0.016**	0.28 (-0.02; 0.58)	0.069
Model 2	**0.11 (0.01; 0.21)**	**0.043**	-0.01 (-0.87; 0.85)	0.977	**0.27 (0.06; 0.47)**	**0.012**	0.28 (-0.02; 0.58)	0.069
Model 3	**0.11 (0.01; 0.21)**	**0.032**	-0.04 (-0.90; 0.83)	0.934	**0.28 (0.07; 0.48)**	**0.011**	0.28 (-0.02; 0.59)	0.068
Triglyceride (mmol/L)								
Model 1	0.23 (-0.04; 0.51)	0.091	0.59 (-1.58; 2.76)	0.584	0.51 (-0.08; 1.11)	0.087	0.02 (-0.76; 0.81)	0.950
Model 2	0.22 (-0.04; 0.47)	0.099	0.48 (-1.65; 2.61)	0.647	0.47 (-0.07; 1.01)	0.086	0.04 (-0.75; 0.82)	0.921
Model 3	0.21 (-0.04; 0.47)	0.099	0.49 (-1.65; 2.64)	0.644	0.47 (-0.08; 1.01)	0.090	0.04 (-0.76; 0.84)	0.925
HDL-c (mmol/L)								
Model 1	**0.48 (0.05; 0.90)**	**0.031**	-0.32 (-3.85; 3.21)	0.856	**1.23 (0.33; 2.13)**	**0.009**	**1.55 (0.41; 2.70)**	**0.009**
Model 2	0.34 (-0.11; 0.80)	0.132	-1.52 (-5.20; 2.16)	0.407	0.91 (-0.02; 1.84)	0.055	**1.96 (0.78; 3.14)**	**0.002**
Model 3	0.34 (-0.12; 0.79)	0.139	-1.48 (-5.19; 2.23)	0.423	0.90 (-0.04; 1.84)	0.060	**1.95 (0.75; 3.15)**	**0.002**
LDL-c (mmol/L)								
Model 1	0.10 (-0.04; 0.25)	0.158	0.21 (-0.92; 1.34)	0.386	0.19 (-0.13; 0.50)	0.237	0.18 (-0.22; 0.59)	0.362
Model 2	0.13 (-0.01; 0.26)	0.064	0.33 (-0.78; 1.44)	0.549	0.24 (-0.04; 0.53)	0.088	0.17 (-0.23; 0.58)	0.395
Model 3	0.13 (-0.01; 0.26)	0.063	0.33 (-0.79; 1.45)	0.551	0.24 (-0.04; 0.53)	0.091	0.17 (-0.24; 0.59)	0.401
Remnant cholesterol (mmol/L)								
Model 1	0.54 (-0.04; 1.12)	0.069	1.30 (-3.35; 5.95)	0.573	**1.29 (0.05; 2.54)**	**0.042**	0.14 (-1.54; 1.83)	0.866
Model 2	0.49 (-0.07; 1.04)	0.082	1.04 (-3.52; 5.60)	0.645	**1.17 (0.04; 2.31)**	**0.044**	0.18 (-1.51; 1.86)	0.833
Model 3	0.49 (-0.06; 1.03)	0.080	1.04 (-3.56; 5.64)	0.648	**1.17 (0.03; 2.32)**	**0.045**	0.18 (-1.53; 1.89)	0.835
Glucose (mmol/L)								
Model 1	0.23 (-0.02; 0.47)	0.073	-0.30 (-2.28; 1.69)	0.764	0.38 (-0.17; 0.93)	0.168	0.04 (-0.68; 0.75)	0.919
Model 2	**0.23 (0.01; 0.46)**	**0.049**	-0.29 (-2.23; 1.65)	0.761	0.39 (-0.10; 0.89)	0.115	0.04 (-0.68; 0.75)	0.919
Model 3	0.23 (-0.01; 0.46)	0.051	-0.28 (-2.23; 1.68)	0.776	0.39 (-0.11; 0.89)	0.122	0.03 (-0.69; 0.76)	0.926

Bold: Significant associations; CI: confidence interval; HDL-c: high density lipoprotein cholesterol; LDL-c: low density lipoprotein cholesterol. All models adjusted by maternal age (years), pre-pregnancy body mass index (kg/m^2), unsaturated fat and sugar intake (g) at mid pregnancy. Model 1: Additionally, adjusted by average mid pregnancy moderate-to-vigorous physical activity (min/day) only; Model 2: Additionally, adjusted by gestational weight gain (kg) at mid pregnancy only; Model 3: Additionally, adjusted by average mid pregnancy moderate-to-vigorous physical activity and gestational weight gain at mid pregnancy.

Appendix Table 4C (manuscript *in prep*).
Associations between late gestation maternal serum lipid and glucose profile with neonate outcomes (n=40).

	Birthweight (kg)		Body fat (%)		Weight-for-age percentile		Weight-for-length z-score	
	β (95% CI)	p-value	β (95% CI)	p-value	β (95% CI)	p-value	β (95% CI)	p-value
Total cholesterol (mmol/L)								
Model 1	0.07 (-0.03; 0.16)	0.162	-0.42 (-1.15; 0.32)	0.256	0.15 (-0.03; 0.34)	0.106	**0.33 (0.09; 0.57)**	**0.008**
Model 2	0.07 (-0.02; 0.15)	0.140	-0.40 (-1.11; 0.31)	0.255	0.14 (-0.04; 0.31)	0.114	**0.33 (0.09; 0.56)**	**0.008**
Model 3	0.07 (-0.02; 0.16)	0.145	-0.42 (-1.14; 0.31)	0.249	0.16 (-0.02; 0.33)	0.080	**0.33 (0.09; 0.57)**	**0.009**
Triglyceride (mmol/L)								
Model 1	0.08 (-0.10; 0.26)	0.353	0.40 (-0.99; 1.78)	0.561	**0.35 (0.01; 0.69)**	**0.045**	0.22 (-0.27; 0.70)	0.368
Model 2	0.06 (-0.12; 0.23)	0.520	0.24 (-1.13; 1.61)	0.723	0.28 (-0.05; 0.61)	0.095	0.22 (-0.28; 0.71)	0.377
Model 3	0.06 (-0.12; 0.23)	0.528	-0.24 (-1.16; 1.63)	0.732	0.29 (-0.04; 0.62)	0.085	0.22 (-0.29; 0.72)	0.388
HDL-c (mmol/L)								
Model 1	0.21 (-0.11; 0.54)	0.185	-0.02 (-2.57; 2.53)	0.988	0.44 (-0.21; 1.09)	0.178	0.81 (-0.04; 1.66)	0.062
Model 2	0.18 (-0.13; 0.49)	0.241	-0.23 (-2.72; 2.25)	0.849	0.38 (-0.23; 0.99)	0.216	0.80 (-0.06; 1.66)	0.067
Model 3	0.18 (-0.13; 0.50)	0.245	-0.22 (-2.75; 2.31)	0.859	0.36 (-0.25; 0.97)	0.243	0.81 (-0.06; 1.68)	0.068
LDL-c (mmol/L)								
Model 1	0.09 (-0.03; 0.20)	0.140	-0.58 (-1.48; 0.33)	0.207	0.14 (-0.10; 0.38)	0.244	**0.35 (0.04; 0.65)**	**0.027**
Model 2	0.10 (-0.01; 0.20)	0.074	-0.47 (-1.35; 0.40)	0.279	0.14 (-0.08; 0.36)	0.193	**0.35 (0.05; 0.65)**	**0.025**
Model 3	0.10 (-0.01; 0.21)	0.074	-0.51 (-1.41; 0.40)	0.261	0.17 (-0.05; 0.39)	0.118	**0.36 (0.04; 0.67)**	**0.027**
Remnant cholesterol (mmol/L)								
Model 1	0.06 (-0.31; 0.43)	0.733	0.14 (-2.74; 3.00)	0.922	0.42 (-0.31; 1.16)	0.249	0.48 (-0.51; 1.48)	0.331
Model 2	0.02 (-0.33; 0.37)	0.907	-0.10 (-2.88; 2.69)	0.945	0.29 (-0.41; 0.98)	0.406	0.48 (-0.52; 1.48)	0.334
Model 3	0.02 (-0.34; 0.38)	0.911	-0.12 (-2.95; 2.72)	0.935	0.32 (-0.37; 1.01)	0.354	0.48 (-0.54; 1.50)	0.348
Glucose (mmol/L)								
Model 1	0.11 (-0.19; 0.42)	0.452	-0.39 (-2.76; 1.98)	0.740	0.07 (-0.55; 0.69)	0.829	0.64 (-0.17; 1.44)	0.118
Model 2	0.10 (-0.19; 0.39)	0.491	-0.47 (-2.76; 1.82)	0.680	0.01 (-0.57; 0.59)	0.977	0.64 (-0.17; 1.44)	0.118
Model 3	0.10 (-0.20; 0.39)	0.499	-0.48 (-2.82; 1.85)	0.677	0.03 (-0.55; 0.61)	0.924	0.63 (-0.19; 1.46)	0.126

Bold: Significant associations; CI: confidence interval; HDL-c: high density lipoprotein cholesterol; LDL-c: low density lipoprotein cholesterol. All models adjusted by maternal age (years), pre-pregnancy body mass index (kg/m^2), unsaturated fat and sugar intake (g) at late pregnancy. Model 1: Additionally, adjusted by late pregnancy moderate-to-vigorous physical activity (min/day) only; Model 2: Additionally, adjusted by gestational weight gain (kg) at late pregnancy only; Model 3: Additionally, adjusted by late pregnancy moderate-to-vigorous physical activity and gestational weight gain at late pregnancy.