

Maternal Phenotype, Directly Measured Physical Activity and Associations with Placenta  
Nutrient Transport Related Gene Expression

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## **Abstract**

The intrauterine environment plays an important role in fetal development and downstream health. Given the rise in maternal obesity and the incidence of babies being born large-for-gestational-age, research is needed exploring the mechanisms through which maternal obesity and health behaviours affect the delivery of nutrients to the fetus. This thesis includes three manuscripts in the pursuit of two objectives: 1) To determine whether there are changes in placenta nutrient transport-related gene expression in response to obesity, excess gestational weight gain, and variations physical activity and diet, and 2) To examine whether the Pregnancy Physical Activity Questionnaire is a reliable estimate of physical activity during the second trimester of pregnancy. In manuscript 1, we found that maternal obesity was not related to placenta nutrient transport-related gene expression, with the exception of lower placental *mTOR* expression in obese women who delivered male offspring, however, gestational weight gain was related to the gene expression of key proteins in the placenta. In manuscript 2, it was determined that the Pregnancy Physical Activity Questionnaire significantly overestimates physical activity and is not correlated with direct measures of activity and thus should not be used in future research. In manuscript 3, we found that physical activity and diet modify the expression of the genes involved in placenta nutrient transport. Meeting physical activity guidelines was associated with lower expression of a fatty acid transporter and higher expression of an amino acid transporter, while sugar intake was related to the expression of a glucose transporter. Together, the studies that make up this thesis suggest that there are numerous factors that may be contributing to placenta nutrient transport-related gene expression in humans and that future research on the placenta ought to include direct measures of physical activity and maternal diet, as well as account for gestational weight gain with respect to the guidelines and fetal sex.

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# Part I

## 1.1 General Introduction

The intrauterine environment plays a critical role in fetal growth and it is an important predictor of long term health. The Developmental Origins of Health and Disease hypothesis, developed by the late David Barker, suggests that environmental assaults early in life (i.e. *in utero*) can alter fetal development such that there is a long term impact on health and future disease risk [1,2]. Furthermore, size at birth is an important predictor of health and it is associated with increased risk of metabolic disease [3]. For example, intra-uterine growth restriction is associated with increased risk of cardiovascular disease [1,4]. It is due to the lasting impact of these intrauterine threats that the prenatal period is such an important area of research. Traditionally, research was focused on the downstream health consequences of intrauterine growth restriction, however, there is evidence of a J- or U-shaped relationship between birth weight and downstream disease risk [3,5], and given the rise in obesity, the research focus is shifting towards the impacts of high birth weight. Different exposures during the prenatal period, including the maternal phenotype (i.e. obesity) and maternal lifestyle (i.e. gestational weight gain, physical activity, and diet) may alter the intrauterine environment, thus potentially modifying disease risk. There is accumulating evidence of the detriments of maternal obesity and excess GWG, and the benefits of PA and a healthy diet during pregnancy, nonetheless there is still limited knowledge of the mechanisms through which these effects occur. The placenta connects the mother and fetus, and it is thought that changes in pregnancy outcome and disease risk might be mediated through changes in the placenta. Altered nutrient transport across the placenta has been identified in fetal growth restriction and fetal over-growth due to maternal

diabetes [6-11], and it is hypothesized that changes in placenta nutrient transport also occur in pregnancies complicated by obesity. Although certain modifiable risk factors, such as gestational weight gain, physical activity and diet, are often the targets of interventions that aim to improve pregnancy outcomes, the interplay between the placenta and these behaviours is still largely unexplored. The following will provide an overview of placenta nutrient transport and will highlight the maternal factors identified as potential modifiers of this process that are the focus of this thesis.

## **1.1 Nutrient Transport across the Placenta**

The placenta plays a fundamental role in the growth and development of the fetus, and fetal growth is directly related to the placenta's ability to transport nutrients from maternal circulation to the developing fetus. To reach fetal circulation, nutrients from the maternal circulation must cross through the placenta, which occurs mainly through specific nutrient transporters. The following review provides an extensive overview of the role of the placenta in nutrient transport to the fetus and explores how an altered maternal environment, such as common pregnancy pathologies and maternal lifestyle, affect the expression and activity of these transporters. The molecular signaling pathways linking maternal nutrient availability and placenta nutrient transport are also discussed.

### ***1.1.1 Maternal-Fetal Nutrient Transport in Pregnancy Pathologies: The role of the Placenta***

The following article has been published in the International Journal of Molecular Sciences and has been formatted according to their requirements.

Review

## Maternal–Fetal Nutrient Transport in Pregnancy Pathologies: The Role of the Placenta

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**Abstract:** Appropriate *in utero* growth is essential for offspring development and is a critical contributor to long-term health. Fetal growth is largely dictated by the availability of nutrients in maternal circulation and the ability of these nutrients to be transported into fetal circulation via the placenta. Substrate flux across placental

gradients is dependent on the accessibility and activity of nutrient-specific transporters. Changes in the expression and activity of these transporters is implicated in cases of restricted and excessive fetal growth, and may represent a control mechanism by which fetal growth rate attempts to match availability of nutrients in maternal circulation. This review provides an overview of placenta nutrient transport with an emphasis on macro-nutrient transporters. It highlights the changes in expression and activity of these transporters associated with common pregnancy pathologies, including intrauterine growth restriction, macrosomia, diabetes and obesity, as well as the potential impact of maternal diet. Molecular signaling pathways linking maternal nutrient availability and placenta nutrient transport are discussed. How sexual dimorphism affects fetal growth strategies and the placenta's response to an altered intrauterine environment is considered. Further knowledge in this area may be the first step in the development of targeted interventions to help optimize fetal growth.

**Keywords:** placental transport; pregnancy; maternal; glucose; amino acids; fatty acids; fetal growth

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## **1. Introduction**

Pregnancy is a critical period of physiological change for both the mother and the fetus. As gestational age increases, so too does the need for energy to meet the nutritional demands of fetal development. Although in humans, only a modest increase of 340 and 450 kcal/day is required for the mother in the second and third trimester of pregnancy, respectively [1], maternal consumption must support her own basal metabolic function and continuously supply nutrients to the fetus. Pregnancy represents a natural state of maternal insulin resistance and the difference in maternal–fetal glucose concentration that increases with advancing gestation facilitates increased fetal macronutrient uptake [2]. Consequently, the metabolic needs of the growing fetus are met in part by the glucose concentration gradient across the maternal–fetal interface [3]. With advancing gestation, increases in fetal body weight are accompanied by changes in body composition such that there is a reduction in total body water concentration and large gains in white adipose tissue from the second trimester onwards [4,5]. The energy demands of fetal growth are substantial given the large caloric requirement associated with fat deposition, which accounts for 90% of energy deposited near term; the total estimated caloric requirement of a human fetus at term is 90–100 kcal/kg/day [6,7]. Energy intakes that diverge from the

appropriate energy requirement may alter the fetal phenotype through epigenetic processes that alter expression of the genotype, such that insufficient or excess energy intake may cause growth restriction and overgrowth, respectively. Placental dysfunction can also restrict fetal growth by limiting nutrient supply to the fetus [8,9]. Intrauterine growth restricted (IUGR) fetuses are often born with depleted fat and glycogen stores [10,11]. In contrast, those born large-for-gestational-age (LGA), from mothers with obesity or to mothers who gain excessive weight during pregnancy, have increased adiposity [12–14] compared to average birth size newborns and mothers who gain the appropriate amount of weight, respectively.

In order to sustain appropriate fetal development the mother must provide glucose, amino acids and fatty acids, which are transported to the fetus across the placenta. There is increasing evidence that maternal factors, including body mass index, gestational weight gain, lifestyle behaviors (e.g., physical activity, smoking), as well as placenta-mediated diseases, can affect fetal growth and pregnancy outcomes. Although the precise mechanisms through which these factors affect fetal growth have yet to be fully elucidated, changes in placental nutrient transport to the fetus are implicated. This review provides an overview of placental nutrient transport, it explores how pregnancy-specific pathologies and maternal health behaviors may affect transporter expression and activity, and describes the molecular signaling pathways implicated in these changes.

## **2. Placenta Nutrient Transport**

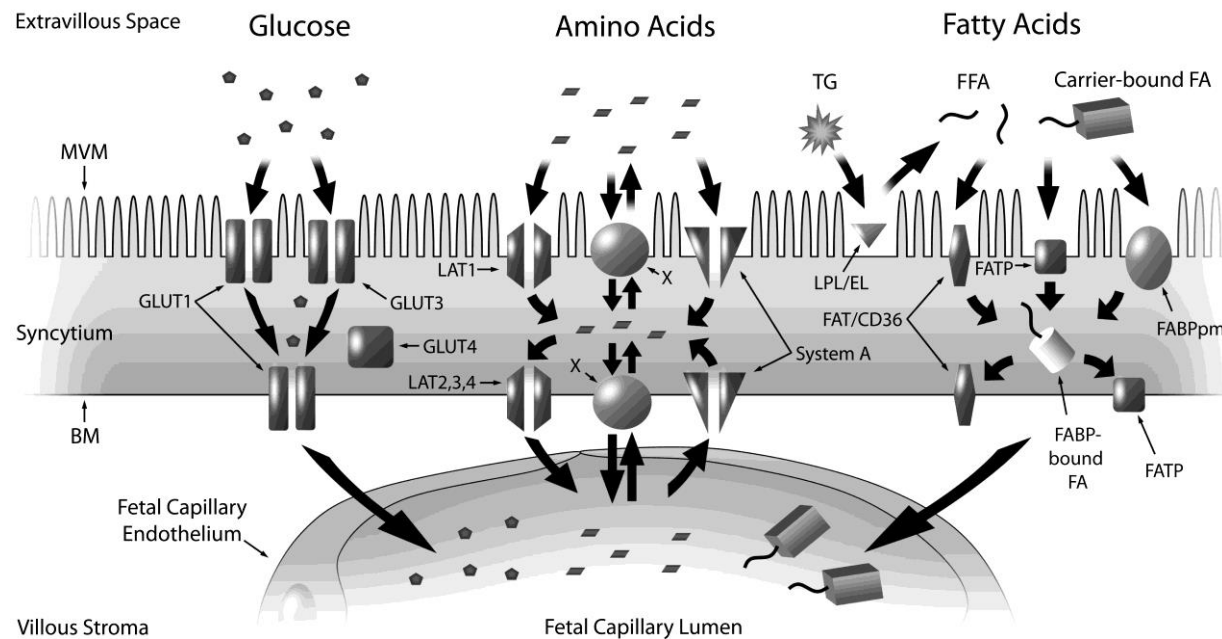
Fetal growth is directly related to maternal nutrient availability and the placenta's ability to transport these nutrients from maternal circulation to the fetus. The anatomical configuration of the placenta prevents direct contact of maternal and fetal blood, highlighting the importance of transport proteins, electrochemical gradients and diffusion channels for substrate exchange across the interface. Nutrient transport across the placenta and into fetal circulation is complex. There are two layers in the placental villi through which substrates, gases and water from maternal circulation must cross in order to reach the fetus [15,16]. The first layer, closest to maternal circulation, is made up of trophoblasts called syncytiotrophoblasts (SCTB), which line the villi. The SCTB constitute the transporting epithelium of the placenta, with two polarized membranes, the microvillous membrane (MVM) facing maternal circulation and the basal plasma membrane (BM) facing the fetal capillary. After passage across the SCTB membranes, substrates must cross the second layer of cells, the fetal capillary epithelium, before entry into the fetal circulation is complete (Figure 1). The fetal capillary endothelium is selectively permeable to molecules, such as amino acids and glucose, based on the size of the solute, and it is a relatively restrictive barrier against the diffusion of larger molecules [17,18]. Only smaller

solutes are highly permeable through the MVM and BM, and thus the SCTB constitutes a barrier and rate-limiting step of the transport of nutrients into fetal circulation.

Complete maternal–fetal exchange across the SCTB relies on facilitated diffusion and active transport against concentration gradients to drive electrochemical potential and nutrient flux [19–22]. Consequently, the transport of nutrients and solutes across the SCTB occurs via a number of passive and active processes including flow-limited diffusion, transcellular diffusion, facilitated diffusion/protein-mediated transfer and endocytosis/exocytosis [21]. Nutrients predominantly enter fetal circulation through nutrient-specific transport proteins located within the MVM and BM. The types of transporter (e.g., facilitated, active, passive, uni- or bi-directional, *etc.*), the subtypes expressed in the placenta, and localization to the MVM and/or BM, have been thoroughly reviewed by others [23,24].

Placenta nutrient transport is dependent on placental size, morphology (exchange zone surface area and tissue thickness), nutrient transporter capacity/availability, and utero- and fetoplacental blood flow [25,26]. With respect to placental size, placenta weight is a marker of the available surface area for maternal–fetal nutrient exchange. Placental weight is an important determinant of both birth weight and fetal growth [27], and fetal and placenta weight are positively correlated near term [28]. If the placenta fails to achieve an adequate size it may be unable to support the development of the fetus [29]. On the contrary, an association between large placentas and poor neonatal outcomes including hypoxia [29] and macrosomia [28] has also been reported. A marker of placental nutrient transporter efficiency is the fetal to placenta weight ratio (birth weight: placenta weight; in grams) [30]. This ratio can be altered by changes in placenta weight, fetal weight or both (reviewed by Fowden *et al.*) [31]. A lighter placenta and a higher fetal to placenta weight ratio is considered more efficient as it is consistent with the fetal drive to obtain nutrients from the placenta [31,32]. A lower fetal to placenta weight ratio may indicate below average placenta nutrient transport efficiency, and has been associated with increased pregnancy complications such as pre-eclampsia, c-section delivery and spontaneous pre-term delivery [28].

**Figure 1.** Nutrient transport across the placenta, featuring the SCTB and the fetal endothelium, and the location of key proteins involved in macronutrient (glucose, amino acids, fatty acids) transport at the MVM and BM. The SCTB is bathed in maternal blood on the apical surface instigating substrate transport at the MVM. This is followed by movement of the nutrients through the cytoplasm of the intermembrane space and interaction with the BM prior to uptake by the fetal capillary endothelium on the opposing side. Glucose is transported across the MVM and BM primarily by GLUT1. The accumulative transporters, System A, mediate the uptake of small neutral amino acids across the MVM and BM into the syncytium. Amino acids are transported across the BM towards the fetal capillary by System L facilitated transporters (TAT1, LAT2, 3 and 4) and exchangers. The exchangers, transport one amino acid in exchange for another, and thus they are dependent on the activity of the accumulative and facilitative transporters. LPL and EL hydrolyze maternal (TG) into FFA that cross the MVM through FATPs, FAT/CD36 and FABPpm. FFAs are trafficked through the cytosol via FABPs and across the BM by FATPs and FAT/CD36. Abbreviations: SCTB—syncytiotrophoblast; MVM—microvillous membrane; BM—basal membrane; GLUT—glucose transporter; LAT—large neutral amino acid transport; TG—triglycerides; LPL—lipoprotein lipase; EL—endothelial lipase; FFA—fatty acid; FAT/CD36—fatty acid translocase; FATP—fatty acid transport protein; FABP—fatty acid binding protein; FABPpm—plasma membrane fatty acid binding protein; X—exchangers.



With regard to nutrient-specific transporters, the placenta's capacity for nutrient transport can be altered by changes in the number, density, distribution or activity of these transporters [33–35]. Glucose, amino acids, free fatty acids (FFAs) and cholesterol are the essential macronutrients for adequate fetal growth, and each nutrient crosses the SCTB through specific transporters (Figure 1). Seminal work from Jansson and Powell has added experimental evidence to support the hypothesis that the placenta functions as a nutrient sensor [36]. Alterations in placental nutrient transport are thought to represent a control mechanism by which the fetal growth rate is matched with the availability of nutrients in maternal circulation—restricting growth when nutrition is limited and accelerating growth when nutrients are in excess [36]. To demonstrate, amino acid transport is down-regulated prior to the development of IUGR in rats fed a low protein diet [33,37], highlighting that maternal malnutrition can affect nutrient delivery and growth of the fetus. An alternate hypothesis suggests that the placenta may respond in a compensatory manner by up- or down-regulating transporter activity in response to low or high substrate levels, respectively, in an effort to maintain normal fetal growth. In normal pregnancies, smaller babies had higher amino acid transport activity [38], meanwhile, glucose transport activity was reduced in a hyperglycemic mouse model [39]. This “adaptive regulation” may serve to protect the placenta and fetus from under or excessive exposure to nutrients [40]. This review will focus on the first hypothesis, that the placenta functions as a nutrient sensor, and how it relates to common pregnancy pathologies. Placental nutrient transport phenotypes have been well-described in the context of IUGR and diabetic pregnancies [36] yet the proteins involved in nutrient transport are not sufficiently characterized, especially with respect to pregnancy complicated by obesity.

### *2.1. Glucose*

Glucose is the primary energy substrate required for growth of the fetus and placenta. Fetal gluconeogenesis is minimal [41], and the fetus is almost entirely dependent on glucose from maternal circulation. Placental glucose transport occurs by facilitated diffusion along a concentration gradient through members of the glucose transporter (GLUT) family [3]. There are 12 members of the GLUT family, however GLUT1 is the only isoform abundantly expressed in early pregnancy and at term, and is the primary placental glucose transporter in humans [42]. There is an asymmetrical distribution of GLUT1 across the placental membrane, with a greater prevalence of GLUT1 on the MVM compared to the BM, suggesting that the rate limiting step of human placental glucose transport may occur at the BM [43]. Insulin like growth factor (IGF) 1, a known regulator of fetal growth [44], increases GLUT1 protein expression and glucose uptake at the BM but not the MVM [45]. GLUT3 and GLUT4 are present in first trimester placentas suggesting a possible role in glucose uptake early in pregnancy. GLUT3 is primarily localized at the MVM of the SCTB, although it is also expressed in the cytotrophoblast and endothelium [46]. GLUT3 expression decreases substantially in the second and third trimesters such that the level in the third trimester is only 34% of that observed in the first trimester [46]. The insulin-sensitive GLUT4 is localized in the cytosol of the SCTB [47], and at term the

expression of GLUT4 is markedly reduced [47], suggesting a minimal role in glucose uptake from maternal circulation at term.

## *2.2. Amino Acids*

Amino acids play a critical role in the development of fetal tissue. The plasma concentrations of most amino acids are higher in fetal circulation compared to maternal circulation [48], indicating active transport of amino acids across the SCTB [49]. The placenta expresses over 15 different amino acid transporters, and each is responsible for the uptake of several different amino acids [49]. The two most studied amino acid transport systems in the placenta are System A and System L [49]. System A is a sodium-dependent accumulative transport system which facilitates the transport of small neutral amino acids (SNAT) such as alanine, serine and glycine into the cell [49]. System A activity is present at both SCTB membranes, but is more highly expressed at the MVM [50]. The third trimester placenta expresses three isoforms of System A: SNAT1, SNAT2, and SNAT4 [51]. System A activity is stimulated by insulin, leptin, IGF1, and interleukin 6 [52–54]. System L is a sodium-independent exchanger for large neutral amino acid transport (LAT); it exchanges non-essential amino acids for predominantly essential amino acids with branched or bulky side chains, such as leucine [55]. System L is stimulated by glucose and insulin [54] and its activity depends on the activity of the other systems to provide the amino acids that drive the system L exchange function [55]. Different isoforms of system L are found on the MVM (LAT1) and the BM (LAT2, LAT3, LAT4) [56,57]. The rate limiting step in amino acid transport is believed to be across the MVM [58]. The transport of amino acids across the BM into fetal circulation occurs via facilitated diffusion down their concentration gradients through the transporters LAT3, LAT4 and TAT1, as well as exchangers [56].

## *2.3. Fatty Acids*

Fatty acids serve many critical roles in fetal growth including brain development and fat accretion. In maternal circulation, lipids are mainly found as triglycerides (TGs), phospholipids and cholesterol esters. TGs cannot cross the SCTB and are first broken down into FFAs by placental TG lipases [59]. The FFAs are then available for uptake into the placenta through FFA transport proteins [60]. Lipoprotein lipase (LPL) and endothelial lipase are both located at the MVM and hydrolyze TGs in the maternal circulation [61–63]. Endothelial lipase is also able to metabolize HDL, LDL and VLDL lipids [61,64]. The proteins associated with FFA transport include fatty acid transport proteins (FATP), fatty acid translocase (FAT/CD36), plasma membrane fatty acid binding protein (FABPpm), and fatty acid binding proteins (FABP).

FATPs are integral membrane proteins that are important for the uptake of long chain fatty acids [65]. There are six members of the FATP family, five of which have been identified in placental trophoblasts (FATP1–4, and 6) [66]. FATP1 and FATP4 are frequently studied in placental tissue as

their expression correlates with docosahexanoic levels in maternal plasma, cord blood and placental phospholipids, suggesting an important role in the transfer of long chain polyunsaturated fatty acids [67]. The FATPs and FAT/CD36 are located on the MWM and BM and are involved in the transport of FFAs across the entire SCTB [68,69]. In contrast, FABPpm, which has a high affinity for long chain polyunsaturated fatty acids, is exclusively located on the MVM [68,69]. Five members of the FABP family (FABP1-5) have been identified in the trophoblast cells of the placenta and are localized in the cytoplasm of the SCTB [69]. The FABPs are responsible for cytosolic trafficking of FFAs to sites for esterification, beta-oxidation and subsequent transfer to the fetus [69]. The expression and activity of the proteins involved in fatty acid transport are influenced by insulin, IGF1 and leptin [70–72]. It remains unclear as to which step in the process limits the rate of placental fatty acid transport to the fetus.

#### *2.4. Cholesterol/Lipoproteins*

Cholesterol has an important role in fetal development, as it is an essential component of cell membranes and a precursor for steroid hormones. The fetus can synthesize cholesterol endogenously [73], but the placenta also transports cholesterol from maternal circulation to the fetus through cholesterol-carrying lipoproteins, such as low density lipoproteins (LDL), high density lipoproteins (HDL) and very low density lipoproteins (VLDL) [74]. The SCTB expresses lipoprotein specific receptors: LDL receptor (LDLR), scavenger receptor class B type I (SRBI) and VLDL receptor (VLDLR) [75–77]. Cholesterol from the placenta is transported to the fetus through specialized transporters, binding cassette transporter A1 and G1 (ABCA1 and ABCG1), located in the endothelial cells of the fetal vessels [78], as well as the MVM (ABCA1) and BM (ABCG1) [79,80].

### **3. Placental Nutrient Transport in Altered Fetal Growth**

Growth restricted infants typically have poor neonatal outcomes, and thus the earliest work on placental nutrient transport focused on IUGR. This was followed by research on fetal overgrowth (e.g., macrosomia) in pregnancies complicated with diabetes. With the rise in maternal overweight and obesity, more recent work has focused on the impact of obesity on nutrient transport and fetal overgrowth (Tables 1 and 2).

**Table 1.** Changes in expression level (protein or mRNA) and activity of the glucose, amino acid and fatty acid transporters in the human placenta associated with different pregnancy conditions <sup>1,2</sup>.

Nutrient Transporter	IUGR—Placental Dysfunction	Type 1 Diabetes	GDM	Obesity
GLUT1	— [43,81]	▲* (BM) (birth weight > control) [82] — (MVM) (birth weight > control) [82]	— (with and without LGA) [83]. ▲ (normal weight mothers; insulin controlled; no difference in birth weight) [84] — (normal weight mothers; diet controlled; no difference in birth weight) [84] — (obese mothers; diet or insulin controlled; no fetal overgrowth) [84]	— (no fetal over growth) [84]
GLUT3	▲ [85]			
GLUT4	— [85]		▼ (normal weight mothers; insulin controlled; no difference in birth weight) [84] ▼ mRNA (obese mothers; diet or insulin controlled; no fetal overgrowth) [84]	▼ mRNA (no fetal over growth) [84]
System A (SNAT1,2,4)	▼* (MVM) [58,86] —* (BM) [81]	▼* (MVM) (macrosomic) (no maternal BMI) [87] ▲* (MVM) (independent of fetal growth, similar maternal BMI) [88]	▲* (MVM) (independent of fetal over growth) [81]	▼* SNAT4 (no difference in birth weight) [89] — SNAT1, SNAT2 (no difference in birth weight) [89] —* (no difference in birth weight) [90] * positive correlation to birth weight [90] ▲ SNAT2 (no difference in birth weight; positive correlation to birth weight) [90]

Table 1. Cont.

Nutrient Transporter	IUGR—Placental Dysfunction	Type 1 Diabetes	GDM	Obesity
System L (LAT1-4)	▼* [91]		▼* (MVM) (fetal overgrowth) [81]	—* (no difference in birth weight) [90]
LPL	▼* (preterm) [92] ▲ mRNA (preterm) [93]	▲* (macrosomic) [92] — (macrosomic) [92] ▼ mRNA (birth weight > control; not macrosomic) [94]	—* ( macrosomic) [92] ▼ mRNA (birth weight > control; not macrosomic) [94]	▲* (no difference in birth weight) [95] — mRNA (birth weight > control; not macrosomic) [96]
Endothelial Lipase	▲ mRNA (preterm) [93]	▲ (birth weight > control; not macrosomic) [97].	— (no fetal over growth) [98] ▲ (with obesity) (no fetal over growth) [98]	
FATP4				▼ (no difference in birth weight) [95] — mRNA (birth weight > control; not macrosomic) [96]
FAT/CD36				▲ (no difference in birth weight) [95] ▼ mRNA (male) (birth weight > control; not macrosomic) [96] — mRNA (female) (birth weight > control; not macrosomic) [96]
FABP1		▲ (macrosomic) [92]	▲ ( macrosomic) [92]	▼(no difference in birth weight) [95]
FABP3				— (no difference in birth weight) [99] ▼(no difference in birth weight) [95]
FABP4		▲ mRNA (birth weight > control; not macrosomic) [94]	▲ mRNA (birth weight > control; not macrosomic) [94]	— (no difference in birth weight) [99] ▲ (with diabetes) (birth weight > control; not macrosomic) [99] — mRNA (birth weight > control; not macrosomic) [96]

Table 1. Cont.

Nutrient Transporter	IUGR—Placental Dysfunction	Type 1 Diabetes	GDM	Obesity
FABP5			▲ mRNA (birth weight > control; not macrosomic) [94]	▲ mRNA (with diabetes) (birth weight > control; not macrosomic) [99] — (no difference in birth weight) [99] ▼ mRNA (male) (birth weight > control; not macrosomic) [96] — mRNA (female) (birth weight > control; not macrosomic) [96]
FABPpm				— (no difference in birth weight) [99] — mRNA (birth weight > control; not macrosomic) [96]

<sup>1</sup> Legend: — no change in protein or mRNA expression, ▲ increase in protein expression (unless mRNA is indicated), ▼ decrease in protein expression (unless mRNA is indicated), \* change in the activity of the transporter. If the box is left blank, there is currently no information on this transporter in this specific condition. Gender is specified only when a difference exists between the sexes; <sup>2</sup> IUGR—intrauterine growth restriction; GDM—gestational diabetes mellitus; GLUT—glucose transporter; SNAT—small neutral amino acid transporters; LAT—large neutral amino acid transporter; LPL—lipoprotein lipase; FATP—fatty acid transporter; FAT/CD36—fatty acid translocase; FABP—fatty acid binding protein; FABPpm—plasma membrane fatty acid binding protein.

**Table 2.** Changes in expression level (protein or mRNA) and activity of the glucose, amino acid and fatty acid transporters in the placenta in animal models of different pregnancy conditions <sup>1,2</sup>.

Nutrient Transporter	IUGR—Nutrient Restriction	Maternal Diet
GLUT1	Mice § ▼ (no change in fetal weight) [100] Mice ¥ ▲ (reduced fetal weight) [100] Sheep § ▲ (reduced fetal weight) [101] Baboon ¥ ▼ (reduced fetal weight) [102]	Mice ▲ (high fat) (increased fetal weight) [103]
GLUT3		Mice ▲ (high fat, high sugar) (§ reduced fetal weight; ¥ no change in fetal weight) [104]
System A (SNAT1, 2, 4)	Mice ¥ ▲ SNAT1 (reduced fetal weight) [100] Mice ¥ ▼ (reduced fetal weight) SNAT4 [100] Sheep § (reduced fetal weight) [101] Baboon ¥ ▼ SNAT2 (reduced fetal weight) [102]	Mice ▲ SNAT2 (high fat) (increased fetal weight) [103] Mice (male) ▲ SNAT2 (“cafeteria” diet) (no change in fetal weight) [105] Mice (female) ▲ SNAT4 (“cafeteria” diet) (no change in fetal weight) [105] Mice ▲ SNAT2 (high fat, high sugar) (§ reduced fetal weight; ¥ no change in fetal weight)[104]
System L (LAT1–4)	Baboon ¥ ▼ LAT1/2 (reduced fetal weight) [102]	
FATP4	Sheep § ▲ (reduced fetal weight) [101]	
FAT/CD36	Sheep § ▲ (reduced fetal weight) [101]	

<sup>1</sup> Legend: — no change in protein or mRNA expression, ▲ increase in protein expression (unless mRNA is indicated), ▼ decrease in protein expression (unless mRNA is indicated), \* change in the activity of the transporter, ¥ End of gestation in animal study, § Mid gestation in animal study. If the box is left blank, there is currently no information on this transporter in this specific condition; <sup>2</sup> IUGR—intrauterine growth restriction; GDM—gestational diabetes mellitus; GLUT—glucose transporter; SNAT—small neutral amino acid transporters; LAT—large neutral amino acid transporter; FATP—fatty acid transporter; FAT/CD36—fatty acid translocase.

### *3.1. Intrauterine Growth Restriction*

IUGR is characterized by the fetus not reaching its predetermined growth potential and can result from a multitude of causes, including placental dysfunction and maternal under-nutrition. With placental dysfunction, the nutrient supply to the fetus is insufficient despite adequate nutrient availability in the mother. Insufficient remodeling of the spiral arteries during placentation is the key physiological change that contributes to inadequate blood flow to the fetus, resulting in a reduction in nutrient and oxygen delivery and IUGR [8,9]. In maternal under-nutrition, there may be inadequate food supply or deliberate calorie restriction and thus there is insufficient availability of nutrients in maternal circulation, often resulting in nutritionally-induced IUGR [106]. In a small subset of the population, maternal obesity can also increase the risk of IUGR [107–109]. However, the mechanisms involved in the development of IUGR in the context of maternal obesity are not well understood and lie outside the focus of this review.

#### 3.1.1. IUGR—Placental Dysfunction

With respect to glucose flux, although fetal hypoglycemia has been implicated in the pathophysiological mechanism of IUGR, this is not due to reduced glucose uptake or expression of the GLUT1 transporter at the SCTB [43,81]. However, there is increased expression of GLUT3 protein on the maternal aspect of the placenta in late-term IUGR compared to normal pregnancy, with no changes in GLUT1 or GLUT4 [85]. In this study, GLUT3 was expressed in the cytotrophoblast, and to a lesser degree in the SCTB, and it is thought that the increased expression of GLUT3 in the cytotrophoblast may contribute to an increased consumption of glucose by the placenta itself [85].

Placental amino acid transport in IUGR pregnancies has previously been reviewed [36,110,111], and reduced expression and activity of the amino acid transporters is consistently identified. This is intuitive, provided that amino acid concentrations in the cord blood in IUGR are significantly reduced compared to normal pregnancies [48]. Specifically, in IUGR the activity of System A is reduced in the MVM [58,86], but unaltered in the BM [81]. In preterm IUGR, System A activity in the MVM is reduced to a greater degree [81]. Additionally, the activity of the System L leucine transporter is reduced at the MVM and the BM [91], and taurine transporter activity is reduced at the MVM [112]. Taken together, diminished amino acid transporter activity, independent of altered expression, may be an adaptation by which the placenta responds to a suboptimal *milieu* in an attempt to regulate growth without compromising development of vital organs (brain and heart). Thus, down-regulation of amino acid transport capacity and efficiency to the fetus is likely an important contributing factor to the restricted fetal growth of these pregnancies.

In the case of fat transport, the activity of LPL was found to be decreased by 47% in preterm IUGR placentas, compared with preterm controls, with no differences observed in term IUGR [92]. On the contrary, Gauster *et al.* found that LPL mRNA expression was increased by greater than two-fold in preterm IUGR compared to normal term placentas, in conjunction with a 30% reduction in

mRNA expression of endothelial lipase [93]. Altered lipoprotein receptor expression has also been identified in IUGR: an increase in LDLR protein and a reduction in SRBI protein compared to average-for-gestational-age controls [113]. These changes in TG hydrolases and lipoprotein receptors may contribute to the reduced adiposity that is typical in IUGR infants.

### 3.1.2. IUGR—Maternal Nutrient Restriction—Evidence from Animal Models

Under-nutrition during pregnancy frequently occurs in developing countries and is also related to eating disorders, famine caused by natural disasters, food insecurity and voluntary calorie restriction to maintain a certain body image. Maternal nutrient restriction can alter placentation, and these effects may depend on the timing of the nutrient restriction. During the Dutch famine, babies who were exposed in mid to late gestation had less efficient placentas (birth weight adjusted for placenta area), in contrast, babies exposed in early gestation or who were conceived after the famine had ended had efficient placentas [114]. However, the influence of deliberate maternal nutrient restriction on placental nutrient transfer has only been investigated in animal models.

Using a murine model of nutrient restriction (80% of control diet), Coan *et al.* generated mice with reduced birth and placental weight as well as reduced fetal to placental weight ratio, compared to control mice, [100]. At Day 16 in the nutrient restricted mice, the gene expression of placental GLUT1 was reduced 83% to that of the controls, although, by Day 19, GLUT1 gene expression was significantly greater than in the controls suggesting an attempted compensatory response of a highly plastic system [100]. However, nutrient restriction did not alter unidirectional materno-fetal clearance of tracer glucose at either time point. There was no difference in amino acid transporter expression or activity at Day 16, however, at Day 19, nutrient restriction increased the gene expression of SNAT1 and decreased the gene expression of SNAT4, which corresponded with increased amino acid clearance [100]. Work by Ma *et al.* demonstrated that deliberate maternal nutrient restriction to 50% of the control diet during the first half of gestation could alter placenta nutrient transport in sheep [101]. At mid-gestation, protein and mRNA expression of GLUT1, FATP4 and FAT/CD36 were elevated in the placentomes of nutrient restricted ewes, likely to maintain placental efficiency and fetal weight, however, despite this adaptation, placental and fetal weights were reduced compared to controls [101]. In ewes that were nutrient restricted for the first half of pregnancy but were fed 100% of the control diet during the second half of pregnancy, only FATP4 mRNA and protein levels were increased compared to controls at the end of gestation, and there was no difference in fetal or placental weight [101].

Collectively, these studies demonstrate placental plasticity to adapt to maternal nutrient restriction by altering its phenotype in an effort to maintain normal fetal growth. However, changes such as increased transport of fatty acids and glucose have the potential to alter the fetal body composition which could continue to impact the offspring in postnatal life [115]. Recently, Kavitha *et al.* were the first to explore changes in placenta nutrient transporter expression in response to maternal nutrient restriction in a non-human primate [102]. Maternal nutrient restriction during gestation (70% of control diet) in baboons

reduced fetal weight, as well as the MVM protein expression of GLUT1 and the amino acid transporters TAUT, SNAT2, and LAT1/2 compared to the controls [102]. Given the similarities between human and primate reproductive physiology and placenta structure, this study is highly relevant for human health, nutrient deprivation and altered fetal growth, as it is not ethical to subject pregnant women to experimental nutrient restriction.

### 3.2. Fetal Overgrowth

Although not always the case, pathologies such as diabetes and obesity may result from positive energy balance, a net surplus of hormones (e.g., insulin), and/or growth dysregulated factors (e.g., IGF) [116,117], thereby increasing the risk of fetal overgrowth. Diabetes during pregnancy is associated with maternal hyperglycemia and hyperlipidemia, and thus increased glucose supply and altered lipid delivery to the fetus [118,119], whereas obesity during pregnancy is associated with elevated maternal lipid levels [59]. Obese mothers, and those who gain excess gestational weight, tend to birth infants with increased neonatal fat mass and body fat percentage [12,13,120], providing evidence that maternal adiposity predicts neonatal fat mass and not simply overall mass. It is thought that the increased availability of nutrients in maternal circulation stimulates the placenta to increase transport of these nutrients thus resulting in fetal overgrowth.

#### 3.2.1. Diabetes

In pregnancies complicated by maternal diabetes, the alterations in nutrient transport differ between type 1 diabetes and GDM, and this has been extensively studied and documented in the seminal work by Jansson and Powell and colleagues [83,88,92]. In type 1 diabetes, studies show altered System A activity. For instance, System A activity was reduced by 49% in the MVM in macrosomic infants born to women with type 1 diabetes [87], in contrast, an increase in System A activity by 65%–80% has also been reported in type 1 diabetes, independent of fetal over-growth [88]. Of note, maternal weight was not accounted for in the former study [87], but maternal body weight was similar across groups in the latter [88], and thus the pathology of the diabetes in the mother may be different across studies affecting the results. Type 1 diabetes is associated with increased GLUT1 expression and increased glucose uptake at the BM, compared to healthy pregnancies, but no alterations were found at the MVM [82]. Compared to appropriate-for-gestational-age infants born to healthy mothers, type 1 diabetes was also associated with an increase in LPL activity (but no difference in protein expression), an increase in FABP1 protein expression [92], and an increased expression of endothelial lipase [97]. Data gathered through microarray profiling of placenta samples has indicated that type 1 diabetes is associated with a 2.4-fold up-regulation of FABP4 compared to control, however, LPL expression was down-regulated nearly 3.4-fold [94]. The difference in LPL expression could be related to the fetal outcomes or how LPL was measured; Magnusson *et al.* observed higher LPL activity with macrosomic infants (but no change in protein expression) [92], conversely, Radaelli *et al.* found that LPL expression

was down-regulated, but the infants were not macrosomic [94]. These inconsistencies highlight the importance of comparing homogeneous populations (*i.e.*, accounting for maternal and fetal characteristics), as well as exploring all aspects of expression (gene, protein, activity).

The work conducted in GDM pregnancies is more difficult to interpret than type 1 diabetes, as treatment is not consistent across patients. Some women regulate their GDM with diet and exercise alone, while other women are required to take insulin. In most studies examining placenta nutrient transport characteristics, the mode of diabetes treatment is not considered and the groups contain both diet and insulin treated women, which might influence the pathology. In an earlier study of GDM pregnancies (22% insulin treated; 78% diet controlled; no record of maternal BMI), it was found that compared to pregnancies not complicated by diabetes, the expression and activity of GLUT1 was unaltered [83]. However, in studies that accounted for treatment modality and maternal BMI, the results differed according to treatment and BMI. In non-obese women, those with insulin controlled-GDM had higher protein and mRNA expression of GLUT1 when compared to non-obese diet controlled-GDM and healthy controls (protein only), as well as lower protein and mRNA expression of GLUT4 when compared to non-obese diet controlled-GDM (mRNA only) and healthy controls [84]. Furthermore, in the obese women with insulin controlled-GDM, GLUT4 mRNA expression was less than that of obese women with diet controlled-GDM and the obese, non-diabetic controls, but there was no change in protein expression [84]. Thus demonstrating the importance of considering maternal BMI and the manner in which the GDM is treated.

With regards to amino acid transport, System A activity was higher at the MVM in women with GDM, with and without LGA babies, when compared to healthy controls, whereas amino acid transport was unaltered in cases of fetal over-growth alone [81]. This suggests that the change in System A activity is a response to the diabetic environment and not a feature of fetal over-growth. Additionally, we can infer that the type of treatment for GDM likely does not influence the pathology, as only seven women with GDM were treated with insulin (1/10 in the GDM group, 6/10 in GDM/LGA group), yet both groups exhibited similar levels of System A activity. System L activity was also higher at the MVM in GDM pregnancies with fetal over-growth [88]. In obese women with GDM (dietary regimen or insulin therapy), endothelial lipase expression was greater, however, obesity or GDM (dietary regimen or insulin therapy) alone had no effect on its expression [98]. In one study, the protein expression of FABP1 was 64% higher in GDM (insulin therapy ( $n = 3$ ), diet only ( $n = 5$ )) when compared to appropriate-for-gestational-age infants born to healthy women, however, the activity of LPL was unaltered [92]. Using microarray profiling, GDM (all insulin therapy) preferentially activated genes related to lipid metabolism, with a two-fold up-regulation of FABP4 and FABP5 compared to control, however, LPL expression was down-regulated nearly three-fold [94]. In placental explants of women with GDM (all insulin therapy), fatty acid oxidation was reduced by 30%, and the TG accumulation was three-fold higher *vs.* non-diabetic control [121]. These alterations in placental lipid

metabolism are thought to be a regulatory step contributing to the fetal fat accumulation and macrosomia that often accompanies GDM.

Gestational diabetes mellitus can also alter the expression of placental cholesterol transport proteins. At the SCTB, the LDLR mRNA and protein expression was significantly higher in women with GDM (all insulin therapy), independent of their BMI, compared to healthy controls. VLDLR expression was higher in overweight/obese women with GDM compared to the normal weight women with GDM and the healthy controls, and SRBI expression was significantly higher in normal weight women with GDM compared to the other groups [118]. The expression of ABCA1 was significantly lower in women with GDM independent of BMI, meanwhile, the expression of ABCG1 was significantly lower only in overweight/obese women with GDM [118]. On the whole, these findings suggest that maternal diabetes may disrupt normal placenta nutrient transporter expression and activity, which may contribute to the accelerated fetal growth in these pregnancies.

### 3.2.2. Obesity

There is limited research on the effects of maternal obesity on placenta nutrient transport, particularly in normoglycemic humans. To our knowledge, there is only one study examining the impact of obesity on glucose transporters in the human term placenta. This study found no difference in GLUT1 mRNA or protein expression and only a significantly lower GLUT4 mRNA but not protein expression in the obese, normoglycemic patients, compared to healthy weight, normoglycemic patients [84]. However, there was no difference in birth weight between groups, raising the possibility that differences may not be present due to the similar fetal outcomes. Similar transport dynamics were also explored using high fat fed mice as a model of obesity [103]. Compared to the control mice, the high fat fed mice had increased fetal weight, an increased rate of glucose clearance and increased GLUT1 expression in the placenta [103]. This discrepancy between rodent and human models highlights the importance of considering whether the human subjects fully represent the population you aim to study. For instance, in this case, did the obese women with healthy weight children truly represent the obese phenotype that the authors wished to study?

The impact of obesity has been explored on placental amino acid transport in humans, and decreased activity and expression of SNAT4 was shown in obese women compared to lean women, despite no difference in infant birth weight, meanwhile, SNAT1 and SNAT2 expression was unchanged [89]. These results were contrary to the author's hypothesis and raised the possibility that reduced amino acid transfer and increased transport of FFA or glucose across the placenta (not measured) might occur in the obese women. We theorize that this could result in offspring of similar weight in the obese and lean women, but with increased adiposity and lower lean mass in the infants from obese mothers [13]. At term, the contribution of SNAT4 to amino acid transport is reduced, while SNAT1 and SNAT2 are believed to be the predominant contributors to System A transport [51,122]. Thus is it interesting that SNAT activity was decreased in the obese women despite similar expression of the key System A

transporters at term. It is possible that the similar expression of SNAT1 and SNAT2 may have contributed to the similarities in birth weight between groups [89]. Conversely, recent work in humans found no difference in the activity of System A or System L transporters when comparing normal weight to overweight/obese women (with no differences in birth weight), however, MVM System A activity was positively correlated with birth weight, but not maternal BMI [90]. Furthermore, the expression of SNAT2 was positively correlated with maternal BMI and birth weight, but neither SNAT1 or SNAT4 were correlated to birth weight or BMI [90]. Given that System A activity was positively correlated with birth weight, it is possible that differences between groups might have been observed had the women with obesity given birth to macrosomic infants. Despite the contradictory results there is some evidence to suggest a possible link between maternal obesity, altered amino acid transport and increased fetal growth. Further work should consider using a more concise definition of the phenotype associated with pregnancies complicated by obesity (*i.e.*, high maternal BMI, LGA fetus) to better understand the underlying mechanisms linking maternal habitus to fetal size at birth.

There is a paucity of research on the impact of maternal obesity and the expression or activity of FFA transport proteins in human pregnancies, although evidence from an obese ovine model suggests that obesity alters placental fatty acid transport through changes in transporter levels and not TG hydrolysis. In this study of sheep, Zhu *et al.* found elevated fetal blood levels of cholesterol and TG, and increased FATP1 and FATP4 protein levels, but no difference in LPL expression [123]. In 2011, Scifres *et al.* found that placentas from obese-diabetic women exhibited an increase in FABP4 and FABP5 mRNA, and FABP4 protein expression, but no change in FABP3 or FABPpm, when compared to obese, non-diabetic or normal weight women [99]. However there were no differences between the obese non-diabetic and the normal weight women [99]. Furthermore, in 2012 Dubé *et al.* found that maternal obesity was associated with significantly higher FAT/CD36 mRNA and protein levels and LPL activity, but a lower expression of FATP4, FABP1 and FABP3 protein compared to the normal weight women [95]. However, the most recent evidence suggests that the susceptibility of the placenta to maternal factors may be fetal sex specific, and it may be important to explore outcomes in a sex specific fashion [96,124,125]. In particular, Brass *et al.* found the rate of placental oleic acid uptake was 43% lower in male offspring and 73% higher in female offspring born to obese women, compared to lean women [96]. Changes in placenta mRNA levels were also dependent on fetal sex, with lower FAT/CD36 and FABP5 expression among male offspring from obese women, whereas gene expression levels were unchanged in female placentas, regardless of maternal BMI [96]. This study also found no detectable differences in placental gene expression of LPL, FATP4, FABP4 and FABPpm between groups [96]. However, in the first two studies there was no difference in birth weight in the infants born to the lean and obese women [95,99]), and in the latter study, the infants born to the women with obesity were heavier than the infants born to the lean women, but they were not macrosomic [96]. It is possible that greater changes in transporter expression and activity would have been observed had the impact of the maternal obesity been more apparent on the child (*i.e.*, macrosomia). Given the paucity

of work in this area and the inconsistent results, it remains unclear if obesity is associated with specific alterations in placental nutrient transport and it is evident that further work is needed examining the sex-specific differences.

#### **4. The Impact of Maternal Diet**

The high incidence of obesity and metabolic diseases is largely attributed to our unhealthy modern food environment, which promotes high calorie, but low nutrient quality diets. Given that fetal growth is closely related to the maternal nutrient supply, it may be important to explore the impact of unhealthy, excessive and unbalanced dietary composition on placental nutrient transport. Preliminary work has explored this topic in animal models. In a mouse model, the *ad libitum* consumption of a high fat diet (32% fat, 52% carbohydrate, 16% protein) for 8 weeks before mating and during gestation increased placenta transport of glucose (5-fold) and neutral amino acids (10-fold), and increased the expression of GLUT1 (five-fold) and SNAT2 (9-fold) [103]. In another mouse model, *ad libitum* access to a high fat, high sucrose “cafeteria diet” (58% fat, 25.5% sucrose, 16.4% protein) increased the expression of SNAT2 in male placentas and SNAT4 in female placentas [105]. Additionally, a high sugar, high fat diet (30% fat, 17% protein, 53% carbohydrate) during pregnancy in mice reduced placenta weight, increased placenta glucose and amino acid transport, and increased the expression of GLUT3 and SNAT2 [104]. Diets with varying compositions of fat and fiber have also altered the placenta mRNA expression of GLUT1, GLUT3 and SNAT4 in mice [126]. Although only explored in rodent models, it is important to appreciate that the absolute caloric quantity in addition to maternal diet composition (*i.e.*, diet quality) may play an important role in altering placenta nutrient transport to the fetus.

#### **5. Molecular Mechanisms Regulating Altered Nutrient Transport**

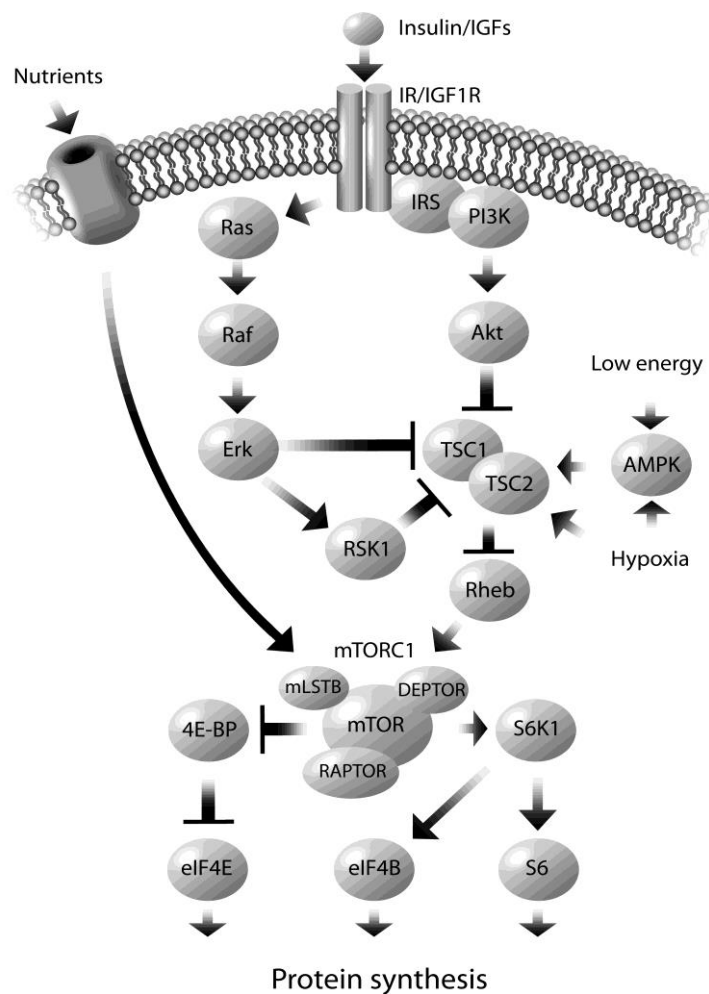
The molecular mechanisms responsible for alterations in placental nutrient transport are largely understood to involve the mammalian target of rapamycin (mTOR) signaling pathway. The placenta nutrient sensing model, proposed by Jansson and Powell, suggests that mTOR, located in the trophoblasts cells, serves as the integrator of signals from maternal supply and fetal demand [20,127]. The mTOR pathway integrates various signals to regulate growth, including growth factors, stress, energy status, oxygen and amino acids [128]. mTOR receives maternal signals (e.g., insulin, leptin) at the MVM and transmits this information downstream to alter gene transcription and protein translation, resulting in up/down regulation of nutrient transport proteins implicated in fetal growth. The positive regulators of mTOR include the maternal hormones insulin, leptin and IGF1 [52,54,129,130], while adiponectin and hypoxia inhibit mTOR [129,131,132]. In particular, in cultured primary human trophoblast cells, the stimulation of System A activity by insulin and IGF1 is dependent on mTOR signaling [54]. Pregnancy complications, such as obesity, that alter maternal nutrient supply, adipokine,

cytokine and hormone levels can alter the mTOR pathway, thus leading to modifications in placenta nutrient transport and consequently modify fetal growth.

mTOR is an atypical serine/threonine protein kinase that interacts with several proteins to form two distinct complexes named mTOR complex 1 (mTORC1) and 2 (mTORC2); albeit mTORC1 is better characterized in placenta nutrient sensing (Figure 2). mTOR is controlled by the intermediate Rheb which is regulated by the Tuberous Sclerosis Complex (TSC1/2). Various upstream effector kinases, such as protein kinase B (Akt/PKB), extracellular-signal-regulated kinase 1/2 (ERK1/2), and MAPK-activated, p90 ribosomal S6 kinase 1(RSK1) act on the TSC1/2 complex, thus affecting mTOR activity [128]. It is through these pathways that TSC1/2 transmits many of the upstream signals that act on mTOR, including growth factors and cytokines. For instance, growth factors, such as insulin and IGF1, signal through the IRS/PI3K and Akt/PKB pathway, which inactivates TSC1/2, thus activating mTORC1 in the presence of anabolic, growth promoting, signals. When activated, the ERK1/2 and RSK1 pathways have been shown to inhibit TSC1/2, thus activating mTOR. Pro-inflammatory cytokines, such as TNF $\alpha$ , may also lead to activation of mTORC1 through a mechanism similar to that of the growth factors by phosphorylating and inhibiting TSC1/2. Although, the mechanism is not fully elucidated, amino acids appear to activate mTORC1 independently of TSC1/2 [128]. mTOR also engages in extensive cross-talk with AMP activated kinase (AMPK) in response to cellular stress and energy depletion [133,134]. AMPK phosphorylation (activation) occurs via an accumulation of AMP and hence detects energy depletion (low ATP levels) which inhibits mTOR [133,135].

Protein synthesis is the best-characterized process that is mediated by mTORC1 activation. In primary villous explants and cultured primary human trophoblasts, mTOR is a positive regulator of System A and System L amino acid transporters [136,137]. This occurs predominantly at the post translational level by altering the transporter abundance at the plasma membrane through mTOR activation [138]; which may increase the cell surface abundance of amino acid transporters. The downstream proteins directly phosphorylated by mTORC1 are p70 ribosomal S6 kinase 1 (S6K1) and the eukaryotic initiation factor 4E-binding protein 1 (4EBP1), regulate translation [128]. The phosphorylation status of S6K1 and 4EBP1 are used as an index of mTORC1 activity. Amino acid deprivation inhibits the activity of mTORC1, evident by the dephosphorylation (inactivation) of S6K1 and 4EBP1 [139].

**Figure 2.** Regulation of the mTORC1. Various upstream kinases (Akt/PI3K, ERK1/2, RSK1) converge on TSC1/2, which regulates mTOR through Rheb. Activation of mTORC1 leads to the phosphorylation of S6K and the dissociation of eIF4E from 4E-BP, which in turn promotes protein synthesis. Insulin/ IGF phosphorylates Akt, which inhibits TSC2, thus releasing the inhibition of Rheb by TSC1/2. Activated Rheb stimulates mTORC1 signaling. AMPK, in response to low energy levels or hypoxia, phosphorylates TSC2, and thus inhibits mTORC1. Nutrients, specifically amino acids, activate mTORC1, independently of TSC1/2. Abbreviations: mTOR—mammalian target of rapamycin; mTORC1—mTOR complex 1; TSC—tuberous sclerosis complex; Akt/PKB—protein kinase B, ERK—extracellular-signal-regulated kinase, RSK1—MAPK-activated, p90 ribosomal S6 kinase 1; IGF—insulin like growth factor; IRS/PI3K—insulin receptor substrate/phosphoinositide 3-kinase; AMPK—AMP activated kinase; S6K1—p70 ribosomal S6 kinase 1; 4EBP1—eukaryotic initiation factor 4E-binding protein; eIF4E—eukaryotic initiation factor 4E; eIF4B—eukaryotic initiation factor 4B; S6—ribosomal protein S6



Alterations in the mTORC1 signaling pathway have been identified in pregnancies associated with abnormal fetal growth. Although substantial mechanistic evidence is available from *in vitro* work using trophoblast and BeWo cell line models of placenta physiology [136–138,140], we have focused on evidence from animal and human studies. When maternal nutrient availability is restricted, the activity of placental mTOR is decreased, such as in human IUGR [136,141], in protein restriction in the rat [33,142] and in nutrient restricted baboons [102]. Specifically, in the baboon at the end of gestation, maternal nutrient restriction is associated with decreased phosphorylation of the upstream (IRS1, Akt, ERK1/2, RSK1) and downstream (4EBP1, S6K1) proteins in the mTOR signaling pathway, as well as reduced placental expression of glucose and amino acid transporters and reduced fetal weights [102]. The down-regulation of mTOR signaling and nutrient transport in response to maternal nutrient restriction suggests that the placenta is matching fetal growth with the availability of nutrients, such that the offspring is smaller and thus a better match for an environment with limited nutritional resources. In nutrient restricted sheep at mid-gestation, the activity of placental AMPK and ERK1/2 was increased, as were GLUT1, FATP4, and FAT/CD36 protein levels, and fetal weight was reduced, however, the activity of mTOR and Akt signaling were not altered [101]. mTOR is activated by ERK1/2, but inhibited by AMPK, and these conflicting signals may contribute to the similarities between groups in mTOR activity. After re-alimentation to the control diet, the nutrient restricted fetuses reached similar weights to the control group at the end of gestation [101], although these offspring had greater adiposity and reduced insulin sensitivity [115]. This suggests that in certain circumstances where nutrients are restricted, that the placenta stimulates several mechanisms which may act independent of the mTOR pathway, in an effort to augment nutrient transport (*i.e.*, glucose and fatty acids) to optimize fetal growth in less than favorable environmental conditions.

When maternal nutrients are in excess, the mTORC1 signaling pathway is activated, as demonstrated in large for gestational age (LGA) babies born to obese women [90], and in high fat fed overweight rats [143]. In the obese women who gave birth to LGA babies, the activity of AMPK (which inhibits mTORC1), was decreased likely due to an excess of nutrients, and the insulin/IGF1 signaling pathway (which activates mTORC1) was activated, in association with increasing BMI and birth weight [90]. Additionally, the phosphorylation of the downstream targets of mTORC1 (S6K1 and 4EBP1) were positively correlated to early pregnancy BMI and birth weight [90]. This suggests that up-regulation of the mTOR signaling pathway with increasing maternal BMI may contribute to the increased amino acid transport and birth weight of these LGA babies [90]. In contrast, in obese, over-nourished sheep at mid-gestation, there was a reduction in total and phosphorylated AMPK, as well as reductions in total mTOR and ERK1/2, and phosphorylated Akt, mTOR and ERK1/2[144], with no difference in fetal weight at the end of gestation [123]. Overall, an excess of nutrients might inhibit the mTOR pathway, potentially through a negative feedback loop [145], in an effort to restrict excessive nutrient transport to optimize fetal growth. To illustrate, an excess of nutrients might result in continuous activation of mTOR-S6K1 signaling, which induces a negative feedback loop to attenuate PI3K signaling

by inhibiting IRS [145]. Collectively, this evidence suggests that dysregulated placental mTOR is implicated in abnormal fetal growth and that a complex interplay between maternal nutrient status and fetal growth is tightly regulated through molecular mediators that may be altered in human pregnancy pathologies.

## **6. Sex Dependent Regulation of Fetal Programming**

A discussion about the regulators of fetal growth would be incomplete without considering fetal sex. In 2007, a review by Di Renzo and colleagues identified male sex as an independent risk factor for adverse pregnancy outcomes, including a higher rate of preterm birth, gestational diabetes mellitus (GDM), and macrosomia, with evidence suggesting that females have a better outcome in the perinatal period, particularly after preterm birth [146]. In 2010, David Barker's lab proposed that "boys live dangerously in the womb", due to their risky growth strategy, characterized by a quicker rate of fetal growth with less investment in placental growth, thus increasing their vulnerability to under-nutrition [147]. These differences in growth and survival suggest that male and female fetuses may not have identical responses to intrauterine stressors, and evidence suggests that this differential susceptibility to fetal programming insults is potentially mediated by sex specific differences in the placenta. In fact, adverse conditions in pregnancy appear to have a sexually dimorphic effect on fetal outcomes, with greater placental adaptation observed in female offspring [148]. Indeed, the Dutch Famine is associated with changes in placental size and later risk for hypertension in men, but not in women [149]. Sexual dimorphism in the human placenta has been noted in cytokine expression, the insulin-like growth factor pathways, and the response to cortisol in relation to asthma during pregnancy (reviewed by Clifton 2010) [124], and recently in preeclampsia, with significantly higher pro-inflammatory cytokine production and apoptosis in the male placentas [125]. In rabbits, a high fat diet compared to a control diet during gestation induced sex specific adaptations in the placenta, including fatty acid accumulation in the female placenta thus protecting the fetus from dyslipidemia, and a down-regulation of the gene LXR $\alpha$  (liver X receptor; involved in cholesterol exchange) in male placentas [150]. In a primate model (baboons), maternal nutrient restriction during gestation did not result in a synchronized molecular response in the placenta when fetal sex was not accounted for. However, when the sexes were treated as separate groups, the female placentas exhibited a highly coordinated response to the nutrient restriction which was absent in the males [151]. This adaptive response of the female placenta was also recently observed in humans. Walker and colleagues found a strong negative correlation between gestational weight gain and placental glucose uptake in the female placentas, but no significant relationship was observed in the males [152], suggesting that the female placentas were able to adapt to the excess gestational weight gain in order to optimize glucose supply to the fetus. It has been proposed that sex-specific placental adaptations attempt to cope with the same adverse maternal environment, thus underlying the importance of considering fetal sex when designing and analyzing placental tissue experiments [124].

## **7. Conclusions and Future Directions**

There are immediate and long-term health consequences associated with fetal under- and over-growth. The placenta plays a pivotal role in offspring growth and adequate nutrient transport is critical to support this development. A thorough understanding of nutrient transport is vital to elucidating the mechanisms contributing to altered fetal growth. In addition, having a greater understanding of placenta metabolism of glucose, lipids and amino acids with respect to obesity, excessive gestational weight gain, GDM and fetal overgrowth is an important area of study. While some pregnancy complications and nutrient transporters have been extensively studied, much of the research remains inconclusive regarding how certain transporters (*i.e.*, fatty acid transporters) are altered as a result of common pathologies (e.g., obesity). There are inconsistencies in the research findings concerning mechanisms of placenta transport in response to metabolic pathologies. We believe that these discrepant research findings are largely due to the heterogeneous methodology and populations explored (*i.e.*, the populations are not properly characterized).

With respect to GDM, the mode of treatment (diet controlled *vs.* insulin controlled) is an important consideration as the treatment likely influences the maternal glycemic control, and thus influences the severity of the insult to the intrauterine environment. For instance, as previously mentioned, in normal weight mothers with GDM, treatment affected the expression of GLUT1 [84]. Future studies in GDM pregnancies should consider the independent effect of diet and pharmacological (insulin or metformin) control of blood glucose, as different treatments modalities may influence the pathology. Moreover, it is also important to consider maternal BMI as the combination of GDM and obesity might alter the response [99]. Similarly, fetal birth weight is a crucial consideration when studying pregnancy pathologies that are related to fetal overgrowth (*i.e.*, diabetes and maternal obesity). For instance, if there is no difference in birth weight between the infants born to the lean and obese women, than it is important to consider the sample size of the population to ensure sufficient power to detect changes in addition to assessing for potential confounders including maternal behaviors (diet and physical activity) and clinical parameters such as severity of impaired glycemia and dyslipidemia. It is possible that if there are insufficient differences in the fetal outcomes (*i.e.*, birth weight), then there may not have been substantial variation in nutrient transport across the placenta. To appropriately identify contributors to altered fetal growth, it is likely necessary that differences in fetal growth exist between the populations being compared. In pregnancies complicated by maternal obesity, selecting only those with fetal macrosomia may be the ideal method to compare an obese to a lean pregnancy. Ensuring that the subject groups are sufficiently different with no other underlying pathology will ensure as representative a human model as possible.

Additionally, recent evidence suggests that male and female placentas might respond in different ways to an adverse intrauterine environment, and thus future research must consider sex-related differences of the infant when exploring changes in the placenta to avoid masking potential important

findings. It has been proposed that ignoring the sex of the placenta is no longer sound scientific practice [124] as one cannot assume that the male and female fetus and placenta respond to environmental and maternal insults in the same manner.

Furthermore, maternal dietary composition during pregnancy likely affects placental nutrient delivery to the fetus and thus warrants further exploration. Similarly, maternal energy balance is an important consideration. We propose that future work in humans should consider controlling for maternal energy balance, including precise quantification of caloric intake (quality and quantity of diet using diet record analysis), and directly measured physical activity (using accelerometers), so as not to confound the results and make appropriate recommendations based on properly phenotyped subjects. Indeed, Lewis *et al.* reported lower System A activity in women with lower arm muscle mass and those who reported strenuous exercise during pregnancy [153]. Equally important is gestational weight gain, a known contributor to fetal growth [154]. The total and rate of gestational weight gain should be accounted for in all work in which fetal growth is an important outcome as excessive gestational weight gain may have a greater influence on birth weight than an underlying pathology (*i.e.*, obesity) [155]. Moreover, the body composition of both mother and infant, and how it pertains to the outcomes of interest, should be considered. Overall, if maternal dietary intake, hormones/growth factors, physical activity and gestational weight gain are associated with fetal growth parameters all future human experimental trials examining placenta transport should undertake due diligence and best account for these confounders.

Overall, a better understanding of how the placenta responds to the altered maternal milieu will be especially important in today's transformed obesogenic environment, which includes a rise in maternal obesity, poor dietary quality, a lack of physical activity and the propensity for women to gain more than the recommended gestational weight gain independent of pregravid BMI. Further knowledge in this area may be the first step in the development of targeted interventions to help optimize fetal growth.

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### **Author Contributions**

K.E.B. is the main author and contributed substantially to the writing and revisions of the manuscript. Z.M.F. is the second major contributor to the writing and revisions of the manuscript. J.Y. created the figures and contributed to the written sections directly related to the figures. A.G. and K.B.A. were instrumental in revising the manuscript.

### **Conflicts of Interest**

The authors declare no conflict of interest.

## References

1. Otten, J.; Pitzel Hellig, J.; Meyers, L. *Dietary Reference Intakes: The Essential Guide to Nutrient Requirements*; National Academic Press: Washington, DC, USA, 2006.
2. Marconi, A.M.; Paolini, C.; Buscaglia, M.; Zerbe, G.; Battaglia, F.C.; Pardi, G. The impact of gestational age and fetal growth on the maternal–fetal glucose concentration difference. *Obstet. Gynecol.* **1996**, *87*, 937–942.
3. Baumann, M.U.; Deborde, S.; Illsley, N.P. Placental glucose transfer and fetal growth. *Endocrine* **2002**, *19*, 13–22.
4. Freinkel, N. Banting Lecture 1980. Of pregnancy and progeny. *Diabetes* **1980**, *29*, 1023–1035.
5. Poissonnet, C.M.; Burdi, A.R.; Garn, S.M. The chronology of adipose tissue appearance and distribution in the human fetus. *Early Hum. Dev.* **1984**, *10*, 1–11.
6. Haggarty, P. Fatty acid supply to the human fetus. *Annu. Rev. Nutr.* **2010**, *30*, 237–255.
7. Sparks, J.W.; Girard, J.R.; Battaglia, F.C. An estimate of the caloric requirements of the human fetus. *Biol. Neonate* **1980**, *38*, 113–119.
8. Gerretsen, G.; Huisjes, H.J.; Elema, J.D. Morphological changes of the spiral arteries in the placental bed in relation to pre-eclampsia and fetal growth retardation. *Br. J. Obstet. Gynaecol.* **1981**, *88*, 876–881.
9. Khong, T.Y.; de Wolf, W.B.; Robertson, W.B.; Brosens, I. Inadequate maternal vascular response to placentation in pregnancies complicated by pre-eclampsia and by small-for-gestational age infants. *Br. J. Obstet. Gynaecol.* **1986**, *93*, 1049–1059.
10. Clandinin, M.T.; Chappell, J.E.; Heim, T.; Swyer, P.R.; Chance, G.W. Fatty acid utilization in perinatal *de novo* synthesis of tissues. *Early Hum. Dev.* **1981**, *5*, 355–366.
11. Clandinin, M.T.; Chappell, J.E.; Heim, T.; Swyer, P.R.; Chance, G.W. Fatty acid accretion in fetal and neonatal liver: Implications for fatty acid requirements. *Early Hum. Dev.* **1981**, *5*, 7–14.
12. Hull, H.R.; Thornton, J.C.; Ji, Y.; Paley, C.; Rosenn, B.; Mathews, P.; Navder, K.; Yu, A.; Dorsey, K.; Gallagher, D. Higher infant body fat with excessive gestational weight gain in overweight women. *Am. J. Obstet. Gynecol.* **2011**, *205*, 211–217.
13. Sewell, M.F.; Huston-Presley, L.; Super, D.M.; Catalano, P. Increased neonatal fat mass, not lean body mass, is associated with maternal obesity. *Am. J. Obstet. Gynecol.* **2006**, *195*, 1100–1103.
14. Josefson, J.L.; Hoffmann, J.A.; Metzger, B.E. Excessive weight gain in women with a normal pre-pregnancy BMI is associated with increased neonatal adiposity. *Pediatr. Obes.* **2013**, *8*, e33–e36.
15. Jansson, T.; Illsley, N.P. Osmotic water permeabilities of human placental microvillous and basal membranes. *J. Membr. Biol.* **1993**, *132*, 147–155.
16. Johnson, L.W.; Smith, C.H. Glucose transport across the basal plasma membrane of human placental syncytiotrophoblast. *Biochim. Biophys. Acta* **1985**, *815*, 44–50.

17. Firth, J.A.; Leach, L. Not trophoblast alone: A review of the contribution of the fetal microvasculature to transplacental exchange. *Placenta* **1996**, *17*, 89–96.
18. Eaton, B.M.; Leach, L.; Firth, J.A. Permeability of the fetal villous microvasculature in the isolated perfused term human placenta. *J. Physiol.* **1993**, *463*, 141–155.
19. Jansson, T.; Myatt, L.; Powell, T.L. The role of trophoblast nutrient and ion transporters in the development of pregnancy complications and adult disease. *Curr. Vasc. Pharmacol.* **2009**, *7*, 521–533.
20. Jansson, T.; Powell, T.L. Role of placental nutrient sensing in developmental programming. *Clin. Obstet. Gynecol.* **2013**, *56*, 591–601.
21. Sibley, C.P.; Birdsey, T.J.; Brownbill, P.; Clarson, L.H.; Doughty, I.; Glazier, J.D.; Greenwood, S.L.; Hughes, J.; Jansson, T.; Mylona, P.; *et al.* Mechanisms of maternofetal exchange across the human placenta. *Biochem. Soc. Trans.* **1998**, *26*, 86–91.
22. Smith, C.H.; Moe, A.J.; Ganapathy, V. Nutrient transport pathways across the epithelium of the placenta. *Annu. Rev. Nutr.* **1992**, *12*, 183–206.
23. Lager, S.; Powell, T.L. Regulation of nutrient transport across the placenta. *J. Pregnancy* **2012**, *2012*, 179827.
24. Larque, E.; Ruiz-Palacios, M.; Koletzko, B. Placental regulation of fetal nutrient supply. *Curr. Opin. Clin. Nutr. Metab. Care* **2013**, *16*, 292–297.
25. Fowden, A.L.; Ward, J.W.; Wooding, F.P.; Forhead, A.J.; Constancia, M. Programming placental nutrient transport capacity. *J. Physiol.* **2006**, *572*, 5–15.
26. Higgins, L.; Greenwood, S.L.; Wareing, M.; Sibley, C.P.; Mills, T.A. Obesity and the placenta: A consideration of nutrient exchange mechanisms in relation to aberrant fetal growth. *Placenta* **2011**, *32*, 1–7.
27. Roland, M.C.; Friis, C.M.; Voldner, N.; Godang, K.; Bollerslev, J.; Haugen, G.; Henriksen, T. Fetal growth *versus* birthweight: The role of placenta *versus* other determinants. *PLoS One* **2012**, *7*, e39324.
28. Wallace, J.M.; Horgan, G.W.; Bhattacharya, S. Placental weight and efficiency in relation to maternal body mass index and the risk of pregnancy complications in women delivering singleton babies. *Placenta* **2012**, *33*, 611–618.
29. Naeye, R.L. Do placental weights have clinical significance? *Hum. Pathol.* **1987**, *18*, 387–391.
30. Wilson, M.E.; Ford, S.P. Comparative aspects of placental efficiency. *Reprod. Suppl.* **2001**, *58*, 223–332.
31. Fowden, A.L.; Sferruzzi-Perri, A.N.; Coan, P.M.; Constancia, M.; Burton, G.J. Placental efficiency and adaptation: Endocrine regulation. *J. Physiol.* **2009**, *587*, 3459–3472.
32. Salafia, C.M.; Zhang, J.; Miller, R.K.; Charles, A.K.; Shrout, P.; Sun, W. Placental growth patterns affect birth weight for given placental weight. *Birth Defects Res. A* **2007**, *79*, 281–288.

33. Jansson, N.; Pettersson, J.; Haafiz, A.; Ericsson, A.; Palmberg, I.; Tranberg, M.; Ganapathy, V.; Powell, T.L.; Jansson, T. Down-regulation of placental transport of amino acids precedes the development of intrauterine growth restriction in rats fed a low protein diet. *J. Physiol.* **2006**, *576*, 935–946.
34. Jansson, T.; Cetin, I.; Powell, T.L.; Desoye, G.; Radaelli, T.; Ericsson, A.; Sibley, C.P. Placental transport and metabolism in fetal overgrowth—A workshop report. *Placenta* **2006**, *27*, S109–S113.
35. Johansson, M.; Karlsson, L.; Wennergren, M.; Jansson, T.; Powell, T.L. Activity and protein expression of Na<sup>+</sup>/K<sup>+</sup> ATPase are reduced in microvillous syncytiotrophoblast plasma membranes isolated from pregnancies complicated by intrauterine growth restriction. *J. Clin. Endocrinol. Metab.* **2003**, *88*, 2831–2837.
36. Jansson, T.; Powell, T.L. IFPA 2005 Award in Placentology Lecture. Human placental transport in altered fetal growth: Does the placenta function as a nutrient sensor?—A review. *Placenta* **2006**, *27*, S91–S97.
37. Malandro, M.S.; Beveridge, M.J.; Kilberg, M.S.; Novak, D.A. Effect of low-protein diet-induced intrauterine growth retardation on rat placental amino acid transport. *Am. J. Physiol.* **1996**, *271*, C295–C303.
38. Godfrey, K.M.; Matthews, N.; Glazier, J.; Jackson, A.; Wilman, C.; Sibley, C.P. Neutral amino acid uptake by the microvillous plasma membrane of the human placenta is inversely related to fetal size at birth in normal pregnancy. *J. Clin. Endocrinol. Metab.* **1998**, *83*, 3320–3326.
39. Ogura, K.; Sakata, M.; Yamaguchi, M.; Kurachi, H.; Murata, Y. High concentration of glucose decreases glucose transporter-1 expression in mouse placenta *in vitro* and *in vivo*. *J. Endocrinol.* **1999**, *160*, 443–452.
40. Jones, H.N.; Powell, T.L.; Jansson, T. Regulation of placental nutrient transport—A review. *Placenta* **2007**, *28*, 763–774.
41. Kalhan, S.; Parimi, P. Gluconeogenesis in the fetus and neonate. *Semin. Perinatol.* **2000**, *24*, 94–106.
42. Illsley, N.P. Glucose transporters in the human placenta. *Placenta* **2000**, *21*, 14–22.
43. Jansson, T.; Wennergren, M.; Illsley, N.P. Glucose transporter protein expression in human placenta throughout gestation and in intrauterine growth retardation. *J. Clin. Endocrinol. Metab.* **1993**, *77*, 1554–1562.
44. Chiesa, C.; Osborn, J.F.; Haass, C.; Natale, F.; Spinelli, M.; Scapillati, E.; Spinelli, A.; Pacifico, L. Ghrelin, leptin, IGF-1, IGFBP-3, and insulin concentrations at birth: Is there a relationship with fetal growth and neonatal anthropometry? *Clin. Chem.* **2008**, *54*, 550–558.
45. Baumann, M.U.; Schneider, H.; Malek, A.; Palta, V.; Surbek, D.V.; Sager, R.; Zamudio, S.; Illsley, N.P. Regulation of human trophoblast GLUT1 glucose transporter by insulin-like growth factor I (IGF-I). *PLoS One* **2014**, *9*, e106037.
46. Brown, K.; Heller, D.S.; Zamudio, S.; Illsley, N.P. Glucose transporter 3 (GLUT3) protein expression in human placenta across gestation. *Placenta* **2011**, *32*, 1041–1049.

47. Ericsson, A.; Hamark, B.; Powell, T.L.; Jansson, T. Glucose transporter isoform 4 is expressed in the syncytiotrophoblast of first trimester human placenta. *Hum. Reprod.* **2005**, *20*, 521–530.
48. Cetin, I.; Marconi, A.M.; Corbetta, C.; Lanfranchi, A.; Baggiani, A.M.; Battaglia, F.C.; Pardi, G. Fetal amino acids in normal pregnancies and in pregnancies complicated by intrauterine growth retardation. *Early Hum. Dev.* **1992**, *29*, 183–186.
49. Jansson, T. Amino acid transporters in the human placenta. *Pediatr. Res.* **2001**, *49*, 141–147.
50. Hoeltzli, S.D.; Smith, C.H. Alanine transport systems in isolated basal plasma membrane of human placenta. *Am. J. Physiol.* **1989**, *256*, C630–C637.
51. Desforges, M.; Mynett, K.J.; Jones, R.L.; Greenwood, S.L.; Westwood, M.; Sibley, C.P.; Glazier, J.D. The SNAT4 isoform of the system A amino acid transporter is functional in human placental microvillous plasma membrane. *J. Physiol.* **2009**, *587*, 61–72.
52. Jansson, N.; Greenwood, S.L.; Johansson, B.R.; Powell, T.L.; Jansson, T. Leptin stimulates the activity of the system A amino acid transporter in human placental villous fragments. *J. Clin. Endocrinol. Metab.* **2003**, *88*, 1205–1211.
53. Jones, H.N.; Jansson, T.; Powell, T.L. IL-6 stimulates system A amino acid transporter activity in trophoblast cells through STAT3 and increased expression of SNAT2. *Am. J. Physiol. Cell Physiol.* **2009**, *297*, C1228–C1235.
54. Roos, S.; Lagerlof, O.; Wennergren, M.; Powell, T.L.; Jansson, T. Regulation of amino acid transporters by glucose and growth factors in cultured primary human trophoblast cells is mediated by mTOR signaling. *Am. J. Physiol. Cell Physiol.* **2009**, *297*, C723–C731.
55. Verrey, F.; System, L. Heteromeric exchangers of large, neutral amino acids involved in directional transport. *Pflugers Arch.* **2003**, *445*, 529–533.
56. Cleal, J.K.; Glazier, J.D.; Ntani, G.; Crozier, S.R.; Day, P.E.; Harvey, N.C.; Robinson, S.M.; Cooper, C.; Godfrey, K.M.; Hanson, M.A. Facilitated transporters mediate net efflux of amino acids to the fetus across the basal membrane of the placental syncytiotrophoblast. *J. Physiol.* **2011**, *589*, 987–997.
57. Kudo, Y.; Boyd, C.A. Characterisation of L-tryptophan transporters in human placenta: A comparison of brush border and basal membrane vesicles. *J. Physiol.* **2001**, *531*, 405–416.
58. Glazier, J.D.; Cetin, I.; Perugino, G.; Ronzoni, S.; Grey, A.M.; Mahendran, D.; Marconi, A.M.; Pardi, G.; Sibley, C.P. Association between the activity of the system A amino acid transporter in the microvillous plasma membrane of the human placenta and severity of fetal compromise in intrauterine growth restriction. *Pediatr. Res.* **1997**, *42*, 514–519.
59. King, J.C. Maternal obesity, metabolism, and pregnancy outcomes. *Annu. Rev. Nutr.* **2006**, *26*, 271–291.
60. Duttaroy, A.K. Transport of fatty acids across the human placenta: A review. *Prog. Lipid Res.* **2009**, *48*, 52–61.

61. Jaye, M.; Lynch, K.J.; Krawiec, J.; Marchadier, D.; Maugeais, C.; Doan, K.; South, V.; Amin, D.; Perrone, M.; Rader, D.J. A novel endothelial-derived lipase that modulates HDL metabolism. *Nat. Genet.* **1999**, *21*, 424–428.
62. Lindegaard, M.L.; Olivecrona, G.; Christoffersen, C.; Kratky, D.; Hannibal, J.; Petersen, B.L.; Zechner, R.; Damm, P.; Nielsen, L.B. Endothelial and lipoprotein lipases in human and mouse placenta. *J. Lipid Res.* **2005**, *46*, 2339–2346.
63. Waterman, I.J.; Emmison, N.; Dutta-Roy, A.K. Characterisation of triacylglycerol hydrolase activities in human placenta. *Biochim. Biophys. Acta* **1998**, *1394*, 169–176.
64. McCoy, M.G.; Sun, G.S.; Marchadier, D.; Maugeais, C.; Glick, J.M.; Rader, D.J. Characterization of the lipolytic activity of endothelial lipase. *J. Lipid Res.* **2002**, *43*, 921–999.
65. Kazantzis, M.; Stahl, A. Fatty acid transport proteins, implications in physiology and disease. *Biochim. Biophys. Acta* **2012**, *1821*, 852–857.
66. Schaiff, W.T.; Bildirici, I.; Cheong, M.; Chern, P.L.; Nelson, D.M.; Sadovsky, Y. Peroxisome proliferator-activated receptor-gamma and retinoid X receptor signaling regulate fatty acid uptake by primary human placental trophoblasts. *J. Clin. Endocrinol. Metab.* **2005**, *90*, 4267–4275.
67. Larque, E.; Demmelmair, H.; Klingler, M.; de Jonge, S.; Bondy, B.; Koletzko, B. Expression pattern of fatty acid transport protein-1 (FATP-1), FATP-4 and heart-fatty acid binding protein (H-FABP) genes in human term placenta. *Early Hum. Dev.* **2006**, *82*, 697–701.
68. Campbell, F.M.; Bush, P.G.; Veerkamp, J.H.; Dutta-Roy, A.K. Detection and cellular localization of plasma membrane-associated and cytoplasmic fatty acid-binding proteins in human placenta. *Placenta* **1998**, *19*, 409–415.
69. Cunningham, P.; McDermott, L. Long chain PUFA transport in human term placenta. *J. Nutr.* **2009**, *139*, 636–639.
70. Mousiolis, A.V.; Kollia, P.; Skentou, C.; Messinis, I.E. Effects of leptin on the expression of fatty acid-binding proteins in human placental cell cultures. *Mol. Med. Rep.* **2012**, *5*, 497–502.
71. Magnusson-Olsson, A.L.; Hamark, B.; Ericsson, A.; Wennergren, M.; Jansson, T.; Powell, T.L. Gestational and hormonal regulation of human placental lipoprotein lipase. *J. Lipid Res.* **2006**, *47*, 2551–2561.
72. Lager, S.; Jansson, N.; Olsson, A.L.; Wennergren, M.; Jansson, T.; Powell, T.L. Effect of IL-6 and TNF- $\alpha$  on fatty acid uptake in cultured human primary trophoblast cells. *Placenta* **2011**, *32*, 121–127.
73. Herrera, E. Lipid metabolism in pregnancy and its consequences in the fetus and newborn. *Endocrine* **2002**, *19*, 43–55.
74. Woollett, L.A. Review: Transport of maternal cholesterol to the fetal circulation. *Placenta* **2011**, *32*, S218–S221.
75. Wittmaack, F.M.; Gafvels, M.E.; Bronner, M.; Matsuo, H.; McCrae, K.R.; Tomaszewski, J.E.; Robinson, S.L.; Strickland, D.K.; Strauss, J.F., 3rd. Localization and regulation of the human very

- low density lipoprotein/apolipoprotein-E receptor: Trophoblast expression predicts a role for the receptor in placental lipid transport. *Endocrinology* **1995**, *136*, 340–348.
76. Furuhashi, M.; Seo, H.; Mizutani, S.; Narita, O.; Tomoda, Y.; Matsui, N. Expression of low density lipoprotein receptor gene in human placenta during pregnancy. *Mol. Endocrinol.* **1989**, *3*, 1252–1256.
77. Wadsack, C.; Hammer, A.; Levak-Frank, S.; Desoye, G.; Kozarsky, K.F.; Hirschmugl, B.; Sattler, W.; Malle, E. Selective cholesteryl ester uptake from high density lipoprotein by human first trimester and term villous trophoblast cells. *Placenta* **2003**, *24*, 131–143.
78. Stefulj, J.; Panzenboeck, U.; Becker, T.; Hirschmugl, B.; Schweinzer, C.; Lang, I.; Marsche, G.; Sadjak, A.; Lang, U.; Desoye, G.; *et al.* Human endothelial cells of the placental barrier efficiently deliver cholesterol to the fetal circulation via ABCA1 and ABCG1. *Circ. Res.* **2009**, *104*, 600–608.
79. Aye, I.L.; Waddell, B.J.; Mark, P.J.; Keelan, J.A. Placental ABCA1 and ABCG1 transporters efflux cholesterol and protect trophoblasts from oxysterol induced toxicity. *Biochim. Biophys. Acta* **2010**, *1801*, 1013–1024.
80. Nikitina, L.; Wenger, F.; Baumann, M.; Surbek, D.; Korner, M.; Albrecht, C. Expression and localization pattern of ABCA1 in diverse human placental primary cells and tissues. *Placenta* **2011**, *32*, 420–430.
81. Jansson, T.; Ylven, K.; Wennergren, M.; Powell, T.L. Glucose transport and system A activity in syncytiotrophoblast microvillous and basal plasma membranes in intrauterine growth restriction. *Placenta* **2002**, *23*, 392–399.
82. Jansson, T.; Wennergren, M.; Powell, T.L. Placental glucose transport and GLUT 1 expression in insulin-dependent diabetes. *Am. J. Obstet. Gynecol.* **1999**, *180*, 163–168.
83. Jansson, T.; Ekstrand, Y.; Wennergren, M.; Powell, T.L. Placental glucose transport in gestational diabetes mellitus. *Am. J. Obstet. Gynecol.* **2001**, *184*, 111–116.
84. Colomiere, M.; Permezal, M.; Riley, C.; Desoye, G.; Lappas, M. Defective insulin signaling in placenta from pregnancies complicated by gestational diabetes mellitus. *Eur. J. Endocrinol.* **2009**, *160*, 567–578.
85. Janzen, C.; Lei, M.Y.; Cho, J.; Sullivan, P.; Shin, B.C.; Devaskar, S.U. Placental glucose transporter 3 (GLUT3) is up-regulated in human pregnancies complicated by late-onset intrauterine growth restriction. *Placenta* **2013**, *34*, 1072–1078.
86. Mahendran, D.; Donnai, P.; Glazier, J.D.; D'Souza, S.W.; Boyd, R.D.; Sibley, C.P. Amino acid (system A) transporter activity in microvillous membrane vesicles from the placentas of appropriate and small for gestational age babies. *Pediatr. Res.* **1993**, *34*, 661–665.
87. Kuruvilla, A.G.; D'Souza, S.W.; Glazier, J.D.; Mahendran, D.; Maresh, M.J.; Sibley, C.P. Altered activity of the system A amino acid transporter in microvillous membrane vesicles from placentas of macrosomic babies born to diabetic women. *J. Clin. Investig.* **1994**, *94*, 689–695.

88. Jansson, T.; Ekstrand, Y.; Bjorn, C.; Wennergren, M.; Powell, T.L. Alterations in the activity of placental amino acid transporters in pregnancies complicated by diabetes. *Diabetes* **2002**, *51*, 2214–2219.
89. Farley, D.M.; Choi, J.; Dudley, D.J.; Li, C.; Jenkins, S.L.; Myatt, L.; Nathanielsz, P.W. Placental amino acid transport and placental leptin resistance in pregnancies complicated by maternal obesity. *Placenta* **2010**, *31*, 718–724.
90. Jansson, N.; Rosario, F.J.; Gaccioli, F.; Lager, S.; Jones, H.N.; Roos, S.; Jansson, T.; Powell, T.L. Activation of placental mTOR signaling and amino acid transporters in obese women giving birth to large babies. *J. Clin. Endocrinol. Metab.* **2013**, *98*, 105–113.
91. Jansson, T.; Scholtbach, V.; Powell, T.L. Placental transport of leucine and lysine is reduced in intrauterine growth restriction. *Pediatr. Res.* **1998**, *44*, 532–537.
92. Magnusson, A.L.; Waterman, I.J.; Wennergren, M.; Jansson, T.; Powell, T.L. Triglyceride hydrolase activities and expression of fatty acid binding proteins in the human placenta in pregnancies complicated by intrauterine growth restriction and diabetes. *J. Clin. Endocrinol. Metab.* **2004**, *89*, 4607–4614.
93. Gauster, M.; Hiden, U.; Blaschitz, A.; Frank, S.; Lang, U.; Alvino, G.; Cetin, I.; Desoye, G.; Wadsack, C. Dysregulation of placental endothelial lipase and lipoprotein lipase in intrauterine growth-restricted pregnancies. *J. Clin. Endocrinol. Metab.* **2007**, *92*, 2256–2263.
94. Radaelli, T.; Lepercq, J.; Varastehpour, A.; Basu, S.; Catalano, P.M.; Hauguel-de, M.S. Differential regulation of genes for fetoplacental lipid pathways in pregnancy with gestational and type 1 diabetes mellitus. *Am. J. Obstet. Gynecol.* **2009**, *201*, 209.
95. Dube, E.; Gravel, A.; Martin, C.; Desparois, G.; Moussa, I.; Ethier-Chiasson, M.; Forest, J.C.; Giguère, Y.; Masse, A.; Lafond, J. Modulation of fatty acid transport and metabolism by maternal obesity in the human full-term placenta. *Biol. Reprod.* **2012**, *87*, 14, 1–11.
96. Brass, E.; Hanson, E.; O'Tierney-Ginn, P.F. Placental oleic acid uptake is lower in male offspring of obese women. *Placenta* **2013**, *34*, 503–509.
97. Lindegaard, M.L.; Damm, P.; Mathiesen, E.R.; Nielsen, L.B. Placental triglyceride accumulation in maternal type 1 diabetes is associated with increased lipase gene expression. *J. Lipid Res.* **2006**, *47*, 2581–2588.
98. Gauster, M.; Hiden, U.; van Poppel, M.; Frank, S.; Wadsack, C.; Hauguel-de Mouzon, S.; Desoye, G. Dysregulation of placental endothelial lipase in obese women with gestational diabetes mellitus. *Diabetes* **2011**, *60*, 2457–2464.
99. Scifres, C.M.; Chen, B.; Nelson, D.M.; Sadovsky, Y. Fatty acid binding protein 4 regulates intracellular lipid accumulation in human trophoblasts. *J. Clin. Endocrinol. Metab.* **2011**, *96*, E1083–E1091.

100. Coan, P.M.; Vaughan, O.R.; Sekita, Y.; Finn, S.L.; Burton, G.J.; Constancia, M.; Fowden, A.L. Adaptations in placental phenotype support fetal growth during undernutrition of pregnant mice. *J. Physiol.* **2010**, *588*, 527–538.
101. Ma, Y.; Zhu, M.J.; Uthlaut, A.B.; Nijland, M.J.; Nathanielsz, P.W.; Hess, B.W.; Ford, S.P. Upregulation of growth signaling and nutrient transporters in cotyledons of early to mid-gestational nutrient restricted ewes. *Placenta* **2011**, *32*, 255–263.
102. Kavitha, J.V.; Rosario, F.J.; Nijland, M.J.; McDonald, T.J.; Wu, G.; Kanai, Y.; Powell, T.L.; Nathanielsz, P.W.; Jansson, T. Down-regulation of placental mTOR, insulin/IGF-I signaling, and nutrient transporters in response to maternal nutrient restriction in the baboon. *FASEB J.* **2014**, *28*, 1294–1305.
103. Jones, H.N.; Woollett, L.A.; Barbour, N.; Prasad, P.D.; Powell, T.L.; Jansson, T. High-fat diet before and during pregnancy causes marked up-regulation of placental nutrient transport and fetal overgrowth in C57/BL6 mice. *FASEB J.* **2009**, *23*, 271–278.
104. Sferruzzi-Perri, A.N.; Vaughan, O.R.; Haro, M.; Cooper, W.N.; Musial, B.; Charalambous, M.; Pestana, D.; Ayyar, S.; Ferguson-Smith, A.C.; *et al.* An obesogenic diet during mouse pregnancy modifies maternal nutrient partitioning and the fetal growth trajectory. *FASEB J.* **2013**, *27*, 3928–3937.
105. King, V.; Hibbert, N.; Seckl, J.R.; Norman, J.E.; Drake, A.J. The effects of an obesogenic diet during pregnancy on fetal growth and placental gene expression are gestation dependent. *Placenta* **2013**, *34*, 1087–1090.
106. Prada, J.A.; Tsang, R.C. Biological mechanisms of environmentally induced causes of IUGR. *Eur. J. Clin. Nutr.* **1998**, *52*, S21–S27.
107. Radulescu, L.; Munteanu, O.; Popa, F.; Cirstoiu, M. The implications and consequences of maternal obesity on fetal intrauterine growth restriction. *J. Med. Life* **2013**, *6*, 292–298.
108. Rajasingam, D.; Seed, P.T.; Briley, A.L.; Shennan, A.H.; Poston, L. A prospective study of pregnancy outcome and biomarkers of oxidative stress in nulliparous obese women. *Am. J. Obstet. Gynecol.* **2009**, *200*, 395–399.
109. Perlow, J.H.; Morgan, M.A.; Montgomery, D.; Towers, C.V.; Porto, M. Perinatal outcome in pregnancy complicated by massive obesity. *Am. J. Obstet. Gynecol.* **1992**, *167*, 958–962.
110. Sibley, C.P.; Turner, M.A.; Cetin, I.; Ayuk, P.; Boyd, C.A.; D’Souza, S.W.; Glazier, J.D.; Greenwood, S.L.; Jansson, T.; Powell, T. Placental phenotypes of intrauterine growth. *Pediatr. Res.* **2005**, *58*, 827–832.
111. Sibley, C.P. Understanding placental nutrient transfer—Why bother? New biomarkers of fetal growth. *J. Physiol.* **2009**, *587*, 3431–3440.
112. Norberg, S.; Powell, T.L.; Jansson, T. Intrauterine growth restriction is associated with a reduced activity of placental taurine transporters. *Pediatr. Res.* **1998**, *44*, 233–238.

113. Wadsack, C.; Tabano, S.; Maier, A.; Hiden, U.; Alvino, G.; Cozzi, V.; Hüttinger, M.; Schneider, W.J.; Lang, U.; Cetin, I.; *et al.* Intrauterine growth restriction is associated with alterations in placental lipoprotein receptors and maternal lipoprotein composition. *Am. J. Physiol. Endocrinol. Metab.* **2007**, *292*, E476–E484.
114. Roseboom, T.J.; Painter, R.C.; de Rooij, S.R.; van Abeelen, A.F.; Veenendaal, M.V.; Osmond, C.; Barker, D.J. Effects of famine on placental size and efficiency. *Placenta* **2011**, *32*, 395–399.
115. Ford, S.P.; Hess, B.W.; Schwoppe, M.M.; Nijland, M.J.; Gilbert, J.S.; Vonnahme, K.A.; Means, W.J.; Han, H.; Nathanielsz, P.W. Maternal undernutrition during early to mid-gestation in the ewe results in altered growth, adiposity, and glucose tolerance in male offspring. *J. Anim. Sci.* **2007**, *85*, 1285–1294.
116. Catalano, P.M.; Presley, L.; Minium, J.; Hauguel-de, M.S. Fetuses of obese mothers develop insulin resistance in utero. *Diabetes Care* **2009**, *32*, 1076–1080.
117. Luo, Z.C.; Nuyt, A.M.; Delvin, E.; Audibert, F.; Girard, I.; Shatenstein, B.; Cloutier, A.; Cousineau, J.; Djemli, A.; Deal, C.; *et al.* Maternal and fetal IGF-I and IGF-II levels, fetal growth, and gestational diabetes. *J. Clin. Endocrinol. Metab.* **2012**, *97*, 1720–1728.
118. Dube, E.; Ethier-Chiasson, M.; Lafond, J. Modulation of cholesterol transport by insulin-treated gestational diabetes mellitus in human full term placenta. *Biol. Reprod.* **2013**, *88*, 16.
119. Kuhl, C. Etiology and pathogenesis of gestational diabetes. *Diabetes Care.* **1998**, *21*, B19–B26.
120. Hull, H.R.; Dinger, M.K.; Knehans, A.W.; Thompson, D.M.; Fields, D.A. Impact of maternal body mass index on neonate birthweight and body composition. *Am. J. Obstet. Gynecol.* **2008**, *198*, 416.
121. Visiedo, F.; Bugatto, F.; Sanchez, V.; Cozar-Castellano, I.; Bartha, J.L.; Perdomo, G. High glucose levels reduce fatty acid oxidation and increase triglyceride accumulation in human placenta. *Am. J. Physiol. Endocrinol. Metab.* **2013**, *305*, E205–E212.
122. Desforges, M.; Greenwood, S.L.; Glazier, J.D.; Westwood, M.; Sibley, C.P. The contribution of SNAT1 to system A amino acid transporter activity in human placental trophoblast. *Biochem. Biophys. Res. Commun.* **2010**, *398*, 130–134.
123. Zhu, M.J.; Ma, Y.; Long, N.M.; Du, M.; Ford, S.P. Maternal obesity markedly increases placental fatty acid transporter expression and fetal blood triglycerides at midgestation in the ewe. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **2010**, *299*, R1224–R1231.
124. Clifton, V.L. Review: Sex and the human placenta: Mediating differential strategies of fetal growth and survival. *Placenta* **2010**, *31*, S33–S39.
125. Muralimanoharan, S.; Maloyan, A.; Myatt, L. Evidence of sexual dimorphism in the placental function with severe preeclampsia. *Placenta* **2013**, *34*, 1183–1189.
126. Lin, Y.; Zhuo, Y.; Fang, Z.F.; Che, L.Q.; Wu, D. Effect of maternal dietary energy types on placenta nutrient transporter gene expressions and intrauterine fetal growth in rats. *Nutrition* **2012**, *28*, 1037–1043.

127. Roos, S.; Powell, T.L.; Jansson, T. Placental mTOR links maternal nutrient availability to fetal growth. *Biochem. Soc. Trans.* **2009**, *37*, 295–298.
128. Laplante, M.; Sabatini, D.M. mTOR signaling in growth control and disease. *Cell* **2012**, *149*, 274–293.
129. Jones, H.N.; Jansson, T.; Powell, T.L. Full-length adiponectin attenuates insulin signaling and inhibits insulin-stimulated amino acid transport in human primary trophoblast cells. *Diabetes* **2010**, *59*, 1161–1170.
130. Von Versen-Hoyneck, F.; Rajakumar, A.; Parrott, M.S.; Powers, R.W. Leptin affects system A amino acid transport activity in the human placenta: Evidence for STAT3 dependent mechanisms. *Placenta* **2009**, *30*, 361–367.
131. Nelson, D.M.; Smith, S.D.; Furesz, T.C.; Sadovsky, Y.; Ganapathy, V.; Parvin, C.A.; Smith, C.H. Hypoxia reduces expression and function of system A amino acid transporters in cultured term human trophoblasts. *Am. J. Physiol. Cell Physiol.* **2003**, *284*, C310–C315.
132. Rosario, F.J.; Schumacher, M.A.; Jiang, J.; Kanai, Y.; Powell, T.L.; Jansson, T. Chronic maternal infusion of full-length adiponectin in pregnant mice down-regulates placental amino acid transporter activity and expression and decreases fetal growth. *J. Physiol.* **2012**, *590*, 1495–1509.
133. Inoki, K.; Zhu, T.; Guan, K.L. TSC2 mediates cellular energy response to control cell growth and survival. *Cell* **2003**, *115*, 577–590.
134. Xu, J.; Ji, J.; Yan, X.H. Cross-talk between AMPK and mTOR in regulating energy balance. *Crit. Rev. Food Sci. Nutr.* **2012**, *52*, 373–381.
135. Liu, X.; Chhipa, R.R.; Pooya, S.; Wortman, M.; Yachyshin, S.; Chow, L.M.; Kumar, A.; Zhou, X.; Sun, Y.; Quinn, B.; *et al.* Discrete mechanisms of mTOR and cell cycle regulation by AMPK agonists independent of AMPK. *Proc. Natl. Acad. Sci. USA* **2014**, *111*, E435–E444.
136. Roos, S.; Jansson, N.; Palmberg, I.; Saljo, K.; Powell, T.L.; Jansson, T. Mammalian target of rapamycin in the human placenta regulates leucine transport and is down-regulated in restricted fetal growth. *J. Physiol.* **2007**, *582*, 449–459.
137. Roos, S.; Kanai, Y.; Prasad, P.D.; Powell, T.L.; Jansson, T. Regulation of placental amino acid transporter activity by mammalian target of rapamycin. *Am. J. Physiol. Cell Physiol.* **2009**, *296*, C142–C150.
138. Rosario, F.J.; Kanai, Y.; Powell, T.L.; Jansson, T. Mammalian target of rapamycin signalling modulates amino acid uptake by regulating transporter cell surface abundance in primary human trophoblast cells. *J. Physiol.* **2013**, *591*, 609–625.
139. Proud, C.G. Amino acids and mTOR signalling in anabolic function. *Biochem. Soc. Trans.* **2007**, *35*, 1187–1190.
140. Fang, J.; Mao, D.; Smith, C.H.; Fant, M.E. IGF regulation of neutral amino acid transport in the BeWo choriocarcinoma cell line (b30 clone): Evidence for MAP kinase-dependent and MAP kinase-independent mechanisms. *Growth Horm. IGF Res.* **2006**, *16*, 318–325.

141. Yung, H.W.; Calabrese, S.; Hynx, D.; Hemmings, B.A.; Cetin, I.; Charnock-Jones, D.S.; Burton, G.J. Evidence of placental translation inhibition and endoplasmic reticulum stress in the etiology of human intrauterine growth restriction. *Am. J. Pathol.* **2008**, *173*, 451–462.
142. Rosario, F.J.; Jansson, N.; Kanai, Y.; Prasad, P.D.; Powell, T.L.; Jansson, T. Maternal protein restriction in the rat inhibits placental insulin, mTOR, and STAT3 signaling and down-regulates placental amino acid transporters. *Endocrinology* **2011**, *152*, 1119–1129.
143. Gaccioli, F.; White, V.; Capobianco, E.; Powell, T.L.; Jawerbaum, A.; Jansson, T. Maternal overweight induced by a diet with high content of saturated fat activates placental mTOR and eIF2 alpha signaling and increases fetal growth in rats. *Biol. Reprod.* **2013**, *89*, 96.
144. Zhu, M.J.; Du, M.; Nijland, M.J.; Nathanielsz, P.W.; Hess, B.W.; Moss, G.E.; Ford, S.P. Down-regulation of growth signaling pathways linked to a reduced cotyledonary vascularity in placentomes of over-nourished, obese pregnant ewes. *Placenta* **2009**, *30*, 405–410.
145. Wullschleger, S.; Loewith, R.; Hall, M.N. TOR signaling in growth and metabolism. *Cell* **2006**, *124*, 471–484.
146. Di Renzo, G.C.; Rosati, A.; Sarti, R.D.; Cruciani, L.; Cutuli, A.M. Does fetal sex affect pregnancy outcome? *Gend. Med.* **2007**, *4*, 19–30.
147. Eriksson, J.G.; Kajantie, E.; Osmond, C.; Thornburg, K.; Barker, D.J. Boys live dangerously in the womb. *Am. J. Hum. Biol.* **2010**, *22*, 330–335.
148. Aiken, C.E.; Ozanne, S.E. Sex differences in developmental programming models. *Reproduction* **2013**, *145*, R1–R13.
149. Van Abeelen, A.F.; de Rooij, S.R.; Osmond, C.; Painter, R.C.; Veenendaal, M.V.; Bossuyt, P.M.; Elias, S.G.; Grobbee, D.E.; van der Schouw, Y.T. The sex-specific effects of famine on the association between placental size and later hypertension. *Placenta* **2011**, *32*, 694–698.
150. Tarrade, A.; Rousseau-Ralliard, D.; Aubriere, M.C.; Peynot, N.; Dahirel, M.; Bertrand-Michel, J.; Aguirre-Lavin, T.; Morel, O.; Beaujean, N.; Duranthon, V.; *et al.* Sexual dimorphism of the fetoplacental phenotype in response to a high fat and control maternal diets in a rabbit model. *PLoS One* **2013**, *8*, e83458.
151. Cox, L.A.; Li, C.; Glenn, J.P.; Lange, K.; Spradling, K.D.; Nathanielsz, P.W.; Jansson, T. Expression of the placental transcriptome in maternal nutrient reduction in baboons is dependent on fetal sex. *J. Nutr.* **2013**, *143*, 1698–1708.
152. Walker, S.P.; Ugoni, A.M.; Lim, R.; Lappas, M. Inverse relationship between gestational weight gain and glucose uptake in human placenta from female foetuses. *Pediatr. Obes.* **2014**, *9*, e73–e76.
153. Lewis, R.M.; Greenwood, S.L.; Cleal, J.K.; Crozier, S.R.; Verrall, L.; Inskip, H.M.; Cameron, I.T.; Cooper, C.; Sibley, C.P.; Hanson, M.A.; *et al.* Maternal muscle mass may influence system A activity in human placenta. *Placenta* **2010**, *31*, 418–422.

154. Kim, S.Y.; Sharma, A.J.; Sappenfield, W.; Wilson, H.G.; Salihu, H.M. Association of maternal body mass index, excessive weight gain, and gestational diabetes mellitus with large-for-gestational-age births. *Obstet. Gynecol.* **2014**, *123*, 737–744.
155. Ferraro, Z.M.; Barrowman, N.; Prud'homme, D.; Walker, M.; Wen, S.W.; Rodger, M.; Adamo, K.B. Excessive gestational weight gain predicts large for gestational age neonates independent of maternal body mass Index. *J. Matern. Fetal Neonatal Med.* **2012**, *25*, 538–542.

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In summary, this review highlighted numerous gaps in the literature and important considerations for future research. For instance, the research on the effects of maternal obesity on all of the placental nutrient transporters is incomplete and inconclusive. In addition, only two studies were identified that accounted for modifiable maternal behaviours such as GWG [12] and physical activity [13], with no evidence concerning how maternal diet might influence these transporters in the human population. Maternal obesity, gestational weight gain, physical activity and diet during pregnancy are all factors that can alter maternal energy balance, thus potentially influencing the hormonal and substrate exposures in the intrauterine environment. The following sections will summarize the current literature on these topics with the emphasis on why it is believed that these factors should be considered in research on the placenta.

## **1.2 Excess Maternal body weight: Maternal obesity and gestational weight gain**

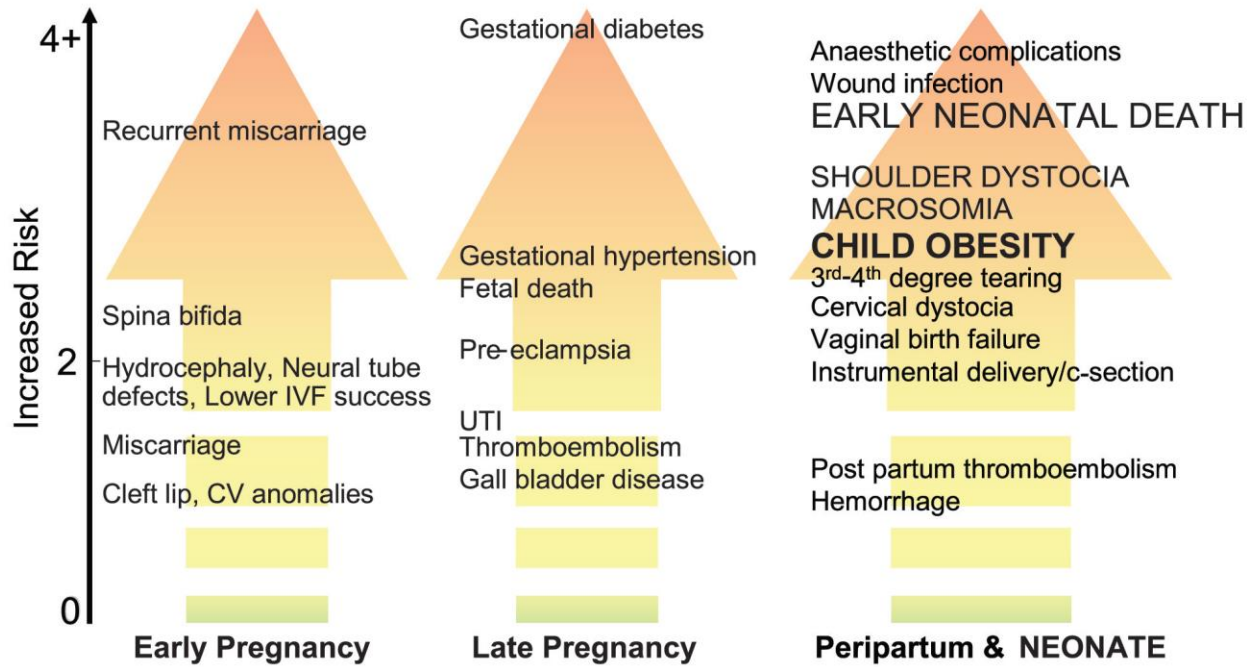
### ***1.2.1 Maternal Obesity***

It is well known that there has been a rise in obesity over the last few decades, and nearly two-thirds of North American women of child-bearing age are either overweight or obese [14]. This is a great concern to public health as there is a considerable amount of evidence linking maternal obesity to numerous adverse pregnancy complications as well as maternal and child health outcomes. These complications are summarised in Figure 1 and include increased risk of miscarriage, GDM, gestational hypertension, preeclampsia, instrumental/C-section delivery and fetal overgrowth (as reviewed by Adamo, Ferraro and Brett) [15,16]. Recently, in a cohort of Canadian women (n=1996), maternal obesity was associated with increased risk of preeclampsia (OR 5.3, 95% CI 3.3-8.5), gestational diabetes (OR 6.5, 95% CI 3.7-11.2) and delivery by C-section (OR 2.2, 95% CI 1.2-4.1) compared to normal weight women [17]. Given that birth weight is a surrogate marker for the health of the intrauterine

environment [18] , it is the association between maternal obesity and the increased risk of fetal overgrowth that is the most relevant to this thesis. Compared to normal weight women, the likelihood of delivering infants that are large-for-gestational-age (LGA) (birth weight >90th percentile) is significantly greater for women who are overweight (OR: 1.99, 95%CI: 1.17-3.37, p=0.01) or obese (OR: 2.64, 95%CI: 1.59-4.39, p<0.001) [19]. A recent systematic review and meta-analysis identified a significant role of obesity during pregnancy and the development of fetal overgrowth. Based on the variations in cut-points for fetal overgrowth, the authors found that maternal obesity was associated with greater odds of Class I macrosomia (birth weight 4000–4499 g) (OR 2.17, 95% CI 1.92-2.45), Class II macrosomia (4500–4999 g) (OR 2.77, 95% CI 2.22-3.45), and birth weight greater than the 90<sup>th</sup> percentile, corrected for gestational age (OR 2.42, 95% CI 2.16-2.72) [20]. In addition to being born larger, infants born to obese women have altered body composition (higher percentage of body fat mass and less lean mass), compared to average for gestational age infants born to normal weight women [21,22].

Unfortunately, infants born LGA predisposes the child to subsequent tracking of excess weight throughout the life-course [23,24], as overweight and obesity have been shown to track very closely from infancy to childhood and to adulthood [25,26]. For instance, a meta-analysis found that being born LGA or macrosomic (>4000g) significantly increased the risk for downstream obesity (OR 2.07, 95% CI: 1.91-2.24), and this relationship persisted from preschool until early adulthood [27]. Pregnancies complicated by obesity are thus thought to represent the start of the intergenerational cycle of obesity, as excess maternal body weight during pregnancy contributes to the greater likelihood that her child will develop obesity later in life [28].

**Figure 1.** Risks associated with pregnancies complicated by overweight or obesity. The x-axis shows the time course and the y-axis illustrates the degree of elevated risk for each outcome based on published literature (IVF = *in vitro* fertilization, CV = cardiovascular, UTI = urinary tract infection).



While there is plenty of epidemiological evidence linking maternal body weight to infant birth weight, there is limited knowledge of the mechanisms that contribute to these relationships. Pregnancies complicated by obesity are associated with increased growth and size of the placenta [29], and maternal hyperlipidemia and hyperinsulinemia [30,31]. Due to this increased availability of substrates in maternal circulation, the placenta is likely exposed to a greater supply of nutrients, namely glucose and free fatty acids, which may alter the transport of these nutrients across the placenta, thus leading to fetal overgrowth [30,32]. Given the impact of maternal obesity on fetal growth and pregnancy outcomes and the limited research on the effects of maternal obesity on placenta nutrient transport [33], it is believed that additional work is needed to determine how maternal BMI influences placental nutrient transport.

### ***1.2.2 Gestational Weight Gain***

Gestational weight gain (GWG) is also tightly linked to birth weight. The GWG guidelines are specific to pre-pregnancy BMI and include an absolute amount and a recommended rate of weight gain (Table 1) [34]. Women who are overweight or obese have a smaller recommended GWG than normal weight women, and due to the more conservative guidelines for women who are overweight or obese, it is easier for these women to exceed the guidelines. Regardless of pre-pregnancy BMI, exceeding the GWG guidelines also increases the risk of delivering a LGA infant [19]. This is concerning, given that as few as 25% of Canadian women meet GWG guidelines [19,35], and overweight/obese women are three times more likely to exceed guidelines compared to normal weight women [36]. Furthermore, women who exceed the GWG guidelines are at increased risk for numerous pregnancy related complications, including gestational diabetes, preeclampsia, increased likelihood of delivery via C-section, postpartum weight retention and an increased likelihood of entering a subsequent pregnancy at a

higher BMI [37-42]. Furthermore, a recent systematic review and meta-analysis demonstrated that exceeding GWG recommendations increases the risk of downstream child overweight/obesity by 40% [43]. Excess GWG is a modifiable risk factor with a high degree of inter-individual variability and it is evident that it is an important contributor to pregnancy outcomes. It is for this reason that it is believed that GWG, especially excess GWG, ought to be considered when studying nutrient transport across the placenta.

**Table 1. Gestational weight gain guidelines**

Pre-Pregnancy BMI	Mean rate of weight gain in the 2nd and 3rd trimester		Recommended total weight gain	
	kg/week	lb/week	kg	lbs
BMI < 18.5	0.5	1	12.5 - 18	28 – 40
BMI 18.5 - 24.9	0.4	1	11.5 - 16	25 - 35
BMI 25.0 - 29.9	0.3	0.6	7 – 11.5	15 - 25
BMI $\geq$ 30.0	0.2	0.5	5 - 9	11 - 20

### 1.3 Physical activity during pregnancy

#### *1.3.1 Benefits of Physical Activity during Pregnancy*

Physical activity (PA) is an important part of a healthy pregnancy for the mother and baby [44,45]. PA is an important mediator of weight maintenance, and in addition to a change in the amount of food intake, a change in PA from pre-pregnancy levels was identified as a predictor of excessive GWG [46]. Prenatal PA does not increase the risk of adverse maternal or neonatal outcomes [47-50],

however, it is still important to monitor the intensity and duration of exercise [51]. Most research indicates that moderate amounts of prenatal PA is safe and does not compromise fetal growth in mothers who do not present any contraindications for exercise [15]. An exercise program consisting of moderate intensity walking (3X/week) initiated between 13 and 20 weeks gestations in previously sedentary women improved maternal fitness level without affecting fetoplacental blood flow, risk of preeclampsia, or fetal growth (e.g. growth restriction, macrosomia, small- or large-for-gestational-age) compared to controls [52]. Most relevant to this thesis, is the finding from a large observational birth cohort (n= 79692) that indicated that participation in regular PA during pregnancy protects against birth weight extremes (i.e. small- and large-for-gestational-age), while increasing the likelihood of birthing an infant whose birth weight is appropriate for their gestational age [53]. In addition, vigorous PA in the first trimester was not detrimental to birth weight, and frequent vigorous PA (>4 times per week) reduced the risk of preterm birth [54]. Furthermore, prenatal PA is associated with reduced neonatal adiposity. In women who continued regular aerobic PA during pregnancy, there was a reduction in birth weight (-310g) compared to the offspring of non-exercising mothers, of which approximately 70% could be explained by a reduction in neonatal fat mass (-220g) [55]. The small reduction in birth weight (fat mass) without increasing the incidence of small-for-gestational-age infants suggests that prenatal exercise may help to optimise, rather than restrict fetal growth. Other benefits of PA include reduced risk of instrumental delivery (e.g. forceps, caesarean section) [56], reduced incidence of GDM [57-60] and preeclampsia [61], less weight gain [62,63], and improved labour tolerance [64]. Due to the known and suspected benefits of prenatal PA, it is often included as a major component in lifestyle interventions during pregnancy, such as those designed to reduce the risk of gestational diabetes or prevent excess GWG. There is still limited knowledge of the mechanisms through which PA impacts the intrauterine

environment and fetal growth, however, since PA affects maternal energy balance, it also likely impacts nutrient transport across the placenta and should be considered in future research on the placenta.

### ***1.3.2 Physical Activity and the placenta***

Although limited, there is evidence that prenatal PA alters placental form and function. Regular weight-bearing exercise (i.e. running) during pregnancy is associated with increased cell proliferation and a greater villous volume in the placenta compared to a non-exercising control population [65]. In response to prenatal PA, there is an increased functional capacity of the placenta via an increase in placental surface area, improvements in blood flow, and an enhanced-perfusion balance [48,66], which may be a compensatory mechanism to increase the delivery of nutrients and oxygen to sustain fetal growth. Furthermore, regular exercise throughout pregnancy is associated with increased parenchymal volume, villous surface area, and vascular volume which suggests increased placental perfusion and improved transport capability [67].

### ***1.3.3 Physical Activity Guidelines***

The current guidelines for PA during pregnancy state that pregnant women with no contraindication to exercise should accumulate 30 minutes or more of moderate to vigorous intensity exercise 4 days per week [49,68,69]. For previously sedentary women, only 3 days per week of moderate intensity PA is recommended with a gradual increase towards 30 minutes [49]. For overweight and obese women, it is recommended that they stay within the target heart rate zones of 102-124bpm (aged 20-29) and 101-120bpm (aged 30-39) [70]. The Canadian Society for Exercise Physiology (CSEP) recommends that adults accumulate 150 minutes of moderate-to-vigorous PA (MVPA) per

week, in bouts of 10 minutes or more, to maintain good health [71]. CSEP's guidelines for the adult population are more stringent, and were conceived from a strong body of evidence-based research [72], and they do not specifically exclude pregnant women from the population that should be meeting these guidelines. The specific details included in CSEP's adult PA guidelines (intensity of PA, bouts of 10+ minutes) provides an advantage over the pregnancy specific guidelines in that these details can easily be measured using accelerometers and statistical software [73].

Unfortunately, most Canadians, do not meet the PA guidelines that are important for good health [74,75], and the pregnant population is less likely than the non-pregnant population to meet PA guidelines [76]. Objectively measured PA and sedentary behaviour from the 2003-2006 NHANES survey found that pregnant women in the US are not meeting the recommendations for PA, in fact they participated in a mean of 12.3min/day of MVPA and also spent on average 57% of their time in sedentary behaviour [77]. Results from the Canadian Community Health Survey also found that pregnant women in Ontario were less likely to meet the PA guidelines than non-pregnant women (23.3% vs. 33.6%,  $P < 0.05$ ) [78]; and given that these results are based on self-reported data, the true proportion of pregnant women meeting the PA guidelines may be lower. Recent work from our lab found that of the pregnant women participating in the Maternal Obesity Management (MOM) trial (ISRCTN #75323409) [79] had poor adherence to both sets of PA guidelines over the course of gestation (Figure 2) [80].

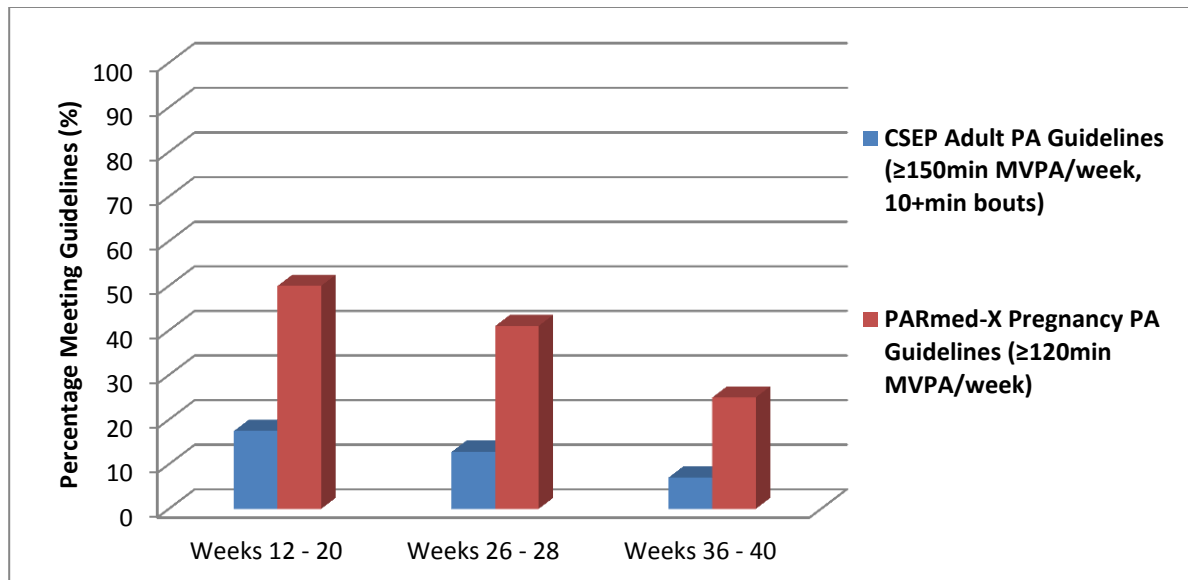


Figure 2. PA guideline adherence from the participants in the Maternal Obesity Management trial

### *1.3.4 Measuring PA during pregnancy*

One issue that became apparent during this review of the literature is that a large portion of the current research on prenatal PA and pregnancy outcomes continues to rely heavily on indirect and self-reported measures of PA during pregnancy, despite evidence of the poor reliability of self-reported PA [81,82]. Many individuals unintentionally overestimate their PA due to issues with perception (i.e. perceived vs. actual time spent engaging in the activity) or a lack of understanding of intensity level, and thus questionnaire are less reliable than direct measures of PA. Previously, prenatal PA measured by questionnaires was shown to correlate poorly with directly measured PA (i.e. accelerometry), with questionnaires often over-predicting moderate intensity PA, and under-predicting light PA or sedentary time [83-85]. Nonetheless, in 2014, there was at least three articles published on the relationships between prenatal PA and GWG that used self-reported estimates of PA [86-88], with reporting periods ranging from  $\leq 14$  weeks gestation [88] to 5 years postpartum [87], and at least four additional studies were published that relied on the Pregnancy Physical Activity Questionnaire (PPAQ) [89-92]. Although

the PPAQ is a ‘pregnancy specific’ questionnaire, it is likely that the PA measures obtained from this tool are imprecise and that direct measures of PA would be more accurate for research purposes.

Relying on data from subjective questionnaires may result in misinterpretation of relationships between prenatal PA and health outcomes.

#### **1.4 Maternal Diet**

Finally, maternal diet is an area that is largely unexplored in relation to its impact on the human placenta. Diet can differ substantially between individuals, and variations in diet quality and quantity have the potential to influence fetal growth, however, as mentioned in the review paper, maternal diet has not previously been considered in any human studies on placental nutrient transport [33]. The number of calories consumed during pregnancy is an important consideration. Maternal dietary intake must meet the needs of both the mother and the growing fetus [93], however, despite popular beliefs, the need for increased energy intake during gestation is minimal [94]. In Canada, the recommended increase in calorie intake for normal weight women is 340kcal/day and 450kcal/day in the second and third trimesters, respectively [95], however, the needs for women who are overweight or obese are currently unknown. Consuming more calories than needed would result in a persistent positive energy balance which will likely contribute to increased GWG. Indeed, a change in the amount of food intake from pre-pregnancy levels has been identified as a predictor of excessive GWG [46]. Meanwhile, interventions that combine physical activity and dietary counseling appear to be successful in lowering GWG [96,97]. In addition to the number of calories consumed, other factors, such as the quality of the diet are important considerations. In a cohort of 1388 women, it was identified that consuming fried foods was directly associated with excessive GWG (OR 3.47, 95% CI 0.91-13.24, per serving per day), while consuming a vegetarian diet in the first trimester was inversely associated with excess GWG (OR

0.46, 95% CI 0.28-0.78) [98]. Furthermore, given that deliberate nutrient restriction and variations in diet composition altered the expression of placenta nutrient transporters in animal models (as reviewed by Brett *et al.*) [33], it is likely that variations in maternal diet would also affect the transport of nutrients in the human placenta. For instance, the placental expression of GLUT1 was different between women with insulin-controlled GDM and diet-controlled GDM [99], and although diet was not measured in this study, one can postulate that the women with diet-controlled GDM consumed less total sugar, which may have contributed to the differences in GLUT1 expression. It is for these reasons that it is believed that diet composition may be an important contributor to the delivery of nutrients to the fetus and that measuring diet should be considered in future research.

## **1.5 Summary**

In summary, this review of the literature presents the importance of the intrauterine environment and the role of the placenta and identifies the gaps in the literature with respect to the impact of the maternal phenotype (i.e. obesity and GWG) and lifestyle (i.e. PA and diet) on nutrient transport across the placenta. A thorough investigation of all the placental nutrient transporters is incomplete in the obese population and there is limited to no evidence concerning how gestational weight gain, maternal PA and diet might influence these transporters. In addition it summarizes the current literature on the detriments of maternal obesity and excess GWG on fetal growth and pregnancy outcomes, and the importance of PA and a healthy diet during pregnancy. Additionally, it was highlighted that prenatal PA research continues to rely on self-reported measures of PA, despite the availability of reliable and accurate tools for directly measuring PA. The following manuscripts aim to address these current gaps in the literature.

## **1.6 Structure of the thesis:**

Given the rising rates of obesity and the significance of the intrauterine environment in fetal development and downstream health, it is important that we improve our understanding of the factors that influence placental nutrient transport, a contributing factor to fetal growth. The general objective of this thesis is to gain a better understanding of the changes in expression patterns of the proteins involved in the regulation of nutrient transport across the placenta that may occur in response to different maternal phenotypes: obesity, excess gestational weight gain, and variations physical activity and diet. In the following articles, first I examined how obesity and gestational weight gain influenced the expression of the key placenta nutrient transporters and members of the mTOR and insulin signaling pathways. Being aware that there are many contributing factors to the maternal phenotype, such as physical activity and diet composition, we sought to explore how these factors influenced the expression of genes in the placenta. However, prior to that, in the second manuscript, I evaluated the accuracy of the commonly used Pregnancy Physical Activity Questionnaire for measuring physical activity during the second trimester. Finally, I conclude with my evaluation of the effects of directly measured physical activity and diet composition on the expression of the key placenta nutrient transporters and members of the mTOR and insulin signaling pathways.

## 1.7 Objectives and Hypotheses

The specific aims of the thesis were accomplished through three manuscripts. These objectives were as follows:

Manuscript 1: To determine if pre-pregnancy maternal obesity or excess gestational weight gain alters the expression of the genes involved in fatty acid, amino acid and glucose transport, and the mTOR and insulin signaling pathways in the placenta of normoglycemic term pregnancies.

Manuscript 2: To evaluate whether the Pregnancy Physical Activity Questionnaire (PPAQ) is a reliable estimate of physical activity during the second trimester by comparing the outcomes of the PPAQ to directly measured physical activity. We also aimed to determine whether there were differences in the accuracy of the PPAQ in women who meet physical activity guidelines during pregnancy compared to those who do not meet guidelines.

Manuscript 3: To determine whether prenatal physical activity or variations in maternal diet alter the expression of genes involved in fatty acid, amino acid and glucose transport, and the mTOR and insulin signaling pathways in the placenta from lean women with term gestations.

The general hypothesis of this thesis was that excess maternal weight during pregnancy, objectively measured physical activity, and maternal diet influence the expression of the genes involved in the regulation of nutrient transport across the placenta. The specific hypotheses, and the manuscripts that they accompany, are found below:

- Manuscript 1: We hypothesized that maternal obesity and excess GWG would increase the expression of key genes involved in placenta nutrient transport, and mTOR and insulin signalling in the human placenta.
- Manuscript 2: We hypothesized that the PPAQ would overestimate physical activity in all of the women, and that the differences between the two measures would be larger in the women who do not meet physical activity guidelines.
- Manuscript 3: We hypothesized that variations in physical activity and diet would influence the expression of the of genes involved in fatty acid, amino acid and glucose transport, and the mTOR and insulin signaling pathways in the human placenta

## Part II

### *2.1 Preamble: Manuscript 1*

After completing the review of the literature it was apparent that the evidence is inconclusive with respect to how maternal obesity alters the expression of nutrient transporters in the placenta. It was discovered that the majority of the previous studies failed to account for gestational weight gain, in particular gestational weight gain with respect to the BMI specific guidelines, despite the mounting evidence to suggest that this factor has a strong influence on fetal growth. Thus an observational study was designed that aimed to test whether maternal obesity and gestational weight gain influenced the gene expression of the key nutrient transporters and members of the mTOR and insulin signaling pathways in the placenta. It was also noted that in a number of the previous studies on maternal obesity and placenta nutrient transport that there was no difference in fetal birth weight between groups, a factor that may have influenced the results. In an effort to ensure the comparison of two distinct populations with limited confounding factors, we specifically selected our groups as follows: women with obesity who gained in excess of the gestational weight gain guidelines with higher birth weight offspring, and lean women who gained within the guidelines with average-for-gestational-age offspring. It was hypothesized that maternal obesity and excess gestational weight gain would increase the expression of key genes involved in the regulation of nutrient transport.

## 2.2 Manuscript 1

Placenta nutrient transport-related gene expression: the impact of maternal obesity and excessive gestational weight gain

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ORIGINAL ARTICLE

## Placenta nutrient transport-related gene expression: the impact of maternal obesity and excessive gestational weight gain

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### Abstract

**Objective:** Maternal obesity and excess gestational weight gain (GWG) increase the risk of delivering large infants. This study examined the associations between maternal obesity and GWG on the expression of genes involved in fatty acid, amino acid and glucose transport, and the mechanistic target of rapamycin (mTOR) and insulin signaling axes in the placenta.

**Methods:** Placenta samples were obtained from lean ( $n = 11$ ) and obese ( $n = 10$ ) women. Gene expression in the placenta was measured using polymerase chain reaction.

**Results:** There were no differences in placenta gene expression between the lean and obese women, with the exception of lower expression of *mTOR* in the women with obesity who delivered male offspring (obese  $n = 6$ ; lean  $n = 7$ ). GWG in excess of the upper limit of the body mass index (BMI) specific guidelines was correlated with increased expression of *SNAT1* and decreased expression of *FABP3*, *mTOR*, *IRS1* and *IGF1R*.

**Conclusions:** Variations in GWG may alter the expression of genes involved in regulating placental nutrient transport. Future research on placental nutrient transport should account for the sex of the offspring and the percentage of GWG that is gained above the upper limit for the pre-pregnancy BMI.

### Keywords

Amino acid, fatty acid, glucose, macrosomia, mTOR

### History

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### Introduction

Maternal obesity and excess gestational weight gain (GWG) during pregnancy increase the risk of delivering large-for-gestational-age or macrosomic (>4000 g) neonates [1,2], increasing the risk of obesity throughout the life-course [3–5]. Altered placental nutrient transport is implicated in intrauterine growth restriction and diabetic pregnancies, and it is also suspected to be altered in maternal obesity [6,7]. The placenta is vital to fetal growth; subsequently understanding how maternal obesity and GWG alter placental nutrient transport is important in determining the mechanisms of fetal overgrowth. The placenta of an obese woman with excess GWG is likely exposed to increased substrate flux, which may alter the expression of key genes involved in nutrient transport and energy signaling.

Research on maternal obesity and placenta nutrient transport is limited [6]. Only one study reported that placental glucose transporter expression is unchanged in women with

obesity [8]. The impact of obesity on placental amino acid and fatty acid transport is inconclusive, with studies reporting increased, decreased and no change in expression or activity of various transporters [9–12]. While reasons for this inconsistency are unknown, evidence suggests that placentas from male fetuses may be more sensitive to maternal obesity [9]. Furthermore, the mechanistic target of rapamycin (mTOR) signaling pathway, an important regulator of fetal growth [13], is activated in the placenta from large-for-gestational-age babies born to obese women [11].

This study examined the associations between maternal obesity and GWG on the expression of genes in the placenta involved in fatty acid, amino acid and glucose transport, and the mTOR and insulin signaling axes, which have been implicated in the regulation of energy balance and fetal growth. It is hypothesized that maternal obesity and excess GWG will increase the expression of key genes involved in nutrient transport, and mTOR and insulin signaling.

### Methods

#### Recruitment

Women were recruited from the Ottawa area (Ontario, Canada). Ethical approval and informed consent was obtained

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from all hospitals and participants. Women who smoked, those with diabetes of any type, fetal growth restriction or hypertensive diseases of pregnancy were excluded.

Maternal and fetal clinical variables were collected through a chart review of the antenatal and birth records. Total GWG was calculated by subtracting pre-pregnancy weight from maternal weight at delivery. GWG was also calculated as a percentage gained above the upper limit of the Institute of Medicine GWG guidelines based on pre-pregnancy body mass index (BMI). The upper limit of GWG for lean and obese women is 16 and 9 kg, respectively [14].

To ensure the comparison of distinct populations with limited confounding factors, the women with obesity were selected to have a high BMI ( $>30 \text{ kg/m}^2$ ) without any other metabolic co-morbidities (i.e. diabetes), and the BMI of the lean women ranged from 18 to  $24.9 \text{ kg/m}^2$ . Given that women with obesity are three times more likely to exceed GWG guidelines than normal weight women [15], we only analyzed samples from women with obesity who exceeded the GWG guidelines ( $n = 10$ ) and from lean women who did not exceed the guidelines ( $n = 11$ ). To ensure the availability of biopsies from patients who presented with these divergent phenotypes, we combined samples from two clinical investigations which included patients who were enrolled prior to delivery ( $n = 21$ ) or at delivery ( $n = 23$ ), as well as vaginal ( $n = 6$ ) and cesarean section ( $n = 15$ ) deliveries. Lean ( $n = 13$ ) and obese ( $n = 8$ ) women were excluded for exceeding or meeting GWG guidelines, respectively, and two samples were excluded due to technical difficulties. Of the patients analyzed, 12 were recruited at delivery and nine were recruited prior to delivery.

#### Placenta collection

The placenta was collected within 30 min of delivery and weighed using a calibrated electronic scale. A biopsy of placental villous tissue, of approximately  $0.5 \text{ cm}^3$ , was obtained from healthy tissue within a 10 cm radius of the cord insertion. During sampling an effort was made to discard the decidual layer in order to obtain only fetal cells. Biopsies were rinsed in PBS and placed in RNeasy<sup>®</sup> (Qiagen Inc, Toronto, Canada). Following refrigeration for 24 h, the RNeasy<sup>®</sup> was removed and samples were stored at  $-80^\circ\text{C}$  for batch analysis.

#### RNA extraction

RNA was extracted from 25 mg of tissue using RNeasy<sup>®</sup> RT (BioShop Canada Inc, Burlington, Canada) following the manufacturer's instructions. RNA concentration and purity was determined by the NanoDrop 2000 (Thermo Fisher Scientific, Waltham, MA). cDNA was synthesized (qScript cDNA SuperMix, Quanta Biosciences, Gaithersburg, MD) and gel electrophoresis and qPCR were used to confirm RNA integrity (Perfecta SYBR Green FastMix, Quanta Biosciences, Gaithersburg, MD).

#### Gene expression

The expression of 28 genes implicated in fatty acid transport, glucose transport, and the mTOR and insulin signaling

pathways was determined using a custom RT<sup>2</sup> Profiler<sup>™</sup> PCR Array (SABiosciences, ON, Canada), with the following housekeeping genes: *UBC*, *YWHAZ*, *ACTB*, *TOPI*, *CYCI*. Cycle parameters for the array were  $95^\circ\text{C}$  for 10 min, followed by 40 cycles of  $95^\circ\text{C}$  for 15 s and  $60^\circ\text{C}$  for 1 min (Eppendorf Mastercycler ep realplex 2S). The expression of the amino acid transporters was determined using QuantiTect Primers Assays (*SLC38A1*, *SLC38A2* and *SLC38A4*, and housekeeping genes: *UBC*, *TOPI* and *YWHAZ*; Qiagen) and Perfecta SYBR Green FastMix (Quanta Biosciences). Cycle parameters for the QuantiTect Primers were:  $95^\circ\text{C}$  for 2 min, followed by 40 cycles of  $95^\circ\text{C}$  for 10 s and  $56^\circ\text{C}$  for 30 s. All samples were analyzed using the web-based data analysis tool from Qiagen, which calculates fold change and the level of significance (Student's *t*-test) between the groups. This analysis tool normalizes the threshold cycle values ( $C_t$ ) relative to the geometric mean of the housekeeping genes. This tool used the  $2^{(-\Delta\Delta C_t)}$  method to determine fold difference in the obese group relative to control, and relative expression level of each gene was calculated using the formula  $2^{(-\Delta C_t)}$ , where  $\Delta C_t = C_t(\text{gene of interest}) - \text{average}(C_t(\text{housekeeping genes}))$ .

#### Data analysis

Data are presented as mean  $\pm$  standard error of the mean. Differences in maternal and fetal characteristics between groups were evaluated using a student's *t*-test or a Mann-Whitney *U* test. Comparisons were made in two ways; (i) including all samples (lean  $n = 11$ ; obese  $n = 10$ ) and (ii) only including samples from male offspring (lean  $n = 7$ ; obese  $n = 6$ ). Due to the small number of female offspring in each group, comparisons were not made using the samples from female offspring only. In cases where data were not normally distributed, a square root transformation was performed. Pearson and Spearman correlations tested potential relations between birth weight and the percentage of GWG gained above the upper limit, and the relative expression of the genes of interest. Partial Pearson and Spearman correlations tested the same associations while controlling for the weight of the placenta. For all analyses,  $p < 0.05$  was considered significant. Analyses were completed using SPSS version 20.0 (SPSS Inc., Chicago, IL).

## Results

### Characteristics of the women and offspring

The characteristics of the women and offspring are presented in Table 1. By design, the women with obesity had significantly higher pre-pregnancy body weights and BMI, and while the absolute amount of GWG was similar between groups, the women with obesity gained a significantly higher percentage of the upper limit of GWG for their BMI. Four macrosomic infants ( $>4000 \text{ g}$ ) were born to women with obesity, and birth weights were higher in this group.

### Gene expression: all women

No differences in gene expression levels were observed between the groups (Table 2). No relationships were observed between birth weight, the percentage gained of GWG and any

Table 1. Maternal and fetal characteristics.

	All women		Women with male offspring only	
	Lean (n = 11)	Obese (n = 10)	Lean (n = 7)	Obese (n = 6)
Maternal age (years)	33.6 ± 1.6	33.6 ± 1.9	35.2 ± 2.2	35.8 ± 2.4
Maternal height (cm)	167.7 ± 2.4	164.1 ± 2.3	166.8 ± 2.6	166.4 ± 2.4
Pre-pregnancy maternal weight (kg)	59.8 ± 1.8	98.3 ± 6.5*	59.9 ± 2.4	105.0 ± 10.0†
Pre-pregnancy BMI (kg/m <sup>2</sup> )	21.3 ± 0.4	36.6 ± 2.8*	21.5 ± 0.4	38.0 ± 4.5†
Maternal weight at delivery (kg)	72.2 ± 2.1	111.7 ± 6.8*	71.4 ± 2.9	119.1 ± 10.3†
Gestational weight gain (kg)	12.4 ± 0.7	13.4 ± 0.9	12.5 ± 1.0	14.2 ± 1.0
Percentage of upper limit of GWG for BMI (%)	77.5 ± 4.7	148.0 ± 10.1*	78.4 ± 6.3	157.0 ± 11.6†
Gestational age at delivery (weeks)	39.0 ± 0.3	38.8 ± 0.3	39.1 ± 0.4	39.0 ± 0.4
Birth weight (g)	3360.8 ± 55.6	3764.0 ± 115.2*	3371.3 ± 89.6	3901.7 ± 134.4†
Number of macrosomic infants	0	4	0	3
Placenta weight (g)	660.3 ± 45.0	751.5 ± 26.6	665.3 ± 68.1	750.3 ± 27.5
Fetal to placenta weight ratio‡	5.3 ± 0.4	5.0 ± 0.2	5.4 ± 0.6	5.2 ± 0.2
Sex of infant (% male)	64	60	100	100
Type of delivery	Vaginal = 5 C-section = 6	Vaginal = 1 C-section = 9	Vaginal = 3 C-section = 4	Vaginal = 1 C-section = 5

\*It is significantly different from the lean value in all women,  $p < 0.05$ .

†It is significantly different from the lean value in only the women with male offspring,  $p < 0.05$ .

‡Calculated as birth weight: placenta weight; in grams.

Table 2. Fold change in the expression of the genes in the obese women compared to the lean women.

Symbol	All women			Women with male offspring only			Description
	Fold change	95% CI	p value	Fold change	95% CI	p value	
<i>SNAT1</i>	1.34	-1.15, 1.80	0.07	1.21	-1.04, 1.45	0.09	Sodium-coupled neutral amino acid transporter 1
<i>SNAT2</i>	1.23	-1.28, 1.68	0.46	-1.08	-1.56, 1.45	0.55	Sodium-coupled neutral amino acid transporter 2
<i>SNAT4</i>	1.60	-2.70, 2.84	0.38	-1.12	-7.69, 1.66	0.93	Sodium-coupled neutral amino acid transporter 4
<i>FABP3</i>	-1.10	-2.04, 1.34	0.61	-1.19	-3.57, 1.4	0.46	Fatty acid binding protein 3
<i>FABP4</i>	1.22	-1.56, 1.79	0.46	1.17	-2.04, 1.86	0.82	Fatty acid binding protein 4
<i>FABP5</i>	-1.02	-1.92, 1.44	0.93	-1.13	-2.78, 1.41	0.62	Fatty acid binding protein 5
<i>LPL</i>	1.91	-100 000, 4.27	0.50	1.88	-100 000, 5.19	0.61	Lipoprotein lipase
<i>FATP2</i>	-1.10	-3.23, 1.51	0.86	-1.69	-12.5, 1.11	0.41	Fatty acid transporter 2
<i>FATP4</i>	-1.01	-1.33, 1.23	0.79	-1.00	-1.49, 1.33	0.79	Fatty acid transporter 4
<i>GLUT4</i>	-1.02	-1.82, 1.41	0.52	1.12	-2, 1.74	0.99	Glucose transporter 4
<i>GLUT1</i>	-1.03	-1.49, 1.28	0.53	1.10	-1.64, 1.6	0.90	Glucose transporter 1
<i>AKT1</i>	-1.15	-1.61, 1.11	0.23	-1.08	-1.79, 1.29	0.49	V-akt murine thymoma viral oncogene homolog 1
<i>IRS1</i>	-1.30	-3.45, 1.24	0.59	-1.52	-7.14, 1.18	0.28	Insulin receptor substrate 1
<i>RPS6KB1</i>	-1.14	-1.61, 1.14	0.40	-1.19	-2, 1.19	0.55	Ribosomal protein S6 kinase, 70 kDa, polypeptide 1
<i>EIF4EBP1</i>	-1.03	-1.64, 1.34	0.91	-1.02	-2.27, 1.52	0.92	Eukaryotic translation initiation factor 4E binding protein 1
<i>RPS6</i>	1.01	-1.32, 1.25	0.68	-1.09	-1.54, 1.19	0.72	Ribosomal protein S6
<i>MTOR</i>	-1.39	-2.7, 1.07	0.19	-2.34	-5, -1.54	0.02*	Mechanistic target of rapamycin
<i>EIF4B</i>	1.23	-1.01, 1.47	0.05	1.25	-1.09, 1.58	0.12	Eukaryotic translation initiation factor 4B
<i>EIF4E</i>	-1.44	-33.33, 1.37	0.65	-1.58	-100 000, 1.63	0.75	Eukaryotic translation initiation factor 4E
<i>MAPK1</i>	1.00	-1.28, 1.23	0.95	1.01	-1.47, 1.34	0.95	Mitogen-activated protein kinase 1
<i>PRKAA1</i>	-1.12	-1.69, 1.19	0.48	-1.16	-2, 1.23	0.68	Protein kinase, AMP-activated, alpha 1 catalytic subunit
<i>TSC1</i>	-1.07	-1.39, 1.14	0.54	-1.12	-1.69, 1.2	0.54	Tuberous sclerosis 1
<i>TSC2</i>	1.06	-1.3, 1.34	0.67	-1.12	-1.61, 1.17	0.42	Tuberous sclerosis 2
<i>IGF1</i>	-1.20	-2.08, 1.18	0.75	-1.27	-2.44, 1.17	0.70	Insulin-like growth factor 1
<i>IGF1R</i>	-1.20	-1.54, 1.02	0.16	-1.19	-1.85, 1.13	0.36	Insulin-like growth factor 1 receptor
<i>PIK3CA</i>	1.39	-1.61, 2.16	0.99	1.70	-1.64, 2.79	0.69	Phosphoinositide-3-kinase, catalytic, alpha polypeptide
<i>PIK3CB</i>	-1.01	-1.41, 1.27	0.70	1.08	-1.28, 1.37	0.59	Phosphoinositide-3-kinase, catalytic, beta polypeptide
<i>PRKAA2</i>	1.17	-1.67, 1.74	0.37	-1.06	-2.7, 1.52	0.73	Protein kinase, AMP-activated, alpha 2 catalytic subunit
<i>PRKAB1</i>	-1.05	-1.28, 1.13	0.62	-1.11	-1.47, 1.12	0.47	Protein kinase, AMP-activated, beta 1 non-catalytic subunit
<i>PPARA</i>	-1.14	-1.67, 1.15	0.68	-1.19	-2.17, 1.22	0.77	Peroxisome proliferator-activated receptor alpha
<i>PPARG</i>	-1.21	-1.47, -1.03	0.06	-1.30	-1.69, -1.06	0.05	Peroxisome proliferator-activated receptor gamma

\* $p < 0.05$  difference in the obese group compared to the lean control.

genes of interest (Table 3). When performing partial correlations, in which we controlled for weight of the placenta, inverse relationships were observed between the percentage gained above GWG guidelines and *IGF1R*, and between birth weight and *FABP3* (Table 3).

#### Gene expression: male offspring

When placenta samples from only male offspring were considered, the women with obesity had lower *mTOR* expression (Table 2). The percentage gained above GWG guidelines was inversely correlated with *mTOR*, and

Table 3. Correlations between the percentage gained above GWG guidelines and birth weight and the expression of genes of interest.

Variable	Gene	All samples		Male offspring only	
		Unadjusted	Adjusted*	Unadjusted	Adjusted*
Percentage gained above GWG guidelines	<i>FABP3</i>	$r = -0.13, p = 0.56$	$r = -0.34, p = 0.15$	$r = -0.38, p = 0.20$	$r = -0.61, p = 0.04†$
	<i>FABP4</i>	$r = 0.34, p = 0.14$	$r = 0.30, p = 0.19$	$r = 0.21, p = 0.49$	$r = 0.18, p = 0.57$
	<i>FABP5</i>	$r = 0.17, p = 0.45$	$r = 0.12, p = 0.62$	$r = 0.05, p = 0.88$	$r = -0.05, p = 0.89$
	<i>LPL</i>	$r = 0.34, p = 0.13$	$r = 0.28, p = 0.23$	$r = 0.30, p = 0.32$	$r = 0.30, p = 0.35$
	<i>FATP2</i>	$r = -0.14, p = 0.55$	$r = -0.30, p = 0.21$	$r = -0.30, p = 0.32$	$r = -0.48, p = 0.12$
	<i>FATP4</i>	$r = 0.10, p = 0.68$	$r = -0.01, p = 0.97$	$r = -0.01, p = 0.96$	$r = -0.19, p = 0.55$
	<i>GLUT1</i>	$r = -0.02, p = 0.95$	$r = -0.06, p = 0.79$	$r = -0.05, p = 0.88$	$r = -0.11, p = 0.73$
	<i>GLUT4</i>	$r = 0.02, p = 0.95$	$r = -0.24, p = 0.32$	$r = -0.05, p = 0.86$	$r = -0.37, p = 0.24$
	<i>SNAT1</i>	$r = 0.41, p = 0.07$	$r = 0.35, p = 0.13$	$r = 0.65, p = 0.02†$	$r = 0.63, p = 0.03†$
	<i>SNAT2</i>	$r = 0.11, p = 0.65$	$r = 0.15, p = 0.53$	$r = 0.18, p = 0.55$	$r = 0.19, p = 0.55$
	<i>SNAT4</i>	$r = 0.12, p = 0.61$	$r = 0.06, p = 0.79$	$r = -0.09, p = 0.76$	$r = -0.11, p = 0.74$
	<i>mTOR</i>	$r = -0.39, p = 0.08$	$r = -0.42, p = 0.06$	$r = -0.65, p = 0.02†$	$r = -0.65, p = 0.02†$
	<i>IRS1</i>	$r = -0.23, p = 0.32$	$r = -0.26, p = 0.26$	$r = -0.51, p = 0.08$	$r = -0.59, p = 0.04†$
	<i>IGF1</i>	$r = -0.19, p = 0.40$	$r = -0.23, p = 0.32$	$r = -0.37, p = 0.22$	$r = -0.39, p = 0.21$
	<i>IGF1R</i>	$r = -0.40, p = 0.08$	$r = -0.54, p = 0.02†$	$r = -0.45, p = 0.12$	$r = -0.64, p = 0.03†$
Birth weight	<i>FABP3</i>	$r = -0.13, p = 0.59$	$r = -0.47, p = 0.04†$	$r = -0.22, p = 0.47$	$r = -0.58, p = 0.05†$
	<i>FABP4</i>	$r = 0.13, p = 0.58$	$r = -0.06, p = 0.81$	$r = -0.15, p = 0.62$	$r = -0.24, p = 0.46$
	<i>FABP5</i>	$r = -0.00, p = 0.99$	$r = -0.13, p = 0.59$	$r = -0.23, p = 0.45$	$r = -0.46, p = 0.13$
	<i>LPL</i>	$r = -0.12, p = 0.59$	$r = -0.11, p = 0.66$	$r = -0.16, p = 0.61$	$r = -0.19, p = 0.55$
	<i>FATP2</i>	$r = -0.01, p = 0.98$	$r = -0.30, p = 0.20$	$r = -0.23, p = 0.44$	$r = -0.54, p = 0.07$
	<i>FATP4</i>	$r = 0.12, p = 0.61$	$r = -0.01, p = 0.98$	$r = 0.08, p = 0.80$	$r = -0.24, p = 0.46$
	<i>GLUT1</i>	$r = 0.37, p = 0.10$	$r = 0.32, p = 0.16$	$r = 0.14, p = 0.64$	$r = 0.04, p = 0.90$
	<i>GLUT4</i>	$r = 0.10, p = 0.68$	$r = -0.34, p = 0.14$	$r = 0.09, p = 0.77$	$r = -0.40, p = 0.20$
	<i>SNAT1</i>	$r = 0.03, p = 0.89$	$r = -0.08, p = 0.73$	$r = 0.28, p = 0.36$	$r = 0.23, p = 0.48$
	<i>SNAT2</i>	$r = 0.10, p = 0.62$	$r = 0.10, p = 0.68$	$r = -0.11, p = 0.72$	$r = -0.02, p = 0.95$
	<i>SNAT4</i>	$r = 0.01, p = 0.96$	$r = -0.21, p = 0.38$	$r = -0.20, p = 0.51$	$r = -0.25, p = 0.44$
	<i>mTOR</i>	$r = -0.24, p = 0.30$	$r = -0.40, p = 0.08$	$r = -0.53, p = 0.06$	$r = -0.54, p = 0.07$
	<i>IRS1</i>	$r = -0.05, p = 0.83$	$r = -0.10, p = 0.68$	$r = -0.22, p = 0.48$	$r = -0.35, p = 0.26$
	<i>IGF1</i>	$r = -0.16, p = 0.50$	$r = -0.22, p = 0.36$	$r = -0.42, p = 0.16$	$r = -0.49, p = 0.11$
	<i>IGF1R</i>	$r = -0.20, p = 0.39$	$r = -0.38, p = 0.10$	$r = -0.31, p = 0.25$	$r = -0.66, p = 0.02†$

\*Partial correlation adjusted for placenta weight.

†Significant at  $p < 0.05$ .

positively correlated with *SNAT1* (Table 3). When the partial correlation controlled for placenta weight, the percentage gained above GWG guidelines was inversely correlated with *FABP3*, *IRS1*, *mTOR*, and *IGF1*, and positively correlated with *SNAT1*, while birth weight was inversely correlated with *FABP3* and *IGF1R* (Table 3).

## Discussion

This study explored the relationship between maternal obesity and excess GWG and gene expression of several nutrient transporters and signaling molecules in the placenta. Contrary to our hypothesis, we found no differences in gene expression in the placenta samples from women with obesity. However, an exception was observed as women with obesity who delivered male offspring had lower placental expression of *mTOR*. Furthermore, correlations were observed when gene expression was examined as a continuous variable across the range of GWG and birth weight.

The study design ensured the comparison of two distinct phenotypes: women with obesity who exceeded GWG guidelines, and lean women who did not exceed GWG guidelines. Although both groups had similar total GWG (i.e. 13 kg), due to the more conservative guidelines for women with obesity (16 kg versus 9 kg) [14] this equates to excessive gain in this group. Infant birth weights were higher in the obese group, and included four macrosomic infants. In a number of previous studies examining placenta

nutrient transport, there was no difference in birth weight between the lean and obese groups [8,10–12,16]. Although women with obesity have greater odds of delivering large-for-gestational-age offspring [1], the majority of the children born to women with obesity are appropriate-for-gestational-age [17].

## Glucose

We did not find differences in *GLUT1* or *GLUT4* expression in relation to maternal obesity or GWG. This aligns with previous reports that obesity has no impact on placental *GLUT1* mRNA and protein expression [8], or glucose transporter activity [18]. Similarly, previous work found no relationship between GWG and glucose uptake in placentas from male offspring [19]. In contrast, one study found lower *GLUT4* mRNA expression in the obese compared to lean women, however, *GLUT4* protein levels did not differ [8]. At term, *GLUT1* is the primary glucose transporter in the human placenta [20], while *GLUT4* expression is markedly reduced [21]. Given the evidence it is unlikely that excess maternal body weight impacts placental glucose transport.

## Amino acids

We also found no difference in amino acid transporter expression between groups. Previously, decreased *SNAT* activity (i.e. amino acid uptake) and decreased *SNAT4* protein expression was found in the placentas from women

with obesity compared to lean women, with no reported differences in SNAT1 and SNAT2 expression [10]. Conversely, no difference in System A activity was observed between lean and overweight/obese women [11], possibly due to the inclusion of overweight women rather than strictly obese women. While inconclusive, this raises the possibility that placental amino acid transporter activity is altered in obesity, despite no changes in RNA expression. Indeed, reduced activity but not protein expression of the taurine transporter was recently observed in the placentas from obese women [22].

Interestingly, in the male offspring, *SNAT1* was positively correlated with the percentage gained above GWG guidelines. This suggests that as weight is progressively gained in excess that there is the potential for increased amino acid transport to the male fetus, if this change in RNA expression is translated into increased protein expression. This supports the theory that the placenta acts as a nutrient sensor [23], where placenta nutrient transport is matched with maternal nutrient availability. This relationship may be present in the samples from male offspring, but not in the combined sample, due to the riskier growth strategy of male offspring [24]. This strategy, characterized by quicker fetal growth and less investment in placental development [24], may be more sensitive to fluctuations in substrate availability.

#### Fatty acids

We found no differences in expression of the fatty acid transporters between groups. Previously, no differences in mRNA expression of *LPL*, *FABP3*, *FABP4*, *FABP5*, *FATP2* and *FATP4* were observed between lean and obese women [9,12,16]. Our null findings differed from previous work with respect to two genes (*FABP5* and *FATP4*): lower mRNA expression of *FABP5* in obese women with male offspring [9], and lower mRNA and protein expression of *FATP4* in obese women [16]. In addition, lower *FABP3* protein expression and higher *LPL* activity were observed in obese women [16]. While our findings suggest that obesity is not associated with changes in fatty acid transporter mRNA expression, it is evident that future work examining protein expression, transporter activity and fetal sex differences is needed. The inverse correlations in the male offspring between *FABP3* and infant birth weight and GWG suggest that as GWG is excessive or as fetal weight increases, the placenta may lower the expression of *FABP3* in an attempt to reduce fatty acid delivery to the fetus. This supports the alternative theory of adaptive regulation [25], which suggests that the placenta will up- or down-regulate nutrient transport in response to low or high nutrient availability, respectively, in an effort to maintain optimal fetal growth.

#### mTOR and insulin signaling

The mTOR axis integrates signals from maternal supply and fetal demand, and it is the pathway largely responsible for alterations in placental nutrient transport [6]. With the exception of lower *mTOR* expression in obese women with male offspring, there were no differences in expression of the genes involved in mTOR and insulin signaling. This corroborates a previous report of no difference in mRNA or protein

expression of *IRS1* or *PIK3CA* between lean and obese women [8]. Lower *mTOR* expression in the placentas from male offspring of obese women may indicate efforts to reduce fetal nutrient delivery in an attempt to maintain normal growth, however, it is unknown whether mTOR activity or protein abundance differed between groups.

Previously, a positive correlation was observed between maternal BMI and activation of the mTOR signaling pathway (i.e. phosphorylated *IRS1*, *4EBP1*), which may have contributed to higher birth weights [11]. However, we did not find any association between obesity and the expression of the mTOR signaling molecules. Nonetheless, the inverse correlation between birth weight and *IGF1R* suggests that as birth weight increases the placenta may attempt to reduce IGF1 or mTOR signaling to limit excess fetal growth. The inverse correlations between the percentage gained above GWG guidelines and *mTOR*, *IGF1R* and *IRS1* support the theory of adaptive regulation. This can be interpreted to mean that as GWG increased, the placenta compensated by reducing the expression of key signaling molecules in an effort to limit surplus nutrient transport to the fetus. The different findings between the studies are likely due to the different measures of expression (protein versus mRNA), and future work ought to include mRNA and protein expression, and transporter activity.

#### Male offspring

The differences that are apparent when examining the samples from the male offspring only suggest that the placentas from male offspring might be more susceptible to perturbations in substrate availability. Male offspring/placentas may respond differently to adverse intrauterine environments [26,27], and it was proposed that “boys live dangerously in the womb” [24]. For instance, in placentas from male offspring fatty acid uptake and *FABP5* expression were lower in obese women compared to lean women, while no differences were observed in female placentas [9]. In contrast, GWG was inversely correlated with glucose uptake in placentas from female offspring, but not male offspring [19], suggesting that the female placenta may have a greater responsive capacity to excess GWG. Unfortunately, the limited number of females born in this cohort prevented exploring the same relationships using only samples from female offspring. Nonetheless, the evidence suggests that the male placenta might respond to obesity and GWG in a distinct manner from female placentas.

#### Healthy obese

It is possible that we did not observe differences in gene expression between the groups due to the absence of metabolic co-morbidities in the women with obesity. The literature suggests that there is a subgroup of obese individuals who do not experience major metabolic abnormalities, known as the “healthy obese” phenotype [28]. In the present study, the women with obesity were selected as normoglycemic with no medical complications, and our findings indicate that the expression of the placental nutrient transport genes is not dysregulated in these healthy obese women. Obesity might only alter the expression of placenta nutrient transporters when it is compounded by additional metabolic insults, such as impaired glucose tolerance. This notion is

supported by a previous finding that placental *FABP4* and *FABP5* expression did not differ between obese-normoglycemic women and lean women, but expression was increased in obese-diabetic women compared to obese-normoglycemic and lean women [22]. The correlations between GWG and *mTOR*, *SNAT1*, *IGF1R*, *IRS1* and *FABP3* expression, support the idea that additional metabolic insults are needed to alter the expression of these genes. Excessive GWG presumably increases the availability of maternal energy stores and may expose the fetus to excess nutrients; thus deviations from optimal GWG, regardless of BMI, may sufficiently stress the intrauterine environment such that the gene expression is altered. Although speculative, if a mother was metabolically healthy, yet exceeded the GWG guidelines, the placenta may alter the expression of nutrient transporters and signaling molecules in an effort to maintain normal fetal growth.

### Strengths and limitations

This is the first study to consider the impact of GWG relative to the BMI-specific guidelines on placental nutrient transport, and our findings suggest that it is an important consideration for future research. One previous study included total GWG as a variable during analysis [19], but did not account for differences in GWG targets between BMI classifications.

Failing to measure maternal or fetal body composition limits this work. BMI does not provide a direct measure of body composition, and it is possible that some of the obese women had an elevated BMI due to a high muscle mass and not an excess of adipose tissue. Meanwhile, infants born to obese women are more likely to have a higher body fat percentage rather than simply an increase in body mass [29,30]. In addition, including samples from vaginal deliveries and cesarean sections may limit the findings, as we cannot exclude the possibility that labor influenced the gene expression. Finally, we did not account for behaviors known to affect substrate availability, specifically physical activity and diet.

In conclusion, we demonstrated that maternal obesity does not alter the gene expression of nutrient transporters or *mTOR* or insulin signaling molecules, with the exception of lower *mTOR* in the obese women with male offspring. We highlight the importance of BMI-specific GWG guidelines and how this may contribute to placental nutrient transport. Future work, with a larger sample size, is warranted to investigate whether protein expression and activity of these molecules is affected by excess GWG. We advise that future research should account for the sex of the offspring and GWG relative to BMI-specific guidelines.

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### Declaration of interest

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### References

1. Ferraro ZM, Barrowman N, Prud'homme D, et al. Excessive gestational weight gain predicts large for gestational age neonates independent of maternal body mass index. *J Matern Fetal Neonatal Med* 2012;25:538–42.
2. Gillman MW, Rifas-Shiman S, Berkey CS, et al. Maternal gestational diabetes, birth weight, and adolescent obesity. *Pediatrics* 2003;111:e221–6.
3. Whitaker RC. Predicting preschooler obesity at birth: the role of maternal obesity in early pregnancy. *Pediatrics* 2004;114:e29–36.
4. Oken E, Taveras EM, Kleinman KP, et al. Gestational weight gain and child adiposity at age 3 years. *Am J Obstet Gynecol* 2007;196:322–8.
5. Catalano PM, Farrell K, Thomas A, et al. Perinatal risk factors for childhood obesity and metabolic dysregulation. *Am J Clin Nutr* 2009;90:1303–13.
6. Brett KE, Ferraro ZM, Yockell-Lelievre J, et al. Maternal-fetal nutrient transport in pregnancy pathologies: the role of the placenta. *Int J Mol Sci* 2014;15:16153–85.
7. Cetin I, Parisi F, Berti C, et al. Placental fatty acid transport in maternal obesity. *J Dev Orig Health Dis* 2012;3:409–14.
8. Colomiere M, Permezel M, Riley C, et al. Defective insulin signaling in placenta from pregnancies complicated by gestational diabetes mellitus. *Eur J Endocrinol* 2009;160:567–78.
9. Brass E, Hanson E, O'Tierney-Ginn PF. Placental oleic acid uptake is lower in male offspring of obese women. *Placenta* 2013;34:503–9.
10. Farley DM, Choi J, Dudley DJ, et al. Placental amino acid transport and placental leptin resistance in pregnancies complicated by maternal obesity. *Placenta* 2010;31:718–24.
11. Jansson N, Rosario FJ, Gaccioli F, et al. Activation of placental *mTOR* signaling and amino acid transporters in obese women giving birth to large babies. *J Clin Endocrinol Metab* 2013;98:105–13.
12. Scifres CM, Chen B, Nelson DM, Sadovsky Y. Fatty acid binding protein 4 regulates intracellular lipid accumulation in human trophoblasts. *J Clin Endocrinol Metab* 2011;96:E1083–91.
13. Roos S, Powell TL, Jansson T. Placental *mTOR* links maternal nutrient availability to fetal growth. *Biochem Soc Trans* 2009;37:295–8.
14. Institute of Medicine. *Weight gain during pregnancy: reexamining the guidelines*. Washington (DC): The National Academies Press; 2009.
15. Weisman CS, Hillemeier MM, Downs DS, et al. Preconception predictors of weight gain during pregnancy: prospective findings from the Central Pennsylvania Women's Health Study. *Womens Health Issues* 2010;20:126–32.
16. Dube E, Gravel A, Martin C, et al. Modulation of fatty acid transport and metabolism by maternal obesity in the human full-term placenta. *Biol Reprod* 2012;87:1–11.
17. Ehrenberg HM, Mercer BM, Catalano PM. The influence of obesity and diabetes on the prevalence of macrosomia. *Am J Obstet Gynecol* 2004;191:964–8.

- ▶18. Acosta O, Ramirez VI, Lager S, et al. Increased glucose and placental GLUT-1 in large infants of obese nondiabetic mothers. *Am J Obstet Gynecol* 2015;212:227.e1-e7.
- ▶19. Walker SP, Ugoni AM, Lim R, Lappas M. Inverse relationship between gestational weight gain and glucose uptake in human placenta from female fetuses. *Pediatr Obes* 2014;9:e73-6.
- ▶20. Illsley NP. Glucose transporters in the human placenta. *Placenta* 2000;21:14-22.
- ▶21. Ericsson A, Hamark B, Powell TL, Jansson T. Glucose transporter isoform 4 is expressed in the syncytiotrophoblast of first trimester human placenta. *Hum Reprod* 2005;20:521-30.
- ▶22. Ditchfield AM, Desforges M, Mills TA, et al. Maternal obesity is associated with a reduction in placental taurine transporter activity. *Int J Obes (Lond)* 2015;39:557-64.
- ▶23. Jansson T, Powell TL. IFPA 2005 Award in Placentology Lecture. Human placental transport in altered fetal growth: does the placenta function as a nutrient sensor? – a review. *Placenta* 2006;27:S91-7.
- ▶24. Eriksson JG, Kajantie E, Osmond C, et al. Boys live dangerously in the womb. *Am J Hum Biol* 2010;22:330-5.
- ▶25. Jones HN, Powell TL, Jansson T. Regulation of placental nutrient transport – a review. *Placenta* 2007;28:763-74.
- ▶26. Clifton VL. Review: sex and the human placenta: mediating differential strategies of fetal growth and survival. *Placenta* 2010; 31:S33-9.
- ▶27. Di Renzo GC, Rosati A, Sarti RD, et al. Does fetal sex affect pregnancy outcome? *Gend Med* 2007;4:19-30.
- ▶28. Sharma AM, Kushner RF. A proposed clinical staging system for obesity. *Int J Obes (Lond)* 2009;33:289-95.
- ▶29. Hull HR, Dinger MK, Knehans AW, et al. Impact of maternal body mass index on neonate birthweight and body composition. *Am J Obstet Gynecol* 2008;198:416.e1-e6.
- ▶30. Sewell MF, Huston-Presley L, Super DM, Catalano P. Increased neonatal fat mass, not lean body mass, is associated with maternal obesity. *Am J Obstet Gynecol* 2006;195:1100-3.

### ***2.3 Preamble: Manuscript 2***

The previous manuscript demonstrated that the ‘healthy obese’ maternal phenotype does not alter the expression of the genes involved in nutrient transporter or the mTOR or insulin signalling pathways, with the exception of lower *mTOR* in the obese women with male offspring. However, correlations were observed between gestational weight gain and some of the genes of interest, suggesting that variations in energy balance might influence placenta nutrient transport. Gestational weight gain is a modifiable risk factor that is influenced by lifestyle factors that are known to affect energy balance and substrate availability, specifically physical activity and diet. Given this evidence it was clear that additional work was needed that examined the influence of physical activity and diet on the expression of the nutrient transporters and signalling molecules in the placenta. With regards to measuring physical activity during pregnancy, there is still a strong reliance on indirect/self-report measures of physical activity, despite the evidence that direct measures are more reliable and accurate. Therefore, it was important to compare the regularly used Pregnancy Physical Activity Questionnaire (PPAQ) to direct measures of physical activity to determine whether this questionnaire could be used as a tool for research examining the relations between physical activity and placental gene expression. The following manuscript compares the PPAQ to directly measured physical activity and examines how well the questionnaire estimates different intensities of physical activity in both active and non-active women. It was hypothesized that the PPAQ would overestimated physical activity in all women, but that the degree of overestimation would be greater in the non-active women.

## 2.4 Manuscript 2

Self-report Pregnancy Physical Activity Questionnaire overestimates physical activity

**K.E. Brett**, S. Wilson, Z.M. Ferraro, K.B. Adamo

The following original article has been accepted for publication in *The Canadian Journal of Public Health*, and has been formatted according to their requirements

**Self-report Pregnancy Physical Activity Questionnaire overestimates physical activity**

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## ABSTRACT

**OBJECTIVES:** Physical activity (PA) research during pregnancy relies heavily on indirect/subjective measures of PA, which may be less accurate than directly measured PA. We tested whether the Pregnancy Physical Activity Questionnaire (PPAQ) could accurately estimate PA by comparing PPAQ results to directly measured PA.

**METHODS:** PA was directly measured in the second trimester of pregnancy using Actical® accelerometers (valid day= 10+ hours; 4-7 valid days) in 29 women who completed the PPAQ. Activity variables from the PPAQ were calculated using all questions, and also by only considering the leisure time section. Women were classified as 'active' or 'non-active' using Canadian PA guidelines for adults (150 minutes moderate-to-vigorous (MVPA)/week, bouts of 10+ minutes). Bonferroni corrections were used to adjust for multiple comparisons. Data are presented as mean  $\pm$  standard deviation or median (interquartile range).

**RESULTS:** The PPAQ overestimated MVPA by 12.12 (14.34) hours/week in the combined sample, and the difference remained substantial when investigating the non-active [overestimate = 11.54 (10.10) hours/week] and the active women [overestimate = 16 $\pm$ 11 hours/week] separately. PPAQ-measured PA variables did not correlate with any of their respective Actical®-measured variables ( $p>0.008$ ). The leisure time PPAQ questions overestimated MVPA by 1 $\pm$ 3 hours/week, with a positive correlation between PPAQ-leisure time MVPA and Actical®-measured MVPA ( $r=0.565$ ,  $p=0.001$ ).

**CONCLUSION:** The PPAQ significantly overestimates MVPA and does not provide an accurate estimate of PA in pregnancy. While PPAQ leisure time questions may help distinguish trends in PA, data from subjective questionnaires may result in misinterpretation of relationships between prenatal PA and health outcomes.

**Key Words:** Maternal Health, accelerometry, Quantitative Evaluation, Qualitative Evaluation, Physical Activity

## **INTRODUCTION**

Physical activity (PA) is an important component of a healthy pregnancy, for both the mother and her child.<sup>1,2</sup> Unfortunately, research exploring the impacts of prenatal PA continues to rely heavily on indirect and self-reported measures of PA, such as questionnaires or PA recalls, despite mounting evidence of the poor reliability of self-reported PA.<sup>3,4</sup> Many individuals unintentionally overestimate their PA (e.g. lack of understanding of intensity), and thus the use of self-reported prenatal PA may be less accurate and may lead to the misinterpretation of health outcomes related to prenatal PA. Numerous self-report PA questionnaires correlate poorly with directly measured PA during pregnancy, with questionnaires often over-predicting time spent in moderate intensity PA, and under-predicting time spent in light PA or sedentary time.<sup>5-7</sup> Despite this evidence, in the past year three papers were published on PA and gestational weight gain (GWG) that used self-reported estimates of PA,<sup>8-10</sup> and at least three studies were published using the Pregnancy and Physical Activity Questionnaire (PPAQ) to measure PA.<sup>11-13</sup> The original development and validation of the PPAQ reports weak to moderate correlations between the PPAQ and directly measured PA,<sup>14</sup> however, p-values were not reported. A second investigation reported moderate correlations between a French-translated PPAQ and directly measured light and moderate intensity PA, but not for sedentary or vigorous activity.<sup>15</sup> Furthermore, both previous studies used uniaxial Actigraphs, and neither study compared the amount of time spent at different PA intensities as measured by the two methods in order to determine whether the PPAQ was over- or under-estimating PA. Since the PPAQ was designed to measure duration, frequency and intensity of activity,<sup>14</sup> it is important to evaluate whether it can accurately measure the time spent in different PA intensities

The purpose of this paper is to compare PPAQ-measured PA to directly measured PA using omniaxial Actical® accelerometers during the second trimester of pregnancy, with a focus on time spent at different PA intensities. Secondly, as it is suspected that women who are regularly physically active may have better comprehension of PA intensity and duration and thus may be more accurate in reporting PA, we aim to determine if there are any differences in the accuracy of the PPAQ in women who meet PA guidelines during pregnancy compared to those who do not meet PA guidelines. It is hypothesized that the PPAQ will overestimated PA in all women, and the differences between the two measures will be larger in the women who do not meet PA guidelines during pregnancy.

## **METHODS**

Women were recruited using flyers posted in medical facilities between weeks 13 and 28 of pregnancy from the Ottawa area as part of study examining the impact of PA on the placenta. This study was approved by the research ethics board (REB#0903E) and written informed consent was obtained from all participants. Women who smoked, as well as those with type 1, type 2 or gestational diabetes, fetal growth restriction or hypertensive diseases of pregnancy were excluded. Completion of the PA readiness medical examination (PARmed-X) for pregnancy was required to participate.<sup>16</sup> Maternal height was directly measured and pre-pregnancy weight was self-reported during their first visit. Prenatal PA was assessed using two methods: the PPAQ,<sup>14</sup> and with an Actical® accelerometer.

### **The PPAQ**

The PPAQ is a self-administered questionnaire that asks respondents to report the time spent participating in 32 activities including household/care giving (13 activities), occupational (5 activities), sports/exercise (8 activities), transportation (3 activities) and inactivity (3 activities).<sup>14</sup> For each

question, respondents select the option that best approximates the amount of time spent engaging in that activity during the current trimester. The PPAQ was completed between weeks 20-28, and women were asked to consider only the second trimester. Each question has six options, and possible time durations range from 0 to 6 or more hours per day and from 0 to 3 or more hours per week. The questionnaire includes an open ended section that allows respondents to add up to two activities that are not included in the list.

The original PPAQ authors previously assigned each activity on the PPAQ to a specific metabolic equivalent (MET) value using field-based measurements in pregnant women<sup>17</sup> and the 2000 update to the compendium-based MET values (1 MET = 1 kcal/kg\*hr).<sup>18</sup> These MET values were used to classify each activity by intensity: sedentary (<1.5 METs), light (1.5 – <3.0 METs), moderate (3.0 – 6.0 METs), and vigorous (>6.0 METs). The duration of time spent per week at each relative intensity of PA was calculated by adding the amount of time spent at each activity within a given intensity level. In congruence with a recent comparison paper,<sup>3</sup> we excluded bicycling, swimming, and weight training from the analysis of the PPAQ, as these activities are not well measured by accelerometry. PPAQ activity variables were calculated using two methods: using all questions, and only considering the leisure time section (8 of the 32 questions). We considered the leisure time section on its own because the authors who developed the PPAQ observed higher correlation coefficients between the leisure time section and the average Actical® counts per minute, compared to the other forms of activity.<sup>14</sup> Additionally, women may be better able to estimate time spent participating in sports/exercise, rather than household/caregiving or occupational activities.

## **Accelerometry**

Free-living PA was assessed for 7 days with the onbiaxial Actical® accelerometer (Mini Mitter Company, Inc., A Respironics Inc. Company, Blend OR., USA), which measures acceleration in all directions. Participants wore the accelerometer on an elastic waistband on the right hip during the day, except during bathing and aquatic activities. Participants kept daily logs to record when and why the Actical® was removed. Data reduction and analysis were harmonized with the Canadian Health Measures Survey, which has been used to measure PA in normal weight, overweight and obese adults.<sup>19,20</sup> In brief, accelerometry data were downloaded as 60 second epochs and signals were reported as counts per minute and translated into steps per minute. Data was processed using standardized quality control and data reduction procedures in SAS version 9.3 (SAS Institute, Cary, USA).<sup>19</sup> Respondents with 4 or more valid days (10+ hours of wear time) were retained for analyses.<sup>20</sup> Standard cut-points were used to measure time spent in various level of movement intensity, including sedentary, light, moderate, and vigorous.<sup>20,21</sup>

Adherence to the Canadian Society for Exercise Physiology's (CSEP) Canadian PA guidelines for adults was used to classify the women as "active" or "non-active". These guidelines recommend 150 minutes MVPA per week accumulated in 10-minute bouts, to maintain good health.<sup>20,22</sup> The more stringent, evidence-based PA guidelines for adults were used instead of pregnancy-specific guidelines in order to guarantee the selection of a very active population and because adherence to these guidelines has previously been measured using Actical® accelerometers.<sup>19,20</sup> Since not all participants completed 7 valid days, adherence to the guidelines was defined as an average daily MVPA > 21.43 minutes, which is equal to 150 minutes divided by 7 days.

## Analysis

We compared the PPAQ results to the Actical®-measured PA in the combined sample of all women and in the active and non-active women separately. We performed these PPAQ variable comparisons using two methods: i) using all questions and, ii) considering only the leisure time section.

Values greater than three standard deviations away from mean were excluded. Non-normally distributed variables were transformed using a logarithmic transformation. Despite transformation attempts, some variables remained non-normally distributed (*all women*: PPAQ-measured VPA, Actical-measured VPA and MVPA; *non-active women*: PPAQ-measured VPA, Actical-measured VPA) and non-parametric methods were employed. Paired sample t-tests and Wilcoxon signed-rank tests were used to test for potential differences in the PA variables between the PPAQ and the Actical® data. Effect sizes were calculated for the normally distributed variables using the Cohen's  $d$ ,<sup>23</sup> and for the non-normally distributed variables we used a modified  $r$  calculation followed by a conversion to Cohen's  $d$ .<sup>24</sup> Independent samples t-tests and Mann-Whitney U tests were used to test for possible differences between the active and the non-active women. Spearman and Pearson correlations tested potential relationships between minutes of light, moderate, and vigorous PA, MVPA, and sedentary time, between the two measures of PA. For the descriptive characteristics,  $p < 0.05$  was considered statistically significant. For the comparisons between methods and the correlations, a Bonferroni correction was used to correct for multiple comparisons, thus  $p < 0.0083$  was considered significant. Statistical analyses were carried out using SPSS version 20.0 (SPSS Inc., Chicago, IL, USA).

## RESULTS

### Characteristics of the women

All women had uncomplicated pregnancies and there were no between group differences in demographic or anthropometric variables (Table 1). Of the 29 participants, 16 women had 7 valid days of Actical® wear time. The number of valid days of Actical® wear time for the remaining women was as follows: 6 days (n=7), 5 days (n=4), and 4 days (n=2).

### Relations between the measurement tools

The time spent in different intensities of PA (sedentary, light PA (LPA), moderate PA (MPA), and vigorous PA (VPA), and MVPA) as measured by the PPAQ and the Actical® are present in Table 2. Relative to the Actical®, the PPAQ significantly over-predicted the number of minutes spent engaging in LPA, MPA and MVPA, with very large effect sizes. Using only the PPAQ leisure time questions still over-predicted MVPA by 1.1 (3.4) hours/week, but to a lesser extent than using the whole PPAQ [12.1 (14.4) hours/week]. In some cases, the PPAQ reported implausibly high levels of activity; five women obtained more than 19 hours/day of activity, with two participant's PPAQ results reporting >24 hours of activity per day.

There are substantial differences in the classification of the women as “active” or “non-active” between the methods (Table 3). The Actical® and the PPAQ agreed in 34.4% of the cases. When only considering the leisure time section of the PPAQ, a large portion of the women were still misclassified as active, and the leisure time PPAQ agreed with the Actical® in 58.6% of cases.

The comparison of the PPAQ to the Actical® when investigating the active and the non-active women separately is in Table 4. In the active women, the PPAQ significantly overestimated LPA, MPA,

and MVPA with very large effect sizes. In the non-active women, the PPAQ significantly overestimated LPA, MPA and MVPA with large to very large effect sizes. When comparing Actical®-measured PA variables between groups, the active women accumulate significantly more minutes of MPA, VPA, and MVPA, and have reduced sedentary time, compared to the non-active women. When comparing PPAQ-measured PA variables, the active women accumulated more minutes of VPA and LT-MVPA, but there was no difference in sedentary time, LPA, MPA or MVPA. The PPAQ overestimated Actical®-measured PA by similar amounts in both groups.

PPAQ variables did not correlate with their respective Actical® measures for sedentary time, LPA, MPA, VPA or MVPA (Table 5). LT-MVPA and Actical®-measured MVPA were positively correlated. When analyzed separately as active and non-active women, there were no correlations between the PPAQ and the Actical® variables (data not shown,  $p>0.05$ ).

## **DISCUSSION**

This analysis reveals discrepancies between Actical-measured PA and the PPAQ. Our data indicates that the PPAQ is not an accurate proxy of PA: it drastically overestimates LPA, MPA and MVPA in all women, and PPAQ-measured PA variables did not correlate with the Actical® measures of sedentary time, LPA, MPA, VPA or MVPA. This suggests that women may have difficulties accurately recalling or quantifying the duration or intensity of their activities, during their second trimester.

These results conflict with the previous publications that concluded that the PPAQ was a reasonably accurate tool for assessing prenatal PA.<sup>14,15</sup> The authors who developed the PPAQ only

reported weak to moderate correlations when comparing the PPAQ to 3 different published cut points for the Actigraph accelerometer [Spearman's  $r=0.08-0.49$  ( $n=54$ )], but failed to report p-values. Thus, it is unknown whether these correlations are statistically significant. In addition, Chandonnet and colleagues, tested a French-translated PPAQ in obese pregnant women, and found that the PPAQ was correlated with Actigraph measures of light and moderate intensity PA [ $r=0.46$  for light,  $r=0.40$  for moderate,  $p<0.01$  ( $n=49$ )], but not sedentary or vigorous activity.<sup>15</sup> Both previous studies used uniaxial Actigraphs rather than omniaxial Acticals, and pooled women from the three trimesters, and these differences may have contributed to the different results observed in the current study. Furthermore, neither of the previous manuscripts directly compared the time spent in different PA intensities as measured by the two methods. Given that only moderate correlations were found between the methods,<sup>14,15</sup> it is unknown whether the PPAQ is under- or over-predicting PA or the magnitude of the difference. Based on our findings, and the missing p-values in the Chasan-Taber paper,<sup>14</sup> we do not believe that the PPAQ is a reliable tool to estimate time spent in different intensities of PA during pregnancy.

According to the Actical®-measured PA, only 10 of the 29 women met the PA guidelines, however according to the PPAQ, all 29 women met, if not vastly exceeded, these guidelines. Using the PPAQ and misclassifying a number of the non-active women as active is problematic, especially if this questionnaire were to be employed in research exploring the health effects of prenatal PA. A questionnaire that overestimates PA may increase the likelihood that spurious relationships will be observed. Using the PPAQ may cause relationships between PA and pregnancy outcomes to be missed if women who are not engaging in sufficient PA are grouped with the highly active women, thus diluting the actual level of PA in an active group of women.

This gross overestimation of PA and the lack of correlation between the PPAQ and the Actical®-measured PA call into question the findings from the recently published studies that used the PPAQ to measure PA during pregnancy.<sup>11-13</sup> Given our findings that the PPAQ significantly overestimates PA, it is possible that the conclusions from the previous studies may not represent true relationships. The use of directly measured PA would have provided more reliable measures for PA and may have altered the results and interpretations.

The over-estimation of PA and the inaccuracies of the PPAQ are consistent with previous work comparing self-report PA to directly measured PA. Bell and colleagues compared two different PA questionnaires to directly measured PA and found that both questionnaires over-estimated MVPA in lean and obese women and showed a poor ability to classify women as active or not (n=59).<sup>5</sup> Additionally, the International Physical Activity Questionnaire (IPAQ) over-predicted MPA, but under-predicted LPA, with poor correlations between the IPAQ and accelerometer measures of PA during pregnancy (n=30).<sup>6</sup> Furthermore, in overweight and obese pregnant women, the self-report Activity Questionnaire for Adults and Adolescents over estimated Actigraph-measured MVPA, and underestimated sedentary time (n=55).<sup>7</sup> Unfortunately, the previous studies comparing the PPAQ to accelerometry data<sup>14,15</sup> only report correlation coefficients and do not directly compare PPAQ-measured and accelerometry-measured time spent in different intensities of PA. Thus, it is unknown how well the PPAQ predicted time spent at different PA intensities in those papers. Collectively, these findings strongly suggest that PA questionnaires are not accurate methods of assessing PA during pregnancy.

When investigating the active and the non-active women separately, by design the active women accumulated significantly more minutes of Actical®-measured MVPA. Contrary to our hypothesis,

there was no difference in how much the PPAQ over- or under-estimated any of the Actical®-measured PA variables between groups. Given that women who are regularly physically active might have a better understanding of the intensity and duration of PA, it was suspected that non-active women would be more likely to over-report their PA. However, when using the PPAQ, both groups over-reported their PA to the same extent.

When only the leisure time section of the PPAQ was considered, the PPAQ continued to overestimate MVPA in all women, but to a lesser extent than the whole questionnaire [1.1(3.4) vs. 12.1(14.4) hours per week]. When the group was divided into active and non-active women, there were no longer significant differences between LT-MVPA and Actical®-measured MVPA, which may have been the result of smaller sample sizes. LT-MVPA was significantly correlated with Actical®-measured MVPA, suggesting that using only the leisure time section of the PPAQ is a more reliable proxy for PA than the whole questionnaire. It is possible that the women were better able to recall or estimate the intensity and time spent participating in leisure time PA rather than all activities of daily living.

## **Limitations**

One limitation is that the measurement collection periods differ. The Actical® was only worn for one week between weeks 14-28 of gestation, while the PPAQ reflects the second trimester. While both measures attempt to capture ‘usual’ levels of PA, it is possible that the women may have been experiencing unpleasant side effects of pregnancy during the week they were issued the Actical®, and may not have engaged in their usual level of PA. Comparatively, since the questionnaire covers the second trimester during which time engaging in PA may become increasingly more difficult as gestation progresses, the women may have had trouble accurately averaging their PA across the trimester. In

addition, since the PPAQ was completed between weeks 20 and 28, the period of time considered as the second trimester varied from 7 weeks to 15 weeks. Furthermore, given the implausibly high levels of activity reported in the results from some of the questionnaires, it is possible that some women were double counting some activities, such as instances where two activities were performed simultaneously. The PPAQ does not include sleeping, eating or personal hygiene, so assuming that the women only slept for 6 hours per day, the total number of hours of sedentary, LPA and MVPA combined should not be higher than 18 hours per day. However, according to the PPAQ, 5 women accumulated more than 19 hours per day of activity.

## **Conclusion**

The PPAQ does not provide a reliable estimate of PA in pregnancy and we caution against the use of this questionnaire for research requiring an accurate measure of maternal energy expenditure during pregnancy. Although PPAQ leisure time questions may help distinguish trends in PA, data from subjective questionnaires may result in misinterpretation of relationships between prenatal PA with health and/or pregnancy outcomes.

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### Conflict of Interests

The authors declare no conflicts of interest.

### Reference List

- (1) Ferraro ZM, Gaudet L, Adamo KB. The potential impact of physical activity during pregnancy on maternal and neonatal outcomes. *Obstet Gynecol Surv* 2012;67(2):99-110.
- (2) Nascimento SL, Surita FG, Cecatti JG. Physical exercise during pregnancy: a systematic review. *Curr Opin Obstet Gynecol* 2012;24(6):387-94.
- (3) Garriguet D, Colley RC. A comparison of self-reported leisure-time physical activity and measured moderate-to-vigorous physical activity in adolescents and adults. *Health Rep* 2014;25(7):3-11.
- (4) Prince SA, Adamo KB, Hamel ME, et al. A comparison of direct versus self-report measures for assessing physical activity in adults: a systematic review. *Int J Behav Nutr Phys Act* 2008;5(1):56-80.
- (5) Bell R, Tennant PW, McParlin C, et al. Measuring physical activity in pregnancy: a comparison of accelerometry and self-completion questionnaires in overweight and obese women. *Eur J Obstet Gynecol Reprod Biol* 2013;170(1):90-5.
- (6) Harrison CL, Thompson RG, Teede HJ, et al. Measuring physical activity during pregnancy. *Int J Behav Nutr Phys Act* 2011;8(19):114-21.
- (7) Oostdam N, van Mechelen W, van Poppel M. Validation and responsiveness of the AQUAA for measuring physical activity in overweight and obese pregnant women. *J Sci Med Sport* 2013;16(5):412-6.
- (8) Harris ST, Liu J, Wilcox S, et al. Exercise During Pregnancy and its Association with Gestational Weight Gain. *Matern Child Health J*. Online First: 10 June 2014 DOI 10.1007/s10995-014-1534-8
- (9) Schlaff RA, Holzman C, Mudd LM, et al. Body Mass Index is Associated With Appropriateness of Weight Gain But Not Leisure-Time Physical Activity During Pregnancy. *J Phys Act Health*. Online First: 11 April 2014 DOI: 10.1123/jpah.2013-0090

- (10) Schlaff RA, Holzman C, Maier KS, et al. Associations among gestational weight gain, physical activity, and pre-pregnancy body size with varying estimates of pre-pregnancy weight. *Midwifery* 2014;30(11):1124-31.
- (11) Chasan-Taber L, Silveira M, Pekow P, et al. Physical activity, sedentary behavior and risk of hypertensive disorders of pregnancy in Hispanic women. *Hypertens Pregnancy*. Online First: 14 August 2014 doi:10.3109/10641955.2014.946616
- (12) Harrod CS, Chasan-Taber L, Reynolds RM, et al. Physical Activity in Pregnancy and Neonatal Body Composition: The Healthy Start Study. *Obstet Gynecol* 2014;124(2):257-64.
- (13) Hawkins M, Chasan-Taber L, Marcus B, et al. Impact of an exercise intervention on physical activity during pregnancy: the behaviors affecting baby and you study. *Am J Public Health* 2014;104(10):e74-e81.
- (14) Chasan-Taber L, Schmidt MD, Roberts DE, et al. Development and validation of a Pregnancy Physical Activity Questionnaire. *Med Sci Sports Exerc* 2004;36(10):1750-60.
- (15) Chandonnet N, Saey D, Almeras N, et al. French Pregnancy Physical Activity Questionnaire Compared with an Accelerometer Cut Point to Classify Physical Activity among Pregnant Obese Women. *PLoS One* 2012;7(6):e38818.
- (16) Canadian Society for Exercise Physiology. Physical activity readiness medical examination (PARmed-X) for pregnancy. Canadian Society for Exercise Physiology 2013. Available at: <http://www.csep.ca/cmfiles/publications/parq/parmed-xpreg.pdf> (Accessed September 9,2014)
- (17) Roberts DE, Fragala D, Pober L, et al. Energy cost of physical activities during pregnancy. *Med Sci Sports Exerc* 2002;34(5):S124.
- (18) Ainsworth BE, Haskell WL, Whitt MC, et al. Compendium of physical activities: an update of activity codes and MET intensities. *Med Sci Sports Exerc* 2000;32(9 Suppl):S498-S504.
- (19) Colley RC, Gorber SC, Tremblay MS. Quality control and data reduction procedures for accelerometry-derived measures of physical activity. *Health Reports* 2010;21(1):63-9.
- (20) Colley RC, Garriguet D, Janssen I, et al. Physical activity of Canadian adults: accelerometer results from the 2007 to 2009 Canadian Health Measures Survey. *Health Rep* 2011;22(1):7-14.
- (21) Wong SL, Colley R, Connor GS, et al. Actical accelerometer sedentary activity thresholds for adults. *J Phys Act Health* 2011;8(4):587-91.
- (22) Tremblay MS, Warburton DE, Janssen I, et al. New Canadian physical activity guidelines. *Appl Physiol Nutr Metab* 2011;36(1):36-46.
- (23) Rosenthal R. Meta-analytic procedures for social research. Newbury Park, CA: Sage; 1991.
- (24) Fritz CO, Morris PE, Richler JJ. Effect size estimates: current use, calculations, and interpretation. *J Exp Psychol Gen* 2012;141(1):2-18.

**Table 1.** Anthropometric and Demographic Characteristics of the Women and their Offspring

	<b>Active</b> <b>(n=10)</b>	<b>Non-Active</b> <b>(n=19)</b>
Maternal Age (years)	31 ± 3	32 ± 3
Maternal height (cm)	166.9 ± 6.1	166.2 ± 6
Married or living with partner	10 (100%)	19 (100%)
White	9 (90%)	19 (100%)
High school education	0 (0%)	1 (5%)
College or University graduate	10 (100%)	18 (95%)
Household income ≥\$50,000	9 (90%)	19 (100%)
Household income <\$50,000	1 (10%)	0 (0%)
Pre-pregnancy Maternal weight (kg)	63.7 ± 8.5	69.0 ± 15.7
Pre-pregnancy BMI (kg/m <sup>2</sup> )	23.0 ± 3.2	25.0 ± 5.8
Maternal weight at delivery (kg)	77.9 ± 7.6	84.4 ± 16.2
Gestational weight gain (kg)	15.0 ± 3.5	13.9 ± 5.0
Weeks of gestation of PPAQ assessment	24.8 ± 2.6	25.4 ± 1.9
PPAQ completed just prior to Actical® <sup>§</sup>	8 (80%)	13 (68%)
Weeks of gestation at Actical® assessment	25.0 ± 2.8	23.0 ± 4.5
Valid days of Actical® assessment <sup>‡</sup>	6.9 ± 0.3	6.0 ± 1.0
Wear time on valid days of Actical® (hours)	13.0 ± 0.3	13.6 ± 1.5
Gestational age at delivery (weeks)	39.6 ± 1.4	39.1 ± 1.5
Birth weight (g)	3669.7 ± 353.0	3352.1 ± 466.4
Sex of infant (% female)	30%	53%

\* $p < 0.05$  was considered significant

† Data are presented as mean  $\pm$  standard deviation or n (%)

‡ Valid day of Actical® wear time = 10 or more hours of wear time

§ In most cases, the Actical® was worn in the week following the completion of the PPAQ. In cases where the Actical® was worn before 20 weeks gestation, the PPAQ was administered in the following weeks (range 4 to 10 weeks after Actical®).

**Table 2.** Comparison of the Amount of Time per Week Spent in Different Intensities of PA as Measured by the Actical® and the PPAQ and How Much the PPAQ Overestimates the Directly Measures of PA in All of the Women

	<b>PPAQ</b> (min/week)	<b>Actical®</b> (min/week)	<b>Effect Size (<i>r</i>)</b>	<b>Over Estimate</b> (hours/week)
Sedentary	3570 (1418)	3045 (319)	0.51	8.6 (21.6)
LPA	1380 (1252)*	897 (304)	1.02	8.8 (17.4)
MPA	885 (893)*	150 (107)	1.76	12.5 (12.9)
VPA	15 (135)	24 (58)	-0.35	0.00 (1.8)
MVPA	885 (810)*	159 (154)	-1.57	12.1 (14.4)
LT-MVPA	255 (293)*	159 (154)	-0.80	1.1 (3.4)

\*  $p < 0.0083$  was considered a significant difference between the methods

† All data (normally and non-normally distributed variables) are presented as median (interquartile range) for ease of comparison.

‡ The mean  $\pm$  standard deviation of the normally distributed variables are as follows: Actical Sedentary ( $3050 \pm 235$ ), sedentary overestimate ( $5.90 \pm 17.35$ ), Actical MPA ( $155 \pm 90$ ), VPA overestimate ( $0.38 \pm 1.24$ ), PPAQ LT-MVPA ( $279 \pm 176$ ), and LT-MVPA overestimate ( $1.39 \pm 2.65$ )

**Table 3.** Classification of the Women as Active or Non-active According to the Actical®, the Whole PPAQ and the Leisure Time Section of the PPAQ

	Active	Non-Active
Actical®	10	19
PPAQ	29	0
Leisure time PPAQ	22	7

\*Active is considered >150 minutes of MVPA per week. For the Actical®, as per standard methods, MVPA was accumulated in bouts of 10 minutes or more.

**Table 4.** Comparison of the Amount of Time per Week Spent at Different Intensities of PA According to the PPAQ and the Actical® and How Much the PPAQ Overestimates Different Intensities of Directly Measured PA in the Active and Non-active Women

	Active				Non-Active			
	PPAQ (min/wk)	Actical® (min/wk)	Effect Size	Over Estimate (hrs/wk)	PPAQ (min/wk)	Actical® (min/wk)	Effect Size	Over Estimate (hrs/wk)
Sedentary	3045 (1968)	2966 (267) <sup>†</sup>	0.14	4.7 (36.1)	3570 (840)	3215 (332) <sup>†</sup>	0.64	8.6 (16.1)
LPA	1995 (1616) <sup>*</sup>	925 (184) <sup>*</sup>	1.17	17.7 (19.7)	1305 (630) <sup>*</sup>	890 (347) <sup>*</sup>	0.81	6.4 (15.6)
MPA	1193 (1073) <sup>*</sup>	233 (149) <sup>*,†</sup>	1.65	14.9 (17.8)	810 (450) <sup>*</sup>	98 (81) <sup>*,†</sup>	1.78	11.3 (9.6)
VPA	135 (146) <sup>‡</sup>	68 (87) <sup>†</sup>	0.26	0.72 (2.6)	0 (15) <sup>‡</sup>	0.9 (23.9) <sup>†</sup>	-0.19	0.00 (0.6)
MVPA	1200 (1208) <sup>*</sup>	347 (224) <sup>*,†</sup>	1.51	14.5 (22.1)	825 (465) <sup>*</sup>	111 (75) <sup>*,†</sup>	1.69	11.5 (10.1)
LT-MVPA	465 (251) <sup>‡</sup>	347 (224) <sup>†</sup>	0.41	1.6 (4.7)	180 (195) <sup>‡</sup>	111 (75) <sup>†</sup>	1.47	1.1 (2.3)

\* p<0.0083 was considered a significant difference between the methods within the women of the same group

† p<0.0083 was considered a significant difference in PA variables from the Actical® between the active and non-active women

‡ p<0.0083 was considered a significant difference in PA variables from the PPAQ between the active and non-active women

§ All data (normally and non-normally distributed variables) are presented as median (interquartile range) for ease of comparison.

|| The mean ± standard deviation of the normally distributed variables are provided in supplemental file 1.

**Table 5.** Correlations Between the PPAQ and the Actical® Measures for the Different intensities of PA in all the Women

	Correlation coefficient	p-value	N
Sedentary	-0.280	0.885	29
LPA	0.275	0.157	28
MPA	0.041	0.835	28
VPA	0.430	0.022	28
MVPA	0.021	0.914	28
LT-MVPA	0.565	0.001*	29

\*  $p < 0.0083$  significant correlation between the PPAQ and the Actical® measure of the same PA variable

Supplemental File 1. Mean and standard deviation for normally distributed variables from Table 4.

	Active			Non-Active		
	PPAQ (min/wk)	Actical® (min/wk)	Over Estimate (hrs/wk)	PPAQ (min/wk)	Actical® (min/wk)	Over Estimate (hrs/wk)
Sedentary	3077 ± 1167	2904 ± 178	2.87 ± 21.17	N/A	N/A	7.49 ± 15.37
LPA	2082 ± 1026	951 ± 171	18.85 ± 16.14	N/A	N/A	8.31 ± 11.07
MPA	1183 ± 566	243 ± 81	15.50 ± 9.39	N/A	108 ± 53	N/A)
VPA	128 ± 92	82 ± 48	0.47 ± 1.76	N/A	N/A	N/A
MVPA	1310 ± 569	344 ± 119	15.85 ± 10.47	N/A	118 ± 57	N/A
LT-MVPA	429 ± 148	344 ± 119	1.41 ± 3.42	201 ± 135	118 ± 57	N/A

\* N/A indicates that the variables is non-normally distributed and thus a mean ± standard deviation is not applicable

### ***2.5 Preamble: Manuscript 3***

The previous manuscript found that the Pregnancy Physical Activity Questionnaire significantly overestimated moderate-to-vigorous physical activity and cautions against the use of this questionnaire for research requiring an accurate measure of physical activity. This finding supports discontinuing the use of this questionnaire and questions the validity of the results from previous research that relied on this questionnaire to measure physical activity. After comparing these two methods of measuring physical activity, coupled with the findings from the first manuscript, the next study was designed to measure the effects of directly measured prenatal physical activity and maternal diet composition on the gene expression of nutrient transporters and insulin and mTOR signaling pathways in the human placenta. There is mounting evidence to suggest that physical activity can influence neonatal outcomes, however, there is limited mechanistic evidence that can explain these observed associations. Furthermore, despite the important contributions of diet towards good health, the research on maternal diet and placenta nutrient transport is limited to animal models. It was hypothesized that variations in physical activity and diet would influence the expression of the of genes involved in fatty acid, amino acid and glucose transport, and the mTOR and insulin signaling pathways in the human placenta.

## 2.6 Manuscript 3

Prenatal physical activity and diet composition affect the expression of nutrient transporters and mTOR signaling molecules in the human placenta

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## Prenatal physical activity and diet composition affect the expression of nutrient transporters and mTOR signaling molecules in the human placenta

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mTOR

## ABSTRACT

**Introduction:** Adequate nutrient delivery to the fetus is essential for optimal growth. Differences in prenatal physical activity level and diet quality influence maternal energy balance and these factors may alter placental nutrient transport. We investigated the associations between meeting physical activity guidelines and the quality of maternal diet on the expression of genes involved in fatty acid, amino acid and glucose transport, and mammalian target of rapamycin (mTOR) and insulin signaling in the placenta from 16 term pregnancies.

**Methods:** Physical activity was directly measured with accelerometry, diet composition was assessed with 24 h dietary recalls, and gene expression was measured with custom polymerase chain reaction (PCR) arrays.

**Results:** Women who met physical activity guidelines had lower gene expression of fatty acid transport protein 4 (*FATP4*), insulin-like growth factor 1 (*IGF1*), and the beta non-catalytic subunit of AMP-activated protein kinase (*AMPK*), and a higher expression of *SNAT2*. There was a strong positive correlation observed between total sugar intake and glucose transporter 1 (*GLUT1*) ( $r = 0.897, p = 0.000, n = 12$ ), and inverse correlations between total sugar and *mTOR* and *IGF1* expression. Percentage of total calories from protein was inversely related to insulin-like growth factor 1 receptor (*IGF1R*) ( $r = -0.605, p = 0.028, n = 13$ ).

**Discussion:** Variations in maternal physical activity and diet composition altered the expression of genes involved in fatty acid, amino acid and glucose transport and mTOR signaling. Future research on placental nutrient transport should include direct measures of maternal PA and dietary habits to help eliminate confounding factors.

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### 1. Introduction

Physical activity (PA) contributes to a healthy pregnancy [1,2]. The Society for Obstetricians and Gynaecologists of Canada (SOGC) and the American College of Obstetricians and Gynecologists

(ACOG) recommend 30 min of aerobic exercise 4 days per week during pregnancy [3–5]; however, the impact of prenatal PA on human placenta gene expression has not been fully investigated. Findings from animal models suggest that prenatal diet composition alters nutrient delivery to the fetus and warrants further investigation in humans [6–8]. Although understanding the impact of maternal lifestyle on the placenta is clinically relevant, research on placental nutrient transport often fails to account for maternal lifestyle behaviors (e.g. physical activity and dietary intake); factors known to modify energy balance, and potentially substrate delivery to the fetus. Only one study has explored the relationship between

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placental amino acid transporter activity and maternal energy balance using indirect measures of physical fitness and diet quality [9]. We are currently unaware of research examining placental nutrient transport using objective measures of PA or a precise qualification of maternal diet. The placenta plays a vital role in offspring growth and understanding which maternal factors influence placental nutrient transport is critical to elucidating the mechanisms contributing to altered fetal growth. While we hypothesize that prenatal PA and maternal diet alter the intrauterine environment, specifically placenta function [10], this area has not been fully explored. In order to begin to fill this knowledge gap the aim of the present study was to examine the associations between PA guideline adherence and maternal diet on the expression of genes involved in fatty acid, amino acid and glucose transport, and mTOR and insulin signaling in the placenta, which have been implicated in the regulation of energy balance and fetal growth.

## 2. Methods

### 2.1. Recruitment

Women with a body mass index (BMI) of 18.5–24.9 kg/m<sup>2</sup> (i.e. normal weight) were recruited from the Ottawa area (Ontario, Canada). Ethical approval and informed consent was obtained from all hospitals and participants. Women who smoked, those with diabetes of any type, fetal growth restriction or hypertensive diseases of pregnancy were excluded. Completion of the physical activity readiness medical examination (PARmed-X) for pregnancy was required to participate in the study [11].

The women visited the lab on two occasions between weeks 13 and 28 of pregnancy. The second trimester was selected as this is considered the most comfortable period for PA during pregnancy [12], and it is the trimester that is most frequently used for PA assessments in pregnant women [12,13]. At the first visit (between weeks 15–25), maternal height was directly measured, pre-pregnancy weight was recorded, and a fasting blood sample was obtained via peripheral venipuncture. At the second visit (between weeks 25–28), aerobic fitness was assessed using the HALO submaximal cardiorespiratory fitness test, for which the details have been previously published [14]. This self-paced walking test measures oxygen consumption (VO<sub>2</sub>) and heart rate (HR) as participants progress through incremental stages (Ultima PF/PFX pulmonary function and exercise system metabolic cart). Peak oxygen consumption (VO<sub>2peak</sub>) was predicted by extrapolating the HR-VO<sub>2</sub> linear relationship to the predicted maximum HR. Maternal and fetal clinical variables were collected through a chart review of the antenatal and birth records.

### 2.2. Diet

Diet was assessed during the 2nd trimester by three 24-h dietary recalls (one weekend day and two week days) using a free online program: Automated Self-administered 24-h (ASA24) recall that was developed and validated by the US National Cancer Institute [15]. Participants recorded everything that they ate or drank over each 24 h period.

### 2.3. Physical activity

Free-living PA was assessed for 7 days with the Actical<sup>®</sup> accelerometer (Mini Mitter Company, Inc., A Respiromics Inc. Company, Blend OR, USA) between weeks 17–28 of gestation. Participants wore the accelerometer on an elastic waistband on the right hip during day time, except during bathing and aquatic activities. The data reduction and analysis were harmonized with the Canadian Health Measures Survey approach [16–18]. In brief, accelerometry data were downloaded as 60 s epochs and signals were reported as counts per minute and translated into steps per minute. Data was processed using standardized quality control and data reduction procedures in SAS version 9.3 (SAS Institute, Cary, USA) [16]. Respondents with 4 or more valid days (10 or more hours of wear time) were retained for analyses [18]. Standard cut-points were used to measure time spent in various levels of movement intensity, including sedentary, light PA (LPA), moderate, and vigorous [18,19]. Adherence to the Adult Canadian physical activity guidelines was used to classify the women as “active” or “non-active”. These guidelines recommend 150 min of moderate-to-vigorous intensity physical activity (MVPA) per week accumulated in 10-min bouts [18,20]. Since not all participants completed 7 valid days, adherence to the guidelines was defined as an average daily MVPA >21.43 min, which is equal to 150 min divided by 7 days. The more stringent PA guidelines for adults were used instead of the pregnancy specific SOGC/ACOG guidelines in order to guarantee the selection of a very active population.

### 2.4. Placenta collection

The placenta was collected within 30 min of delivery. The placenta was weighed using a calibrated electronic scale. A biopsy of placental villous tissue of

approximately 0.5 × 0.5 cm thickness was obtained from healthy, non-necrotic tissue within a 10 cm radius of the cord insertion. Biopsies were rinsed in PBS and placed immediately in RNAlater<sup>®</sup> (QIAGEN Inc., Canada). After refrigeration for 24 h, the RNAlater<sup>®</sup> was removed and the samples were stored at –80 °C for batch analysis.

### 2.5. Blood samples

Following delivery of the placenta, a venous serum sample was obtained from the umbilical cord. Glucose, total, HDL and LDL cholesterol, and triglycerides were measured in the maternal and cord serum samples by oxidase enzymatic methodology using a Cholestech<sup>®</sup> LDX analyzer (Hayward, CA, USA).

### 2.6. RNA extraction

RNA was extracted using RNAzol<sup>®</sup> RT (BioShop Canada Inc., ON, Canada) from 25 mg of tissue following the manufacturer instructions. RNA concentration and purity were measured with the NanoDrop 2000 (Thermo Scientific). RNA integrity was confirmed by gel electrophoresis and qPCR (Perfecta SYBR Green FastMix and qScript cDNA SuperMix, Quanta Biosciences).

### 2.7. Gene expression

The expression of 24 genes implicated in fatty acid transport, glucose transport, and the mTOR and insulin signaling pathways were measured using a custom RT<sup>2</sup> Profiler<sup>™</sup> PCR Array (SABiosciences, ON, Canada). Housekeeping genes (UBC, YWHAZ, ACTB, TOP1, CYC1) were selected due to their stability in the placenta [21,22]. PCR was performed using an Eppendorf Mastercycler ep realplex 2S. Cycle parameters were 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. There were two samples per plate. The expression of three members of the System A amino acid transporter family (SNAT1, SNAT2 and SNAT4) were measured using QuantiTect Primers Assays (SLC38A1, SLC38A2, and SLC38A4; Qiagen) and Perfecta SYBR Green FastMix (Quanta Biosciences). Three housekeeping genes were used (UBC, TOP1, and YWHAZ; Qiagen). Cycle parameters were as follows: 95 °C for 2 min, followed by 40 cycles of 95 °C for 10 s and 56 °C for 30 s. All samples were run in triplicate, with four samples per plate. Threshold cycle values (C<sub>t</sub>) were directly obtained by this methodology. RNA levels were analyzed relative to the geometric mean of the housekeeping genes. Fold change was calculated using the web-based data analysis tool from Qiagen. The fold difference in the active group relative to control was determined by the 2<sup>-(ΔΔC<sub>t</sub>)</sup> method [23]. The relative expression level of each gene in the samples was calculated using the formula 2<sup>-(ΔC<sub>t</sub>)</sup>, where ΔC<sub>t</sub> = C<sub>t</sub>(gene of interest) – average (C<sub>t</sub>(housekeeping genes)).

### 2.8. Statistical analysis

Data are presented as mean ± standard error of the mean. Differences between groups were evaluated using a student's *t*-test. Values that were more than 3 standard deviations above the mean were excluded. Variables that were not normally distributed were transformed using a square root transformation. Pearson and Spearman correlations tested relations between dietary and PA variables and the relative expression of the genes of interest. Partial correlations tested the association between the same variables while controlling for MVPA in the correlations between the dietary variables and the genes of interest, and controlling for total sugar intake in the correlations between MVPA and the genes of interest. For all analyses, *p* < 0.05 was considered significant. Statistical analyses were carried out using SPSS version 20.0 (SPSS Inc., Chicago, IL, USA).

## 3. Results

### 3.1. Characteristics of the women and offspring

Anthropometric, metabolic profile, and fetal outcomes were not different between groups (Table 1). Ethnicity, educational attainment, and household income were similar (data not shown). Two macrosomic (>4000g) infants were born in the non-active group, but when gestational age and sex were considered only one was large-for-gestational-age (90th percentile). All other infants were average-for-gestational-age. Only six women met CSEP's PA guideline for adults. By design, the active women accumulated significantly more MVPA per week (315 min) than the non-active women (60 min), despite less total wear time (Table 2). The active women also took more steps per day, had higher cardiovascular fitness, and accumulated less sedentary time. Only LPA was similar between groups. Dietary intake was similar between groups with the only differences being higher consumptions of fiber and sodium in the active women (Table 3).

**Table 1**  
Maternal and fetal characteristics.

	Active (n = 6)	Non-active (n = 10)
Maternal age (years)	30.5 ± 1.4	31.0 ± 0.9
Maternal height (cm)	169.8 ± 2.1	168.7 ± 2.0
Pre-pregnancy maternal weight (kg)	63.5 ± 2.1	61.9 ± 3.0
Pre-pregnancy BMI (kg/m <sup>2</sup> )	22.3 ± 0.8	21.7 ± 0.8
Maternal weight at delivery (kg)	79.6 ± 3.4	76.6 ± 3.7
Gestational weight gain (kg)	15.9 ± 1.6	14.8 ± 1.4
Percentage of upper limit of GWG for BMI (%)	99.3 ± 10.25	96.4 ± 10.3
Maternal glucose (mmol/L)	3.9 ± 0.1	3.9 ± 0.1
Maternal triglycerides (mmol/L)	1.0 ± 0.2	1.4 ± 0.2
Maternal total cholesterol (mmol/L)	5.1 ± 0.5	4.8 ± 0.2
Maternal HDL cholesterol (mmol/L)	2.0 ± 0.1	1.6 ± 0.1
Maternal LDL cholesterol (mmol/L)	2.9 ± 0.4	2.6 ± 0.3
Maternal Non-HDL cholesterol (mmol/L)	3.4 ± 0.5	3.2 ± 0.2
Gestational age at delivery (weeks)	39.5 ± 0.4	39.8 ± 0.2
Type of birth	Vaginal = 5 C-section = 1	Vaginal = 8 C-section = 2
Birth weight (g)	3684.8 ± 43.3	3548.4 ± 110.9
Placenta weight (g)	644.2 ± 29.3	594.7 ± 30.9
Sex of infant	Male = 4 Female = 2	Male = 6 Female = 4
Cord blood glucose (mmol/L)	4.1 ± 0.3	4.3 ± 0.3
Cord blood HDL cholesterol (mmol/L)	0.6 ± 0.1	0.6 ± 0.1

Abbreviations: BMI = body mass index; GWG = gestational weight gain; HDL = high density lipoprotein; LDL = low density lipoprotein.

### 3.2. Changes in gene expression in response to physical activity

The fold change of the genes in the active women compared to the non-active women is shown in Table 4. The active women had lower expression of *FATP4*, *IGF1*, and *PRKAB1* and a higher expression of *SNAT2* ( $p < 0.05$ ), and a trend towards a lower expression of *IRS1*, *EIF4EBP1*, and *TSC2* ( $p < 0.07$ ). MVPA was inversely correlated with the relative expression of *FATP4*, *mTOR*, and *IGF1* (Fig. 1), but was not related to the other genes of interest (Table 5). When controlling for total sugar intake, the correlation between MVPA and the relative expression of *mTOR* was stronger, but the level of significance weakened. Similarly, the correlation between MVPA and *IGF1* remained, but the  $p$ -value increased. The correlation between MVPA and *FATP4* was reduced when controlling for sugar intake. We suspect that the weaker level of significance in these cases was due to the smaller number of women from whom we had available dietary information. A very strong inverse correlation between MVPA and the relative expression of *GLUT1* emerged when the partial correlation controlled for sugar intake.

**Table 2**  
Maternal physical Activity variables.

	Active (n = 6)	Non-active (n = 10)
Valid days	6.8	6.4
Wear time (hours)	13.0 ± 0.1 <sup>a</sup>	14.1 ± 0.3
MVPA/day (minutes) <sup>b</sup>	45.0 ± 7.4 <sup>a</sup>	8.5 ± 1.8
MPA/day (minutes) <sup>b</sup>	27.7 ± 5.1 <sup>a</sup>	6.4 ± 2.1
VPA/day (minutes) <sup>b</sup>	11.7 ± 3.2 <sup>a</sup>	1.3 ± 0.8
LPA/day (minutes)	191.8 ± 13.1	194.2 ± 13.3
Sedentary time (minutes)	533.4 ± 17.6 <sup>a</sup>	633.2 ± 20.5
Steps per day	11038 ± 878 <sup>a</sup>	6776 ± 327
VO <sub>2</sub> peak (ml/kg/min)	37.7 ± 3.2 <sup>a</sup>	29.3 ± 2.0

Abbreviations: MVPA = moderate-to-vigorous physical activity; MPA = moderate intensity physical activity; VPA = vigorous intensity physical activity; LPA = light intensity physical activity.

<sup>a</sup> Indicates a significant difference between the groups at  $p < 0.05$ .

<sup>b</sup> Accumulated in bouts of 10 min or more.

**Table 3**  
Maternal diet composition.

	Active (n = 5)	Non-active (n = 8)
Energy (kcal)	2609.9 ± 165.4	2456.5 ± 199.5
Carbohydrate (g)	333.9 ± 16.6	338.0 ± 36.6
Calories from carbohydrate (%)	51.9 ± 3.6	54.5 ± 1.5
Protein (g)	102.6 ± 9.3	88.9 ± 7.2
Calories from protein (%)	15.7 ± 0.8	14.6 ± 0.7
Total fat (g)	107.0 ± 14.2	90.1 ± 5.2
Calories from fat (%)	36.4 ± 3.7	33.5 ± 1.6
Total sugar (g)	158.0 ± 11.1	167.9 ± 28.8
Total dietary fiber (g)	41.0 ± 2.3 <sup>a</sup>	26.9 ± 1.6
Total saturated fat (g)	33.8 ± 3.9	31.1 ± 1.6
Total monounsaturated fatty acids (g)	38.7 ± 6.2	31.7 ± 2.1
Total polyunsaturated fatty acids (g)	26.0 ± 5.4	19.7 ± 2.9
Calcium (mg)	1545.1 ± 78.1	1300.6 ± 130.1
Iron (mg)	23.7 ± 2.1	19.7 ± 2.7
Cholesterol (mg)	365.9 ± 89.6	277.3 ± 27.3
Sodium (mg)	4436.8 ± 346.9 <sup>a</sup>	3351.6 ± 199.3

<sup>a</sup> Indicates a significant difference between the groups at  $p < 0.05$ .

### 3.3. Relations between the diet and genes of interest

The correlation coefficients and the partial correlation coefficients from the dietary variables and the genes of interest are shown in Table 6. There was a strong positive correlation observed between total sugar intake and the relative expression of *GLUT1*, and inverse correlations between total sugar and the relative expression of *mTOR* and *IGF1* (Fig. 1). Controlling for MVPA strengthened the correlation between total sugar intake and *GLUT1*, weakened the association between total sugar intake and *mTOR*, but did not alter the association with *IGF1*. Total energy intake was positively correlated with *SNAT4* expression, and there was a trend towards an inverse correlation between total energy intake and *mTOR*, but both relationships disappeared when controlling for MVPA. The percentage of total calories from protein was inversely correlated with the relative gene expression of *IGF1R*, and this association was strengthened when controlling for MVPA. The percentage of total calories from carbohydrates and fat were not related to any of the genes of interest.

## 4. Discussion

To our knowledge, this is the first investigation to explore the impact of objectively measured PA and maternal diet composition on placental gene expression in humans. The results of this investigation suggest that differences in maternal PA and diet quality during the second trimester are associated with altered expression of genes involved in glucose, amino acid and fatty acid transport, and the insulin and mTOR signaling pathways in the human placenta. This suggests that lifestyle factors that influence maternal energy balance may affect subsequent nutrient delivery to the fetus. Thus, we propose that direct measures of PA and objectively quantified dietary intake ought to be measured in future research examining placenta nutrient transport to eliminate confounding factors.

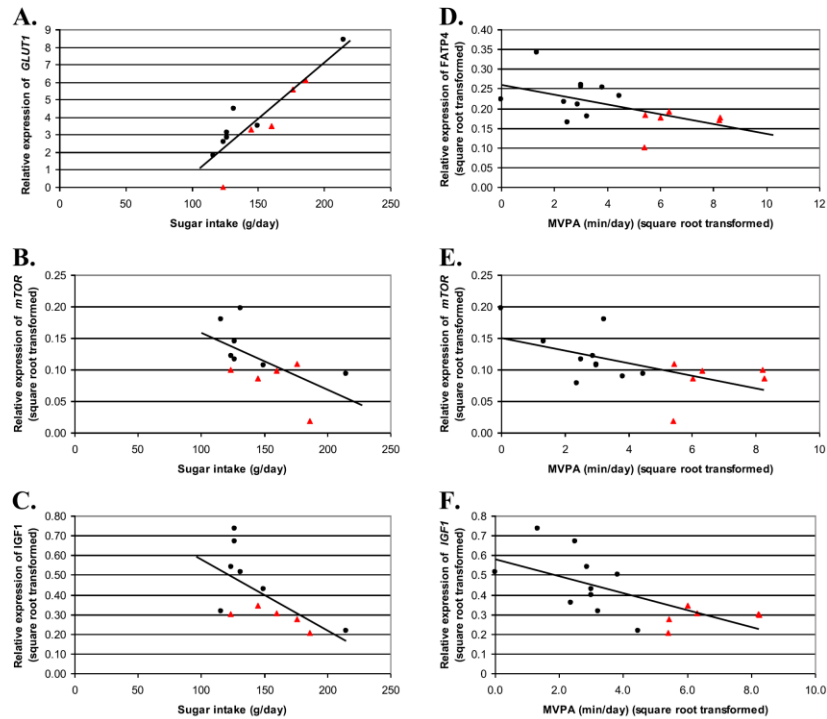
Prenatal PA has been shown to protect against birth weight extremes (i.e. small- or large-for-gestational-age) and increase the likelihood of delivering an average-for-gestational-age infant [24]. Despite substantial differences in PA between the active and non-active women, there were no differences in GWG or birth weight, although, there were two macrosomic infants born to non-active women. GWG is also an important independent contributor to birth weight [25,26], and the similar GWG likely contributed to the similarities in birth weight between groups. However, increasing energy expenditure through prenatal PA is associated with reduced

**Table 4**  
Fold change in the expression of the genes in the active women compared to the non-active women.

Symbol	Fold Change	95% CI	P-value	Description
<i>FATP4</i>	-1.95	-3.57, -1.33	0.022*	Fatty acid transport protein 4
<i>IGF1</i>	-2.37	-5, -1.56	0.031*	Insulin-like growth factor 1
<i>PRKAB1</i>	-1.40	-1.72, -1.19	0.019*	Protein kinase, AMP-activated, beta 1 non-catalytic subunit
<i>SNAT2</i>	1.68	1.02, 2.33	0.030*	Sodium-coupled neutral amino acid transporter 2
<i>IRS1</i>	-1.63	-2.86, -1.15	0.064	Insulin receptor substrate 1
<i>EIF4EBP1</i>	-1.64	-3.23, -1.1	0.059	Eukaryotic translation initiation factor 4E binding protein 1
<i>TSC2</i>	-1.45	-2.08, -1.11	0.058	Tuberous sclerosis 2
<i>FABP3</i>	-1.31	-3.23, 1.21	0.431	Fatty acid binding protein 3
<i>FABP4</i>	-1.34	-12.5, 1.42	0.866	Fatty acid binding protein 4
<i>FABP5</i>	-1.43	-4.35, 1.18	0.252	Fatty acid binding protein 5
<i>LPL</i>	2.20	-100000, 4.52	0.341	Lipoprotein lipase
<i>FATP2</i>	-1.52	-100000, 1.42	0.315	Fatty acid transport protein 2
<i>GLUT1</i>	-2.56	-100000, 1.27	0.831	Glucose transporter 1
<i>SNAT1</i>	1.43	-1.18, 2.00	0.276	Sodium-coupled neutral amino acid transporter 1
<i>SNAT4</i>	1.19	-6.67, 2.23	0.718	Sodium-coupled neutral amino acid transporter 4
<i>MTOR</i>	-2.62	-100000, -1.23	0.080	Mechanistic target of rapamycin
<i>IGF1R</i>	-1.25	-1.85, 1.06	0.214	Insulin-like growth factor 1 receptor
<i>AKT1</i>	-1.28	-2, 1.07	0.188	V-akt murine thymoma viral oncogene homolog 1
<i>RPS6KB1</i>	-1.33	-2.27, 1.06	0.193	Ribosomal protein S6 kinase, 70kDa, polypeptide 1
<i>RPS6</i>	-1.40	-2.33, 1	0.107	Ribosomal protein S6
<i>EIF4B</i>	1.15	-1.16, 1.44	0.260	Eukaryotic translation initiation factor 4B
<i>EIF4E</i>	-1.00	-1.49, 1.33	0.838	Eukaryotic translation initiation factor 4E
<i>PRKAA1</i>	-1.36	-1.96, -1.04	0.084	Protein kinase, AMP-activated, alpha 1 catalytic subunit
<i>TSC1</i>	-1.13	-1.43, 1.06	0.281	Tuberous sclerosis 1
<i>PIK3CA</i>	1.07	-1.85, 1.59	0.732	Phosphoinositide-3-kinase, catalytic, alpha polypeptide
<i>PIK3CB</i>	1.17	-1.33, 1.59	0.608	Phosphoinositide-3-kinase, catalytic, beta polypeptide
<i>PRKAA2</i>	1.23	-1.33, 1.72	0.428	Protein kinase, AMP-activated, alpha 2 catalytic subunit

\* p<0.05 difference in the active group compared to the non-active control

Fold difference was determined by the  $2^{(-\Delta\Delta Ct)}$  method. The arithmetic mean of 5 housekeeping genes was used.



**Fig. 1.** Significant correlations between the average total sugar intake per day and MVPA during the second trimester and the relative expression of the genes of interest in the placenta at term. A. Average total sugar intake per day was positively correlated with the relative expression of *GLUT1* ( $r = 0.897$ ,  $p = 0.000$ ,  $n = 12$ ). B. Average total sugar intake per day was inversely correlated with the relative *mTOR* expression ( $r = -0.603$ ,  $p = 0.038$ ,  $n = 12$ ). C. Average total sugar intake per day was inversely correlated with the relative expression of *IGF1* ( $r = -0.636$ ,  $p = 0.026$ ,  $n = 12$ ). D. MVPA was inversely correlated with the relative expression of *FATP4* ( $r = -0.534$ ,  $p = 0.033$ ,  $n = 16$ ). E. MVPA was inversely correlated with the relative expression of *mTOR* ( $r = -0.566$ ,  $p = 0.022$ ,  $n = 16$ ). F. MVPA was inversely correlated with the relative expression of *IGF1* ( $r = -0.656$ ,  $p = 0.006$ ,  $n = 16$ ). ▲ = Active women; ● = Non-active women.

neonatal adiposity without reducing neonatal fat free mass or absolute birth weight [27]. Thus, it is possible that the infants born to active women had less fat mass than those born to non-active women, despite no differences in birth weight.

According to our data, meeting PA guidelines during the second trimester is associated with a nearly 2-fold lower gene expression of *FATP4*. *FATP4* transports free fatty acids across the placenta [28] and thus lower *FATP4* RNA expression in active women suggests

reduced potential for fatty acid transport to the fetus. Prenatal PA is associated with reduced neonatal fat mass [27,29], consequently it is not surprising that *FATP4* expression is reduced in active women. Meeting the physical activity guidelines was also associated with a 1.68-fold higher expression of *SNAT2*. *SNAT2* is a key amino acid transporter at term [30,31], and our data suggests that the physically active women may have the potential for higher amino acid transport to the fetus. Previously, reduced System A amino acid transport was associated with women who reported strenuous exercise during pregnancy [9], however, this was based on self-reported PA, which is not as reliable as directly measured PA [32,33]. The potential for lower fatty acid transport and higher amino acid transport to the fetus in the active women may explain why there was no difference in birth weight between the active women and the non-active women. Future work should measure the protein expression and activity of *FATP4* and *SNAT2* in active and non-active women, and should include a measure of infant body composition to determine if reduced expression of *FATP4* and higher *SNAT2* in the active women is contributing to improved neonatal body composition.

The active women also had lower expression of members of the insulin and mTOR signaling pathways including *IGF1*, *PRKAB1*, *IRS1*, *EIF4EBP1*, and *TSC2*. In addition, MVPA was inversely correlated with *GLUT1* expression (when controlling for total sugar intake), and MVPA was inversely correlated with *mTOR* and *IGF1*. IGF-I peptides have a vital role in fetal growth [34], and lower *IGF1* suggests lower levels of this growth promoting peptide in the placenta; however, birth weight was similar in the active and non-

**Table 5**  
Correlations between MVPA and the genes of interest.

Independent variable	Gene of interest	Correlation coefficient	p-value	N	Partial correlation controlling for sugar intake (n = 12)	p-value of partial correlation
MVPA (min/day)	<i>mTOR</i>	-0.566	0.022*	16	-0.627	0.052
	<i>FABP3</i>	-0.001	0.997	15	0.002	0.995
	<i>FABP4</i>	-0.118	0.665	16	-0.095	0.795
	<i>FABP5</i>	-0.344	0.192	16	-0.228	0.526
	<i>FATP2</i>	-0.137	0.614	16	0.245	0.496
	<i>FATP4</i>	-0.534	0.033*	16	-0.425	0.220
	<i>GLUT1</i>	-0.032	0.908	16	-0.926	0.000*
	<i>SNAT1</i>	0.021	0.938	16	0.020	0.954
	<i>SNAT2</i>	-0.113	0.677	16	-0.198	0.560
	<i>SNAT4</i>	-0.042	0.878	16	-0.021	0.951
	<i>IRS1</i>	-0.380	0.147	16	-0.277	0.439
	<i>IGF1</i>	-0.656	0.006*	16	-0.648	0.043
	<i>IGF1R</i>	-0.135	0.618	16	-0.260	0.469

\* $p < 0.05$ , significant correlation between the variables.

**Table 6**  
Correlations between dietary variables and the genes of interest.

Independent variable	Gene of interest	Correlation coefficient	p-value	N	Partial correlation controlling for MVPA	p-value of partial correlation
Total energy	<i>mTOR</i>	−0.547	0.053	13	−0.396	0.202
	<i>SNAT1</i>	−0.351	0.240	13	−0.143	0.674
	<i>SNAT2</i>	−0.509	0.075	13	−0.381	0.247
	<i>SNAT4</i>	0.650	0.016*	13	0.376	0.254
Percentage of calories from carbohydrates	<i>mTOR</i>	−0.103	0.728	13	−0.364	0.245
	<i>GLUT1</i>	0.319	0.288	13	0.304	0.336
	<i>IRS1</i>	−0.363	0.223	13	−0.195	0.544
	<i>IGF1</i>	0.206	0.0500	13	0.040	0.901
	<i>IGF1R</i>	0.221	0.468	13	0.150	0.641
Percentage of calories from protein	<i>mTOR</i>	0.050	0.872	13	0.060	0.853
	<i>SNAT1</i>	−0.427	0.146	13	−0.563	0.071
	<i>SNAT2</i>	−0.137	0.655	13	−0.228	0.501
	<i>SNAT4</i>	0.051	0.867	13	0.269	0.424
	<i>IRS1</i>	−0.516	0.071	13	−0.561	0.058
	<i>IGF1</i>	−0.146	0.634	13	−0.211	0.511
Percentage of calories from fat	<i>IGF1R</i>	−0.605	0.028*	13	−0.644	0.024*
	<i>mTOR</i>	0.033	0.915	13	0.414	0.205
	<i>FABP3</i>	−0.234	0.465	12	−0.232	0.493
	<i>FABP4</i>	−0.106	0.730	13	−0.060	0.861
	<i>FABP5</i>	0.091	0.728	13	0.270	0.422
	<i>FATP2</i>	0.285	0.345	13	0.343	0.302
	<i>FATP4</i>	−0.039	0.899	13	0.176	0.604
	<i>IRS1</i>	0.533	0.061	13	0.443	0.172
	<i>IGF1</i>	−0.255	0.401	13	−0.035	0.919
	<i>IGF1R</i>	−0.107	0.727	13	0.025	0.941
	Total sugar per day	<i>mTOR</i>	−0.603	0.038*	12	−0.550
<i>GLUT1</i>		0.897	0.000*	12	0.986	0.000*
<i>IRS1</i>		−0.021	0.948	12	0.193	0.571
<i>IGF1</i>		−0.636	0.026*	12	−0.610	0.046*
<i>IGF1R</i>		−0.394	0.205	12	−0.312	0.350

\*p < 0.05, significant correlation between the variables.

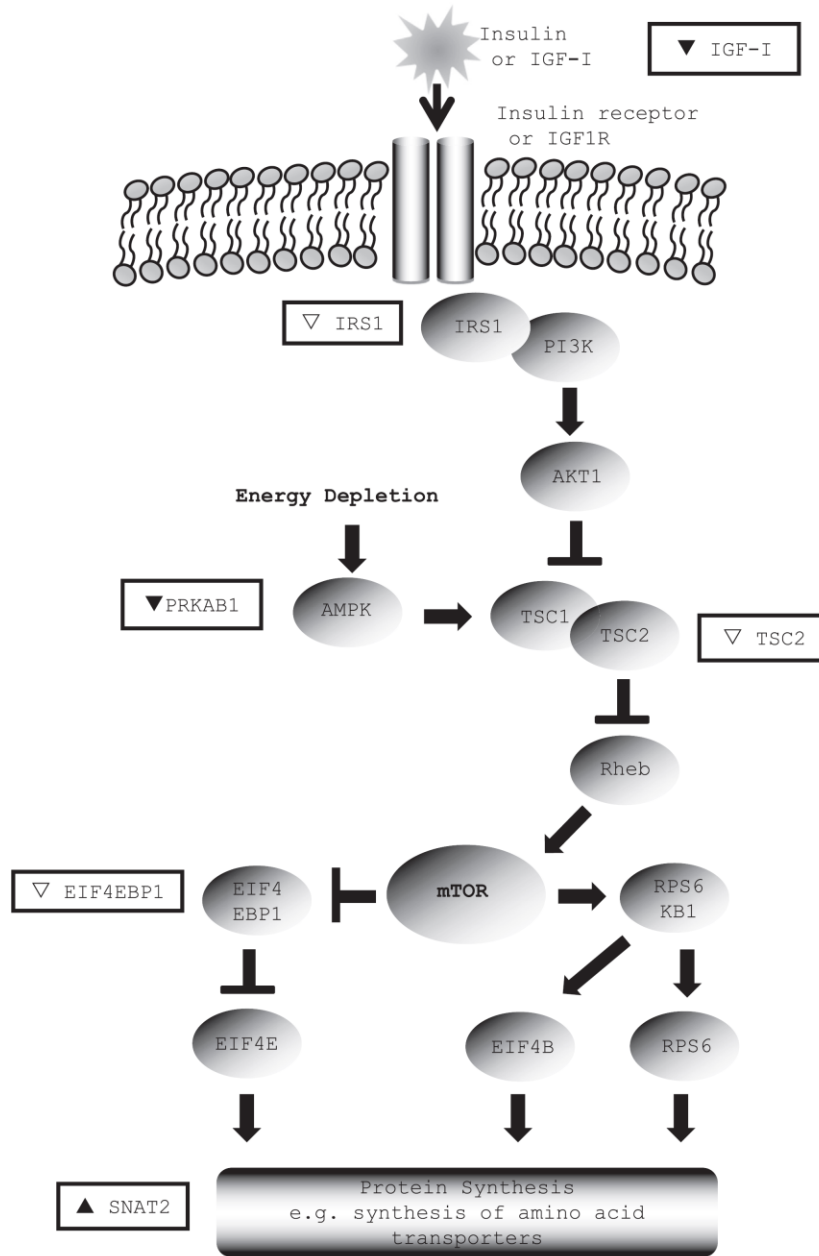
active women. Similarly, studies performed by Hopkins et al. have shown that women who participated in prenatal exercise had offspring with lower cord levels of IGF-I and IGF-II, but no change in birth weight distribution or in maternal IGF axis proteins relative to controls [35,36]. These findings suggest that exercise may have influenced fetal growth via the placenta. In both cases, a reduction in *IGF1* without a major shift in birth weight supports the notion that exercise functions to normalize, rather than reduce fetal growth.

The mTOR pathway integrates various signals (e.g. growth factors, energy status, oxygen) to regulate fetal growth [37]. The inhibition of mTOR significantly reduces the activity of placental amino acid transporters [38,39]. The lower expression of members of the mTOR signaling pathway in the physically active women include both positive (*IGF1* and *IRS1*) and negative (*PRKAB1* and *TSC2*) regulators of mTOR (summarized in Fig. 2). *PRKAB1* is a regulatory subunit of AMPK, and AMPK detects energy depletion and inhibits mTOR [40,41]. *IRS1* transmits signals from the insulin receptor and *IGF1R* [42], the *TSC1/2* complex transmits the upstream signals from the *IRS* pathway to mTOR, and the deactivation of EIF4EBP1 downstream of mTOR promotes protein translation, namely synthesis of amino acid transporters [37]. A reduction in the genes that positively regulate mTOR (i.e. *IGF1* and *IRS1*), if accompanied by reduced protein expression, could lead to a reduction in mTOR signaling. Meanwhile, a reduction in the genes that inhibit mTOR signaling (i.e. *PRKAB1* and *TSC2*), if accompanied by reduced protein expression, may lead to increased mTOR signaling. The reduction in both the positive and negative regulators of mTOR in the active women may explain why the expression of one of the amino acid transporters (*SNAT2*) was increased, while the other two remained unchanged. Although our study was not powered to detect absolute changes in birth weight, given that offspring birth weight was similar between groups, a reduction in the positive and negative regulators of mTOR may have optimized mTOR signaling

and consequently helped maintain normal fetal growth rather than triggering restricted or excess growth.

The dietary variable with the largest effect on gene expression was total sugar intake. *GLUT1* is the primary placental glucose transporter in humans at term [43]. The strong positive correlation suggests that as sugar intake increases so does the expression of *GLUT1*, and therefore there is the potential for increased glucose transport to the fetus. This supports the theory that the placenta functions as a nutrient sensor [44], where placental nutrient transport is matched with the availability of nutrients in maternal circulation. In lean women with GDM, those with insulin-controlled GDM had higher *GLUT1* expression when compared to diet-controlled GDM and healthy controls [45]. Based on our findings, we postulate that the women with diet-controlled GDM may have made greater efforts to reduce total sugar intake, and this may have contributed to the differences in *GLUT1* expression in insulin-vs. diet-controlled GDM; which further emphasizes the importance of measuring diet in this area of research.

An alternative theory of 'adaptive regulation' suggests that in an effort to maintain normal fetal growth the placenta up- or down-regulates nutrient transporter activity in response to low or high substrate levels, respectively [46]. For instance, glucose transport was reduced in a hyperglycemic mouse model [47]. The moderate inverse correlations between total sugar and *mTOR* and *IGF1* reported here support this theory. The placenta might compensate for increased maternal sugar intake with lower expression of members of the mTOR pathway thus helping to moderate nutrient delivery to the fetus despite increased glucose availability. This is further supported by the inverse correlations between the percentage of calories from protein and *IGF1R*, and between total energy intake and *mTOR*. However, the percentage of calories from protein was not related to the expression of any of the amino acid transporters, while total energy intake was positively correlated with *SNAT4*, with a trend towards an inverse



**Fig. 2.** Simplified mTOR signaling pathway with changes in gene expression in active women compared to non-active women. mTOR is controlled by the intermediate Rheb which is regulated by TSC1/2. Insulin and IGF-I phosphorylate AKT1 through IRS1, which inhibits TSC2, thus releasing the inhibition of Rheb by TSC1/2. Activated Rheb stimulates mTOR signalling. Activation of mTOR leads to the phosphorylation of RPS6KB1 and the dissociation of eIF4E from EIF4EBP1, which in turn promotes protein synthesis. AMPK, in response to low energy levels, phosphorylates TSC2 and inhibits mTOR. ▼ = a significant lower expression of this gene in the placenta of active women ( $p < 0.05$ ); ▲ = a significantly higher expression of this gene in the placenta of active women ( $p < 0.05$ ); ▽ = trend towards lower expression of this gene in the placenta of active women ( $p < 0.07$ ). Abbreviations: IGF-I – Insulin-like growth factor 1; IGF1R – Insulin-like growth factor 1 receptor; IRS1 – Insulin receptor substrate 1; AKT1 – protein kinase B; TSC – tuberous sclerosis complex; AMPK – AMP activated kinase; PRKAB1 – beta 1 non-catalytic subunit of AMPK; mTOR – mammalian target of rapamycin; EIF4EBP1 – Eukaryotic translation initiation factor 4E binding protein 1; RPS6KB1 – Ribosomal protein S6 kinase, polypeptide 1; EIF4E – Eukaryotic translation initiation factor 4E; EIF4B – Eukaryotic translation initiation factor 4B; RPS6 – Ribosomal protein S6; SNAT2 – Sodium-coupled neutral amino acid transporter 2.

correlation between total energy intake and SNAT2. At term, SNAT1 and SNAT2 are believed to be the predominant contributors to System A amino acid transport, while the contribution of SNAT4 is reduced [30,31]. Thus, in response to a higher intake of

total energy, the placenta might compensate with reduced expression mTOR and one of the key amino acid transporters (SNAT2), thus potentially reducing the transport of amino acids, in an effort to maintain normal fetal growth. This warrants further

investigation on the protein expression and activity of the members of the mTOR signaling molecules and the amino acid transporters when maternal energy balance (i.e. PA and diet) are objectively measured.

The macronutrient distribution of both groups of women was similar to the acceptable macronutrient distribution ranges recommended for Canadians [48], which could explain why we did not observe associations between the percentage of calories coming from carbohydrates or fat and the genes of interest because greater deviations in macronutrient composition may be needed to alter placental gene expression. For instance, in animal models up-regulation of placental nutrient transporter abundance and activity is a common response to abnormal nutrient availability (e.g. high fat or high sucrose diets) [6–8,49].

Women who met PA guidelines during the second trimester had lower expression of *FATP4* and members of the mTOR pathway, and higher expression of *SNAT2*, compared to non-active women. Variations in the quality and quantity of the diet during the second trimester also affected the expression of nutrient transporters and signaling molecules. These results are limited by the fact that PA and diet were only measured during the second trimester and it is unknown if these variables differed across gestation. Nonetheless, we believe that these findings represent important considerations regarding the importance of accounting for variations in maternal lifestyle and energy balance during pregnancy. Future work with a larger sample size would be valuable to confirm these findings and investigate whether protein expression and activity of these molecules is also affected. The level of PA and diet composition of women can be vastly different and likely alters the intrauterine environment [10]. Based on our findings we advise that future research on placental nutrient transport should include direct measures of maternal PA and dietary habits to help eliminate confounding factors.

#### Conflict of interest statement

There are no conflicts of interest for any authors.

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#### References

- [1] Nascimento SL, Surita FG, Cecatti JG. Physical exercise during pregnancy: a systematic review. *Curr Opin Obstet Gynecol* 2012;24(6):387–94.

- [2] Ferraro ZM, Gaudet L, Adamo KB. The potential impact of physical activity during pregnancy on maternal and neonatal outcomes. *Obstet Gynecol Surv* 2012;67(2):99–110.
- [3] Artal R, O'Toole M. Guidelines of the American College of Obstetricians and Gynecologists for exercise during pregnancy and the postpartum period. *Br J Sports Med* 2003;37(1):6–12.
- [4] Davies GA, Wolfe LA, Mottola ML, Mackinnon C. Joint SOGC/CSEP clinical practice guideline: exercise in pregnancy and the postpartum period. *Can J Appl Physiol* 2003;28(3):330–41.
- [5] Wolfe LA, Davies GA. Canadian guidelines for exercise in pregnancy. *Clin Obstet Gynecol* 2003;46(2):488–95.
- [6] Sferruzzi-Perri AN, Vaughan OR, Haro M, Cooper WN, Musial B, Charalambous M, et al. An obesogenic diet during mouse pregnancy modifies maternal nutrient partitioning and the fetal growth trajectory. *FASEB J* 2013;27(10):3928–37.
- [7] King V, Hibbert N, Seckl JR, Norman JE, Drake AJ. The effects of an obesogenic diet during pregnancy on fetal growth and placental gene expression are gestation dependent. *Placenta* 2013;34(11):1087–90.
- [8] Jones HN, Woollett LA, Barbour N, Prasad PD, Powell TL, Jansson T. High-fat diet before and during pregnancy causes marked up-regulation of placental nutrient transport and fetal overgrowth in C57/BL6 mice. *FASEB J* 2009;23(1):271–8.
- [9] Lewis RM, Greenwood SL, Cleal JK, Crozier SR, Verrall L, Inskip HM, et al. Maternal muscle mass may influence system A activity in human placenta. *Placenta* 2010;31(5):418–22.
- [10] Adamo KB, Ferraro ZM, Brett KE. Can we modify the intrauterine environment to halt the intergenerational cycle of obesity? *Int J Environ Res Public Health* 2012;9(4):1263–307.
- [11] Canadian Society for Exercise Physiology. Physical activity readiness medical examination (PARmed-X) for pregnancy. 2013 [Ref Type: Pamphlet].
- [12] Downs DS, LeMasurier GC, Dinallo JM. Baby steps: pedometer-determined and self-reported leisure-time exercise behaviors of pregnant women. *J Phys Act Health* 2009;6(1):63–72.
- [13] Harrison CL, Thompson RG, Teede HJ, Lombard CB. Measuring physical activity during pregnancy. *Int J Behav Nutr Phys Act* 2011;8:19.
- [14] Breithaupt P, Adamo KB, Colley RC. The HALO submaximal treadmill protocol to measure cardiorespiratory fitness in obese children and youth: a proof of principle study. *Appl Physiol Nutr Metab* 2012;37(2):308–14.
- [15] National Cancer Institute. Automated self-administered 24-Hour recall (ASA24). 2011. Bethesda, MD. 6-1-2014. Ref Type: Internet Communication.
- [16] Colley RC, Gorber SC, Tremblay MS. Quality control and data reduction procedures for accelerometry-derived measures of physical activity. *Health Rep* 2010;21(1):63–9.
- [17] Colley RC, Garriguet D, Janssen I, Craig CL, Clarke J, Tremblay MS. Physical activity of Canadian children and youth: accelerometer results from the 2007 to 2009 Canadian Health Measures Survey. *Health Rep* 2011;22(1):15–23.
- [18] Colley RC, Garriguet D, Janssen I, Craig CL, Clarke J, Tremblay MS. Physical activity of Canadian adults: accelerometer results from the 2007 to 2009 Canadian Health Measures Survey. *Health Rep* 2011;22(1):7–14.
- [19] Wong SL, Colley R, Connor GS, Tremblay M. Actical accelerometer sedentary activity thresholds for adults. *J Phys Act Health* 2011;8(4):587–91.
- [20] Tremblay MS, Warburton DE, Janssen I, Paterson DH, Latimer AE, Rhodes RE, et al. New Canadian physical activity guidelines. *Appl Physiol Nutr Metab* 2011;36(1):36–46.
- [21] Cleal JK, Day P, Hanson MA, Lewis RM. Measurement of housekeeping genes in human placenta. *Placenta* 2009;30(11):1002–3.
- [22] Drewlo S, Levytska K, Kingdom J. Revisiting the housekeeping genes of human placental development and insufficiency syndromes. *Placenta* 2012;33(11):952–4.
- [23] Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* 2001;25(4):402–8.
- [24] Juhl M, Olsen J, Anderson P, Nohr E, Anderson A. Physical exercise during pregnancy and fetal growth measures: a study within the Danish National Birth Cohort. *Am J Obstetrics Gynecol* 2010;202(1):e1–8.
- [25] Ferraro ZM, Barrowman N, Prud'homme D, Walker M, Wen SW, Rodger M, et al. Excessive gestational weight gain predicts large for gestational age neonates independent of maternal body mass index. *J Matern Fetal Neonatal Med* 2012;25(5):538–42.
- [26] Ludwig DS, Currie J. The association between pregnancy weight gain and birthweight: a within-family comparison. *Lancet* 2010;376(9745):984–90.
- [27] Harrod CS, Chasan-Taber L, Reynolds RM, Fingerlin TE, Glueck DH, Brinton JT, et al. Physical activity in pregnancy and neonatal body composition: the healthy start study. *Obstet Gynecol* 2014;124(2):257–64.
- [28] Cunningham P, McDermott L. Long chain PUFA transport in human term placenta. *J Nutr* 2009;139(4):636–9.
- [29] Clapp III JF, Capeless E. Neonatal morphometrics after endurance exercise during pregnancy. *Am J Obstetrics Gynecol* 1990;163(6 1):1805–11.
- [30] Desforges M, Mynett KJ, Jones RL, Greenwood SL, Westwood M, Sibley CP, et al. The SNAT4 isoform of the system A amino acid transporter is functional in human placental microvillous plasma membrane. *J Physiol* 2009;587(Pt 1):61–72.

- [31] Desforges M, Greenwood SL, Glazier JD, Westwood M, Sibley CP. The contribution of SNAT1 to system A amino acid transporter activity in human placental trophoblast. *Biochem Biophys Res Commun* 2010;398(1):130–4.
- [32] Garriguet D, Colley RC. A comparison of self-reported leisure-time physical activity and measured moderate-to-vigorous physical activity in adolescents and adults. *Health Rep* 2014;25(7):3–11.
- [33] Prince SA, Adamo KB, Hamel ME, Hardt J, Connor GS, Tremblay M. A comparison of direct versus self-report measures for assessing physical activity in adults: a systematic review. *Int J Behav Nutr Phys Act* 2008;5:56.
- [34] Chiesa C, Osborn JF, Haass C, Natale F, Spinelli M, Scapillati E, et al. Ghrelin, leptin, IGF-1, IGFBP-3, and insulin concentrations at birth: is there a relationship with fetal growth and neonatal anthropometry? *Clin Chem* 2008;54(3):550–8.
- [35] Hopkins SA, Baldi JC, Cutfield WS, McCowan L, Hofman PL. Exercise training in pregnancy reduces offspring size without changes in maternal insulin sensitivity. *J Clin Endocrinol Metab* 2010;95(5):2080–8.
- [36] Hopkins SA, Baldi JC, Cutfield WS, McCowan L, Hofman PL. Effects of exercise training on maternal hormonal changes in pregnancy. *Clin Endocrinol (Oxf)* 2011;74(4):495–500.
- [37] Laplante M, Sabatini DM. mTOR signaling in growth control and disease. *Cell* 2012;149(2):274–93.
- [38] Roos S, Jansson N, Palmberg I, Saljo K, Powell TL, Jansson T. Mammalian target of rapamycin in the human placenta regulates leucine transport and is down-regulated in restricted fetal growth. *J Physiol* 2007;582(Pt 1):449–59.
- [39] Roos S, Kanai Y, Prasad PD, Powell TL, Jansson T. Regulation of placental amino acid transporter activity by mammalian target of rapamycin. *Am J Physiol Cell Physiol* 2009;296(1):C142–50.
- [40] Liu X, Chhipa RR, Pooya S, Wortman M, Yachyshin S, Chow LM, et al. Discrete mechanisms of mTOR and cell cycle regulation by AMPK agonists independent of AMPK. *Proc Natl Acad Sci U S A* 2014;111(4):E435–44.
- [41] Xu J, Ji J, Yan XH. Cross-talk between AMPK and mTOR in regulating energy balance. *Crit Rev Food Sci Nutr* 2012;52(5):373–81.
- [42] Forbes K, Westwood M. The IGF axis and placental function. A mini review. *Horm Res* 2008;69(3):129–37.
- [43] Illsley NP. Glucose transporters in the human placenta. *Placenta* 2000;21(1):14–22.
- [44] Jansson T, Powell TL. IFPA 2005 award in placentology lecture. Human placental transport in altered fetal growth: does the placenta function as a nutrient sensor? – A review. *Placenta* 2006;27(Suppl. A):S91–7.
- [45] Colomiere M, Permezel M, Riley C, Desoye G, Lappas M. Defective insulin signaling in placenta from pregnancies complicated by gestational diabetes mellitus. *Eur J Endocrinol* 2009;160(4):567–78.
- [46] Jones HN, Powell TL, Jansson T. Regulation of placental nutrient transport—a review. *Placenta* 2007;28(8–9):763–74.
- [47] Ogura K, Sakata M, Yamaguchi M, Kurachi H, Murata Y. High concentration of glucose decreases glucose transporter-1 expression in mouse placenta in vitro and in vivo. *J Endocrinol* 1999;160(3):443–52.
- [48] Health Canada. Dietary reference intakes tables. Canada: Government of Canada; 2010.
- [49] Lin Y, Zhuo Y, Fang ZF, Che LQ, Wu D. Effect of maternal dietary energy types on placenta nutrient transporter gene expressions and intrauterine fetal growth in rats. *Nutrition* 2012;28(10):1037–43.

## **Part III**

### **3.1 Discussion**

The intrauterine environment plays a critical role in fetal development and downstream health. Given the rise in maternal obesity, the propensity for women to gain excess gestational weight and the incidence of babies being born large-for-gestational-age, there is the need for more prenatal research that addresses the mechanisms through which the maternal environment of excess body weight affects fetal growth, as well the potential modifiable factors that may offset these negative influences. These behaviours are often the components of lifestyle interventions targeting women with obesity and include gaining the appropriate amount of weight during gestation, regular physical activity and eating a healthy diet. Although there is accumulating evidence of the detriments of maternal obesity and excess GWG, and the benefits of physical activity and a healthy diet during pregnancy, there is limited knowledge of the mechanisms through which these effects occur. It is proposed that these factors may cause changes in the placenta biology, including transport of nutrients across the placenta, thus mediating some of the associated changes in neonatal phenotype.

The purpose of this thesis was to help address the knowledge gaps and to gain a better understanding of the contribution of the maternal obesity and modifiable risk factors, such as excess GWG, physical activity and diet, on the expression of genes that regulate nutrient transport across the placenta, as well as evaluate the accuracy of the regularly used Pregnancy Physical Activity Questionnaire. This work provides several contributions including:

- i. The discovery that the expression of the genes involved in nutrient transport, and the mTOR and insulin signalling pathways in the placenta do not differ between lean and obese women
  - a. With the exception of lower *mTOR* in the obese women with male offspring
- ii. The discovery that GWG is related to the expression of genes in the placenta, particularly from male offspring (see table below)

	Correlations with the percentage gained above GWG guidelines in placentas (male offspring)	
<b>Gene</b>	<b>Unadjusted</b>	<b>Adjusted for weight of the placenta</b>
FABP3	r= -0.38, p= 0.20	r= -0.61, p= 0.04
SNAT1	r= 0.65, p= 0.02	r= 0.63, p=0.03
mTOR	r= -0.65, p= 0.02	r= -0.65, p= 0.02
IRS1	r= -0.51, p= 0.08	r= -0.59, p= 0.04
IGF1R	r= -0.45, p= 0.12	r= -0.64, p= 0.03

- iii. Determining that the Pregnancy Physical Activity Questionnaire significantly overestimates physical activity and is not correlated with direct measure of activity in both active and non-active women.
- iv. The identification of physical activity and diet as modifiers of placenta nutrient transport-related gene expression
  - a. The discovery that meeting physical activity guidelines was associated with lower expression of a fatty acid transporter (*FATP4*) and higher expression of an amino acid transporter (*SNAT2*), suggesting the potential for altered fatty acid and amino acid delivery to the fetus.
  - b. The discovery that sugar intake was related to the expression of the principal glucose transporter (*GLUT1*), indicating that as there is an increase in the intake of sugar that there exists the potential for higher glucose transport to the fetus.

This thesis provides evidence that there are numerous maternal and fetal factors that may be contributing to placenta nutrient transport-related gene expression in humans and that moving forward it may not be sound scientific practice to ignore these factors when studying the placenta. Previous work on placenta nutrient transport only considered the disease status of the mother (i.e. diabetic or not) and failed to consider modifiable risk factors. To the best of our knowledge this work represents the first set of studies in humans that has considered directly measured PA, maternal diet composition, or whether GWG was gained in accordance with the current guidelines. Variation in factors that modify maternal energy balance, such as GWG, PA and diet, may alter the substrate and hormonal exposure of the placenta, thus influencing the expression of the genes involved in nutrient transport, while fetal sex may affect how the placenta reacts to these variations. There is extensive research to support gaining within the GWG guidelines to promote appropriate fetal growth [19,34], and given the high degree of inter-individual variability for GWG it is unfortunate that previous research on placenta nutrient transport did not consider the effects of GWG with respect to the BMI-specific guidelines. In addition, the observed association between sugar intake and *GLUT1* expression highlights the importance of monitoring maternal diet, a conclusion that is further supported by the recent finding that maternal added sugar intake predicted the accumulation of fetal abdominal fat among pregnant adolescents [100]. Likewise, it should not be surprising that physical activity, a known mediator of health and energy balance, was associated with modifications in placenta nutrient transport-related gene expression.

Considering how drastically different GWG, levels of PA, and sugar intake can be between individuals even if they all fall within the same BMI category (Table 2), failing to account for

variations in these potentially confounding factors might mask important differences. The environmental exposure of the placenta of a mother who gains excess weight, has a sedentary lifestyle and a diet high in sugar is likely very different than that of a mother who gains the recommended amount of weight, is regularly physically active, and limits her sugar intake. Given the associations observed between physical activity, sugar intake and GWG and the genes of interest in Manuscripts 1 and 3, one can postulate that these two women could have very different gene expression profiles. Overall, these findings emphasize the importance of considering the whole picture, rather than focusing on one particular aspect of the pregnancy. This knowledge can be used to guide future research on placental nutrient transport and placenta biology more broadly, by ensuring that all relevant factors which might influence maternal energy balance and substrate transfer to the fetus are considered.

**Table 2.** Range of values for GWG, sugar intake, physical activity and sedentary time in the lean women who participated in the Active MOM study (n=23)

<b>Maternal Variable</b>	<b>Range of values</b>
GWG gained with respect to BMI-specific upper limit (%)	53 - 142
Total sugar intake/day (g)	105 – 354
MVPA/day accumulated in 10 min bouts (min)	0 – 94
Minutes of sedentary time/day (min)	441 - 790

Furthermore, this thesis provides evidence that lends support to the theory that “*boys live dangerously in the womb*”[101], as the placenta from male offspring may respond to intrauterine conditions in a different manner than female offspring, potentially affecting the delivery of nutrients to the fetus. For instance, in the placentas from male offspring we observed a -2.3 fold

change in *mTOR* expression in the women with obesity, and positive and negative correlations between the percentage of GWG gained above the BMI specific guidelines and key nutrient transporters and signalling molecules. The presence of these relationships in the placentas from male offspring but not in the combined sample suggests that the male placentas might be more susceptible to perturbations in maternal weight, or simply that they respond in a different manner to these perturbations than the placentas from female offspring. Given that these relationships were only examined at the level of gene expression, it cannot be concluded that the response to the excess maternal weight of the placentas from the male offspring is indeed ‘dangerous’, however, it does suggest that the placentas from male offspring may be responding discordantly. Thus future work exploring these relationships at the protein and activity level ought to consider offspring sex.

Moreover, the findings from this thesis provide evidence in support of both placenta nutrient transport theories: the ‘nutrient sensor’ hypothesis, whereby nutrient delivery is matched to the availability of nutrients in maternal circulation, and the alternative theory of ‘adaptive regulation’, which suggests that the placenta responds in a compensatory manner by up- or down-regulating nutrient transport in response to low or high levels of nutrients. For instance, in manuscript 1, gaining excess GWG was positively correlated with *SNATI* (nutrient sensing), however it was inversely correlated with *FABP3*, *mTOR*, *IRS1*, and *IGF1R* (adaptive regulation). Similarly, in manuscript 3, sugar intake had a strong positive correlation with *GLUT1* (nutrient sensing), as well as a negative correlation with *mTOR* and *IGF1* (adaptive regulation). Given that both studies support the two conflicting theories, we might postulate that different genes may respond to the same stress in different manners, and that both theories may be correct. The

majority of the previous work focused solely on one nutrient or signalling pathway and the findings only supported one of the theories, thus it is possible that the findings would have differed had more macronutrient transporters and signalling molecules been considered.

This work adds to the current literature on placental nutrient transport in the context of maternal obesity and draws attention to the possibility that a ‘healthy obese’ phenotype exists in pregnancy which may not alter the expression of nutrient transport related genes in the placenta. In the same manner that additional metabolic abnormalities are necessary to increase the mortality risk in an obese individual [102], perhaps additional metabolic co-morbidities, such as impaired glucose tolerance, are required before the gene expression of the placenta nutrient transporters is altered in pregnancies complicated by obesity. In order to isolate the specific effects attributed to obesity, the women in Manuscript 1 were selected as normoglycemic, with no overt metabolic co-morbidities, and it is possible that in this subgroup of healthy obese pregnant women that the metabolic insult to the intrauterine environment was minimal, and thus the gene expression was not dysregulated. Including women with obesity who deliver appropriate-for-gestational-age offspring in Manuscript 1, and in previously published studies, may have contributed to our null and as well as the conflicting findings in the literature. While maternal obesity increases the risk of pregnancy complications and increases the likelihood of birthing a large-for-gestational-age infant, it is important to remember that not all women with obesity will experience pregnancy complications nor will they all birth larger offspring. While there were four macrosomic infants born to the obese women in Manuscript 1, these women had otherwise healthy pregnancies and did not experience any additional pregnancy complications.

Indeed, future work is warranted that focuses on placenta nutrient transport in pregnancies that are compromised by obesity and fetal over growth.

This thesis also reinforces the importance of directly measuring physical activity during pregnancy in order to gain accurate and reliable measures of the intensity and duration of physical activity, rather than relying on questionnaires. The PPAQ substantially overestimated physical activity (moderate-to-vigorous intensity overestimated by 15 hr/week) during the second trimester and misclassified all of the non-active women (as determined by direct measures) as being physically active. Misclassifying non-active women as active is problematic, especially if this questionnaire were to be employed in research exploring the effects of physical activity during pregnancy. If the physical activity level of the women in Manuscript 3 had been measured using the PPAQ then the findings of the study would have been substantially different. According to the PPAQ all 16 subjects in Manuscript 3 met (and vastly exceeded) CSEP's physical activity guidelines (>150 minutes of moderate-to-vigorous physical activity/week) and would have thus been classified as 'active'; this differs from the six active women and 10 non-active women as determined by direct measures using Actical accelerometers. In addition, since none of the PPAQ-measured and Actical-measured physical activity variables were correlated, relying on the PPAQ for Manuscript 3 would have resulted in very different (if any) correlations being observed between the genes of interest and the physical activity variables. Given the inaccuracies of the PPAQ, we advise against any future use of this questionnaire, in addition it is recommended that caution be used when interpreting the findings from previous studies that have relied on the PPAQ to assess physical activity.

### 3.2 Future Directions

These novel findings linking GWG, directly measured physical activity and sugar intake to the expression of genes in the placenta highlight the importance of considering all of the factors that might influence the intrauterine environment rather than focusing on only one aspect of the maternal phenotype (e.g BMI). To more comprehensively examine these concepts, future research with sufficient numbers (i.e. higher sample size) is needed to account for the sex of the offspring and to evaluate whether these factors also affect the protein expression and activity of the placenta nutrient transport-related genes. With respect to physical activity, it is advised that only direct measures, such as Actical® accelerometry, are used to measure energy expenditure. Additionally, given the potential for variability in diet and physical activity patterns across gestation, future work should consider multiple measures of physical activity and diet at different time points throughout pregnancy. Measures of infant body composition would also offer insight regarding whether any of these changes is contributing to altered neonatal body composition and not solely birth weight.

Our lab has plans to continue along this research path, specifically focusing on further characterizing the effects of physical activity on the placenta. I contributed to a proposal for a Research Growth Award from the Children's Hospital of Eastern Ontario Research Institute that our lab was recently awarded in order to pursue this research. In addition, I am helping to draft a CIHR grant that aims to study the effects of physical activity on placenta biology. This project will be a continuation of the *Active MOM* study that I designed for this thesis, and will incorporate various techniques such as nutrient uptake in placenta explants, transcriptional and ribosomal profiling, and metabolome and exosome profiling. The knowledge gained from this thesis will be useful for these future projects with respect to properly selecting and characterizing

the patient populations (i.e. physical activity, diet, GWG, and offspring gender), as well as providing direction on which nutrient pathways may be worthy of further exploration (i.e. fatty acids and amino acid transport).

### **3.3 Conclusion**

This work improves our knowledge of the maternal and fetal factors that influence placenta nutrient transport-related gene expression. While the direct implications of these findings to pregnant women and health care providers are still in their infancy, this work provides a valuable contribution to placenta based research. Considering that intervention studies that aim to optimize fetal growth and improve pregnancy outcomes often focus on eating a healthy diet, regular physical activity and meeting GWG guidelines, it is logical that these factors ought to be considered in observational and mechanistic research. Furthermore, this work emphasized the importance of using direct measures of physical activity during pregnancy in order to be confident that the findings reflect the true nature of the relationships studied. Overall, if the results from this thesis are applied to upcoming research, it will improve the quality of future prenatal research.

## Part IV

### 4.0 Statement of contribution – Review Article

Maternal-Fetal Nutrient Transport in Pregnancy Pathologies: The role of the Placenta  
**Kendra Brett**, Zachary Ferraro, Julien Yockell-Lelievre, Andrée Gruslin, Kristi Adamo.  
*International Journal of Molecular Sciences*. 2014; 15: 16153-16185.

Kendra Brett was responsible for the conception of the paper, she searched the literature, retrieved and evaluated sources, evaluated the evidence, and wrote and revised the manuscript.

Zachary Ferraro contributed to the literature search, and writing and revisions of the manuscript.

Julien Yockell-Lelievre created the figures and contributed to the written sections directly related to the figures.

Andrée Gruslin advised on the content of the manuscript and critically revised the manuscript.

Kristi Adamo supervised the research, critically revised the manuscript, and provided guidance and support.

### 4.1 Statement of contribution – Manuscript 1

Placenta nutrient transport-related gene expression: the impact of maternal obesity and excessive gestational weight gain

**Kendra Brett**, Zachary Ferraro, Martin Holcik, Kristi Adamo  
Submitted to *Journal of Maternal-Fetal and Neonatal Medicine*

Kendra Brett conceived the study, recruited patients, collected samples and clinical data, ran the experiments, analysed and interpreted the data, and drafted the manuscript.

Zachary Ferraro assisted with study conception, recruited patients, helped collect samples and clinical data, and critically reviewed the manuscript.

Martin Holcik provided the technical expertise, helped interpret the data and approved the final version of the manuscript.

Krista Adamo assisted with study conception and critically reviewed the manuscript.

### 4.2 Statement of contribution – Manuscript 2

Self-report Pregnancy Physical Activity Questionnaire overestimates physical activity

**Kendra Brett**, Shanna Wilson, Zachary Ferraro, Kristi Adamo  
Submitted to the *Canadian Journal of Public Health*

Kendra Brett conceived the study, recruited participants, collected the data, analysed and interpreted the data, and drafted the manuscript.

Shanna Wilson assisted with data analysis, helped interpret the data, and critically reviewed the manuscript.

Zachary Ferraro critically reviewed the manuscript.

Kristi Adamo assisted with study conception and critically reviewed the manuscript.

#### **4.3 Statement of contribution – Manuscript 3**

Prenatal physical activity and diet composition affects the expression of nutrient transporters and mTOR signaling molecules in the human placenta

**Kendra Brett**, Zachary Ferraro, Martin Holcik, Kristi Adamo.

*Placenta* 2015; 36:204-212

Kendra Brett conceived the study, recruited the patients, collected the samples and clinical data, ran the experiments, analysed and interpreted the data, and drafted the manuscript.

Zachary Ferraro helped with data collection and critically revised the manuscript.

Martin Holcik provided the technical expertise, helped interpret the data and approved the final version of the manuscript.

Kristi Adamo assisted with study conception and critically reviewed the manuscript.

## Part V

### 5.1 References for Part I and Part III

- (1) Barker DJ, Bull AR, Osmond C, et al. Fetal and placental size and risk of hypertension in adult life. *BMJ* 1990;301(6746):259-62.
- (2) Barker DJ. The fetal and infant origins of adult disease. *BMJ* 1990;301(6761):1111.
- (3) Ong KK. Size at birth, postnatal growth and risk of obesity. *Horm Res* 2006;65 Suppl 3:65-9.
- (4) Barker DJ, Winter PD, Osmond C, et al. Weight in infancy and death from ischaemic heart disease. *Lancet* 1989;2(8663):577-80.
- (5) Pettitt DJ, Jovanovic L. Birth weight as a predictor of type 2 diabetes mellitus: the U-shaped curve. *Curr Diab Rep* 2001;1(1):78-81.
- (6) Jansson T, Ekstrand Y, Wennergren M, et al. Placental glucose transport in gestational diabetes mellitus. *Am J Obstet Gynecol* 2001;184(2):111-6.
- (7) Jansson T, Ekstrand Y, Bjorn C, et al. Alterations in the activity of placental amino acid transporters in pregnancies complicated by diabetes. *Diabetes* 2002;51(7):2214-9.
- (8) Jansson T, Cetin I, Powell TL, et al. Placental transport and metabolism in fetal overgrowth -- a workshop report. *Placenta* 2006;27 Suppl A:S109-S113.
- (9) Magnusson AL, Waterman IJ, Wennergren M, et al. Triglyceride hydrolase activities and expression of fatty acid binding proteins in the human placenta in pregnancies complicated by intrauterine growth restriction and diabetes. *J Clin Endocrinol Metab* 2004;89(9):4607-14.
- (10) Sibley CP, Turner MA, Cetin I, et al. Placental phenotypes of intrauterine growth. *Pediatr Res* 2005;58(5):827-32.
- (11) Sibley CP. Understanding placental nutrient transfer--why bother? New biomarkers of fetal growth. *J Physiol* 2009;587(Pt 14):3431-40.
- (12) Walker SP, Ugoni AM, Lim R, et al. Inverse relationship between gestational weight gain and glucose uptake in human placenta from female foetuses. *Pediatr Obes* 2014;9(3):e73-e76.
- (13) Lewis RM, Greenwood SL, Cleal JK, et al. Maternal muscle mass may influence system A activity in human placenta. *Placenta* 2010;31(5):418-22.

- (14) Yogev Y, Catalano PM. Pregnancy and obesity. *Obstet Gynecol Clin North Am* 2009;36(2):285-300, viii.
- (15) Adamo KB, Ferraro ZM, Brett KE. Can we modify the intrauterine environment to halt the intergenerational cycle of obesity? *Int J Environ Res Public Health* 2012;9(4):1263-307.
- (16) Adamo KB, Ferraro ZM, Brett KE. Pregnancy is a Critical Period for Prevention of Obesity and Cardiometabolic Risk. *Canadian Journal of Diabetes* 2014;36(3):133-41.
- (17) Vinturache A, Moledina N, McDonald S, et al. Pre-pregnancy Body Mass Index (BMI) and delivery outcomes in a Canadian population. *BMC Pregnancy Childbirth* 2014;14(1):422.
- (18) Whitaker RC, Dietz WH. Role of the prenatal environment in the development of obesity. *J Pediatr* 1998;132(5):768-76.
- (19) Ferraro ZM, Barrowman N, Prud'homme D, et al. Excessive gestational weight gain predicts large for gestational age neonates independent of maternal body mass index. *J Matern Fetal Neonatal Med* 2012;25(5):538-42.
- (20) Gaudet L, Ferraro ZM, Wen SW, et al. Maternal Obesity and Occurrence of Fetal Macrosomia: A Systematic Review and Meta-Analysis. *BioMed Research International*. Online First: 2014 <http://dx.doi.org/10.1155/2014/640291>
- (21) Hull HR, Dinger MK, Knehans AW, et al. Impact of maternal body mass index on neonate birthweight and body composition. *Am J Obstet Gynecol* 2008;198(4):416.
- (22) Sewell MF, Huston-Presley L, Super DM, et al. Increased neonatal fat mass, not lean body mass, is associated with maternal obesity. *Am J Obstet Gynecol* 2006;195(4):1100-3.
- (23) Oken E. Maternal and child obesity: the causal link. *Obstet Gynecol Clin North Am* 2009;36(2):361-x.
- (24) Parsons TJ, Power C, Manor O. Fetal and early life growth and body mass index from birth to early adulthood in 1958 British cohort: longitudinal study. *BMJ* 2001;323(7325):1331-5.
- (25) Magarey AM, Daniels LA, Boulton TJ, et al. Predicting obesity in early adulthood from childhood and parental obesity. *Int J Obes Relat Metab Disord* 2003;27(4):505-13.
- (26) Whitaker RC. Predicting preschooler obesity at birth: the role of maternal obesity in early pregnancy. *Pediatrics* 2004;114(1):e29-e36.
- (27) Yu ZB, Han SP, Zhu GZ, et al. Birth weight and subsequent risk of obesity: a systematic review and meta-analysis. *Obes Rev* 2011;12(7):525-42.

- (28) Catalano PM. Obesity and pregnancy--the propagation of a viscous cycle? *J Clin Endocrinol Metab* 2003;88(8):3505-6.
- (29) Swanson LD, Bewtra C. Increase in normal placental weights related to increase in maternal body mass index. *J Matern Fetal Neonatal Med* 2008;21(2):111-3.
- (30) Heerwagen MJ, Miller MR, Barbour LA, et al. Maternal obesity and fetal metabolic programming: a fertile epigenetic soil. *Am J Physiol Regul Integr Comp Physiol* 2010;299(3):R711-R722.
- (31) King JC. Maternal obesity, metabolism, and pregnancy outcomes. *Annu Rev Nutr* 2006;26:271-91.
- (32) Jansson T, Myatt L, Powell TL. The role of trophoblast nutrient and ion transporters in the development of pregnancy complications and adult disease. *Curr Vasc Pharmacol* 2009;7(4):521-33.
- (33) Brett KE, Ferraro ZM, Yockell-Lelievre J, et al. Maternal-fetal nutrient transport in pregnancy pathologies: the role of the placenta. *Int J Mol Sci* 2014;15(9):16153-85.
- (34) Institute of Medicine. *Weight Gain During Pregnancy: Reexamining the Guidelines*. Washington, DC, USA: The National Academies Press; 2009.
- (35) Begum F, Colman I, McCargar LJ, et al. Gestational weight gain and early postpartum weight retention in a prospective cohort of Alberta women. *J Obstet Gynaecol Can* 2012;34(7):637-47.
- (36) Weisman CS, Hillemeier MM, Downs DS, et al. Preconception predictors of weight gain during pregnancy: prospective findings from the Central Pennsylvania Women's Health Study. *Womens Health Issues* 2010;20(2):126-32.
- (37) Denison FC, Roberts KA, Barr SM, et al. Obesity, pregnancy, inflammation, and vascular function. *Reproduction* 2010;140(3):373-85.
- (38) Nehring I, Schmoll S, Beyerlein A, et al. Gestational weight gain and long-term postpartum weight retention: a meta-analysis. *Am J Clin Nutr* 2011;94(5):1225-31.
- (39) Gunderson EP, Abrams B, Selvin S. The relative importance of gestational gain and maternal characteristics associated with the risk of becoming overweight after pregnancy. *Int J Obes Relat Metab Disord* 2000;24(12):1660-8.
- (40) Luke B, Hediger ML, Scholl TO. Point of diminishing returns: when does gestational weight gain cease benefiting birthweight and begin adding to maternal obesity? *J Matern Fetal Med* 1996;5(4):168-73.
- (41) Villamor E, Cnattingius S. Interpregnancy weight change and risk of adverse pregnancy outcomes: a population-based study. *Lancet* 2006;368(9542):1164-70.

- (42) Ehrlich SF, Hedderson MM, Feng J, et al. Change in body mass index between pregnancies and the risk of gestational diabetes in a second pregnancy. *Obstet Gynecol* 2011;117(6):1323-30.
- (43) Mamun AA, Mannan M, Doi SA. Gestational weight gain in relation to offspring obesity over the life course: a systematic review and bias-adjusted meta-analysis. *Obes Rev* 2014;15(4):338-47.
- (44) Ferraro ZM, Gaudet L, Adamo KB. The potential impact of physical activity during pregnancy on maternal and neonatal outcomes. *Obstet Gynecol Surv* 2012;67(2):99-110.
- (45) Nascimento SL, Surita FG, Cecatti JG. Physical exercise during pregnancy: a systematic review. *Curr Opin Obstet Gynecol* 2012;24(6):387-94.
- (46) Olson CM, Strawderman MS. Modifiable behavioral factors in a biopsychosocial model predict inadequate and excessive gestational weight gain. *J Am Diet Assoc* 2003;103(1):48-54.
- (47) Clapp III JF. Oxygen consumption during treadmill exercise before, during, and after pregnancy. *American Journal of Obstetrics and Gynecology* 1989;161(6):1458-64.
- (48) Clapp JF, Capeless E. The changing glycemic response to exercise during pregnancy. *American Journal of Obstetrics and Gynecology* 1991;165(6 I):1678-83.
- (49) Davies GA, Wolfe LA, Mottola ML, et al. Joint SOGC/CSEP clinical practice guideline: exercise in pregnancy and the postpartum period. *Canadian Journal of Applied Physiology* 2003;28(3):330-41.
- (50) Kramer MS, McDonald SW. Aerobic exercise for women during pregnancy. *Cochrane Database of Systematic Reviews* 2006;19(3):CD000180.
- (51) Penney DS. The effect of vigorous exercise during pregnancy. *J Midwifery Womens Health* 2008;53(2):155-9.
- (52) de Oliveria Melo AS, Silva JL, Tavares JS, et al. Effect of a physical exercise program during pregnancy on uteroplacental and fetal blood flow and fetal growth: a randomized controlled trial. *Obstet Gynecol* 2012;120(2 Pt 1):302-10.
- (53) Juhl M, Olsen J, Anderson P, et al. Physical exercise during pregnancy and fetal growth measures: a study within the Danish National Birth Cohort. *American Journal of Obstetrics and Gynecology* 2010;202(1):e1-e8.
- (54) Jukic AM, Evenson KR, Daniels JL, et al. A prospective study of the association between vigorous physical activity during pregnancy and length of gestation and birthweight. *Matern Child Health J* 2012;16(5):1031-44.
- (55) Clapp III JF, Capeless E. Neonatal morphometrics after endurance exercise during pregnancy. *American Journal of Obstetrics and Gynecology* 1990;163(6 I):1805-11.

- (56) Morgan KL, Rahman MA, Hill RA, et al. Physical activity and excess weight in pregnancy have independent and unique effects on delivery and perinatal outcomes. *PLoS One* 2014;9(4):e94532.
- (57) Artal R, Catanzaro RB, Gavard JA, et al. A lifestyle intervention of weight-gain restriction: diet and exercise in obese women with gestational diabetes mellitus. *Appl Physiol Nutr Metab* 2007;32(3):596-601.
- (58) Dempsey JC, Sorensen TK, Williams MA, et al. Prospective study of gestational diabetes mellitus risk in relation to maternal recreational physical activity before and during pregnancy. *Am J Epidemiol* 2004;159(7):663-70.
- (59) Dempsey JC, Butler CL, Sorensen TK, et al. A case-control study of maternal recreational physical activity and risk of gestational diabetes mellitus. *Diabetes Res Clin Pract* 2004;66(2):203-15.
- (60) Ong MJ, Guelfi KJ, Hunter T, et al. Supervised home-based exercise may attenuate the decline of glucose tolerance in obese pregnant women. *Diabetes Metab* 2009;35(5):418-21.
- (61) Aune D, Saugstad OD, Henriksen T, et al. Physical activity and the risk of preeclampsia: a systematic review and meta-analysis. *Epidemiology* 2014;25(3):331-43.
- (62) Sui Z, Grivell RM, Dodd JM. Antenatal exercise to improve outcomes in overweight or obese women: A systematic review. *Acta Obstet Gynecol Scand* 2012;91(5):538-45.
- (63) Choi J, Fukuoka Y, Lee JH. The effects of physical activity and physical activity plus diet interventions on body weight in overweight or obese women who are pregnant or in postpartum: a systematic review and meta-analysis of randomized controlled trials. *Prev Med* 2013;56(6):351-64.
- (64) Artal R. Exercise and pregnancy. *Clin Sports Med* 1992;11(2):363-77.
- (65) Bergmann A, Zygmunt M, Clapp JF, III. Running throughout pregnancy: effect on placental villous vascular volume and cell proliferation. *Placenta* 2004;25(8-9):694-8.
- (66) Clapp JF, III. The effects of maternal exercise on fetal oxygenation and feto-placental growth. *Eur J Obstet Gynecol Reprod Biol* 2003;110 Suppl 1:S80-S85.
- (67) Jackson MR, Gott P, Lye SJ, et al. The effects of maternal aerobic exercise on human placental development: placental volumetric composition and surface areas. *Placenta* 1995;16(2):179-91.
- (68) Artal R, O'Toole M. Guidelines of the American College of Obstetricians and Gynecologists for exercise during pregnancy and the postpartum period. *Br J Sports Med* 2003;37(1):6-12.

- (69) Wolfe LA, Davies GA. Canadian guidelines for exercise in pregnancy. *Clin Obstet Gynecol* 2003;46(2):488-95.
- (70) Davenport MH, Charlesworth S, Vanderspank D, et al. Development and validation of exercise target heart rate zones for overweight and obese pregnant women. *Appl Physiol Nutr Metab* 2008;33(5):984-9.
- (71) Tremblay MS, Warburton DE, Janssen I, et al. New Canadian physical activity guidelines. *Appl Physiol Nutr Metab* 2011;36(1):36-46.
- (72) Warburton DE, Charlesworth S, Ivey A, et al. A systematic review of the evidence for Canada's Physical Activity Guidelines for Adults. *Int J Behav Nutr Phys Act* 2010;7:39.
- (73) Colley RC, Gorber SC, Tremblay MS. Quality control and data reduction procedures for accelerometry-derived measures of physical activity. *Health Reports* 2010;21(1):63-9.
- (74) Colley RC, Garriguet D, Janssen I, et al. Physical activity of Canadian children and youth: accelerometer results from the 2007 to 2009 Canadian Health Measures Survey. *Health Rep* 2011;22(1):15-23.
- (75) Colley RC, Garriguet D, Janssen I, et al. Physical activity of Canadian adults: accelerometer results from the 2007 to 2009 Canadian Health Measures Survey. *Health Rep* 2011;22(1):7-14.
- (76) Petersen AM, Leet TL, Brownson RC. Correlates of physical activity among pregnant women in the United States. *Med Sci Sports Exerc* 2005;37(10):1748-53.
- (77) Evenson KR, Wen F. Prevalence and correlates of objectively measured physical activity and sedentary behavior among US pregnant women. *Prev Med* 2011;53(1-2):39-43.
- (78) Gaston A, Vamos CA. Leisure-Time Physical Activity Patterns and Correlates Among Pregnant Women in Ontario, Canada. *Matern Child Health J* 2012.
- (79) Adamo KB, Ferraro ZM, Goldfield G, et al. The Maternal Obesity Management (MOM) Trial Protocol: a lifestyle intervention during pregnancy to minimize downstream obesity. *Contemp Clin Trials* 2013;35(1):87-96.
- (80) Brett KE, Ferraro ZM, Wilson S, et al. Associations between objectively measured physical activity, pre-pregnancy body mass index and gestational weight gain. *Appl Physiol Nutr Metab* 2014;39(S1):S7.
- (81) Garriguet D, Colley RC. A comparison of self-reported leisure-time physical activity and measured moderate-to-vigorous physical activity in adolescents and adults. *Health Rep* 2014;25(7):3-11.
- (82) Prince SA, Adamo KB, Hamel ME, et al. A comparison of direct versus self-report measures for assessing physical activity in adults: a systematic review. *Int J Behav Nutr Phys Act* 2008;5(1):56-80.

- (83) Bell R, Tennant PW, McParlin C, et al. Measuring physical activity in pregnancy: a comparison of accelerometry and self-completion questionnaires in overweight and obese women. *Eur J Obstet Gynecol Reprod Biol* 2013;170(1):90-5.
- (84) Harrison CL, Thompson RG, Teede HJ, et al. Measuring physical activity during pregnancy. *Int J Behav Nutr Phys Act* 2011;8(19):114-21.
- (85) Oostdam N, van Mechelen W, van Poppel M. Validation and responsiveness of the AQuAA for measuring physical activity in overweight and obese pregnant women. *J Sci Med Sport* 2013;16(5):412-6.
- (86) Harris ST, Liu J, Wilcox S, et al. Exercise During Pregnancy and its Association with Gestational Weight Gain. *Matern Child Health J*. Online First: 10 June 2014 DOI 10.1007/s10995-014-1534-8
- (87) Schlaff RA, Holzman C, Mudd LM, et al. Body Mass Index is Associated With Appropriateness of Weight Gain But Not Leisure-Time Physical Activity During Pregnancy. *J Phys Act Health*. Online First: 11 April 2014 DOI: 10.1123/jpah.2013-0090
- (88) Schlaff RA, Holzman C, Maier KS, et al. Associations among gestational weight gain, physical activity, and pre-pregnancy body size with varying estimates of pre-pregnancy weight. *Midwifery* 2014;30(11):1124-31.
- (89) Chasan-Taber L, Silveira M, Pekow P, et al. Physical activity, sedentary behavior and risk of hypertensive disorders of pregnancy in Hispanic women. *Hypertens Pregnancy*. Online First: 14 August 2014 doi:10.3109/10641955.2014.946616
- (90) Chasan-Taber L, Silveira M, Lynch KE, et al. Physical activity and gestational weight gain in Hispanic women. *Obesity (Silver Spring)* 2014;22(3):909-18.
- (91) Harrod CS, Chasan-Taber L, Reynolds RM, et al. Physical Activity in Pregnancy and Neonatal Body Composition: The Healthy Start Study. *Obstet Gynecol* 2014;124(2):257-64.
- (92) Hawkins M, Chasan-Taber L, Marcus B, et al. Impact of an exercise intervention on physical activity during pregnancy: the behaviors affecting baby and you study. *Am J Public Health* 2014;104(10):e74-e81.
- (93) King JC. Physiology of pregnancy and nutrient metabolism. *Am J Clin Nutr* 2000;71(5 Suppl):1218S-25S.
- (94) Butte NF, Wong WW, Treuth MS, et al. Energy requirements during pregnancy based on total energy expenditure and energy deposition. *Am J Clin Nutr* 2004;79(6):1078-87.
- (95) Otten J, Pitzel Hellig J, Meyers L. Dietary Reference Intakes: The Essential Guide to Nutrient Requirements. Washington, DC: National Academic Press; 2006.

- (96) Streuling I, Beyerlein A, von KR. Can gestational weight gain be modified by increasing physical activity and diet counseling? A meta-analysis of interventional trials. *Am J Clin Nutr* 2010;92(4):678-87.
- (97) Thangaratinam S, Rogozinska E, Jolly K, et al. Effects of interventions in pregnancy on maternal weight and obstetric outcomes: meta-analysis of randomised evidence. *BMJ* 2012;344:e2088.
- (98) Stuebe AM, Oken E, Gillman MW. Associations of diet and physical activity during pregnancy with risk for excessive gestational weight gain. *Am J Obstet Gynecol* 2009;201(1):58.
- (99) Colomiere M, Permezel M, Riley C, et al. Defective insulin signaling in placenta from pregnancies complicated by gestational diabetes mellitus. *Eur J Endocrinol* 2009;160(4):567-78.
- (100) Whisner CM, Young BE, Pressman EK, et al. Maternal diet but not gestational weight gain predicts central adiposity accretion in utero among pregnant adolescents. *Int J Obes (Lond)* 2014.
- (101) Eriksson JG, Kajantie E, Osmond C, et al. Boys live dangerously in the womb. *Am J Hum Biol* 2010;22(3):330-5.
- (102) Sharma AM, Kushner RF. A proposed clinical staging system for obesity. *Int J Obes (Lond)* 2009;33(3):289-95.

## 5.2 Appendices

### Appendix A - Qiagen Online data analysis tool for Manuscript 1 and 3

<http://pcrdataanalysis.sabiosciences.com/pcr/arrayanalysis.php>

RT<sup>2</sup> Profiler PCR Array Data Analysis version 3.5

SABiosciences  
A QIAGEN Company

Upload Data | Analysis Setup | Analysis | Plots & Charts | Export Data | What's Next

Experiment Performed Using:

- Standard RT<sup>2</sup> PCR Array
- Custom RT<sup>2</sup> PCR Array
- Single or Multi-Gene qPCR Assays

File:  No file chosen   
\* File must be a MS Excel Sheet (in .XLS format, not .XLSX).

Excel Templates for Formatting your Experimental Data:

- [Cataloged PCR Array](#)
- [Custom PCR Array](#)
- [Single/Multi-Gene Assays](#)

For Custom PCR Arrays - Data Analysis Patches  
Convert your custom array raw data into the compatible format for your customized Data Analysis Template.

96-well	384-well
8 genes x 12 samples	8 genes x 48 samples

New to RT<sup>2</sup> Data Analysis? Learn more by:

- [Taking a test run](#)
- [Playing a movie guide \(Flash\)](#)
- [Attending a webinar](#)

Notes:

1. Please note that you must complete all of your work with the PCR Array Data Analysis Web Portal in the same session. Your data is not stored on a server, so all work is lost once the session (or your web browser) is closed. Be sure to export all processed data and results to an Excel file saved on your local computer.
2. Please set your screen resolution to **1024 X 768** or greater, if possible.
3. Turn off any window pop-up blockers. The software will launch separate windows for viewing the plots and charts.

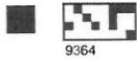
Instructions

1. Choose the experiment that was performed: Standard/Cataloged RT<sup>2</sup> Profiler PCR Array, Custom RT<sup>2</sup> PCR Array or individual assays
  - a. If you selected Standard RT<sup>2</sup> PCR Array then Enter the PCR Array Pathway Number from the drop-down list.
  - b. If you selected Custom RT<sup>2</sup> PCR Array then Enter the Custom Array ID (ex. CAPX###) in the text field.
  - c. If your experiment used individual primer assays then select Single or Multi-Gene qPCR Assays.
2. Browse and select the MS Excel file containing your PCR data with a maximum number of 100 samples. Click "Upload".
3. Analysis Setup page:

### Instructions

1. Choose the experiment that was performed: Standard/Cataloged RT<sup>2</sup> Profiler PCR Array, Custom RT<sup>2</sup> PCR Array or individual assays
2. Browse and select the MS Excel file containing your PCR data with a maximum number of 100 samples. Click "Upload".
3. Analysis Setup page:
  - a. In the "Basic Setup" section, assign samples to different groups. At least two groups are needed, where one of those groups must be the control group. Click "Update" when finished. You may exclude samples from the analysis by selecting "Exclude" on the drop down menu.
  - b. Review the "Data QC" section to assess each groups' PCR reproducibility, reverse transcription efficiency, and the presence of genomic DNA contamination.
  - c. The "Select Housekeeping Genes" section allows you to remove or add preferred housekeeping genes for data normalization by clicking the appropriate checkboxes. Click "Update" when finished.
  - d. Review the "Data Overview" section to see each group's distribution of threshold cycle values and the average of the raw data in each group.
4. Analysis page:
  - a. See the "Average Ct", "2<sup>-</sup>(- Ct)", "Fold Change", "p-value", and "Fold Regulation" sections for the results processed by the software from your data. The "Fold Change" and "p-value" results are used by the software in subsequent graphical analyses.
5. Click "Export Data" to download a MS Excel file containing all raw and processed data from the "Readout" and "Analysis Result" sections.

*Appendix B. Pregnancy Physical Activity Questionnaire (PPAQ) used in Manuscript 2*



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**Pregnancy Physical Activity Questionnaire**


**Instructions:**  
 Please use an ordinary No. 2 pencil. Fill in the circles completely. The Question will be read by a machine so if you need to change your answer, erase the incorrect mark **completely**. If you have comments, please write them on the back of the questionnaire.

**Example:** During this trimester, when you are NOT at work, how much time do you usually spend:

*If you take care of your mom for 2 hours each day, then your answer should look like this...* →

**E1. Taking care of an older adult**

- None
- Less than 1/2 hour per day
- 1/2 to almost 1 hour per day
- 1 to almost 2 hours per day
- 2 to almost 3 hours per day
- 3 or more hours per day



It is very important you tell us about yourself honestly. There are no right or wrong answers. We just want to know about the things you are doing during this trimester.

1. Today's Date:  /  /
2. What was the first day of your last period?  /  /   I don't know
3. When is your baby due?  /  /   I don't know

During this trimester, when you are NOT at work, how much time do you usually spend:

4. **Preparing meals (cook, set table, wash dishes)**
  - None
  - Less than 1/2 hour per day
  - 1/2 to almost 1 hour per day
  - 1 to almost 2 hours per day
  - 2 to almost 3 hours per day
  - 3 or more hours per day
5. **Dressing, bathing, feeding children while you are sitting**
  - None
  - Less than 1/2 hour per day
  - 1/2 to almost 1 hour per day
  - 1 to almost 2 hours per day
  - 2 to almost 3 hours per day
  - 3 or more hours per day





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During this trimester, when you are NOT at work, how much time do you usually spend:

6. Dressing, bathing, feeding children while you are standing
- None
  - Less than 1/2 hour per day
  - 1/2 to almost 1 hour per day
  - 1 to almost 2 hours per day
  - 2 to almost 3 hours per day
  - 3 or more hours per day

7. Playing with children while you are sitting or standing
- None
  - Less than 1/2 hour per day
  - 1/2 to almost 1 hour per day
  - 1 to almost 2 hours per day
  - 2 to almost 3 hours per day
  - 3 or more hours per day

8. Playing with children while you are walking or running
- None
  - Less than 1/2 hour per day
  - 1/2 to almost 1 hour per day
  - 1 to almost 2 hours per day
  - 2 to almost 3 hours per day
  - 3 or more hours per day

9. Carrying children
- None
  - Less than 1/2 hour per day
  - 1/2 to almost 1 hour per day
  - 1 to almost 2 hours per day
  - 2 to almost 3 hours per day
  - 3 or more hours per day

10. Taking care of an older adult
- None
  - Less than 1/2 hour per day
  - 1/2 to almost 1 hour per day
  - 1 to almost 2 hours per day
  - 2 to almost 3 hours per day
  - 3 or more hours per day

11. Sitting and using a computer or writing, while not at work
- None
  - Less than 1/2 hour per day
  - 1/2 to almost 1 hour per day
  - 1 to almost 2 hours per day
  - 2 to almost 3 hours per day
  - 3 or more hours per day



12. Watching TV or a video
- None
  - Less than 1/2 hour per day
  - 1/2 to almost 2 hours per day
  - 2 to almost 4 hours per day
  - 4 to almost 6 hours per day
  - 6 or more hours per day

13. Sitting and reading, talking, or on the phone, while not at work
- None
  - Less than 1/2 hour per day
  - 1/2 to almost 2 hours per day
  - 2 to almost 4 hours per day
  - 4 to almost 6 hours per day
  - 6 or more hours per day

14. Playing with pets
- None
  - Less than 1/2 hour per day
  - 1/2 to almost 1 hour per day
  - 1 to almost 2 hours per day
  - 2 to almost 3 hours per day
  - 3 or more hours per day

15. Light cleaning (make beds, laundry, iron, put things away)
- None
  - Less than 1/2 hour per day
  - 1/2 to almost 1 hour per day
  - 1 to almost 2 hours per day
  - 2 to almost 3 hours per day
  - 3 or more hours per day


16. Shopping (for food, clothes, or other items)
- None
  - Less than 1/2 hour per day
  - 1/2 to almost 1 hour per day
  - 1 to almost 2 hours per day
  - 2 to almost 3 hours per day
  - 3 or more hours per day



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During this trimester, when you are NOT at work, how much time do you usually spend:

- 17. Heavier cleaning (vacuum, mop, sweep, wash windows) 
  - None
  - Less than 1/2 hour per week
  - 1/2 to almost 1 hour per week
  - 1 to almost 2 hours per week
  - 2 to almost 3 hours per week
  - 3 or more hours per week
- 18. Mowing lawn while on a riding mower
  - None
  - Less than 1/2 hour per week
  - 1/2 to almost 1 hour per week
  - 1 to almost 2 hours per week
  - 2 to almost 3 hours per week
  - 3 or more hours per week
- 19. Mowing lawn using a walking mower, raking, gardening
  - None
  - Less than 1/2 hour per week
  - 1/2 to almost 1 hour per week
  - 1 to almost 2 hours per week
  - 2 to almost 3 hours per week
  - 3 or more hours per week

## Going Places...

During this trimester, how much time do you usually spend:

- 20. Walking slowly to go places (such as to the bus, work, visiting)   
*Not for fun or exercise*
  - None
  - Less than 1/2 hour per day
  - 1/2 to almost 1 hour per day
  - 1 to almost 2 hours per day
  - 2 to almost 3 hours per day
  - 3 or more hours per day
- 21. Walking quickly to go places (such as to the bus, work, or school)   
*Not for fun or exercise*
  - None
  - Less than 1/2 hour per day
  - 1/2 to almost 1 hour per day
  - 1 to almost 2 hours per day
  - 2 to almost 3 hours per day
  - 3 or more hours per day
- 22. Driving or riding in a car or bus
  - None
  - Less than 1/2 hour per day
  - 1/2 to almost 1 hour per day
  - 1 to almost 2 hours per day
  - 2 to almost 3 hours per day
  - 3 or more hours per day

## For Fun or Exercise...

During this trimester, how much time do you usually spend:

- 23. Walking slowly for fun or exercise
  - None
  - Less than 1/2 hour per week
  - 1/2 to almost 1 hour per week
  - 1 to almost 2 hours per week
  - 2 to almost 3 hours per week
  - 3 or more hours per week
- 24. Walking more quickly for fun or exercise
  - None
  - Less than 1/2 hour per week
  - 1/2 to almost 1 hour per week
  - 1 to almost 2 hours per week
  - 2 to almost 3 hours per week
  - 3 or more hours per week
- 25. Walking quickly up hills for fun or exercise
  - None
  - Less than 1/2 hour per week
  - 1/2 to almost 1 hour per week
  - 1 to almost 2 hours per week
  - 2 to almost 3 hours per week
  - 3 or more hours per week



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During this trimester, how much time do you usually spend:

26. Jogging

- None, Less than 1/2 hour per week, 1/2 to almost 1 hour per week, 1 to almost 2 hours per week, 2 to almost 3 hours per week, 3 or more hours per week

27. Prenatal exercise class

- None, Less than 1/2 hour per week, 1/2 to almost 1 hour per week, 1 to almost 2 hours per week, 2 to almost 3 hours per week, 3 or more hours per week

28. Swimming

- None, Less than 1/2 hour per week, 1/2 to almost 1 hour per week, 1 to almost 2 hours per week, 2 to almost 3 hours per week, 3 or more hours per week

29. Dancing

- None, Less than 1/2 hour per week, 1/2 to almost 1 hour per week, 1 to almost 2 hours per week, 2 to almost 3 hours per week, 3 or more hours per week

Doing other things for fun or exercise? Please tell us what they are.

30. Name of Activity

- None, Less than 1/2 hour per week, 1/2 to almost 1 hour per week, 1 to almost 2 hours per week, 2 to almost 3 hours per week, 3 or more hours per week

31. Name of Activity

- None, Less than 1/2 hour per week, 1/2 to almost 1 hour per week, 1 to almost 2 hours per week, 2 to almost 3 hours per week, 3 or more hours per week

Please fill out the next section if you work for wages, as a volunteer, or if you are a student. If you are a homemaker, out of work, or unable to work, you do not need to complete this last section.

At Work...

During this trimester, how much time do you usually spend:

32. Sitting at working or in class

- None, Less than 1/2 hours per day, 1/2 to almost 2 hours per day, 2 to almost 4 hours per day, 4 to almost 6 hours per day, 6 or more hours per day



33. Standing or slowly walking at work while carrying things (heavier than a 1 gallon milk jug)

- None, Less than 1/2 hour per day, 1/2 to almost 2 hours per day, 2 to almost 4 hours per day, 4 to almost 6 hours per day, 6 or more hours per day

34. Standing or slowly walking at work not carrying anything

- None, Less than 1/2 hours per day, 1/2 to almost 2 hours per day, 2 to almost 4 hours per day, 4 to almost 6 hours per day, 6 or more hours per day

35. Walking quickly at work while carrying things (heavier than a 1 gallon milk jug)

- None, Less than 1/2 hour per day, 1/2 to almost 2 hours per day, 2 to almost 4 hours per day, 4 to almost 6 hours per day, 6 or more hours per day

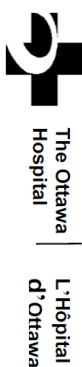
36. Walking quickly at work not carrying anything

- None, Less than 1/2 hour per day, 1/2 to almost 2 hours per day, 2 to almost 4 hours per day, 4 to almost 6 hours per day, 6 or more hours per day

Thank You



**Appendix C. Actical log sheet for Manuscript 2 and Manuscript 3**



Date: (dd/mm/yyyy):

<input type="text"/>	<input type="text"/>	/	<input type="text"/>	<input type="text"/>	/	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
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ID:

<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
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**Activity Monitor Log Sheet**

	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
<b>On at Wake-up Time</b> (time you first put the Monitor on for the day)							
<b>Off time</b> (give reason)							
<b>On Time</b>							
<b>Off at Bedtime</b> (time you take off the Monitor before bed)							

The seven days of monitoring end on \_\_\_\_\_ at \_\_\_\_\_. After this time you are no longer required to wear the monitor. Please return the **log sheet** and **monitor** back to Kendra Brett along with your diet record when you return for your second visit.

**NOTES**

1. The activity monitor is to be worn securely on the right hip with the blue dot facing up.
2. The monitor can be worn over or under your clothing (your choice) just to ensure that it is secure over the right hip area.
3. The monitors are not-waterproof therefore; please do not wear them while swimming or any other water activities.
4. Although the monitors are fairly durable, care should be taken when wearing them as they are quite expensive.

## Appendix D - PARmed-X for Pregnancy for Manuscript 2 and Manuscript 3

Physical Activity Readiness  
Medical Examination for  
Pregnancy (2002)

# PARmed-X for PREGNANCY PHYSICAL ACTIVITY READINESS MEDICAL EXAMINATION

**PARmed-X for PREGNANCY is a guideline for health screening  
prior to participation in a prenatal fitness class or other exercise.**

Healthy women with uncomplicated pregnancies can integrate physical activity into their daily living and can participate without significant risks either to themselves or to their unborn child. Postulated benefits of such programs include improved aerobic and muscular fitness, promotion of appropriate weight gain, and facilitation of labour. Regular exercise may also help to prevent gestational glucose intolerance and pregnancy-induced hypertension.

The safety of prenatal exercise programs depends on an adequate level of maternal-fetal physiological reserve. PARmed-X for PREGNANCY is a convenient checklist and prescription for use by health care providers to evaluate pregnant patients who want to enter a prenatal fitness program and for ongoing medical surveillance of exercising pregnant patients.

Instructions for use of the 4-page PARmed-X for PREGNANCY are the following:

1. The patient should fill out the section on PATIENT INFORMATION and the PRE-EXERCISE HEALTH CHECKLIST (PART 1, 2, 3, and 4 on p. 1) and give the form to the health care provider monitoring her pregnancy.
2. The health care provider should check the information provided by the patient for accuracy and fill out SECTION C on CONTRAINDICATIONS (p. 2) based on current medical information.
3. If no exercise contraindications exist, the HEALTH EVALUATION FORM (p. 3) should be completed, signed by the health care provider, and given by the patient to her prenatal fitness professional.

In addition to prudent medical care, participation in appropriate types, intensities and amounts of exercise is recommended to increase the likelihood of a beneficial pregnancy outcome. PARmed-X for PREGNANCY provides recommendations for individualized exercise prescription (p. 3) and program safety (p. 4).

**NOTE:** Sections A and B should be completed by the patient before the appointment with the health care provider.

<h3 style="margin: 0;">A PATIENT INFORMATION</h3> <p>NAME _____</p> <p>ADDRESS _____</p> <p>TELEPHONE _____ BIRTHDATE _____ HEALTH INSURANCE No. _____</p> <p>NAME OF PRENATAL FITNESS PROFESSIONAL _____ PRENATAL FITNESS PROFESSIONAL'S PHONE NUMBER _____</p>																																																																																																			
<h3 style="margin: 0;">B PRE-EXERCISE HEALTH CHECKLIST</h3> <p><b>PART 1: GENERAL HEALTH STATUS</b></p> <p>In the past, have you experienced (check YES or NO):</p> <table style="width: 100%; border: none;"> <thead> <tr> <th style="width: 80%;"></th> <th style="width: 10%; text-align: center;">YES</th> <th style="width: 10%; text-align: center;">NO</th> </tr> </thead> <tbody> <tr> <td>1. Miscarriage in an earlier pregnancy?</td> <td style="text-align: center;"><input type="checkbox"/></td> <td style="text-align: center;"><input type="checkbox"/></td> </tr> <tr> <td>2. Other pregnancy complications?</td> <td style="text-align: center;"><input type="checkbox"/></td> <td style="text-align: center;"><input type="checkbox"/></td> </tr> <tr> <td>3. I have completed a PAR-Q within the last 30 days.</td> <td style="text-align: center;"><input type="checkbox"/></td> <td style="text-align: center;"><input type="checkbox"/></td> </tr> </tbody> </table> <p>If you answered YES to question 1 or 2, please explain: _____</p> <p>Number of previous pregnancies? _____</p> <p><b>PART 2: STATUS OF CURRENT PREGNANCY</b></p> <p>Due Date: _____</p> <p>During this pregnancy, have you experienced:</p> <table style="width: 100%; border: none;"> <thead> <tr> <th style="width: 80%;"></th> <th style="width: 10%; text-align: center;">YES</th> <th style="width: 10%; text-align: center;">NO</th> </tr> </thead> <tbody> <tr><td>1. Marked fatigue?</td><td style="text-align: center;"><input type="checkbox"/></td><td style="text-align: center;"><input type="checkbox"/></td></tr> <tr><td>2. Bleeding from the vagina ("spotting")?</td><td style="text-align: center;"><input type="checkbox"/></td><td style="text-align: center;"><input type="checkbox"/></td></tr> <tr><td>3. Unexplained faintness or dizziness?</td><td style="text-align: center;"><input type="checkbox"/></td><td style="text-align: center;"><input type="checkbox"/></td></tr> <tr><td>4. Unexplained abdominal pain?</td><td style="text-align: center;"><input type="checkbox"/></td><td style="text-align: center;"><input type="checkbox"/></td></tr> <tr><td>5. Sudden swelling of ankles, hands or face?</td><td style="text-align: center;"><input type="checkbox"/></td><td style="text-align: center;"><input type="checkbox"/></td></tr> <tr><td>6. Persistent headaches or problems with headaches?</td><td style="text-align: center;"><input type="checkbox"/></td><td style="text-align: center;"><input type="checkbox"/></td></tr> <tr><td>7. Swelling, pain or redness in the calf of one leg?</td><td style="text-align: center;"><input type="checkbox"/></td><td style="text-align: center;"><input type="checkbox"/></td></tr> <tr><td>8. Absence of fetal movement after 6<sup>th</sup> month?</td><td style="text-align: center;"><input type="checkbox"/></td><td style="text-align: center;"><input type="checkbox"/></td></tr> <tr><td>9. Failure to gain weight after 5<sup>th</sup> month?</td><td style="text-align: center;"><input type="checkbox"/></td><td style="text-align: center;"><input type="checkbox"/></td></tr> </tbody> </table> <p>If you answered YES to any of the above questions, please explain: _____</p>		YES	NO	1. Miscarriage in an earlier pregnancy?	<input type="checkbox"/>	<input type="checkbox"/>	2. Other pregnancy complications?	<input type="checkbox"/>	<input type="checkbox"/>	3. I have completed a PAR-Q within the last 30 days.	<input type="checkbox"/>	<input type="checkbox"/>		YES	NO	1. Marked fatigue?	<input type="checkbox"/>	<input type="checkbox"/>	2. Bleeding from the vagina ("spotting")?	<input type="checkbox"/>	<input type="checkbox"/>	3. Unexplained faintness or dizziness?	<input type="checkbox"/>	<input type="checkbox"/>	4. Unexplained abdominal pain?	<input type="checkbox"/>	<input type="checkbox"/>	5. Sudden swelling of ankles, hands or face?	<input type="checkbox"/>	<input type="checkbox"/>	6. Persistent headaches or problems with headaches?	<input type="checkbox"/>	<input type="checkbox"/>	7. Swelling, pain or redness in the calf of one leg?	<input type="checkbox"/>	<input type="checkbox"/>	8. Absence of fetal movement after 6 <sup>th</sup> month?	<input type="checkbox"/>	<input type="checkbox"/>	9. Failure to gain weight after 5 <sup>th</sup> month?	<input type="checkbox"/>	<input type="checkbox"/>	<p><b>PART 3: ACTIVITY HABITS DURING THE PAST MONTH</b></p> <p>1. List only regular fitness/recreational activities: _____</p> <table style="width: 100%; border: none;"> <thead> <tr> <th style="width: 20%;">INTENSITY</th> <th colspan="3" style="width: 40%;">FREQUENCY (times/week)</th> <th colspan="3" style="width: 40%;">TIME (minutes/day)</th> </tr> <tr> <th></th> <th style="width: 13.3%;">1-2</th> <th style="width: 13.3%;">2-4</th> <th style="width: 13.4%;">4+</th> <th style="width: 13.3%; text-align: center;">&lt;20</th> <th style="width: 13.3%; text-align: center;">20-40</th> <th style="width: 13.4%; text-align: center;">40+</th> </tr> </thead> <tbody> <tr> <td>Heavy</td> <td style="text-align: center;">—</td> <td style="text-align: center;">—</td> <td style="text-align: center;">—</td> <td style="text-align: center;">—</td> <td style="text-align: center;">—</td> <td style="text-align: center;">—</td> </tr> <tr> <td>Medium</td> <td style="text-align: center;">—</td> <td style="text-align: center;">—</td> <td style="text-align: center;">—</td> <td style="text-align: center;">—</td> <td style="text-align: center;">—</td> <td style="text-align: center;">—</td> </tr> <tr> <td>Light</td> <td style="text-align: center;">—</td> <td style="text-align: center;">—</td> <td style="text-align: center;">—</td> <td style="text-align: center;">—</td> <td style="text-align: center;">—</td> <td style="text-align: center;">—</td> </tr> </tbody> </table> <p>2. Does your regular occupation (job/home) activity involve:</p> <table style="width: 100%; border: none;"> <thead> <tr> <th style="width: 80%;"></th> <th style="width: 10%; text-align: center;">YES</th> <th style="width: 10%; text-align: center;">NO</th> </tr> </thead> <tbody> <tr><td>Heavy Lifting?</td><td style="text-align: center;"><input type="checkbox"/></td><td style="text-align: center;"><input type="checkbox"/></td></tr> <tr><td>Frequent walking/stair climbing?</td><td style="text-align: center;"><input type="checkbox"/></td><td style="text-align: center;"><input type="checkbox"/></td></tr> <tr><td>Occasional walking (&gt;once/hr)?</td><td style="text-align: center;"><input type="checkbox"/></td><td style="text-align: center;"><input type="checkbox"/></td></tr> <tr><td>Prolonged standing?</td><td style="text-align: center;"><input type="checkbox"/></td><td style="text-align: center;"><input type="checkbox"/></td></tr> <tr><td>Mainly sitting?</td><td style="text-align: center;"><input type="checkbox"/></td><td style="text-align: center;"><input type="checkbox"/></td></tr> <tr><td>Normal daily activity?</td><td style="text-align: center;"><input type="checkbox"/></td><td style="text-align: center;"><input type="checkbox"/></td></tr> </tbody> </table> <p>3. Do you currently smoke tobacco? <input type="checkbox"/> YES <input type="checkbox"/> NO</p> <p>4. Do you consume alcohol? <input type="checkbox"/> YES <input type="checkbox"/> NO</p> <p><b>PART 4: PHYSICAL ACTIVITY INTENTIONS</b></p> <p>What physical activity do you intend to do? _____</p> <p>Is this a change from what you currently do? <input type="checkbox"/> YES <input type="checkbox"/> NO</p> <div style="border: 1px solid black; padding: 5px; margin-top: 10px;"> <p><b>*NOTE: PREGNANT WOMEN ARE STRONGLY ADVISED NOT TO SMOKE OR CONSUME ALCOHOL DURING PREGNANCY AND DURING LACTATION.</b></p> </div>	INTENSITY	FREQUENCY (times/week)			TIME (minutes/day)				1-2	2-4	4+	<20	20-40	40+	Heavy	—	—	—	—	—	—	Medium	—	—	—	—	—	—	Light	—	—	—	—	—	—		YES	NO	Heavy Lifting?	<input type="checkbox"/>	<input type="checkbox"/>	Frequent walking/stair climbing?	<input type="checkbox"/>	<input type="checkbox"/>	Occasional walking (>once/hr)?	<input type="checkbox"/>	<input type="checkbox"/>	Prolonged standing?	<input type="checkbox"/>	<input type="checkbox"/>	Mainly sitting?	<input type="checkbox"/>	<input type="checkbox"/>	Normal daily activity?	<input type="checkbox"/>	<input type="checkbox"/>
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# PARmed-X for PREGNANCY PHYSICAL ACTIVITY READINESS MEDICAL EXAMINATION

## **C** CONTRAINDICATIONS TO EXERCISE: to be completed by your health care provider

Absolute Contraindications			Relative Contraindications		
<i>Does the patient have:</i>			<i>Does the patient have:</i>		
1. Ruptured membranes, premature labour?	YES <input type="checkbox"/>	NO <input type="checkbox"/>	1. History of spontaneous abortion or premature labour in previous pregnancies?	YES <input type="checkbox"/>	NO <input type="checkbox"/>
2. Persistent second or third trimester bleeding/placenta previa?	<input type="checkbox"/>	<input type="checkbox"/>	2. Mild/moderate cardiovascular or respiratory disease (e.g., chronic hypertension, asthma)?	<input type="checkbox"/>	<input type="checkbox"/>
3. Pregnancy-induced hypertension or pre-eclampsia?	<input type="checkbox"/>	<input type="checkbox"/>	3. Anemia or iron deficiency? (Hb < 100 g/L)?	<input type="checkbox"/>	<input type="checkbox"/>
4. Incompetent cervix?	<input type="checkbox"/>	<input type="checkbox"/>	4. Malnutrition or eating disorder (anorexia, bulimia)?	<input type="checkbox"/>	<input type="checkbox"/>
5. Evidence of intrauterine growth restriction?	<input type="checkbox"/>	<input type="checkbox"/>	5. Twin pregnancy after 28th week?	<input type="checkbox"/>	<input type="checkbox"/>
6. High-order pregnancy (e.g., triplets)?	<input type="checkbox"/>	<input type="checkbox"/>	6. Other significant medical condition?	<input type="checkbox"/>	<input type="checkbox"/>
7. Uncontrolled Type I diabetes, hypertension or thyroid disease, other serious cardiovascular, respiratory or systemic disorder?	<input type="checkbox"/>	<input type="checkbox"/>	Please specify: _____		
NOTE: Risk may exceed benefits of regular physical activity. The decision to be physically active or not should be made with qualified medical advice.					
PHYSICAL ACTIVITY RECOMMENDATION:			<input type="checkbox"/> Recommended/Approved <input type="checkbox"/> Contraindicated		

## Prescription for Aerobic Activity

**RATE OF PROGRESSION:** The best time to progress is during the second trimester since risks and discomforts of pregnancy are lowest at that time. Aerobic exercise should be increased gradually during the second trimester from a minimum of 15 minutes per session, 3 times per week (at the appropriate target heart rate or RPE) to a maximum of approximately 30 minutes per session, 4 times per week (at the appropriate target heart rate or RPE).

**WARM-UP/COOL-DOWN:** Aerobic activity should be preceded by a brief (10-15 min.) warm-up and followed by a short (10-15 min.) cool-down. Low intensity calisthenics, stretching and relaxation exercises should be included in the warm-up/cool-down.

**PRESCRIPTION/MONITORING OF INTENSITY:** The best way to prescribe and monitor exercise is by combining the heart rate and rating of perceived exertion (RPE) methods.

F	I	T	T
FREQUENCY	INTENSITY	TIME	TYPE
Begin at 3 times per week and progress to four times per week	Exercise within an appropriate RPE range and/or target heart rate zone	Attempt 15 minutes, even if it means reducing the intensity. Rest intervals may be helpful	Non weight-bearing or low-impact endurance exercise using large muscle groups (e.g., walking, stationary cycling, swimming, aquatic exercises, low impact aerobics)

TARGET HEART RATE ZONES	
The heart rate zones shown below are appropriate for most pregnant women. Work during the lower end of the HR range at the start of a new exercise program and in late pregnancy.	
Rate	Heart
Age	Range
< 20	140-155
20-29	135-150
30-39	130-145
≥ 40	125-140

RATING OF PERCEIVED EXERTION (RPE)	
Check the accuracy of your heart rate target zone by comparing it to the scale below. A range of about 12-14 (somewhat hard) is appropriate for most pregnant women.	
6	
7	Very, very light
8	
9	Somewhat light
10	
11	Fairly light
12	
13	Somewhat hard
14	
15	Hard
16	
17	Very hard
18	
19	Very, very hard
20	

**"TALK TEST"** - A final check to avoid overexertion is to use the "talk test". The exercise intensity is excessive if you cannot carry on a verbal conversation while exercising.

The original PARmed-X for PREGNANCY was developed by L.A. Wolfe, Ph.D., Queen's University. The muscular conditioning component was developed by M.F. Mottola, Ph.D., University of Western Ontario. The document has been revised based on advice from an Expert Advisory Committee of the Canadian Society for Exercise Physiology chaired by Dr. N. Gledhill, with additional input from Drs. Wolfe and Mottola, and Gregory A.L. Davies, M.D.,FRCS(C) Department of Obstetrics and Gynaecology, Queen's University, 2002.

**No changes permitted. Translation and reproduction in its entirety is encouraged.**

Disponible en français sous le titre «Examen médical sur l'aptitude à l'activité physique pour les femmes enceintes (X-AAP pour les femmes enceintes)»

Additional copies of the PARmed-X for PREGNANCY, the PARmed-X and/or the PAR-Q can be downloaded from: <http://www.csep.ca/forms>

For more information contact the:

Canadian Society for Exercise Physiology  
370 - 18 Louisa St Ottawa, Ontario CANADA K1R 6Y6  
tel.: 1-877-651-3755 FAX (613) 234-3565 www.csep.ca

# PARmed-X for PREGNANCY PHYSICAL ACTIVITY READINESS MEDICAL EXAMINATION

## Prescription for Muscular Conditioning

It is important to condition all major muscle groups during both prenatal and postnatal periods.

**WARM-UPS & COOL DOWN:**  
*Range of Motion:* neck, shoulder girdle, back, arms, hips, knees, ankles, etc.

*Static Stretching:* all major muscle groups

**(DO NOT OVER STRETCH!)**

### EXAMPLES OF MUSCULAR STRENGTHENING EXERCISES

CATEGORY	PURPOSE	EXAMPLE
Upper back	Promotion of good posture	Shoulder shrugs, shoulder blade pinch
Lower back	Promotion of good posture	Modified standing opposite leg & arm lifts
Abdomen	Promotion of good posture, prevent low-back pain, prevent diastasis recti, strengthen muscles of labour	Abdominal tightening, abdominal curl-ups, head raises lying on side or standing position
Pelvic floor ("Kegels")	Promotion of good bladder control, prevention of urinary incontinence	"Wave", "elevator"
Upper body	Improve muscular support for breasts	Shoulder rotations, modified push-ups against a wall
Buttocks, lower limbs	Facilitation of weight-bearing, prevention of varicose veins	Buttocks squeeze, standing leg lifts, heel raises

### PRECAUTIONS FOR MUSCULAR CONDITIONING DURING PREGNANCY

VARIABLE	EFFECTS OF PREGNANCY	EXERCISE MODIFICATIONS
Body Position	<ul style="list-style-type: none"> <li>in the supine position (lying on the back), the enlarged uterus may either decrease the flow of blood returning from the lower half of the body as it presses on a major vein (inferior vena cava) or it may decrease flow to a major artery (abdominal aorta)</li> </ul>	<ul style="list-style-type: none"> <li>past 4 months of gestation, exercises normally done in the supine position should be altered</li> <li>such exercises should be done side lying or standing</li> </ul>
Joint Laxity	<ul style="list-style-type: none"> <li>ligaments become relaxed due to increasing hormone levels</li> <li>joints may be prone to injury</li> </ul>	<ul style="list-style-type: none"> <li>avoid rapid changes in direction and bouncing during exercises</li> <li>stretching should be performed with controlled movements</li> </ul>
Abdominal Muscles	<ul style="list-style-type: none"> <li>presence of a rippling (bulging) of connective tissue along the midline of the pregnant abdomen (diastasis recti) may be seen during abdominal exercise</li> </ul>	<ul style="list-style-type: none"> <li>abdominal exercises are not recommended if diastasis recti develops</li> </ul>
Posture	<ul style="list-style-type: none"> <li>increasing weight of enlarged breasts and uterus may cause a forward shift in the centre of gravity and may increase the arch in the lower back</li> <li>this may also cause shoulders to slump forward</li> </ul>	<ul style="list-style-type: none"> <li>emphasis on correct posture and neutral pelvic alignment. Neutral pelvic alignment is found by bending the knees, feet shoulder width apart, and aligning the pelvis between accentuated lordosis and the posterior pelvic tilt position.</li> </ul>
Precautions for Resistance Exercise	<ul style="list-style-type: none"> <li>emphasis must be placed on continuous breathing throughout exercise</li> <li>exhale on exertion, inhale on relaxation using high repetitions and low weights</li> <li>Valsalva Manoeuvre (holding breath while working against a resistance) causes a change in blood pressure and therefore should be avoided</li> <li>avoid exercise in supine position past 4 months gestation</li> </ul>	



## PARmed-X for Pregnancy - Health Evaluation Form

(to be completed by patient and given to the prenatal fitness professional after obtaining medical clearance to exercise)

I, \_\_\_\_\_ PLEASE PRINT (patient's name), have discussed my plans to participate in physical activity during my current pregnancy with my health care provider and I have obtained his/her approval to begin participation.

Signed: \_\_\_\_\_  
(patient's signature)

Date: \_\_\_\_\_

HEALTH CARE PROVIDER'S COMMENTS:

Name of health care provider: \_\_\_\_\_

Address: \_\_\_\_\_

Telephone: \_\_\_\_\_

(health care provider's signature)

## Advice for Active Living During Pregnancy

Pregnancy is a time when women can make beneficial changes in their health habits to protect and promote the healthy development of their unborn babies. These changes include adopting improved eating habits, abstinence from smoking and alcohol intake, and participating in regular moderate physical activity. Since all of these changes can be carried over into the postnatal period and beyond, pregnancy is a very good time to adopt healthy lifestyle habits that are permanent by integrating physical activity with enjoyable healthy eating and a positive self and body image.

### Active Living:

- see your doctor before increasing your activity level during pregnancy
- exercise regularly but don't overexert
- exercise with a pregnant friend or join a prenatal exercise program
- follow FITT principles modified for pregnant women
- know safety considerations for exercise in pregnancy

### Healthy Eating:

- the need for calories is higher (about 300 more per day) than before pregnancy
- follow Canada's Food Guide to Healthy Eating and choose healthy foods from the following groups: whole grain or enriched bread or cereal, fruits and vegetables, milk and milk products, meat, fish, poultry and alternatives
- drink 6-8 glasses of fluid, including water, each day
- salt intake should not be restricted
- limit caffeine intake i.e., coffee, tea, chocolate, and cola drinks
- dieting to lose weight is not recommended during pregnancy

### Positive Self and Body Image:

- remember that it is normal to gain weight during pregnancy
- accept that your body shape will change during pregnancy
- enjoy your pregnancy as a unique and meaningful experience

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For more detailed information and advice about pre- and postnatal exercise, you may wish to obtain a copy of a booklet entitled *Active Living During Pregnancy: Physical Activity Guidelines for Mother and Baby* © 1999. Available from the Canadian Society for Exercise Physiology, 370-18 Louisa Street, Ottawa, Ontario Canada K1R 6Y6 Tel. 1-877-651-3755 Fax: (613) 234-3565 Email: info@csep.ca (online: www.csep.ca). Cost: \$11.95

For more detailed information about the safety of exercise in pregnancy you may wish to obtain a copy of the Clinical Practice Guidelines of the Society of Obstetricians and Gynaecologists of Canada and Canadian Society for Exercise Physiology entitled *Exercise in Pregnancy and Postpartum* © 2003. Available from the Society of Obstetricians and Gynaecologists of Canada online at www.sogc.org

For more detailed information about pregnancy and childbirth you may wish to obtain a copy of *Healthy Beginnings: Your Handbook for Pregnancy and Birth* © 1998. Available from the Society of Obstetricians and Gynaecologists of Canada at 1-877-519-7999 (also available online at www.sogc.org) Cost \$12.95.

For more detailed information on healthy eating during pregnancy, you may wish to obtain a copy of *Nutrition for a Healthy Pregnancy: National Guidelines for the Childbearing Years* © 1999. Available from Health Canada, Minister of Public Works and Government Services, Ottawa, Ontario Canada (also available online at www.hc-sc.gc.ca).

### SAFETY CONSIDERATIONS

- ◆ Avoid exercise in warm/humid environments, especially during the 1<sup>st</sup> trimester
- ◆ Avoid isometric exercise or straining while holding your breath
- ◆ Maintain adequate nutrition and hydration — drink liquids before and after exercise
- ◆ Avoid exercise while lying on your back past the 4<sup>th</sup> month of pregnancy
- ◆ Avoid activities which involve physical contact or danger of falling
- ◆ Know your limits — pregnancy is not a good time to train for athletic competition
- ◆ Know the reasons to stop exercise and consult a qualified health care provider immediately if they occur

### REASONS TO STOP EXERCISE AND CONSULT YOUR HEALTH CARE PROVIDER

- ◆ Excessive shortness of breath
- ◆ Chest pain
- ◆ Painful uterine contractions (more than 6-8 per hour)
- ◆ Vaginal bleeding
- ◆ Any "gush" of fluid from vagina (suggesting premature rupture of the membranes)
- ◆ Dizziness or faintness

## Appendix E - ASA24 online tool for 3 day dietary record in Manuscript 3

The screenshot shows the National Cancer Institute website header with the text "National Cancer Institute" and "at the National Institutes of Health | www.cancer.gov". Below this is a navigation bar with "APPLIED RESEARCH" and "CANCER CONTROL AND POPULATION SCIENCES". A search bar is visible on the right. The main content area shows a breadcrumb trail: "Browse by Resource Type > Data Collection Instruments > Automated Self-Administered 24-hour Dietary Recall (ASA24)".

**ASA24™**  
ASA24 Automated Self-administered 24-hour Recall

**ASA24™**  
ASA24 is a freely available Web-based tool that enables automated self-administered 24-hour recalls. ASA24 consists of a [Respondent Web site](#) used to collect recall data and a [Researcher Web site](#) used to manage study logistics and obtain data analyses. Two Respondent Web sites (English and Spanish) are currently available for registering new studies: **ASA24-2014** (for adults) and **ASA24-Kids-2014**. Still available until March of 2015 are two previous versions of the Respondent Web site: **ASA24-2011** (for adults) and **ASA24-Kids-2012**. Further information on the Respondent and Researcher Web sites is provided in the sections below.

**BACKGROUND**

- [Background](#)

**RESPONDENT WEB SITES**

- [Methodology](#)
- [Features](#)
- [Evaluation & Validation](#)
- [Costs](#)
- [Available Versions](#)

**Announcements**

- [Dietary Assessment Primer](#)
- [ASA24-2014](#) and [ASA24-Kids-2014](#) were released!

**Quick Links**

- [Respondent Web Site Demonstration \(ASA24-2014 and ASA24-Kids-2014\)](#)
- [Respondent Web Site Demonstration \(ASA24-2011 and ASA24-Kids-2012\)](#)
- [Researcher Web Site](#)
- [Join the ASA24 Listserv](#)

**Meal Details**  
Enter the details of the first meal or snack you would like to report.

Meal or snack:  
Select a meal or snack

Time of meal or snack:  
HH:MM  AM  PM

Location:  
Select a location

Next

**Actions**  
Select an action below to edit your foods and drinks.

- Add a meal or snack
- Delete a meal or snack
- Edit a meal or snack
- Delete a food or drink
- Move a food or drink
- Copy a food or drink
- Edit a food or drink
- Done entering all meals and snacks
- Undo
- Finish later

**Find a Food or Drink**  
Browse the categories or search using the box below.

Enter search term

- Beans, peas, nuts, soy products
- Beverages
- Breads, other baked goods
- Cereals and energy bars
- Chicken, turkey, poultry
- Dairy, dairy substitutes
- Desserts and sweets
- Eggs
- Fats, Oils, Dressings, Spreads
- Fish, shellfish
- Fruit
- Meat
- Miscellaneous
- Mixtures, Chinese, Mexican, Chili, Other
- Pancakes, waffles, crepes
- Pasta, noodles, and spaghetti

No Match Found

**My Foods and Drinks**  
What I ate and drank Yesterday, Monday, December 02

Breakfast - 07:00 AM

**Cheerios: How much did you actually eat?**

Select the image that best represents the amount you ate at breakfast at 07:00 AM.

Amount eaten:  
1 1/2 cups

Previous Don't Know Next

**Did you add anything to your Cheerios that you haven't already reported?**

Select all that apply.

Search or browse to find foods added to your Cheerios at breakfast at 07:00 AM. Use the arrows to add or remove additions. If nothing was added or you have already reported the additions, select **Nothing Added**.

Enter search term	1% milk
<ul style="list-style-type: none"> <li>Cold cereals</li> <li>Fruit</li> <li>Milk</li> <li>Sugars and sugar substitutes</li> </ul>	<ul style="list-style-type: none"> <li>Common Additions</li> <li>2% milk</li> <li>Buttermilk</li> <li>Skim milk</li> <li>Soy milk</li> <li>Whole milk</li> <li>Dry milk (1% fat)</li> <li>Other Additions</li> <li>Acidophilus milk</li> <li>Calcium fortified milk (skim or nonfat)</li> <li>Coconut milk</li> <li>Dry milk (lowfat)</li> <li>Dry milk (skim or nonfat)</li> <li>Dry milk (unknown type)</li> </ul>

Previous Don't Know Nothing Added Next

**FREQUENTLY FORGOTTEN FOODS**

Certain foods and drinks are frequently forgotten. Did you forget to report any of the following foods and drinks? Please respond to each item by selecting **Yes** or **No**.

In addition to the foods and drinks you already reported, did you have any:

Water, including tap, faucet, bottled, water fountain?  Yes  No

Coffee, tea, soft drinks, milk or juice?  Yes  No

Beer, wine, cocktails or other drinks?  Yes  No

Cookies, candy, ice cream or other sweets?  Yes  No

Chips, crackers, popcorn, pretzels, nuts or other snack foods?  Yes  No

Fruits, vegetables or cheese?  Yes  No

Breads, rolls or tortillas?  Yes  No

Anything else?  Yes  No

Next

*Appendix F - HALO sub-maximal fitness test log sheet (Manuscript 3)*



Date:

Protocol:

**HALO Submax Protocol**

<b>ID #:</b>	<b>DOB (DD-MMM-YY):</b>	<b>Age:</b>
<b>Height:</b>	<b>Test Date:</b>	<b>Predicted HRmax:</b>
<b>Weight:</b>		<b>85% HRmax:</b>

Resting HR: \_\_\_\_\_bpm

25% Resting HR: \_\_\_\_\_bpm

Chosen spw: \_\_\_\_\_mph

HR @ 85%max

Time:

Time (min)	Test Time (sec)	Speed (mph)	Grade %	HR (bpm)	RPE
0-4	-4		0		
4-8	30 (0:30)		3		
	60 (1:00)		3		
	90 (1:30)		3		
	120 (2:00)		3		
	150 (2:30)		3		
	180 (3:00)		3		
	210 (3:30)		3		
	240 (4:00)		3		
8-12	30 (0:30)		6		
	60 (1:00)		6		
	90 (1:30)		6		
	120 (2:00)		6		
	150 (2:30)		6		
	180 (3:00)		6		
	210 (3:30)		6		
	240 (4:00)		6		

Version: May 2012

Name of Person Conducting Test: \_\_\_\_\_

*Appendix G - Consent form for lean vs. obese placenta study*

**Patient Information and Consent Form**



The Ottawa | L'Hôpital  
Hospital | d'Ottawa



**MECHANISM OF PLACENTAL DYSFUNCTION IN OBESE MOTHERS**

**WHY ARE YOU BEING GIVEN THIS FORM?**

This form will provide information about the study. This form will also (1) Allow your obstetrician to run additional tests on your blood and take blood from your umbilical cord after delivery; (2) Allow your obstetrician to conduct ultrasounds during your pregnancy and to collect and examine your placenta after delivery (3) Allow you to decide if you would like to participate in the study described below.

*DELETED  
11/15*

**PROJECT TEAM**

Dr. A. Gruslin (Maternal-Fetal Medicine Specialist), Ottawa Hospital-General Campus  
Dr. Q. Qiu (Research Scientist), Ottawa Hospital  
Mylène Gagné (3<sup>rd</sup> year Medical Student), University of Ottawa  
Kristina Arendas (Postgraduate Year 2), Ottawa Hospital-General Campus  
Sharron Lafrenière (Research Nurse) Ottawa Hospital-General Campus  
Lindsay Patrick (Research Assistant) Ottawa Hospital-General Campus  
Zachary Ferraro (3<sup>rd</sup> year PhD Student), University of Ottawa

**WHY IS THE STUDY BEING DONE?**

In the past decade, obesity has greatly increased in the general population, and doctors are seeing more and more obese pregnant women in their clinics. This is important because many reports explain that obesity during pregnancy increases the risk of pregnancy complications.

When a woman is obese certain changes can occur in her body that may cause changes to a vital tissue during pregnancy, the placenta. The placenta, the connection between the mother and baby, is essential to both fetal growth and health. If the placenta is dysfunctional (because of obesity for example), the baby and mother can suffer serious complications.

Our knowledge is very limited as to the link between obesity and fetal complications. We know that in order to have a healthy placenta and baby the mother needs a certain protein called insulin growth factor II (IGF-II). This

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Updated on September 23, 2010

## Patient Information and Consent Form

protein is made by the body and is present normally in all women but we believe that obesity might cause it to decrease. A decrease in IGF-II may lead to placental dysfunction and pregnancy complications. The overall objective of this study is to examine whether changes in IGF-II may be involved with observed placental dysfunction and pregnancy complications.

### WHAT WILL YOU BE ASKED TO DO?

If you agree to participate

- (1) three blood tests (5mL each), two during your pregnancy and one at delivery
- (2) three ultrasounds during your pregnancy
- (3) taking cord blood (5mL)
- (4) sending your placenta to the laboratory for analysis after delivery
- (5) have a small sample of placenta tissue stored and subsequently analyzed for **non-clinical** genetic markers of metabolism (i.e, IGF axis protein expression).
- (6) record your baby's weight as well as the weight of the placenta.

Results from the genetic component of this study will be preliminary and the clinical implications of any findings may not be understood for many years. These genetic markers have NO clinical diagnostic value and thus no individuals, other than study researchers, will have access to these samples or information gained from the samples. Because no information will be provided to participants or others from the examination of this sample, the risk is minimal.

We are **NOT screening for genetic abnormalities but rather potential relationships that exist as a result of excess body weight**. Therefore, the use of the term **non-clinical genetic markers refers to testing ONLY for gene variations** which may provide insight on how nutrients are transferred from mom to baby during pregnancy.

We will conduct our study using all of these results. Blood samples provided will only be analyzed for the purposes of this study and no further testing will be done without your written consent. This will, in no way, interfere with your care. In addition, we will also record whether you have any characteristics that may affect our research project (e.g. diabetes, hypertension). That information will be given a code for research, and any papers with your identity will be kept in a locked cabinet by the senior physician (Dr. A. Gruslin).

### POTENTIAL HARMS

There is no risk to you or your baby.

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Updated on September 23, 2010

## **Patient Information and Consent Form**

### **RISKS OF INSURABILITY AND EMPLOYABILITY**

We will take all reasonable steps to keep your research information confidential. Should someone not involved in the research find out that you took part in this research study, or if you choose to share your results (if they are provided to you), there is a possibility that this could affect your insurance or employment.

### **BENEFITS**

Our study will help us determine if maternal obesity is related to placental dysfunction and if this dysfunction is associated with changes in IGF-II. This study could provide information for the development of useful clinical screening tools to better understand and therefore better treat obese pregnant women. Although this may not benefit you directly, this knowledge will benefit others in the future.

### **ALTERNATIVES TO PARTICIPATING IN THE PROJECT**

You do not have to participate in this research study to receive standard of care.

### **PRIVACY AND CONFIDENTIALITY**

All information provided regarding you and your baby, including blood and placenta tissue, will be coded with a numeric identifier, will not contain your name and will be kept under lock and key. A list of names and matching codes will be stored separately in the office of Dr. Gruslin so that no identifying information will be present in research files. Only the staff involved in this research study will have access to the records. Overall results may be published for scientific purposes, but participant identity will remain confidential. All results of the study will be kept confidential and will not be communicated to any third parties such as employers, governmental organizations or insurance companies unless you provide specific authorization, or where the law requires, or a court order has been obtained. This includes your spouse, other members of your family and your physician. These results will not appear in your medical chart.

The placenta tissue samples will become property of Dr. A. Gruslin and used for academic research purposes only. The results of this study will not be exploited for commercial use.

No identifiable information will leave the Ottawa Hospital. All information relating to this study will be kept in Dr. Gruslin's office in a locked cabinet for 15 years. After this time, the papers copies will be shredded and the electronic files will be deleted to ensure that all information is destroyed. All tissue and blood samples taken will be kept until termination of the study under lock and key and stored in

## **Patient Information and Consent Form**

a -80 freezer in the laboratory of a member of the project team. Following completion of the study all unused samples will be disposed of as medical waste by the University of Ottawa's usual method.

All the information regarding you or your baby will be kept confidential at the Ottawa Hospital unless release is required by law. Representatives of the Ottawa Hospital Ethics Board (OHREB) and the Ottawa Health Research Institute (OHRI) may review your relevant records for audit purposes under the supervision of Dr Gruslin's staff, for audit purposes.

### **COMPENSATION FOR INJURY, LEGAL RIGHTS**

You are not waiving your legal rights by agreeing to participate in this study. The doctors and the hospital still have their legal and professional responsibilities.

### **REIMBURSEMENT OF EXPENSES/PAYMENTS FOR PARTICIPATING**

There are no expenses or payments associated with the participation in this project.


### **YOU HAVE THE RIGHT TO CHANGE YOUR MIND**

Your participation in this study is entirely voluntary. You may choose not to participate or to withdraw from the study at any time without providing the investigator with a reason. Your decision will not affect the care you and your baby receive at this institution now or in the future.

### **NEW INFORMATION DURING THE STUDY?**

If any new information during the study becomes available that might affect your willingness to participate, you will be informed as soon as possible.

### **WHO TO CONTACT IF YOU HAVE ANY FURTHER CONCERNS OR QUESTIONS**

If you have any concerns or questions regarding the study, you may reach Dr. A. Gruslin at 

### **ETHICS REVIEW**

If you have any questions about your rights as a research participant, you may contact the Chairperson of the Ottawa Hospital Research Ethics Board at 613-798-5555, extension 14902.

## Patient Information and Consent Form

### STATEMENT OF CONSENT – PRINT AND SIGN NAME

I, \_\_\_\_\_, ( \_\_\_\_\_ )  
have read, or have been read the information given in this 4-page informed consent and all my questions have been answered to my satisfaction. I have had sufficient time to consider whether to participate in this study. My participation in this study is entirely voluntary and I may withdraw from the study at any time without penalty.

I voluntarily consent to participate in this study and allow my placental tissue to be sampled. I will receive a signed copy of this form for my records.

### TEAM MEMBER WHO INTERACTED WITH THE SUBJECT - PRINT AND SIGN NAME

To the best of my knowledge, the information in this consent form, and the information that I, \_\_\_\_\_ ( \_\_\_\_\_ )  
have provided in the response to any questions, fairly represents the project. I am committed to conducting this study in compliance with all the ethical standards that apply to projects that involve human subjects. I will ensure that the subject receives a copy of this consent form.

\_\_\_\_\_  
Participant's name

\_\_\_\_\_  
Participant's signature

\_\_\_\_\_  
Date

\_\_\_\_\_  
Investigator/Delegate's name

\_\_\_\_\_  
Investigator/Delegate's signature

\_\_\_\_\_  
Date

*Appendix H - Active MOM consent form (Manuscript 2 and 3)*



**Active MOM: an observational study on the influence of exercise on the placenta**

**Informed consent form**

Investigators:

**Dr. Kristi Adamo**                      **Healthy Active Living and Obesity Research Group, CHEO**

**Kendra Brett**                         **Healthy Active Living and Obesity Research Group, CHEO**

**Background & Rationale**

The Active MOM study is designed to examine how physical activity during pregnancy can influence how nutrients (sugar, protein and fat) are transferred to the developing fetus. Regular exercise is an important component of a healthy pregnancy, and prenatal exercise does not negatively affect pregnancy or neonatal outcomes. The benefits of regular physical activity during pregnancy include a lower risk of gestational diabetes and pre-eclampsia (high blood pressure related to pregnancy), while reducing the risk of birthing infants that are too small or too large for their gestational age. Physical activity is an important part of a healthy lifestyle, however, most Canadians, pregnant and non-pregnant, do not meet the guidelines for physical activity.

Prenatal exercise may alter the environment in which the fetus develops. This may cause changes to a vital tissue during pregnancy, the placenta. The placenta is the connection between the mother and baby and is essential for fetal growth and health. The placenta controls the transfer of nutrients (sugar, protein and fat) and oxygen to the fetus. If the placenta is not functioning properly, the baby may receive too few or too many nutrients. It is not well known how exercise affects the transfer of nutrients to the fetus.

The goal of this study is to examine whether there are differences in the placenta's ability to transfer nutrients in women who exercised regularly during pregnancy compared to women who did not exercise regularly.

**Purpose**

You are being asked to participate in a study that will examine the effects of prenatal physical activity on the placenta. This study will compare samples of the placenta from women who were physically active throughout their pregnancy and those who were not. We will explore the impact of physical activity on the genes that control the transfer of nutrients across the placenta to the fetus. Genes are the building blocks or instructions found inside cells, which contribute to the different features you have such as the colour of your eyes. The knowledge we gain from this information may be helpful in designing preventive health strategies for pregnancy.

Participation in this study is voluntary. Your care at CHEO or the Ottawa Hospital will not be affected if you choose not to participate. You should discuss any questions you have about this study with the people who explain it to you.

## **Study Procedures and Assessments**

If you consent to participate in our study, you will be asked to come to CHEO on two occasions at a specific time and date which will be arranged between you and the study's research coordinator. The estimated length of each visit is 1 hour. Visits will take place during the week or on the weekend at a time that is convenient for you. You will be compensated for the cost of parking at CHEO or bus tickets for both visits.

The following will occur during to the initial visit:

- 1) We will measure your height and weight and ask you to recall your pre-pregnancy weight. You will fill out two questionnaires that ask questions about your socio-demographic information, your health, and your physical activity habits.
- 2) You will have a fasting blood sample taken by a nurse. The blood sample will occur in the morning following a twelve (12) hour fast. This will be used to assess markers associated with nutrient transport, fetal growth, and weight gain. We will take 10mL or 2 teaspoon of blood. This blood will not be used for secondary, unrelated uses.
- 3) You will be given the PARmed-x for pregnancy questionnaire to complete. We ask that you bring this to your doctor to ensure that it is safe for you to exercise during pregnancy.
- 4) You will be asked to complete three 24-hour dietary recalls in the week following the initial visit which will take about 20 minutes/day. You will be supplied with a small physical activity monitor to wear for 1-week and instructions on how to use it.

### **Details of the Activity Monitor**

The physical activity monitor that you will be asked to wear for one week is a 'smart' pedometer that works like the motion sensor (counting steps) but additionally provides information regarding the person's speed and other movement patterns. It is always on but it is activated only by movement. This will give us an idea of your typical physical activity patterns. The monitor (1" square) is safe, relatively small, non-invasive and is attached to the body by a soft belt around the waist either under or over clothing and will not impact day-to-day activities.

The following will occur during the second visit:

1) You will be asked to undergo a fitness test on a treadmill. You will choose the speed of the treadmill by selecting a comfortable walking pace. The incline of the treadmill will increase at the start of each 4 minute stage. The test will take 20-25 minutes to complete. Your heart rate will be monitored during this test. The test will stop before you reach 85% of your maximum heart rate.

2) You will be given a questionnaire that will ask you to recall how often you ate or drank certain foods over the past year. You will be asked to complete the questionnaire one month prior to your delivery. The questionnaire will take about 20 minutes.

The final component of the study will occur on the day of your delivery, and we are asking permission to obtain and bank samples of your cord blood and placenta tissue.

The following will occur on the day of your delivery:

1) After delivery of your child and placenta, and after the placenta is no longer attached to you or your baby, we will take a blood sample from the umbilical cord and tissue samples from the placenta. This will not involve any pain and will not involve sticking your baby with a needle. These samples will be used to assess markers associated with nutrient transport, growth and development. We will take 10mL or 2 teaspoons of blood. This blood will not be used for secondary, unrelated uses.

This will not prevent you from taking part in a private cord blood storage program, if you so desire. However, the blood sample that we store for research purposes will not be available to you for any other purposes.

No other genetic studies will be performed using your blood sample or that of your child without your consent.

All samples will be kept in a locked  $-80^{\circ}\text{C}$  freezer located in the Healthy Active Living and Obesity research group's lab in the Research Institute at CHEO. After having completed all the analyses, the University of Ottawa will take all unused blood samples and will destroy them according to their usual method.

We will be accessing your medical charts from your delivery so that we can gain information on a variety of measures that are done including the birth weight of your child, the weight of your placenta, level of labour pain and any complications that arose during delivery.

## **Risks**

If you are eligible to participate, there is little risk to you or your baby by participating in this study.

Blood drawing causes some pain and may cause bruising, bleeding or infections at the site of the needle stick. Care will be taken to avoid these complications.

The exercise test will occur in a safe environment and will incorporate the most recent evidence for exercise guidelines during pregnancy. CPR and first aid trained exercise physiologists, specially trained to perform exercise testing, will coordinate and monitor the testing. Heart rate will be continually monitored to ensure that you do not reach an unsafe heart rate. Research staff will make sure the treadmill is adjusted properly for each subject, and will conduct a proper warm-up and cool-down to prevent injuries.

The risk of an adverse event is minimized through medical screening using the PARmed-x for physical activity during pregnancy and the supervision of testing by qualified personnel. In the unlikely event that you experience an injury, medical or psychological crisis (i.e. chest pains, heart attack, panic attack, etc) during the fitness test, a safety protocol is in place and the hospital emergency response team will be contacted immediately. The CHEO emergency response team and study staff will immediately accompany participants to the emergency room at the General Hospital and the study staff will stay with them until they are assessed.

We will take all reasonable steps to keep your research information confidential. Should someone not involved in the research find out that you took part in this research study, or if you choose to share your results (if they are provided to you), there is a possibility that this could affect your insurance or employment.

## **Benefits**

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

At the conclusion of the research study, you can receive a summary of the results if you so wish.

## **Confidentiality**

Only study personnel, and scientists working directly with us, will have access to the data collected. The data collected in this study will be kept under lock and key in a safe place. All information that you provide will be coded with a study number and will not contain your name. A list of names and matching codes will be stored separately so that no identifying information will be present in your research file from this study to ensure confidentiality. Only the staff involved in this research study will have access to the records, with the exception of the CHEO Research Ethics Board, who have access to records for auditing purposes. Overall results may be published for scientific purposes, but participant identity will remain confidential. Your personal information will be kept confidential except as required or permitted by law. Limits of this confidentiality include situations of suspected child abuse, concerns of harm to self or others, and any request for information by court order. The study data will be kept for 15 years after termination of the study and then will be destroyed.



All information gathered during the course of the study will be completely confidential. If you choose not to participate in this study, you/your child's future care at CHEO or the Ottawa Hospital will not be affected in any way. You are free to withdraw from the study at any time and you/your child will receive the same future care at CHEO and the Ottawa Hospital. You should contact the study coordinator or the investigator if you wish to withdraw from the study.

This study is being conducted by Kendra Brett (PhD student),

Questions about the Study

Kendra Brett Graduate Student, CHEO

Research Coordinator: Research Coordinator, CHEO

Dr. Kristi Adamo: Research Scientist, CHEO

If nobody is able to answer your call, a voice messaging system is available.

This study has been reviewed and approved by the CHEO Research Ethics Board. The CHEO Research Ethics Board is a committee of the hospital that includes individuals from different professional backgrounds. The Boards reviews all human research that takes place at the hospital. Their goals are to ensure the protection of the rights and welfare of people participating in research. The Boards' work is not intended to replace a parent or child's judgment about what decisions and choices are best for them. You may contact the Chair of the CHEO Research Ethics Board, for information regarding patient's rights in research studies at  although this person cannot provide any health-related information about the study. These Boards could review your study records in fulfilling its roles and responsibilities.

**Active MOM: an observational study on the influence of exercise on the placenta**

**Informed consent form**

I have read the explanation about this study in this 5-page consent form. I have been given the opportunity to discuss it and my questions have been answered to my satisfaction. I hereby consent to:

Yes  No Take part in this study. I realize that my participation is voluntary and I am free to withdraw from the study at any time.

Yes  No Have my blood/DNA and placenta tissue samples stored and used for future, ethics approved, research on physical activity, pregnancy outcomes and weight regulation.

Yes  No Have my child's cord blood/DNA samples banked and used for future, ethics approved, research on pregnancy outcomes.

Yes  No Give access to my medical charts that are related to my pregnancy and the birth records of my offspring to the study researchers for a minimal risk chart review to ensure that the necessary physical and physiological characteristics of my pregnancy are recorded.

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

**Contact Information:**

Daytime and evening telephone number(s): \_\_\_\_\_

Email address: \_\_\_\_\_

I will be given a copy of the consent form for my records.

Name of Participant	Signature of Participant	Date
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I have explained this study to the person authorized to sign above and they have been given the opportunity to ask questions.

\_\_\_\_\_  
Name of Investigator or Delegate

\_\_\_\_\_  
Signature

\_\_\_\_\_  
Date

*Appendix I - MOM trial consent form (Active MOM is a sub-study of MOM trial)*



The Ottawa Hospital  
L'Hôpital d'Ottawa

**The MOM Trial- A pregnancy Specific Randomized Control Trial**

**Informed consent form**

**Investigators:**

Dr. Kristi Adamo      Healthy Active Living and Obesity Research Group, CHEO

Dr. Erin Keely      Endocrinology and Metabolism, Riverside Campus

**Background & Rationale**

Between 1993 and 2003, the number of women entering pregnancy with a greater than recommended weight increased by 69%. This is alarming as a mother's pre-pregnancy body mass index (BMI) and the amount of weight gained during pregnancy (gestational weight gain) have been recognized as vital contributing factors to child health issues including downstream obesity. Children born to overweight and obese mothers are significantly more likely to be large at birth, as well as being obese as infants and as young children compared to children of normal weight mothers. Unfortunately children who are heavier than their peers are more likely remain so as adults. Additionally, higher pre-pregnancy BMI and higher pregnancy weight gain are both associated with greater incidence of pre-eclampsia (high blood pressure related to pregnancy which can be associated with serious complications to mother and/or baby) and significantly increased risk of developing diabetes during pregnancy- known as gestational diabetes. The presence of gestational diabetes provides a less than favorable intrauterine environment – the environment in which the fetus grows - which has been shown to play a critical role in the development of obesity and type 2 diabetes in the offspring.

This situation, if not addressed properly, will have significant impact on the quality of life of the future Canadian population and on public health services. Preventive interventions during pregnancy may be critical to reduce childhood obesity and its associated health complications.

**Purpose**

You are being asked to participate in a study that will examine the effects of a structured prenatal physical activity and nutrition intervention program that will be provided to pregnant women during their 2<sup>nd</sup> and 3<sup>rd</sup> trimester. Your child's participation will be requested following birth up until the age of 2 years. We will be examining the effects of the intervention on gestational weight gain, infant birth weight, the occurrence of pregnancy related complications, psychosocial functioning, maternal physical activity and dietary habits, as well as the effect on the mother and child's BMI and body composition up to 2-years after birth.

Healthy eating and physical activity habits are important for avoiding weight gain and allowing for weight maintenance in people of all ages and have been specifically identified as predictors of excessive weight gain during pregnancy. Furthermore, a balanced maternal diet provides health benefits for both the mother and developing child.

Regular prenatal exercise does not negatively affect pregnancy or neonatal outcomes but rather, is an important component of a healthy pregnancy. Failure to exercise may be associated with decreases in fitness, excessive weight gain during pregnancy, varicose veins, low-back pain, gestational diabetes and pregnancy-related high blood pressure. Given that women who exercise before and during pregnancy tend to weigh less, gain less weight, have improved labour pain tolerance and deliver smaller babies than those who do not, it is reasonable to expect that a program combining healthy eating and activity habits would lead to healthy fetal growth and development, resulting in fewer pregnancy related complications, normal weight offspring, and less maternal weight gain and retention.

Your care at CHEO, the Ottawa Hospital or the Queensway Carleton Hospital or the Montfort Hospital will not be affected if you choose not to participate. You should discuss any questions you have about this study with the people who explain it to you.

### **Study Procedures and Assessments**

If you consent to participate in our study, you will be asked to come to the Children's Hospital of Eastern Ontario (CHEO) at a specific time and date which will be arranged between you and the study's research coordinator, at your convenience. You will need to arrive fasted (not having anything to eat or drink except water for 12 hours). The total estimated length of the visit is 2 hours and will take place during the week or on the weekend in the morning.

The following measurements (1 & 2) will, in addition to the initial visit, be repeated at the end of your 2<sup>nd</sup> trimester and before the end of your 3<sup>rd</sup> trimester:

1) The research coordinator will measure your height and weight and will have you fill out questionnaires that ask a series of questions relating to stress, social support, depression and attitudes towards pregnancy. You will be provided with appropriate medical advice and or attention should your responses indicate a need. You will also be required to have a blood sample taken that we will use to help assess markers associated with nutrient transport, growth and development and weight gain at those three time points. At each sampling, we will take 5mL or 1 teaspoon of blood. This blood will not be used for secondary, unrelated uses.

2) In order to assess dietary and physical activity patterns, you will be asked to complete a 7-day dietary recall (this will take approximately 15 minutes/day) and a 7-day physical activity recall (this will take approximately 5 minutes/day). You will also be supplied with a small physical activity monitor to wear for 1-week and instructions on how to use it.

We will be accessing your medical charts from your delivery so that we can gain information on a variety of measures that are done including the birth weight of your child, the weight of your placenta, level of labour pain and any complications that arose during delivery.

At the initial visit you will be randomized to either the Standard Care Control Group or to the Intervention Group. Like flipping a coin, you will have a 50/50 chance of being selected to partake in the control group or the intervention group. Neither you nor your doctor can choose the group to which you will be assigned. All participants will undergo the measures described above, and in addition:

### **Standard Care Control Group**

If you are randomized to the control group, you will receive the normal prenatal care as recommended by your health care practitioner as well as “A Sensible Guide to a Healthy Pregnancy” which is a booklet produced by Health Canada.

### **Intervention Group**

If you are participating in the intervention group you will be asked to come in fasted (nothing to eat or drink except water for 12 hours) for your initial visit and undergo a measure of your resting energy expenditure (REE) sometimes referred to as metabolic rate. This is done lying down, following a 10 minute rest period, while you wear a face mask and it takes about 15-20 minutes. The results from the REE measurement will let us know how many calories you use and we use this information to adjust your diet.

You will be given a Healthy Gestation Workbook that will provide helpful suggestions and contain ‘tear-out’ food record and physical activity log forms and, all intervention subjects will have a baseline session with a nutrition specialist immediately after recruitment (beginning of 2<sup>nd</sup> trimester) to discuss weight, diet history and healthy eating strategies for during pregnancy following Health Canada’s nutritional guidelines. Your partner is encouraged to attend this session to support you, but it is not required. Nutrition education classes will be scheduled each month over the course of the program in the form of 3 modules (transition between 1<sup>st</sup> and 2<sup>nd</sup> trimester, transition between 2<sup>nd</sup> and 3<sup>rd</sup> trimester and mid 3<sup>rd</sup> trimester) and will take place following an exercise class at the University of Ottawa Lees campus facility gymnasium. You will also receive a post-card mail-out every 4 weeks reviewing your nutritional needs at that stage of pregnancy (7 in total) and reinforcing your goals. A final personalized session with the nutrition specialist will take place at the beginning of the 3<sup>rd</sup> trimester to take place following an evening exercise session at the Lees campus.

You will also be expected to attend two, 45-60 minute (including warm-up and cool-down) supervised evening exercise classes each week during your 2<sup>nd</sup> and 3<sup>rd</sup> trimester of pregnancy. These will take place on Tuesday and Thursday at 5:30pm – 6:30pm. Safe and specifically designed exercise classes for pregnant women will be offered and lead by certified exercise specialists with a specialization in maternal health will be offered through the University of Ottawa – Lees Avenue campus facility gymnasium. Classes will incorporate both aerobic and resistance exercises that minimize the risk of loss of balance and fetal trauma. All nutrition and exercise classes will be offered in the evening. In addition, women will be encouraged to perform 30 minutes of aerobic activity (i.e. walking), independently, at least 3 other days/week thereby meeting the physical activity guidelines of 30 minutes or more of moderate intensity physical activity on most, if not all, days of the week.

The University of Ottawa Lees campus facility gymnasium and its staff meet the required standards of the Canadian Safety Council. Its staff are trained and certified in their area of expertise, in first aid and CPR and in the facility’s Emergency Action Plan.

### Intervention and Standard Care After Birth Measurements

At 3, 6 and 12 months post-delivery you will have your weight, dietary habits and physical activity measured as described previously, and you will also be asked about your infant feeding practices (home visits can be made for the 3 month follow-up). At 12 months, we will measure your body composition using dual x-ray absorptiometry (DEXA) which is the method of choice for assessing body composition (bone density, body fat percentage and muscle mass). It takes approximately 10 minutes and is a painless procedure requiring you to lie still for the duration of the test. This test will be performed at CHEO.

At these time points as well as at 24 months post-delivery we will also measure the height and weight of your baby as well as their body composition using the common and traditional skin fold caliper method. We will also assess their physical activity by placing, for 24-hours, an activity monitor on their right ankle using an ankle band and another activity monitor will be placed just above your child's right hip using medical tape to hold it in place.

As with the study in general, you and your child can opt out of this at any time and not have your care at CHEO, the Ottawa Hospital or the Queensway Carleton Hospital or the Montfort Hospital affected in any way.

### **Risks**

If you are eligible to participate, there is little risk to taking part in this study. All exercise and nutrition sessions will be led and monitored by trained specialists in a safe environment and will incorporate the evidence-based Society of Gynecologists of Canada and Canadian Society for Exercise Physiology's National Guidelines for exercise during pregnancy and postpartum. The American College of Obstetricians and Gynecologists' guidelines for heart rate intensity during pregnancy will be followed and you will be instructed on how to monitor your HR and be encouraged to stay within age-specific, modified target zones.

Blood drawing causes some pain and may cause bruising, bleeding or infection at the site of the needle stick.

The DEXA scan, performed by a Medical Radiation Technologist, is safe and fast (<10 minutes) with minimal radiation exposure equivalent to 1 day of natural background radiation.

The physical activity monitor that is placed just above your child's right hip will be secured using hypoallergenic medical tape along with a liquid barrier application that will help further reduce the chance of irritation.

### **Benefits**

You may or may not receive benefit from this study but its results will help define the potential role for nutrition and physical activity in overweight or obese pregnant women who, together with their offspring, are at risk for negative health consequences. The results from this study will be shared with health care professionals including general practitioners, obstetricians and gynecologists, exercise and nutrition professionals as well as policy makers and health care planners.

At the conclusion of the research study, you can receive a summary of the results if you so wish by contacting the primary investigator, Dr. Kristi Adamo:

kadamo@cheo.on.ca

### **Confidentiality**

Only study personnel, and scientists working directly with us, will have access to the data collected. The data collected in this study will be kept under lock and key in a safe place. All information that you provide will be coded with a study number and will not contain your name. A list of names and matching codes will be stored separately so that no identifying information will be present in your research file from this study to ensure confidentiality. Only the staff involved in this research study will have access to the records, with the exception of the CHEO Research Ethics Board, the Ottawa Hospital Research Ethics Board, the Queensway Carleton Hospital Research Ethics Board, the Montfort Hospital Research Ethics Committee (Comité d'éthique de la recherche), the Ottawa Health Research Institute and the University of Ottawa Research Board ho have access to records for auditing purposes. Overall results may be published for scientific purposes, but participant identity will remain confidential. Your personal information will be kept confidential except as required or permitted by law. Limits of this confidentiality include situations of suspected child abuse, concerns of harm to self or others, and any request for information by court order. The study data will be kept for 15 years after termination of the study and then will be destroyed.

All information gathered during the course of the study will be completely confidential. If you choose not to participate in this study, you/your child's future care at CHEO, the Ottawa Hospital, the Queensway Carleton Hospital or the Montfort Hospital will not be affected in any way. You are free to withdraw from the study at any time and you/your child will receive the same future care at CHEO, the Ottawa Hospital, the Queensway Carleton Hospital, and the Montfort Hospital. You should contact the study coordinator or the investigator if you wish to withdraw from the study.

This study is being conducted by Dr. Kristi Adamo (Research Scientist),

### Questions about the Study

Dr. Kristi Adamo:

Research Scientist, CHEO

MOM Trial Research Coordinator:

Research Coordinator, CHEO

If nobody is able to answer your call, a voice messaging system is available.

This study has been reviewed and approved by the CHEO Research Ethics Board, the Ottawa Hospital Research Ethics Board, the Queensway Carleton Research Ethics Board and the Montfort Hospital

Research Ethics Committee (Comité d'éthique de la recherche). The CHEO Research Ethics Board and the Ottawa Hospital Research Ethics Board are committees of the hospital that includes individuals from different professional backgrounds. The Boards reviews all human research that takes place at the hospital. Their goals are to ensure the protection of the rights and welfare of people participating in research. The Boards' work is not intended to replace a parent or child's judgment about what decisions and choices are best for them. You may contact the Chair of the CHEO Research Ethics Board, for information regarding patient's rights in research studies at [redacted] the Chairman of the Ottawa Hospital Research Ethics Board at [redacted] or the Chair of the Queensway Carleton Hospital Research Ethics Board at [redacted] President of the Montfort Hospital Research Ethics Committee (Comité d'éthique de la recherche) at [redacted], although this person cannot provide any health-related information about the study. These Boards could review your study records in fulfilling its roles and responsibilities.

**Maternal Obesity Management:  
A pregnancy Specific Randomized Control Trial – The MOM Trial**

**Informed consent form**

I have read the explanation about this study in this 7-page consent form. I have been given the opportunity to discuss it and my questions have been answered to my satisfaction. I hereby consent to:

Take part in this study and be randomized to either the Standard Care Control or the Intervention Group. I realize that my participation is voluntary and I am free to withdraw from the study at any time.

Giving access to my medical charts that are related to my pregnancy and the birth records of my offspring to the study researchers for a minimal risk chart review to ensure that the necessary physical and physiological characteristics of my pregnancy are recorded.

Having various measurements repeated until my child is 2 years old. I realize that my participation is voluntary and I am free to withdraw from the study at any time.

Yes  No      Allowing the research team to send a summary of the study results when data collection is complete as well as potentially contacting me for optional additional assessments following my involvement in the trial until my child is of preschool age.

**Contact Information:**

Daytime and evening telephone number(s): \_\_\_\_\_

I will be given a copy of the consent form for my records.

Name of Participant	Signature of Participant	Date

I have explained this study to the person authorized to sign above and they have been given the opportunity to ask questions.

\_\_\_\_\_  
Name of Investigator or  
Delegate

\_\_\_\_\_  
Signature

\_\_\_\_\_  
Date

## Appendix J - MOM trial genetics consent form



### The MOM Trial: A pregnancy Specific Randomized Control Trial

#### Genetics Informed consent form

#### PART II

#### Investigators:

Dr. Kristi Adamo *Healthy Active Living and Obesity Research Group, CHEO*

Dr. Frédérique Tesson-Rulko: *Associate Professor; Health Sciences  
University of Ottawa*

#### Background

The MOM trial is a study designed to examine how fetal exposures and events can influence a baby's health later in life. For example, the presence of Gestational Diabetes Mellitus (GDM) provides a less than favorable intrauterine environment – the environment in which the fetus grows, and has been shown to play a critical role in the development of obesity and type 2 diabetes in the offspring.

#### Purpose

This study is an optional part of the MOM trial to which you have already consented to participate in. With this part of the study we would like to study the nutritional, environmental, socioeconomic and genetic factors that may affect pregnancy by studying the relationship between genes/DNA and natural processes that occur during pregnancy which determine how a baby grows while in the womb and after birth. Genes are the building blocks or instructions found inside cells, which contribute to the different features you have such as the colour of your hair and eyes. The knowledge we gain from this information may be helpful in designing preventive health strategies for all women of childbearing age.

Your care at CHEO, the Ottawa Hospital, the Queensway Carleton Hospital or the Montfort Hospital will not be affected if you choose not to participate in this optional part of the study, and you will be able to continue participating in the Maternal Obesity Management trial. You should discuss any questions you have about this study with the people who explain it to you.

#### Study Procedures and Assessments

If you agree to participate in this genetic study, we are asking your permission to obtain and bank samples of your blood/DNA and placenta tissue. You may also allow us to obtain and bank samples of your baby's blood/DNA for research related to weight regulation. Participation in this portion of the study will not prevent you from taking part in private cord blood storage program, if you so desire.

However, the blood sample that we store for research purposes will not be available to you for any other purposes. We will take the baby's sample from the umbilical cord once it is cut away from the baby after delivery and no longer attached to you or your baby. Similarly, the placenta tissue sample will be taken after it has been delivered and thus will not involve any pain whatsoever nor will these procedures involve sticking your baby with a needle. No other genetic studies will be performed using your DNA sample or that of your child without your consent.

As recommended, all the samples will be kept in a locked  $-80^{\circ}\text{C}$  freezer located in the Healthy Active Living and Obesity research group's lab in the Research Institute at CHEO. After having completed all the analyses, the University of Ottawa will take all unused blood samples and will destroy them according to their usual method.

### **Risks**

There is little risk for you or your baby by participating in this study. In obtaining your blood work, you may develop a small bruise at the site of the blood test, which would disappear after a few days.

We will take all reasonable steps to keep your research information confidential. Should someone not involved in the research find out that you took part in this research study, or if you choose to share your results (if they are provided to you), there is a possibility that this could affect your insurance or employment.

### **Benefits**

The results of these tests may not be directly beneficial to you and your baby. However, the knowledge gained from it may offer the promise of new diagnostic and treatment avenues and may be helpful in designing preventative strategies for all women of childbearing age.

At the conclusion of the research study, you can receive a summary of the results if you so wish.

### **Confidentiality**

Only study personnel, and scientists working directly with us, will have access to the data collected. The data collected in this study will be kept under lock and key in a safe place. All information that you provide will be coded with a study number and will not contain your name. A list of names and matching codes will be stored separately so that no identifying information will be present in your research file from this study to ensure confidentiality. Only the staff involved in this research study will have access to the records, with the exception of the CHEO Research Ethics Board, the Ottawa Hospital Research Ethics Board, the Montfort Hospital Research Ethics Committee (Comité d'éthique de la recherche), the Queensway Carleton Hospital Research Ethics Board, the Ottawa Health Research Institute, and the University of Ottawa Research Board who have access to records for auditing purposes. Overall results may be published for scientific purposes, but participant identity will remain confidential. All results of the



# The MOM Trial- A pregnancy Specific Randomized Control Trial

## Genetics Informed consent form

### PART II

I have read the explanation about this study in this 4 page consent form. I have been given the opportunity to discuss it and my questions have been answered to my satisfaction. I hereby consent to:

Yes  No      Having my blood/DNA and placenta tissue samples stored and used for future ethics approved research on pregnancy outcomes and weight regulation.

#### Contact Information:

Daytime and evening telephone number(s): \_\_\_\_\_

I will be given a copy of the consent form for my records.

Name of Participant	Signature of Participant	Date
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I have explained this study to the person authorized to sign above and they have been given the opportunity to ask questions.

\_\_\_\_\_  
Name of Investigator  
or Delegate

\_\_\_\_\_  
Signature

\_\_\_\_\_  
Date

**Appendix K - Research Ethics approval for Placenta study in Obese women**



**Ottawa Hospital Research Ethics Boards / Conseils d'éthique en recherches**

725 Parkdale Avenue, Box 411, Ottawa, Ontario K1Y 4E9 613-798-5555 ext. 14902 Fax: 613-761-4311  
<http://www.ohri.ca/ohreb>

August 14, 2012

Dr. Andree Gruslin  
Ottawa Hospital - General Campus  
Department of Obstetrics/Gynecology/Newborn Care  
501 Smyth Road, Room 8420  
Ottawa, ON K1H 8L6

Dear Dr. Gruslin:

**RE: Protocol# - 2008450-01H Mechanism of Placental Dysfunction in Obese Mothers**

**Renewal Expiry Date - August 13, 2013**

I am pleased to inform you that your Annual Renewal Request (listed above) was reviewed by the Ottawa Hospital Research Ethics Board (OHREB) and is approved. No changes, amendments or addenda may be made in the protocol without the OHREB's review and approval.

Also approved is the Protocol Amendment Report dated July 13, 2012, extending the study end date to July 2013, and adding Kendra Brett as staff. Also acknowledged is the removal of Dr. Arendas, Mr. Qiu and Ms. Lafreniere as Co-Investigators. The file has been updated on all accounts.

Renewal is valid for a period of one year. Approximately one month prior to that time, a single renewal form should be sent to the OHREB office.

The Tri-Council Policy Statement requires a greater involvement of the OHREB in studies over the course of their execution. As well, you must inform the Board of adverse events encountered during the study, here or elsewhere, or of significant new information which becomes available after the Board review, either of which may impinge on the ethics of continuing the study. The OHREB will review the new information to determine if the protocol should be modified, discontinued, or should continue as originally approved.

Yours sincerely,

Ottawa Hospital Research Ethics Board

/kd

**Appendix L - Research Ethics approval for MOM trial**



**Research Ethics Board  
2014 Annual Renewal (Delegated)**

**Principal Investigator:** Dr. Kristi Adamo

**REB Protocol No:** 09/03E

**Romeo File No:** 10000159

**Project Title:** Maternal Obesity Management: a pregnancy specific RCT – The MOM Trial (SOMET)

**Primary Affiliation:** HALO\HALO

**Contingencies:** None

**Protocol Status:** Active

**Approval Date:** June 13, 2014

**Approval Valid Until:** June 15, 2015

**Annual Renewal Submission Deadline:** May 15, 2015

**Documents Reviewed & Approved:**

Document Name	Comments	Version Date
Other Document	Protocol paper for Canadian Journal of Diabetes	
Other Document	Abstract for Canadian Obesity Network	
Other Document	Book chapter	

This is to notify you that the CHEO REB has granted approval to the renewal for the above named research study for a period of one year. The renewal was reviewed and approved by the Chair only. Decisions made by the Chair under delegated review are ratified by the full Board at its subsequent meeting.

In fulfilling its mandate, the CHEO REB is guided by: Tri-Council Policy Statement; ICH Good Clinical Practice Practices; Consolidated Guideline; Applicable laws and regulations of Ontario and Canada (e.g., Health Canada Division 5 of the Food and Drug Regulations & the Food and Drugs Act - Medical Devices Regulations).

Approval is granted with the understanding that the investigator agrees to comply with the following requirements:

1. The investigator must conduct the study in compliance with the protocol and any additional conditions set out by the Board.

2. The investigator must not implement any deviation from, or changes to, the protocol without the approval of the REB except where necessary to eliminate an immediate hazard to the research subject, or when the change involves only logistical or administrative aspects of the study (e.g., change of telephone number or research staff). As soon as possible, however, the implemented deviation or change, the reasons for it and, if appropriate, the proposed protocol amendment(s) should be submitted to the Board for review.
3. The investigator must, prior to use, submit to the Board changes to the study documentation, e.g., changes to the informed consent letters, recruitment materials. Should major revisions to the consent form be made, the investigator agrees to re-consent those subjects who have originally consented to the study and who wish to continue on the study.
4. For clinical drug or device trials, investigators must promptly report to the REB all adverse events that are both serious and unexpected (SAEs). For SAE reports on CHEO patients, the investigator must also comply with the hospital-wide Policy regarding Procedures For Considering Medical Error In The Differential Diagnosis of Severe Adverse Events (SAE) Associated with the Drugs Administered in a Clinical Trial (see [http://cheonet/data/1/rec\\_docs/3792\\_Medical%20Error%20Policy%20revised%20january%2020061.doc](http://cheonet/data/1/rec_docs/3792_Medical%20Error%20Policy%20revised%20january%2020061.doc)).
5. For all other research studies, investigators must promptly report to the REB all unexpected and untoward occurrences (including the loss or theft of study data and other such privacy breaches).
6. Investigators must promptly report to the REB any new information regarding the safety or research subject (e.g., changes to the product monograph or investigator's brochure of drug trials). Where available, any reports produced by Data Safety Monitoring Board should be submitted to the REB.
7. Investigators must notify the REB of any study closures (temporary, premature or permanent), in writing along with an explanation of the rationale for such action.
8. Investigators must submit an annual renewal report to the REB 30 days prior to the expiration date stated on the final approval letter.
9. Investigators must submit a final report at the conclusion of the study.
10. Investigators must provide the Board with French version of the consent form, unless a waiver has been granted.

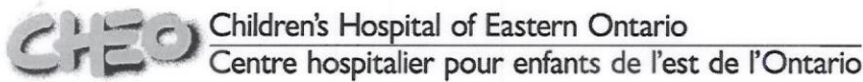
If you have any questions, pertaining to this letter, please contact Natalie Anderson, Research Ethics Board office at (613) 737-7600, ext. 3350 or [nanderson@cheo.on.ca](mailto:nanderson@cheo.on.ca).

Regards,

**Dr. Carole Gentile**  
**Chair, Research Ethics Board**  
**Présidente, Comité d'éthique de la recherche**  
**401 Smyth Road, Ottawa, ON K1H 8L1**  
**Tel: (613) 737-7600 ext. 3624 | Fax/Télé: (613) 738-4202 |**

**Appendix M - Research Ethics approval for Active MOM study (missing)**

*file*  
*09/03E*



**Research Ethics Board**  
**Approval of Major Modification**

Principal Investigator	Dr. Kristi Adamo
REB Protocol Number	09/03E
Protocol Title	Maternal Obesity Management: a pregnancy specific RCT – The MOM Trial (SOMET)
Department or PSU	HALO
Date Reviewed & Approved by the REB	September 5, 2012
Contingencies	<input checked="" type="checkbox"/> Not Applicable <input type="checkbox"/> Applicable ~ Date contingencies were met:
Documents Reviewed & Approved	<ul style="list-style-type: none"> <li>▪ Protocol (version: August 2012)</li> <li>▪ Consent Form (English) (version: July 2012)</li> <li>▪ Consent Form (French) (version: le 5 juillet 2012)</li> <li>▪ Appendix 1: Justification for the use of ASA24 (version: August 2012)</li> <li>▪ Appendix 2: Justification for the pregnancy physical activity questionnaire (PPAQ) (version: August 2012)</li> <li>▪ Appendix 3: Justification for the sub-maximal exercise protocol (version: August 2012)</li> <li>▪ Appendix 4: Details of the Harvard food frequency questionnaire (FFQ) (version: August 2012)</li> <li>▪ Sub-study vs. MOM trial comparison chart</li> <li>▪ Recruitment flyer (English)</li> <li>▪ Recruitment flyer (French)</li> <li>▪ PPAQ (English)</li> <li>▪ PPAQ (French)</li> <li>▪ Harvard FFQ (English)</li> <li>▪ 3 day food record (English) (version: August 2012)</li> <li>▪ 3 day food record (French) (version: Août 2012)</li> <li>▪ Actical log sheet (English) (version: August 2012)</li> <li>▪ Actical log sheet (French) (version: Août 2012)</li> <li>▪ Pregnancy and weight gain attitude scale (English) (version: August 2012)</li> <li>▪ Pregnancy and weight gain attitude scale (French) (version: Août 2012)</li> <li>▪ Diet History Questionnaire (English) (version: August 2012)</li> <li>▪ Diet History Questionnaire (French) (version: Août 2012)</li> <li>▪ Socio-demographic information (English) (version: August 2012)</li> <li>▪ Socio-demographic information (French) (version: Août 2012)</li> </ul>

This is to notify you that the CHEO REB has granted full board approval to the modification for the above named research study. The protocol was approved at a meeting of the REB in which the quorum rules were met and only those REB members who were independent of the investigator(s) conducting the study voted on the final decision.

In fulfilling its mandate, the CHEO REB is guided by: Tri-Council Policy Statement (TCPS); ICH Good Clinical Practice Practices: Consolidated Guideline; Applicable laws and regulations of Ontario and Canada (e.g., Health Canada Division 5 of the Food and Drug Regulations & the Food and Drugs Act - Medical Devices Regulations).

Regulations require that subjects be provided with significant new information/findings developed during the course of research, which may affect a subject's willingness to continue participation [45 CFR 46.116(b)(5) and

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21CFR50.25(b)(5)]. This is listed in section 4.8.2 of the ICH Good Clinical Practice Practices: Consolidated Guidelines.

**Approval is granted with the understanding that the investigator agrees to comply with the following requirements:**

- The investigator must conduct the study in compliance with the protocol and any additional conditions set out by the Board.
- The investigator must not implement any deviation from, or changes to, the protocol without the approval of the REB except where necessary to eliminate an immediate hazard to the research subject, or when the change involves only logistical or administrative aspects of the study (e.g., change of telephone number or research staff). As soon as possible, however, the implemented deviation or change, the reasons for it, and, if appropriate, the proposed protocol amendment(s) should be submitted to the Board for review.
- The investigator must, prior to use, submit to the Board changes to the study documentation, e.g., changes to the informed consent letters, recruitment materials. Should major revisions to the consent form be made, the investigator agrees to re-consent those subjects who have originally consented to the study and who wish to continue on the study.
- For clinical drug or device trials, investigators must promptly report to the REB all adverse events that are both serious and unexpected (SAEs). For SAE reports on CHEO patients, the investigator must also comply with the hospital-wide Policy regarding, Procedures For Considering Medical Error In The Differential Diagnosis of Severe Adverse Events (SAE) Associated with the Drugs Administered in a Clinical Trial (see [http://cheonet/data/1/rec\\_docs/3792\\_Medical%20Error%20Policy%20revised%20january%2020061.doc](http://cheonet/data/1/rec_docs/3792_Medical%20Error%20Policy%20revised%20january%2020061.doc)).
- For all other research studies, investigators must promptly report to the REB all unexpected and untoward occurrences (including the loss or theft of study data and other such privacy breaches).
- Investigators must promptly report to the REB any new information regarding the safety of research subjects (e.g., changes to the product monograph or investigator's brochure for drug trials). Where available, any reports produced by Data Safety Monitoring Board should be submitted to the REB.
- Investigators must notify the REB of any study closures (temporary, premature or permanent), in writing along with an explanation of the rationale for such action.
- Investigators must submit an annual renewal report to the REB 30 days prior to the expiration date stated on the final approval letter.
- Investigators must submit a final report at the conclusion of the study.
- Investigators must provide the Board with French version of the consent form, unless a waiver has been granted.

Reg

Dr.  
Chair, Research Ethics Board

c.c. Kendra Brett, Research Coordinator

## *Appendix N - List of Publications during PhD*

### **Published Manuscripts**

**K.E. Brett**, Z.M. Ferraro, M. Holcik, K.B. Adamo. Prenatal physical activity and diet composition affects the expression of nutrient transporters and mTOR signaling molecules in the human placenta. *Placenta*. 2015; 36: 204-212.

**K.E. Brett**, Z.M. Ferraro, J. Yockell-Lelievre, A. Gruslin, K.B. Adamo. Maternal-Fetal Nutrient Transport in Pregnancy Pathologies: The role of the Placenta. *International Journal of Molecular Sciences*. 2014; 15: 16153-16185.

K.B. Adamo, **K.E. Brett**. Parental Perceptions and childhood dietary quality. *Maternal and Child Health Journal*. 2014;18(4): 978-995.

K.B. Adamo, Z.M. Ferraro, **K.E. Brett**. Pregnancy is a critical period for prevention of obesity and cardiometabolic risk. *Canadian Journal of Diabetes*. 2012; 36: 133-141

K.B. Adamo, Z.M. Ferraro, **K.E. Brett**. Can we modify the intrauterine environment to halt the intergenerational cycle of obesity? *International Journal of Environmental Research and Public Health*. 2012; 9:1263-1307

K.B. Adamo, K.A. Langlois, **K.E. Brett**, R.C. Colley. Young Children and Parental Physical Activity Levels: Findings from the Canadian Health Measures Survey. *American Journal of Preventative Medicine*. 2012;43(2):168 –175

### **Book Chapters**

K.B. Adamo & **K.E. Brett**. Parental perceptions and childhood diet quality: who holds the reins? In: V.R. Preedy, L. Hunter, V.B. Patel, ed. Kings College London *Diet Quality – An Evidence-Based Approach, Volume 1*. Springer New York; 2013. ISBN: 978-1-4614-7338-1 (Print).

### **Published Abstracts**

**K.E. Brett**, Z.M. Ferraro, S. Wilson, H. Ockenden, K. Nerenberg, G. Goldfield, E. Keely, K.B. Adamo. Associations between objectively measured physical activity, pre-pregnancy body mass index and gestational weight gain. *Applied Physiology, Nutrition, and Metabolism*. 2014, 39(S1): S7

**K.E. Brett**, Z.M. Ferraro, S. Wilson, K.B. Adamo. Self-report questionnaire overestimates physical activity in pregnancy. *Applied Physiology, Nutrition, and Metabolism*. 2014, 39(S1): S7

Z.M. Ferraro, S. Wilson, **K.E. Brett**, K. Nerenberg, G. Goldfield, E. Keely, K.B. Adamo. Associations between objectively measured physical activity, sedentary time and maternal social support and depression indices. *Applied Physiology, Nutrition, and Metabolism*. 2014, 39(S1): S17

**K.E. Brett**, D. Tessier, Z.M. Ferraro, , A. Gruslin, K.B. Adamo. The Characterization of Fatty Acid Transport Protein 4 in Third Trimester Placentas from Lean and Obese Pregnancies. *Canadian Journal of Diabetes*. 2013; 37 (S2): S232.

Z.M. Ferraro, **K.E. Brett**, A. Gruslin, K.B. Adamo. Maternal Lipid Metabolism Correlates With Surrogate Markers of Insulin-Like Growth Factor Bioavailability in Maternal and Umbilical Cord Serum. *Canadian Journal of Diabetes*. 2013; 37 (S2):S232.

K.B. Adamo, K.A. Langlois, **K.E. Brett**, R.C. Colley. Does having children make us inactive? *Obesity* 2011; 19(S1):S58.

## *Appendix P - List of Presentations during PhD*

### **Oral Presentations**

**K.E. Brett**, Z.M. Ferraro, S. Wilson, H. Ockenden, K. Nerenberg, G. Goldfield, E. Keely, K.B. Adamo. Associations between objectively measured physical activity, pre-pregnancy body mass index and gestational weight gain. Canadian Society for Exercise Physiology Annual General Meeting, St. John's, NL, October 2014.

**K.E. Brett**, Z.M. Ferraro, S. Wilson, K.B. Adamo. Self-report questionnaire overestimates physical activity in pregnancy. Canadian Society for Exercise Physiology Annual General Meeting, St. John's, NL, October 2014.

**K.E. Brett**, D. Tessier, Z.M. Ferraro, , A. Gruslin, K.B. Adamo. The Characterization of Fatty Acid Transport Protein 4 in Third Trimester Placentas from Lean and Obese Pregnancies. 3<sup>rd</sup> Canadian Obesity Summit, Vancouver, BC, May 2013. 4<sup>th</sup> Annual SOMET-MONET Research Day, Montreal, QC, May 2013.

**K.E. Brett**, A. Gruslin. The Placenta: an instigator. Obesity & Fetal Programming: How Important Is 9 Months Anyway. 3<sup>rd</sup> Canadian Obesity Summit, Vancouver, BC, May 2013.

**K.E. Brett**. Exploring the impact of maternal obesity and exercise on placental fatty acid transporter expression. 4<sup>th</sup> Annual Human Kinetics Graduate Student Conference, University of Ottawa, Ottawa, ON, April 2012.

**K.E. Brett**. Maternal obesity and the placenta – are changes in nutrient transport contributing to fetal overgrowth. 2<sup>nd</sup> Annual SOMET-MONET Research Day. Women and body weight changes during hormonal transitions: sharing evidence from bench through clinical trials to action. Institut de recherches clinique de Montreal, Montreal, QC, May 2011.

**K.E. Brett**. Exploring the impact of maternal obesity on the expression of fat transport proteins in the placenta. 3<sup>rd</sup> Annual Human Kinetics Graduate Student Conference, University of Ottawa, Ottawa, ON, April 2011.

### **Poster Presentations**

**K.E. Brett**, D. Tessier, Z.M. Ferraro, , A. Gruslin, K.B. Adamo. Characterization of the insulin-like growth factor binding protein-4 (IGFBP-4) in third trimester placentae from lean and obese pregnancies. 7<sup>th</sup> International DIP Symposium on Diabetes, Hypertension, Metabolic Syndrome and Pregnancy, Florence, Italy, March 2013.