Physical Activity-Associated Differences in Placental Lipid Profiles and Docosahexaenoic Acid Uptake: Insights from Lipidomics and an *in vitro* Exercise Model

Meaghan MacDonald, HBSc. Kinesiology

Thesis submitted to the University of Ottawa
in partial fulfillment of the requirements for the
degree of Master of Science, Human Kinetics

School of Human Kinetics
Faculty of Health Sciences
University of Ottawa, Canada

uOttawa

Table of Contents

Preface

Approvals: Placenta tissue samples analyzed in this thesis originated from two distinct trials; Dr. Kristi Adamo's *PLACENTA* project in Ottawa, Ontario, and Dr. Leanne Redman's *Expecting Success* project in Baton Rouge, Louisiana. The *PLACENTA* project was approved by the Ottawa Health Science Network Research Ethics Board (20160178-01H) and the University of Ottawa Research Ethics Board (H11-15-29). The *Expecting Success* project was approved by the Pennington Biomedical Research Centre Institutional Review Board (NCT01610752). All participants provided informed consent.

Contributions: This thesis was conceptualized, executed and written by me (Meaghan MacDonald) and supervised by Dr. Kristi Adamo. For the first manuscript in this thesis, extractions and benchwork was aided by Anahita Yadegari, Justine Chittaro, and Dr. Arthur Dantas. Dr. Jeff Smith, Karl Wasslen, and Christian Rosales managed the mass spectrometer and provided assistance with statistical analysis. All authors contributed to reviewing and editing the manuscript. For the second manuscript in this thesis, Dr. Martin Gauster and Dr. Michael Gruber helped guide the experimental design. Lena Neuper and Dr. Michael Gruber contributed to performing experiments.

Acknowledgements

This work would not have been possible without the village of support I am lucky to be surrounded with. To my supervisor, Dr. Kristi Adamo, thank you for your endless advice, encouragement, and feedback through every step of this process. Your confidence in my capabilities has pushed me to become a better scientist. I am deeply grateful to all the lab members who took me under their wings and gave me the opportunity to contribute to their work. Vini, Jess, Abbey, Chris, you have all given me invaluable guidance and companionship. Jess, thank you for always lifting me up, looking out for me, and visiting me on the other side of the world. Abbey and Chris, you are the most encouraging run buddies I could ask for. You are all the best!

Thank you to Dr. Arthur Dantas, and my committee members, Dr. Michael De Lisio and Dr. Dylan Burger, for your guidance throughout my thesis. I am sincerely grateful for all the time and knowledge that you shared with me.

I extend my deepest gratitude to everyone at the Medical University of Graz, and especially Dr. Martin Gauster and Dr. Michael Gruber, for taking a chance on me and giving me the opportunity to learn from your group. Lena, Kaja, and Signe, thank you for friendship when I was far from home. The kindness you all showed me will stick with me forever.

To every pregnant participant who gave their time and energy (and placenta) to our lab's projects; this literally could not have been done without you. Thank you for your dedication to research and willingness to participate at such a pivotal time in your life. This work is for you.

To my family and friends, for listening to countless presentations on repeat and with only minimal complaints, I wouldn't be here without you. Dad, you have always believed in me, from my astronaut aspirations to the very end. I wish you were still around to see what I have been able to achieve with the lessons you instilled in me. Mom and Cail, thanks for always answering the phone and listening to me vent or yap about science. You are my biggest cheerleaders. Lastly, to

my partner, Tom; I am forever grateful for your endless patience and ability to make me laugh at any moment. Having you by my side makes everything feel lighter.

Abstract

Background: Gestational physical activity (PA) has been associated with a multitude of positive outcomes for both the gestational parent and the offspring. Given the placenta's central role in supporting pregnancy, it is likely involved in mediating these benefits, though the mechanistic explanations have yet to be elucidated. Placental lipid composition and uptake can provide a wealth of information on placental and fetal lipid availability, and their downstream pathways. Docosahexaenoic acid (DHA) is a fatty acid of particular importance for fetal brain and retinal development, and its proper delivery to the fetus is critical. The influence of gestational PA on these factors has not been explored.

Methods: The first objective of this thesis was to characterize the lipid profile of placentas from pregnancies classified as physically active or inactive. Thirty-six term placenta samples were analyzed using liquid chromatography mass spectrometry (LC-MS/MS). PA levels were objectively measured using accelerometry. Major facilitator superfamily domain containing 2a (MFSD2a) protein expression in placental tissue was measured semi-quantitatively using Western blotting. The second objective was to evaluate DHA uptake *in vitro.* Primary trophoblast cells were exposed to bouts of intermittent hypoxia (IH) and high shear stress as proxies for PA. Hypoxia was considered 3% and normoxia 10% O_2. Low shear stress was defined as 1 dyn/cm^2 and high shear stress as 3 dyn/cm^2. DHA was added to the cell culture media, and uptake into cells was quantified by gas chromatography - mass spectrometry (GC-MS).

Results: A total of 192 lipid species were annotated in placenta samples, of which 34 differed significantly between active and inactive groups ($p < 0.05$; $|\log_2 FC| \geq 0.58$). Seven of the 17 lipids containing DHA were higher in the active group. Multivariate analyses demonstrated modest separation between active and inactive cohorts. MFSD2a expression did not differ between groups,

however, infant weight-to-length percentile was negatively correlated with MFSD2a (r = -0.40; p = 0.019). Contrary to our hypothesis, DHA uptake was significantly greater in cells exposed to normoxia + static conditions compared to IH + high shear stress (p = 0.008), and IH + low shear stress (p = 0.003).

Conclusions: There is a modest relationship between gestational PA and the placental lipidome, with several lipids differentially expressed between groups. Although MFSD2a (a main DHA transporter) expression did not differ, DHA-containing lipids were higher in placentas from physically active pregnancies. Our *in vitro* model of PA impacted intracellular DHA uptake, though not in the expected direction. This unanticipated finding may reflect limitations in the application of IH and SS to proxy PA, or an inability of the experiment to fully model fetal DHA delivery *in vivo*. Collectively, these findings provide a basis for targeted lipid analyses, and help guide future *in vitro* modeling of PA.

Abbreviations

β-hCG: beta-human chorionic gonadotropin

AF: ammonium formate

ARA: arachidonic acid

AUROC; AUC: area under receiver operating curve; area under curve

BMI: body mass index

BSA: bovine serum albumin

c-FABP: cytosolic fatty acid binding protein

CE: cholesteryl ester

$CHCl_3$: chloroform

CK7: cytokeratin-7

CTB: cytotrophoblast

DHA: docosahexaenoic acid

DOHaD: developmental origins of health and disease

E-Cad: E-cadherin

EtOH: ethanol

FAT/CD36: fatty acid translocase

FATP: fatty acid transporter protein

FBS: fetal bovine serum

FC: fold-change

GC-MS: gas chromatography mass spectrometry

GDM: gestational diabetes mellitus

GesP: gestational parent

IH: intermittent hypoxia

IPA: isopropanol

LC-MS: liquid chromatography mass spectrometry

LCPUFA: long-chain polyunsaturated fatty acid

LPC: lysophosphatidylcholine

MeOH: methanol

MFSD2a: major facilitator superfamily domain containing 2a

MTBE: methyl tert-butyl ether

MVM: microvillous membrane

NaOH: sodium hydroxide

PA: physical activity

PC: phosphatidylcholine

PCA: principal component analysis

PE: phosphatidylethanolamine

PLS-DA: partial least squares-discriminant analysis

pm-FABP: plasma membrane fatty acid binding protein

PS: phosphatidylserine

PSD: phosphatidylserine decarboxylase

Q: cardiac output

QC: quality control

RT: retention time

SM: sphingomyelin

SS: shear stress

STB: syncytiotrophoblast

TG: triacylglycerol

List of Figures

List of Tables

Chapter 1: Introduction

1.1 Physical Activity and Pregnancy

The science of exercise physiology has been shaped by generations of researchers uncovering the multifaceted connections between physical activity (PA) and health. Until recent years, however, this wealth of knowledge has predominantly been developed through research performed on and by men (1). Women have frequently been excluded from such work, a choice oftentimes attributed to perceived complexities surrounding the menstrual cycle (2, 3). Even more stigmatized is research on PA during pregnancy; historical belief – based on little scientifically rigorous evidence and more reflective of the societal and cultural influences of the past – asserted that movement and upright posture posed threats to the developing fetus (4), and bedrest was thus commonly prescribed (as reviewed by Corson *et al.(1)*).

Recent research has illustrated the harms of physical inactivity during pregnancy (5), reinforcing the importance of gestational PA. The *2019 Canadian guideline for physical activity throughout pregnancy* recommends a minimum of 150 minutes of moderate activity per week, accumulated over three or more days (6). Adherence to this guideline is associated with a multitude of benefits to both the gesP and offspring, including reduced risk of gestational diabetes, preeclampsia, instrumental delivery, excessive gestational weight gain, and metabolic disease in offspring (7–10). Moreover, engagement in gestational PA is linked to improved neonatal adiposity and enhanced maternal mental health (9, 11, 12).

When considered through the lens of the Developmental Origins of Health and Disease (DOHaD) model, which states that the first 1000 days of life are highly influential in shaping long-term health and disease trajectories, PA is a tool to positively impact outcomes far beyond the transient window of pregnancy. While the physiological mechanisms through which PA may

impart such benefits are largely unknown, the placenta, the organ that develops *in utero* to compose the maternal-fetal interface, presents a compelling area for inquiry. The placenta is responsible for several critical functions throughout pregnancy, including nutrient, gas, and waste exchange, toxin filtering, hormonal signaling, and immune functions (13). The outermost layer of multinucleated cells, known as the syncytiotrophoblast (STB), make direct contact with maternal blood flow in the intervillous space. This interface represents the articulation between gesP and fetus. Unlike most organs, the placenta lacks innervation; therefore, gesP cannot transmit PA-related neural signals to the placenta tissue. As a result, any PA-associated benefit must be mediated through drivers that interact directly with the STB, such as oxygenation, shear stress, or circulating metabolites. Consequently, it is at this interface that we focus our search for mechanistic explanations.

Intermittent hypoxia (IH) has been proposed as a key PA-induced stimulus at the level of the placenta (13, 14). During PA, blood flow is redistributed to the working musculature, reducing blood and thereby oxygen delivery to tissues not directly involved in movement (15). While it is not fully understood what occurs during PA at the maternal-fetal junction, evidence supporting the IH hypothesis comes from both animal and non-invasive human experiments; in ewes, exercise bouts reduce uterine blood flow, while in humans, uteroplacental vascular resistance has been shown to increase (16, 17). These findings suggest altered circulation to the intervillous space, which may expose the STB to a transient state of hypoxia. Importantly, these brief hypoxic conditions are not detrimental to fetal oxygen availability, as evidenced by lack of differences in erythropoietin levels (synthesized in response to fetal hypoxemia) in amniotic fluid and cord blood between exercising and non-exercising women (18).

Beyond IH, shear stress may represent another potential mechanistic driver. Following PA, distribution of blood flow returns to baseline, which may result in a temporarily high amount of shear stress exerted on the walls of the intervillous space. In other tissues, PA-induced endothelial shear stress improves vascular function by upregulating endothelial nitric oxide synthase expression and nitric oxide production, both of which have been found to increase in placentas from exercising gesP (19). Cumulatively, these findings warrant further exploration of IH and shear stress as potential mediators of gestational PA-associated benefits.

1.2 Lipids

1.2.1 Lipidomics

Lipids represent a highly diverse group of molecules with a wide range of biological functions, including but not limited to cell membrane composition, fuel, energy storage, cellular signaling, and transport (20). The LIPID MAPS consortium classifies compounds into eight main categories: fatty acyls, glycerolipids, glycerophospholipids, sphingolipids, saccharolipids, polyketides, sterol lipids and prenol lipids (Table 1). These categories are further broken down into classes and subclasses, with thousands of distinct lipid species. The lipidome is, by definition, the entire lipid composition of a given sample, which can provide a wealth of information regarding the health and disease status of a cell, tissue, or organism (20). Lipidomic data are largely influenced by the techniques and tools implemented and never truly capture the entirety of the lipidome, though careful selection of methodology can help optimize output (21).

Table 1. The LIPID MAPS consortium classification of lipid categories.

Lipid Category	Common Biological Functions in Eukaryotes	Examples of Classes
Fatty Acyls	- Energy source for β-oxidation (22) - Regulate intracellular signalling pathways, transcription factor activity, gene expression, and bioactive lipid mediator production (22)	- Fatty acids and conjugates - Eicosanoids
Glycerolipids	- Fat storage within lipid droplets (23) - Precursors for lipid synthesis (23) - Activation of protein kinases (23)	- Diradylglycerols - Triradylglycerols
Glycero-phospholipids	- Largest component of cell membranes (23) - Cellular signalling (23)	- Glycerophosphocholines - Glycerophosphoinositols
Sphingolipids	- Signal transduction (24) - Stress response (24) - Immune reaction (24) - Cell membrane structure (24)	- Ceramides - Phosphosphingolipids
Saccharolipids	- Endotoxin effects (25) - Stimulate immune system (25)	- Acylaminosugars - Acyltrehaloses
Polyketides	- Produced by fungi, bacteria, plants (26) - Used for antibiotics, antifungals, immunosupressants (26)	- Flavonoids - Halogenated acetogenins
Sterol Lipids	- Maintain cell membrane structure (27) - Produce signalling molecules(27)	- Sterols - Steroids
Prenol Lipids	- Antioxidants (28) - Vitamins K and E (28)	- Isoprenoids - Polyprenols

Note. Biological functions within lipid categories are highly heterogenous and not generalizable; the *Common Biological Functions in Eukaryotes* column provides examples from specific subgroups within each category.

Given the numerable functional roles of lipids in humans, it follows that the biological applications of lipidomics are also widespread. Lipid profiles provide insight into disease pathophysiology, including metabolic syndromes, cancers, and neurological disorders (29), and can reveal information related to disease risk, development, and prevention (20). Additionally, lipidomics is used in pharmaceutical, nutrition, and multi-omics approaches (29). This powerful tool can reveal drug targets, screen drug candidates for efficacy, and evaluate drug toxicity; help nutrition scientists understand the effect of dietary interventions on cellular structure and composition; and be combined with proteomic, genomic and broader metabolomic data to provide a whole system biology understanding of health and disease (29, 30).

1.2.2 The Placental Lipidome

Lipids, alongside glucose and amino acids, are one of the major fuel sources for both placenta and fetus. Once taken up by STB, they may remain within the placenta for immediate use or storage, or transfer into fetal circulation to support growth and development (31). The lipidome of the placenta is therefore highly dynamic, with lipid availability differing across gestation. Maternal fat stores accumulate during the first two trimesters of pregnancy, preparing for the third trimester, where maternal lipolysis and hyperlipidemia increase circulating lipids to meet the demands of rapid fetal growth at this stage (32).

To date, much of the placental research has focused on pathological pregnancies, which have highlighted distinct alterations to the placental lipidome in preeclampsia, gestational diabetes, and obesity (33–36).

A recent systematic review found lipidomic signatures linked to adverse pregnancy outcomes that were consistent across data from maternal serum, placentas, cord blood, and fetal serum (37). Perturbations in phosphatidylcholine and phosphatidylethanolamine metabolism were frequently

found in pregnancies complicated by preeclampsia and gestational diabetes (34). As these lipids compose cell membranes, such disruptions may alter placental nutrient transport as well as vascular development (37).

It is evident that harmful environmental exposures, such as those imposed by pathologies, can influence placental lipids. The impact of advantageous gestational environmental exposures, like PA, on the placental lipidome and fetal lipid availability remains to be fully elucidated.

1.2.3 Placental Lipid Transport

The STB, and more specifically the microvillous membrane (MVM), is in direct contact with maternal circulation and is consequently responsible for the uptake and passage of lipids. Free fatty acids may cross the STB through simple diffusion (38), whereas other lipid classes may require active transport. The STB is therefore equipped with several transporters to efficiently perform these functions (Figure 1). Additionally, lipoprotein lipase and endothelial lipase embedded in the MVM hydrolyze circulating lipids, enabling their subsequent diffusion as free fatty acids (38).

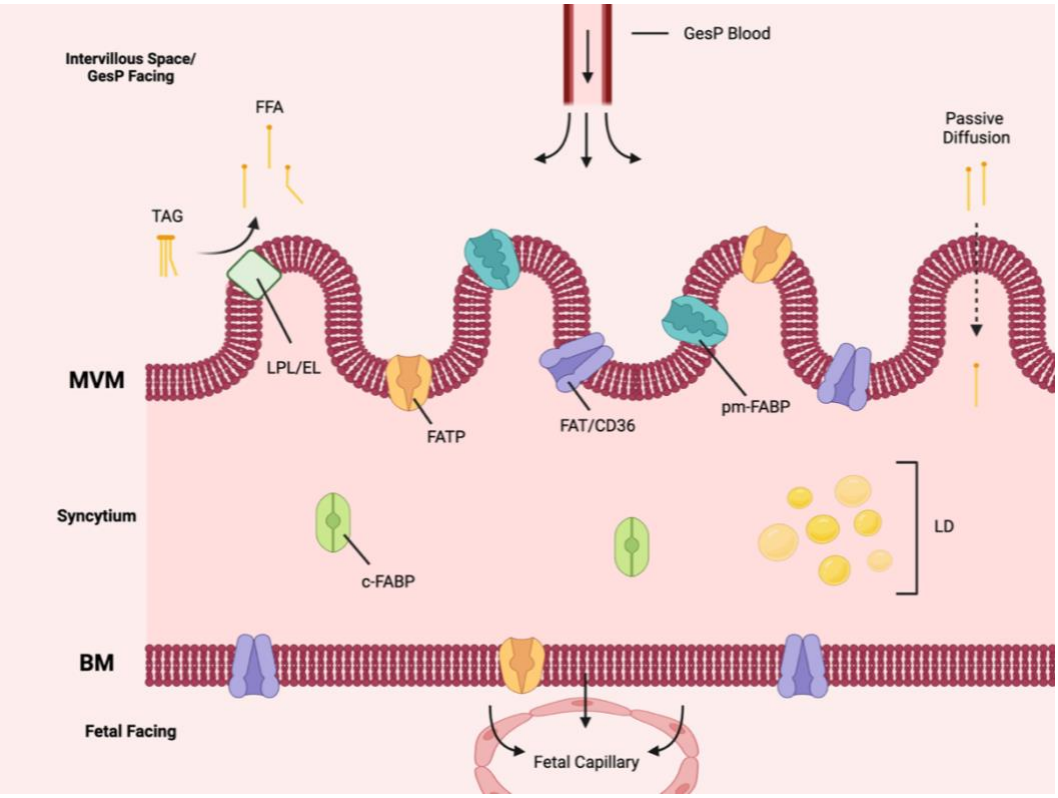


Figure 1. Lipid transport across the STB. *Abbreviations: MVM – microvillous membrane; BM – basal membrane; TAG – triacylglycerol; FFA – free fatty acid; LPL – lipoprotein lipase; EL – endothelial lipase; FATP – fatty acid transporter protein; FAT/CD36 – fatty acid translocase; pm-FABP – plasma membrane fatty acid binding protein; c-FABP – cytoplasmic fatty acid binding protein; LD – lipid droplet*

The major placental lipid transporters are fatty acid transport proteins (FATP), fatty acid translocase (FAT/CD36), plasma membrane fatty acid binding protein (pm-FABP), and cytosolic fatty acid binding protein (c-FABP). The FATPs and FAT are located on both the MVM and the fetal-facing basal membrane, whereas pm-FABP is only on the MVM, and c-FABP resides within the cytoplasm (39, 40). These distinct localizations reflect their unique functions; multimembrane transporters such as FATP and FAT are involved in bidirectional nutrient transport, pm-FABP

facilitates unidirectional transport from parent to fetus, and c-FABP directs intracellular lipid trafficking (41).

Special mechanisms are in place to ensure transport of long chain polyunsaturated fatty acids (LCPUFA), which are particularly critical for healthy fetal growth and development. For instance, pm-FABP, FATP-1 and FATP-4 preferentially bind LCPUFAs, prioritizing their transport over shorter fatty acids (40, 42, 43). Docosahexaenoic acid (DHA) is one such LCPUFA which has been demonstrated *via* placental perfusion to be preferentially transported into fetal circulation (44). Recently, major facilitator domain-containing 2a (MFSD2a), a known transporter of DHA at the blood-brain barrier, has been identified in the placenta, where it is believed to play a similar role (45, 46).

Importantly, placental lipid transporter expression is dynamic, and influenced by environmental factors. In pregnancies complicated by preeclampsia, lower mRNA expression of FATP-1 and -4 were identified in term placenta (47). Gestational diabetes and high pre-pregnancy BMI are also linked to lower FATP-1, FATP-4, and endothelial lipase expression, coinciding with greater FAT and FATP6 expression (48, 49). Intrauterine growth restriction is associated with higher lipoprotein lipase and lower endothelial lipase mRNA expression (50). For a more in-depth review of placental lipid transport in pathology, the reader is referred to Brett *et al.* (51).

Emerging evidence suggests PA may also modulate lipid transporters. Human term placentas from physically active pregnancies have demonstrated higher FATP-4 expression compared to their inactive counterparts (52), while murine models have noted that PA can reverse elevated placental FAT and lipoprotein lipase levels in dams fed high fat diets (53). Collectively, these findings illustrate that placental lipid transporters are responsive to environmental exposures, thereby impacting lipid availability and delivery to both placenta and fetus. Such alterations can

have lasting effects on the health of the pregnancy and the offspring, making a more robust understanding of how different gesP behaviours and conditions impact placental physiology essential.

1.3 Docosahexaenoic Acid and Pregnancy

DHA is an essential omega-3 LCPUFA, meaning it cannot be synthesized *de novo* by the body and therefore must be exogenously supplied. Abundant in fish and other seafood, sufficient levels of DHA are critical to brain, retina, and nervous system function (54). Its highly unsaturated structure makes DHA an essential constituent of cell membranes in these tissues, allowing for proper membrane fluidity (41, 55). DHA is crucial for synaptic transmission, impulse propagation, and signal transduction (41).

During the last trimester of pregnancy, when fetal brain growth peaks, sufficient DHA delivery and accumulation are paramount. Preterm babies, who miss this window of DHA accretion, often have low blood DHA levels coinciding with abnormal eye and brain function in infancy (56). The provision of DHA during the perinatal period is critical, with sufficient supply linked to long-term health measures in offspring such as improved problem-solving skills at 1 year, higher IQ at 4 years, sustained attention at 5 years, and reduced problem behaviour at 7 years old (57–60).

While the deleterious effects of pathological states on placental DHA transport and delivery are well-documented (46, 47, 61, 62), far less is known about how PA influences these processes. At the blood-brain barrier, murine models have demonstrated that PA in tandem with DHA supplementation enhances outcomes beyond DHA supplementation alone, improving functional recovery following traumatic brain injury as well as synaptic plasticity and cognitive function in healthy rats (63–65). Further, PA has also been shown to rescue MFSD2a levels previously

depleted by hypertension in rats (66). Despite these promising findings, most human pregnancy research has focused on DHA supplementation, ignoring its downstream placental uptake and transport. This leaves a critical gap in understanding how fetal DHA accretion, and the developmental benefits it underpins, can be optimized.

1.4 Thesis Aims

This thesis aims to explore the placental lipidome in relation to PA, with a particular interest in DHA, and functionally analyze the role of PA on placental DHA kinetics. To address these objectives, the thesis document is organized around two aims:

Aim 1a: Exploratory analysis of the lipid profile of placentas from physically active and inactive pregnancies through the application of mass spectrometry.

Research question: How does the placental lipidome differ between physically active and inactive individuals?

Aim 1b: Semi-quantitatively analyze MFSD2a expression in placentas from physically active and inactive pregnancies through Western blot.

Hypothesis: Expression of MFSD2a is higher in placentas from physically active compared to inactive pregnancies.

Aim 2: Functionally analyze DHA uptake in cultured primary STB using a combination of IH and high shear stress as proxies for PA.

Hypothesis: STB exposed to IH and high shear stress yield greater DHA uptake than unexposed STB.

1.5 Study Rationale

Despite its integral roles in supporting pregnancy, the placenta remains poorly understood. Its transient and sensitive nature creates an ethically challenging research environment, but the

historical exclusion of women – and especially pregnant women – from scientific investigation has also contributed significantly to this knowledge gap. With the growing prominence of the DOHaD paradigm, pregnancy is now recognized as a crucial window for long-term health programming, and the study of gestational PA is finally gaining the traction it deserves. A deeper understanding of how PA can benefit pregnancy and maternal-fetal outcomes, and what the mechanistic drivers are, is essential to informing pregnancy guidelines, shaping recommendations, and identifying potential downstream therapeutic applications. The placenta represents a prime area of inquiry in this effort.

This research project aims to address the limited understanding of how PA influences placental physiology by generating a more comprehensive view of placental lipid composition and lipid uptake in relation to PA. Lipidomics can provide an abundance of information on placental and fetal lipid availability, storage, and health status. To our knowledge, the lipidome of placentas from physically active, uncomplicated pregnancies has not yet been characterized, making this project the first of its kind. Liquid chromatography-mass spectrometry (LC-MS), a highly sensitive and powerful analytical tool, was employed to ensure robust lipidomic analysis of complex samples.

A specific focus of this thesis is DHA, given its central role in supporting fetal brain and retina development. While evidence suggests a relationship between PA and DHA in the brain, these associations have yet to be examined in the placenta. Alongside LC-MS analysis of DHA-containing lipid species, we applied a functional approach using a novel cell culture model of PA. Specifically, a flow bioreactor exposed primary cells to a combination of IH and high shear stress at physiologically relevant levels, creating a more representative *in vitro* environment in which to

measure DHA uptake following supplementation. Investigation of MFSD2a expression was implemented to further clarify whether PA influences DHA transport routes across the STB.

By elucidating the relationship between PA and DHA uptake and availability at the level of the placenta, we aimed to provide a clearer understanding of how fetal development and long-term offspring outcomes can be optimized. Through the integration of exploratory lipidomic analysis and functional modelling of DHA movement across the STB, this thesis offers a thorough, multi-perspective approach to understanding the placental lipid response to PA.

Preamble to Chapter 2:

The manuscript entitled *Exploring the Placental Lipidome in Physically Active vs. Inactive Pregnancies* is formatted for submission to the *Journal of Applied Physiology*. This manuscript covers the first aim of this thesis. Herein, we aim to characterize and differentiate lipid profiles in active and inactive pregnancies.

Contributions: I was responsible for the conceptual design and primary authorship of this study. I conducted all benchwork with assistance from Justine Chittaro, Anahita Yadegari, and Arthur Dantas, with the exception of mass spectrometer operation, which was performed by Karl Wasslen, Christian Rosales, and Jeff Smith. Additionally, I performed all data processing, statistical analysis, and figure preparation. I drafted the initial manuscript, to which all co-authors contributed revisions.

RESEARCH ARTICLE

Chapter 2: Exploring the Placental Lipidome in Physically Active vs. Inactive Pregnancies

M. MacDonald[1], A. Yadegari[1], J. Chittaro[1], A. Dantas[1], K. Wasslen[2], C. Rosales[2], J. Shearer[3], L. Redman[4], J. Smith[2], K.B. Adamo[1].

[1] School of Human Kinetics, Faculty of Health Sciences, University of Ottawa, Ottawa, ON, Canada

[2] Department of Chemistry, Carleton University, Ottawa, ON, Canada

[3] Department of Biochemistry and Molecular Biology, Cumming School of Medicine & Faculty of Kinesiology; Alberta Children's Hospital Research Institute, University of Calgary, Calgary, AB. Canada

[4] Division of Clinical Sciences Pennington, Biomedical Research Center, Baton Rouge, LA, USA

Correspondence: Kristi B. Adamo (kadamo@uottawa.ca)

Abstract

Physical activity during pregnancy is highly beneficial, but the mechanisms underlying these benefits have not been elucidated. Given its central role in supporting pregnancy, the placenta is a primary target for investigation. We implemented untargeted lipidomic analysis on 36 human placenta samples collected at term from uncomplicated pregnancies. Participant activity levels were objectively measured using accelerometry and participants were categorized as active or inactive. Samples were assessed by LC-MS/MS. The docosahexaenoic acid (DHA) transporter major facilitator superfamily domain-containing 2a (MFSD2a) was analyzed with western blot. A total of 192 lipids were annotated. Among these, 34 lipid species differed significantly between groups ($|\log2FC| \geq 0.58$; FDR-adjusted p value < 0.05). Multivariate analyses demonstrated modest separation between groups. DHA was identified in 17 lipid species, and seven were higher in the active group. Area under the receiver operating characteristic curve (AUROC) revealed five lipids with perfect group separation. Pathway analysis showed enrichment of the phosphatidylserine decarboxylase (PSD) pathway in the active group. No differences in MFSD2a expression were identified. The results demonstrate a relationship between physical activity and the placental lipidome. Several DHA species are higher in the active group, suggesting improved fetal availability for brain and retinal development. Lipids with perfect group separation may represent biomarkers of physical activity. Multiple lipid species and pathways warranting future targeted investigation are highlighted.

Keywords: lipidomics; physical activity; placenta; pregnancy

Introduction

Pregnancy represents a unique physiological state in which lifestyle behaviours can influence short- and long-term health outcomes for both the gestational parent (gesP) and offspring. Physical activity (PA) is one such behaviour widely recognized as a cornerstone of health promotion and disease prevention, with well-established benefits across a range of pathological states (1–3). Historically, however, PA during pregnancy has been hindered by pervasive stigma and a lack of population-specific research, leading to widespread discouragement of its practice throughout gestation (4). Progress in this field has shifted this perspective, identifying pregnancy as a time when PA can yield substantial and long-lasting health benefits. Engagement in gestational PA is associated with reduced risk of several pregnancy and birth complications, such as excessive gestational weight gain, gestational diabetes, hypertension, preeclampsia, preterm delivery, and instrumental delivery (5–7). For offspring, maternal PA has been linked to improved neonatal adiposity and decreased risk of macrosomia and future metabolic disease (8,9). As the strength of the literature base supporting PA throughout gestation continues to grow, and research barriers are alleviated, there is a pressing need to establish the underlying physiological mechanisms driving these positive outcomes.

A central target for mechanistic investigation is the placenta, which comprises the maternal-fetal barrier. As the conduit between gesP and fetus, it regulates gas and nutrient exchange, waste filtering, and endocrine function. The placenta represents a compelling target for exploring how gestational PA may influence developmental trajectories. In particular, placental lipidomics offers a promising avenue for examining molecular adaptations associated with PA exposure during pregnancy.

Lipids are fundamental components of cell membranes and play important roles in energy metabolism and cell signaling (10). Lipidomics is the large-scale study of lipids, covering structural identification, quantification, and functional interactions with other molecules. Disturbances in lipid metabolism are hallmark signs of several diseases (11); thus, the analysis of lipid profiles can uncover crucial information regarding the health status of the organism (10). Pathological lipidomic signatures have been demonstrated within placental tissue, with marked differences in the lipid profile of placentas from pregnancies impacted by preeclampsia, intrauterine growth restriction, and gesP depression compared to nonpathological placentas (12–16).

The placental lipidome also provides insight into downstream lipid availability. For example, placental transfer of docosahexaenoic acid (DHA) – an essential omega-3 fatty acid with roles in fetal brain and retina development – cannot be synthesized by the fetus and therefore must be transported from maternal circulation across the syncytiotrophoblast (STB) (17). Lower DHA levels have been documented in preeclampsia and gestational diabetes (18,19), and in turn, lower fetal DHA levels have been linked to negative long-term cognitive and neural outcomes (20,21). Lipidomic profiling of DHA can therefore provide insight into its placental availability for fetal delivery.

Many lipids rely on active transporters at the STB membrane to be shuttled from gesP blood to fetal circulation, with some evidence to suggest altered transporter expression in response to PA (22–24). One transporter of particular interest is Major Facilitator Superfamily Domain-containing 2a (MSFD2a), a protein involved in the uptake and transfer of DHA at both the blood-brain barrier and the placenta (25). PA has been shown to restore blood-brain barrier MFSD2a

levels in hypertensive rats (26), however the relationship in humans and the placenta has never been examined.

This study aimed to characterize and compare the placental lipidome in physically active and inactive pregnancies using untargeted LC-MS/MS. A secondary objective was to assess placental MFSD2a transporter expression in the same cohort. Together, these analyses strive to provide novel insight into potential mechanisms through which PA benefits gestational outcomes.

Materials and Methods

Participants and Placenta Tissue

Participants included in this study were recruited through two research initiatives: The CIHR-funded *PLACENTA* project in Ottawa, Ontario, approved by the Ottawa Health Science Network Research Ethics Board (20160178-01H) and University of Ottawa Research Ethics Board (H11-15-29); and the NIH-funded *Expecting Success* project in Baton Rouge, Louisiana, approved by the Pennington Biomedical Research Centre Institutional Review Board (NCT01610752), respectively. All participants involved provided informed consent.

GesP were deemed eligible to participate in the *PLACENTA* study if recruitment occurred prior to 28 weeks pregnancy, participants were between 18-40 years of age, and carrying a singleton fetus. Prepregnancy, a BMI between 18.5-29.9 kg/m^2 and stable weight (±5 lbs) for at least six months were requirements. Exclusion criteria were defined as medical complications including diabetes prior to pregnancy, untreated thyroid disease, and medicated hypertension. Moreover, contraindications to PA, inability to communicate in French or English, or plans to put their baby up for adoption rendered gesPs ineligible. Participation in the *Expecting Success* project was considered if gestational parents between 18-40 years recruited during the first trimester were

carrying a singleton fetus and with overweight or obesity (BMI 25.0-39.9 kg/m2). Exclusion criteria reflected those of the *PLACENTA* trial. More information regarding this study has been previously described (27). From the *PLACENTA* project, six active and ten inactive gesP were included. An additional thirteen active and seven inactive gesP were recruited through the *Expecting Success* project, yielding a combined total of 19 active and 17 inactive participants. Full demographic data for study participants are provided in Table 1.

PLACENTA participants visited the lab two to three times throughout pregnancy, depending on gestational age at recruitment; visit 1 (early gestation) occurred in weeks 12-16, visit 2 (mid-gestation) in weeks 24-28, and visit 3 (late gestation) in weeks 34-38. Participants were provided with an Actical® accelerometer (Phillips Respironics, Montreal, QC), and a three-day diet log at each visit. They were instructed to wear the accelerometer on the hip during waking hours for seven consecutive days. *Expecting Success* participants were randomized to no intervention, in-person intervention, or remote intervention. Intervention arms included dietary and exercise advice in combination with behaviour modification counseling. Participants were provided Actigraph GT3X+ accelerometers (Actigraph, LLC) to measure PA.

Active and inactive classifications were made based on accelerometer data analyzed through SAS version 9.4 (SAS Institute, Cary, NC). A minimum of three day's wear was considered valid (28). Participants were classified as active if they met the recommended 150 minutes of moderate PA per week (or 21.4 min per day) as per the *2019 Canadian guideline for physical activity throughout pregnancy* (29). For the purpose of this study, activity status classification was based on third trimester accelerometer data.

Dietary intake data was collected via participant recall and analyzed using the Automated Self-Administered Dietary Assessment Tool (ASA24®), version ASA24-Canada-2018 (National Cancer Institute, Bethesda, MD).

Term placentas were sampled within an hour of delivery. To account for heterogeneity, samples were pooled from both peripheral and central cotyledons. Immediately post sampling, tissue was frozen in liquid nitrogen, then stored at -80°C until further processing.

Lipid Extraction

Placenta tissue samples were powdered on dry ice using a tissue pulverizer to ensure homogeneity. The resulting powdered tissue was stored at -80°C until lipid extraction.

The lipid extraction was performed using a modified Bligh-Dyer protocol, adapted by our lab to optimize downstream analytic performance (30). Approximately 50 ± 5 mg of powdered tissue was transferred into glass Kimble tubes. To initiate extraction, 1.5 mL of LC/MS grade water (Fisher Scientific, Cat.#W6-4) and 2 mL of MS-grade methanol (MeOH) (Thermo Scientific, Cat#047192.K2) were added, followed by three cycles of vortexing (15 s) and icing (10 s), before allowing to rest on ice for three minutes. Chloroform ($CHCl_3$) (Sigma-Aldrich, Cat.#650498) was spiked with internal standards: 20 μM 17:0 cholesteryl ester (Cat#700186), 2 μM 13:0 lyso PC (Cat#8554760), and 2 μM 19:0 PC (Cat#850367) (all from Avanti Research, Alabama, USA). A volume of 1.5 mL of spiked $CHCl_3$ was added to each tube, vortexed for 30 s, and rested on ice for one minute.

Tubes were then centrifuged at 2000 rpm (4°C) for two minutes, producing a biphasic solution. The top (aqueous) phase was retained for a separate metabolomics study. The bottom (nonpolar) phase was carefully collected and evaporated under a constant stream of N_2 gas (2 psi) using a Reacti-Vap™ Evaporator until dry. Lipids were then resuspended in 250 μL of 1:1

MeOH:EtOH, incubated at 30°C for 10 minutes, and centrifuged at 2000 rpm for one minute. Samples were diluted 10-fold in 1:1 MeOH:EtOH and transferred into Agilent HPLC vials (Cat#5188-6592) for subsequent mass spectrometry (MS) analysis.

For quality assurance, both blank controls and pooled quality control (QC) samples were included in the extraction protocol. Process blanks were prepared as described above, omitting the addition of a biological sample. These blanks served to identify background signals produced by extraction reagents and potential contamination introduced during sample processing/handling (31). Pooled QC samples were generated by combining 100 µL subaliquots of the final extraction solution of all biological samples within a single batch. This composite sample was used to monitor variability in data acquisition and pre-processing, providing a benchmark for consistency across the analytical workflow (31).

Mass Spectrometry

Additional LC-MS method parameters are outlined in the Supplemental Materials (MS Additional Supporting Information). All data were acquired on an Agilent 6546 QToF mass spectrometer with an Agilent 1260 HPLC. A Kinetix, C18 2.6 µm, 2.1 mm x 10 mm column heated to 45˚C was used for analyte separation, 5 and 10 µL injection volumes were used for positive and negative polarities, respectively. Mobile phase solvents included ultrapure MilliQ water (Millipore), MeOH (Sigma Lichrosolv, Cat#1.06035), and isopropanol (IPA, VWR BDH Chemicals HiPerSolv CHROMANORM, Cat#BDH20880) as well as the modifier ammonium formate (AF, Sigma Lichropur, Cat#70221). Lipid extractions were injected and separated using a binary mobile phase gradient, solvent A contained water:MeOH (1:1 v/v) with 10 mM AF, and solvent B was MeOH:IPA (1:3 v/v) with 10 mM AF. The flow rate for lipid analysis was constant at 0.4 mL/min, injections in positive polarity used the following HPLC gradient program: 0 min

20% B, 0.35 min 20% B, 0.40 min 32% B, 9.60 min 44% B, 9.70 min 65% B, 11.50 min 65% B, 26.80 min 82%, 27.30 min 87% B, 37 min 96% B, 37.1 min 100% B, 44 min 100% B. Injections in negative polarity used a modified gradient program: 0 min 20% B, 0.35 min 20% B, 0.40 min 32% B, 9.60 min 44% B, 9.70 min 65% B, 11.50 min 65% B, 30 min 86% B, 30.10 min 100% B, 39 min 100% B. The HPLC was run at 20% B for 5 minutes following each injection to re-equilibrate the column.

Technical triplicates at the MS-level were recorded for each sample in both polarities. Blank samples were analyzed every 4 injections to ensure sample carry-over was not observed. Internal standards in each sample were monitored for mass accuracy and RT drift throughout the batch and used to assess the data preprocessing parameters in later steps. Once MS-level only samples were completed, pooled samples from each batch were injected thrice using the same data-dependent analysis (DDA) method with the iterative injections feature activated (excluding features ±20 ppm and 0.15 retention time (RT) in previous injection) to acquire MS/MS spectra on low abundance features. A single fixed collision energy of 28 V for lipid fragmentation.

Western Blotting

Approximately 50 mg of powdered placenta tissue were homogenized in a 1x RIPA buffer supplemented with a 1:100 dilution of protease inhibitor cocktail (Millipore Sigma, Oakville, ON, Cat.#P8340). Lysates were centrifuged at 14000 g for 10 minutes (4°C), and the resulting supernatant was collected. Protein concentration was quantified by DC protein assay (Bio-Rad).

For immunoblotting, 20 ug of total protein was denatured and loaded onto 4-15% Mini-PROTEAN TGX precast gels (Bio-Rad) for electrophoretic separation. Proteins were transferred onto 0.22 μm PVDF membranes, which were then blocked in 5% skim milk for 1 hour at room temperature. Membranes were incubated overnight at 4°C with a 1:1000 dilution of primary

MFSD2a antibody (Abcam, Cat#AB307690). The following day, membranes were washed, then incubated for 1 hour at room temperature with a 1:7000 dilution of HRP-conjugated goat anti-rabbit IgG secondary antibody (Bio-Rad, Cat.#1706516).

Single detection was performed using Clarity Western ECL Blotting Substrates (Bio-Rad, Cat.#1721019) and chemiluminescence was imaged using the ChemiDoc XRS+ Molecular Imager (Bio-Rad). Total protein loading was assessed by Amido Black staining. Band intensities for MFSD2a were quantified by densitometry with JASP software (version 0.18.2) and normalized to total protein.

Data Processing and Analysis

Pooled QC data files for both negative and positive MS/MS runs were run separately through Agilent Lipid Annotator (Version 1.0 build 1.0.54.0) to create annotation lists. Raw data was converted into mzML using MSConvert software (ProteoWizard 3.0 25253 64-bit). All MS1 scans were then uploaded to MZmine (version 4.7.29) for data processing. Mass detection was set at 5E3 (centroid) to filter out noise. Feature detection was applied using Lipid Annotator generated annotation lists to negative and positive MS1 scans respectively. Parameters were set as follows: intensity tolerance 25%, mz tolerance 0 or 10 ppm and rt tolerance 0.1 min. To correct any drift in retention time, featured list files were calibrated using rt tolerance 0.5 min with minimum intensity 1E4. Lastly, files were aligned using the following parameters, then exported: mz tolerance 0 or 10 ppm, rt tolerance 0.2 min, weight for mz 50, and weight for rt 50.

The aligned feature lists were combined into one sheet, then analyzed in Microsoft Excel. Hits appearing in blank extractions were removed from the dataset unless average sample intensity was greater than 3x the blank (32). Any lipids that did not pass the 80% rule (that is, lipid must be present in at least 80% of samples in one group), were removed from the dataset. R (version 4.5.0)

was used for all further processing and data analysis. Data were log transformed and normalized using cyclic loess, and missing values were imputed. Unsupervised principal component analysis (PCA), supervised partial least square-discriminant analysis (PLS-DA), volcano plot, and area under receiver operating characteristic curves (AUROC) were performed at 95% confidence. PLS-DA model was cross-validated using goodness-of-fit (R^2) and predictive ability (Q^2). Fold-change, *p*-value, and FDR-adjusted *p*-values were calculated for each lipid. Significance level was established as $p < 0.05$. Lipids identified as statistically significant were used for pathway analysis with BioPAN.

Anthropometric and demographic characteristics of participants were analyzed by *t*-test for numerical variables and Chi-squared or Fisher's exact test for categorical variables. MFSD2a between active and inactive groups was also measured by *t*-test.

Results

Participant Characteristics

A total of 36 participant samples were analyzed for this study. Characteristics of participants are presented in Table 1. No significant differences were found between study groups regarding age, BMI, gestational weight gain, T3 total caloric or fat intake, placental weight, birth weight or length, or fetal sex. The only significant difference identified between the active and inactive cohort was T3 moderate-to-vigorous PA (34.2 ± 10.2 vs. 10.8 ± 6.9; $p < 0.001$). While c-section rates were higher in the active group, this trend was not significant.

Table 1. Characteristics of gestational parent and offspring

Gestational parent characteristics	**Inactive (n = 17)**	**Active (n = 19)**	***p*-value**
Age (years)	31.2 (4.7)	31.0 (4.2)	0.9058
Pre-pregnancy BMI (kg/m^2)	25.5 (4.2)	26.7 (4.3)	0.4194
BMI category (normal weight/overweight/obese)	7/8/2	7/7/5	0.6216
GWG (kg)	13.9 (4.5)	11.4 (5.7)	0.1488
GWG category (insufficient/appropriate/excessive)	3/3/11	5/6/8	0.5003
MVPA T3 (min/day)	10.8 (6.9)	34.8 (10.2)	**< 0.001**
Total caloric intake T3 (kcal/day)	1710.8 (681.2)	1686.6 (561.8)	0.9152
Fat intake T3 (g/day)	67.1 (33.7)	70.9 (26.0)	0.7227
Birth Characteristics			
Birth method (csection/vaginal)	1/16	6/13	0.0918
Placental weight - untrimmed (g)	589.9 (107.5)	548.6 (97.4)	0.2642
Placental weight – trimmed (g)	478.1 (85.1)	463.9 (81.0)	0.6345
Fetal sex (M/F)	9/8	9/10	1
Birth weight (g)	3345.2 (486.9)	3201.1 (415.5)	0.3448
Birth length (cm)	50.4 (2.6)	49.7 (2.1)	0.3641
Weight/length ratio	66.2 (7.3)	64.3 (6.2)	0.4009
Weight/length percentile	38.3 (26.9)	41.1 (23.6)	0.7374

Note. Results expressed as mean (SD). Significance was accepted as $p < 0.05$. BMI – body mass index; GWG – gestational weight gain; MVPA – moderate to vigorous physical activity; M – male; F – female.

Lipid Composition in Active vs. Inactive Placentas

After data processing and applying the 80% rule, 206 lipid species were detected across the placenta samples in combined positive and negative ionization mode. Missing values accounted for approximately 5.8% of the dataset and were imputed using Quantile Regression Imputation of Left Censored Data. Following filtering of duplicates, 192 unique lipids were retained for statistical analyses. Phosphatidylcholines (PCs) represented the most abundant lipid class comprising 48.3%, followed by sphingomyelins (SM) at 15.9% of total abundance (Figure 1). The most prevalent fatty acids were palmitic acid (C16:0) and arachidonic acid (C20:4).

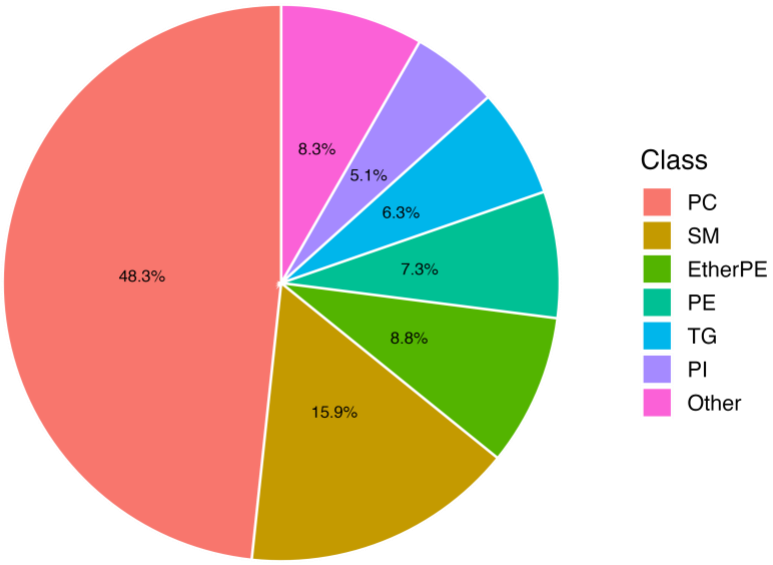


Figure 1. Lipid class composition of placenta samples based on raw intensity values. Classes in the section labeled "other" each represent < 2% of total composition individually. *PC = phosphatidylcholine; SM = sphingomyelin; EtherPE = ether-linked phosphatidylethanolamine; PE = phosphatidylethanolamine; TG = triacylglycerol; PI = phosphatidylinositol.*

Unsupervised principal component analysis (PCA) to visualize group distinctions in overall lipidome revealed modest separation between the active and inactive groups, with PC1 and PC2 explaining 18.6% and 17% of variance, respectively (Figure S1). Supervised partial least squares-discriminant analysis (PLS-DA) further distinguished the groups (PC1 accounting for 14% and PC2 12% of explained variance; Figure 2), with cross-validation indicating strong model performance ($R^2 = 0.836$; $Q^2 = 0.694$), suggesting good predictive accuracy without overfitting. A total of 39 lipid species differed significantly between active and inactive groups (FDR adjusted p-value < 0.05; Figure 3). Applying a $\log_2$FC threshold of ±0.58 (equivalent to a 1.5-fold change)

reduced this to 34 significant lipids. A complete list of significant lipid species can be found in the Supplemental Materials (Table S1). Among 17 DHA-containing (22:6) lipid species, eight were significantly higher in placentas from active participants, with seven exceeding the log_2FC threshold (±0.58). PG 18:0/22:6 was also significantly higher in the active group, but fell just below the threshold (log_2FC = 0.57). The remaining 9 DHA-containing lipids showed no significant group differences.

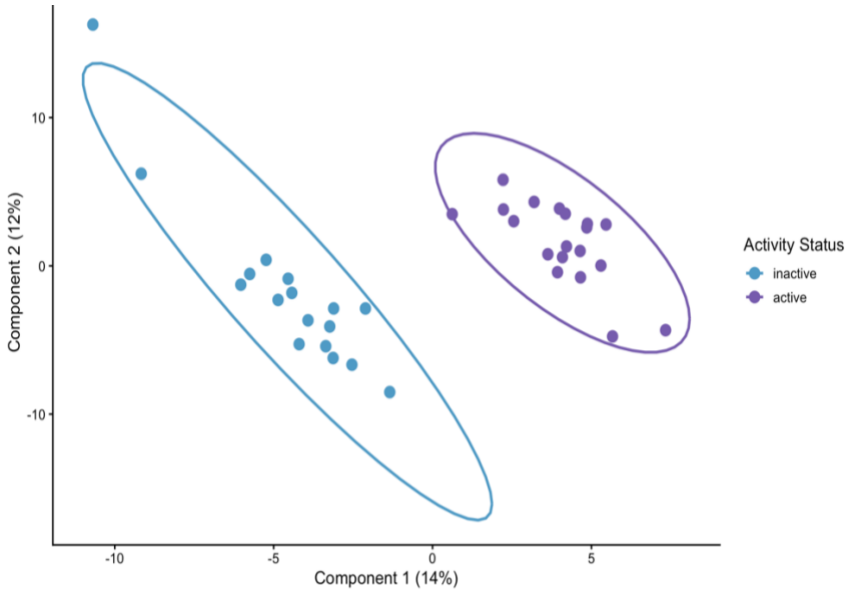


Figure 2. PLS-DA with 95% confidence of placental lipidome in active vs. inactive pregnancies (R^2 = 0.836; Q^2 = 0.694).

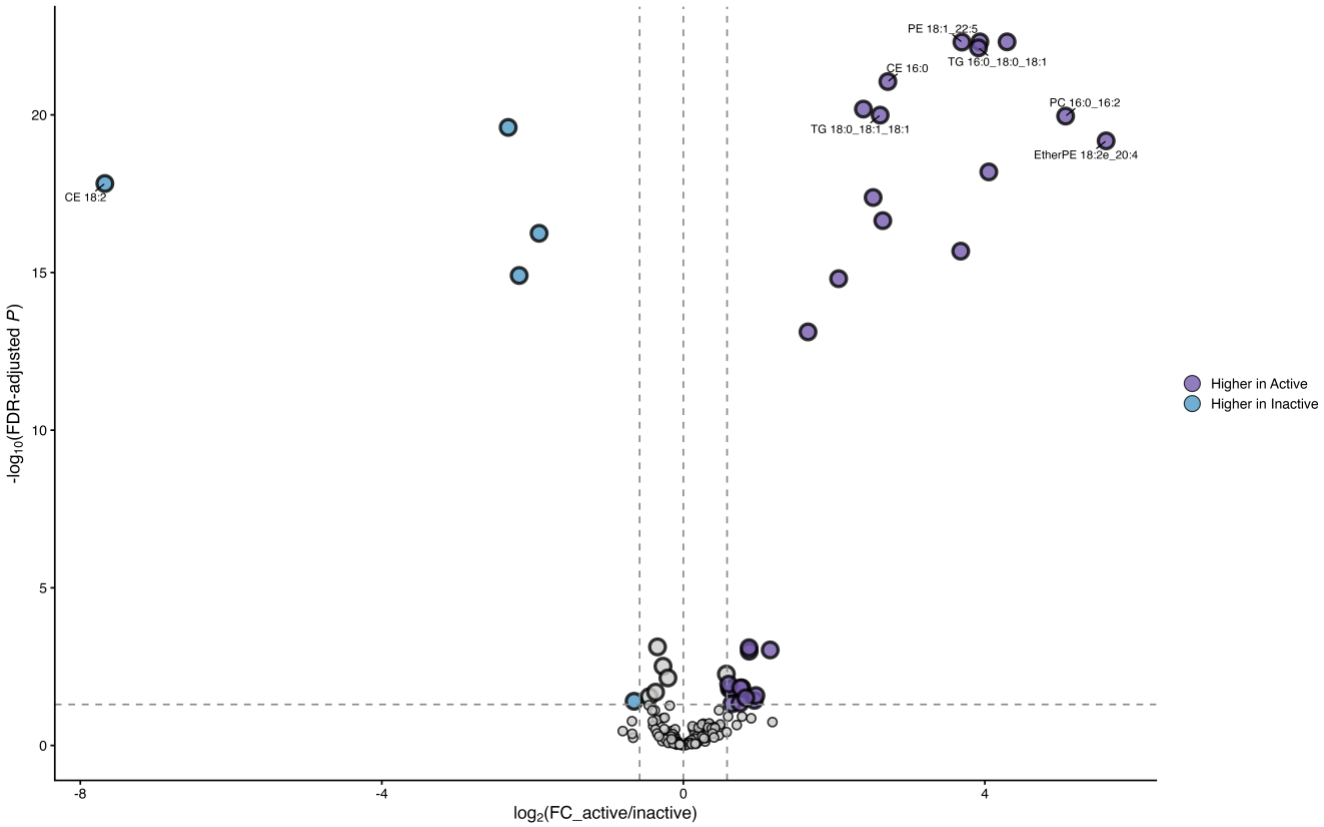


Figure 3. Volcano plot of lipids. The *x*-axis is the $\log_2$ of FC and the *y*-axis is the $-\log_{10}$ of *p*-value. Larger dots represent lipids with *p*-value < 0.05. The right side of the plot represents lipids more present in the active cohort (purple dots); the left side of the plot represents lipids more present in the inactive cohort (blue dots). Outer vertical dashed lines represent FC thresholds ($\log_2$FC ±0.58). Grey dots represent lipids that did not meet significance and/or FC threshold.

Discrimination for Physical Activity Status

AUROC curve analyses were conducted to assess predictive abilities of individual lipid species as potential biomarkers of PA status. AUC values were interpreted as follows: 1 indicated perfect discrimination; 0.90-0.99, excellent; 0.80-0.89, good; 0.70-0.79, fair; and < 0.70, non-informative (Carter et al., 2016). Five lipids (CE 16:0, PC 16:0/16:2, PE 18:1/22:5, TG

16:0/18:0/18:1, and TG 18:0/18:1/18:1) scored an AUC of 1, indicating perfect distinction between active and inactive groups. Violin plots for these five lipids are shown in Figure 4. In total, 33 lipid species had an AUC > 0.70, indicating fair to excellent predictive capacity for PA status.

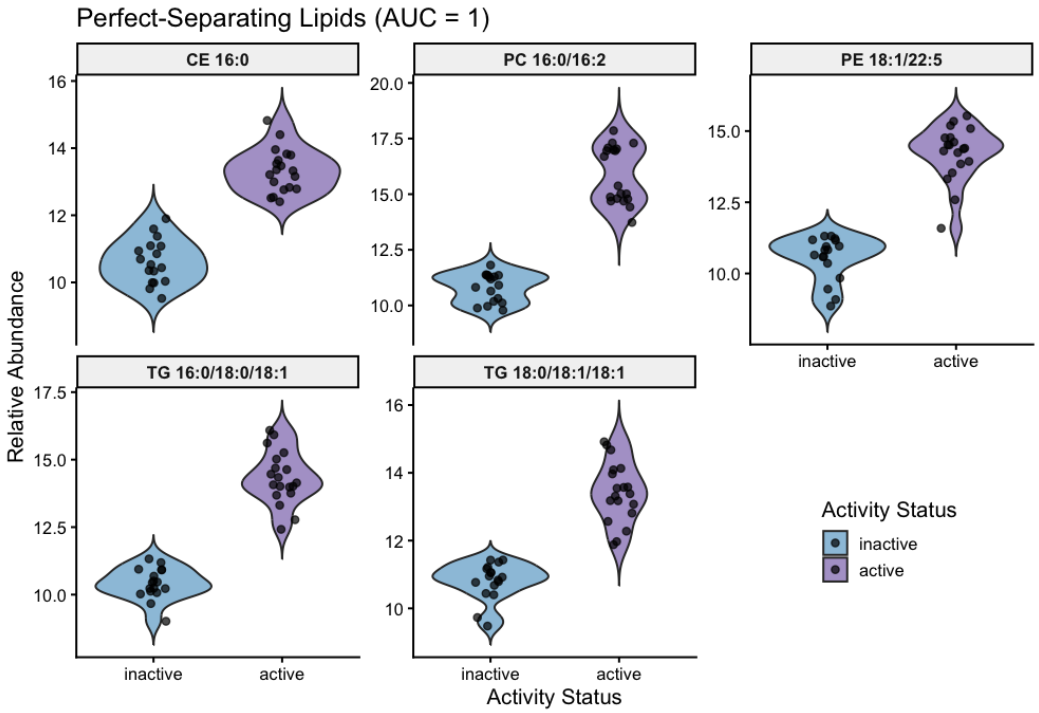


Figure 4. Violin plots of all lipids scoring an AUC = 1. The *x*-axis represents activity status whereas the *y*-axis represents lipid abundance (log-transformed values) as measured by mass spectrometry.

Pathway Analysis

To assess potential differences in lipid pathways, differentially expressed lipid species were analyzed using BioPAN (34). The software identified a significant elevation of the PS to PE pathway in the active group, driven by PS 40:6 and PE 40:6 specifically (z-score = 2.199; $p <$

0.05). BioPAN predicted the involvement of the phosphatidylserine decarboxylase (PISD) gene in this reaction.

MFSD2a Expression in Active vs. Inactive Placentas

No significant differences were observed in MFSD2a expression between active and inactive groups (Figure 5). However, after adjusting for PA status and gestational weight gain category, MFSD2a expression was inversely associated with infant weight-to-length ratio (β = -0.93; p = 0.019). Additionally, MFSD2a expression showed a moderate negative correlation with infant weight-to-length percentiles (r = -0.40; p = 0.019).

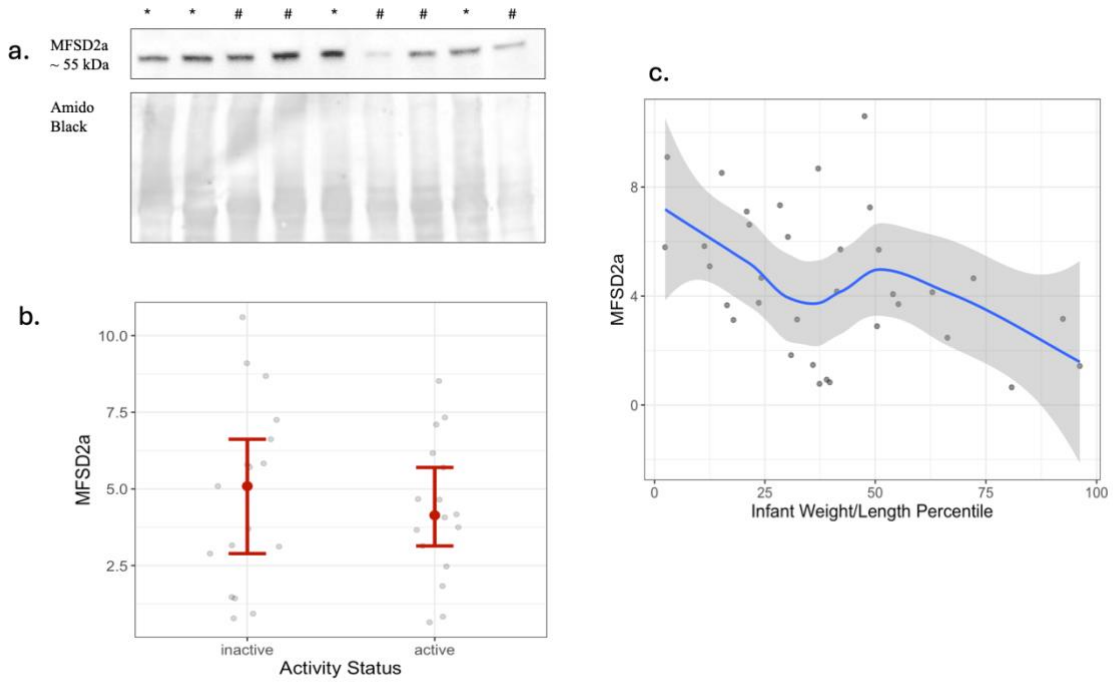


Figure 5. a) Representative Western blot of MFSD2a in placentas from active and inactive pregnancies. Band density was normalized to total lane protein density as measured by Amido Black staining. * = *active; # = inactive*. b) MFSD2a expression (arbitrary units) in placentas from

active and inactive pregnancies. c) Correlation between infant weight to length percentile and MFSD2a (arbitrary units). A moderate negative correlation is observed (r = -0.40; $p = 0.019$).

Discussion

This study explored the placental lipidome of active and inactive gesP from the *PLACENTA* and the *Expecting Success* trials, offering insight into potential mechanisms through which gestational PA may alter maternal and/or fetal outcomes. The modest separation observed in PLS-DA aligns with expectations, given that all participants experienced healthy, uncomplicated pregnancies. These results suggest that PA may selectively modulate placental lipid composition, potentially shifting fuel availability, fetal nutrient transport, and other lipid-related functions.

Among the lipid species with the greatest fold-changes between groups were EtherPE 18:2e/20:4 and CE 18:2. EtherPE 18:2e/20:4, an ether phosphatidylethanolamine containing arachidonic acid (ARA), was approximately 48.8-fold more abundant in placentas from active pregnancies ($log_2FC = 5.61$, $p < 0.001$). ARA, a long-chain polyunsaturated fatty acid, is critical to the structure of the nervous system and inflammatory signalling via eicosanoid and leukotrienes (Duttaroy, 2009). Placental ether PE species are considered reservoirs for ARA (35); therefore, their upregulation in placentas from active pregnancies may indicate a greater fetal supply. Notably, Powell *et al.* found that while placental levels of ether PE containing ARA were decreased in obesity, cord blood levels of ARA remained unchanged in female fetuses, suggesting a sex-specific protective mechanism against obesity-induced deficiencies (35). Future analysis of cord blood ARA in active and inactive pregnancies may clarify the functional implications of our findings and establish their relations to fetal supply.

CE 18:2, or cholesteryl linoleate, showed the greatest absolute fold-change ($log_2FC = -7.67$), with ~ 203.7-fold higher abundance in placentas from the inactive cohort. CE 18:2 is a

component of low-density lipoproteins with pro-atherogenic properties (36,37). Although its role in placenta biology is not well characterized, association between atherosclerosis and pregnancy disorders such as preeclampsia has been established (38). The lower abundance of CE 18:2 in placentas from active pregnancies could therefore be indicative of a protective mechanism of PA against vascular pathology. Nevertheless, a FC of this magnitude should be interpreted with caution; as imputation of zero raw intensity values, likely reflecting levels below detection, may have artificially inflated the calculated FC. Such cases warrant careful consideration before inferring biological significance.

AUROC analyses revealed five lipid species that demonstrated perfect discriminatory power (AUC = 1) based on PA status. These lipids, CE 16:0, TG 16:0/18:0/18:1, TG 18:0/18:1/18:1, PC 16:0/16:2, and PE 18:1/22.5, are all more abundant in the active group. CE 16:0, or cholesteryl palmitate, is a cholesterol ester previously linked to respiratory distress syndrome in offspring, as well as gestational diabetes in cases of low concentrations in amniotic fluid (39,40). Its role in the placenta remains unexplored, though the current findings provide a compelling basis for inquiry. TG 18:0/18:1/18:1 and TG 16:0/18:0/18:1 are both triacylglycerols which, to our knowledge, have not been studied in the context of placental biology. Interestingly, Mamtani *et al.* found plasma concentrations of TG 16:0/18:0/18:1 to be predictive of future type 2 diabetes (41). Any relation of this species to gestational diabetes risk and placental levels is currently unknown. PC 16:0/16:2 is a phosphatidylcholine, the predominant subclass of glycerophospholipid comprising cell membranes (42). Although this specific species has not been previously reported in placenta, its sum composition (PC 32:2) has been noted in cord plasma (43) – potentially offering novel insight into the placental lipid response to PA. Lastly, PE 18:1/22:5 belongs to the phosphatidylethanolamine subclass, the second most abundant glycerophospholipid

in cell membranes (42). This study is among the first to report differential abundance of this lipid species in the placenta, and its functional role remains largely unexamined. Prior work has noted that mitochondrial PEs contain a greater percentage of polyunsaturated fatty acids in the *sn*-2 position compared to whole cell or ER membranes (44), suggesting mitochondrial membrane enrichment. While within our data the functional interpretation of this group difference in PE 18:1/22:5 remains speculative, its structure aligns with this profile and may reflect a shift in mitochondrial membrane composition.

Pathway analysis revealed an enrichment of the phosphatidylserine decarboxylase (PSD) pathway in the active cohort. This mitochondrial pathway converts PS to PE via the PISD gene (44) and represents one of two major metabolic pathways responsible for PE production. One study found PS abundance to be greater in syncytiotrophoblast microvesicles from placentas affected by preeclampsia and recurrent miscarriage; PS species contribute to heightened apoptosis and coagulation, which are characteristic traits of these conditions (45). GDM may also be associated with this pathway, with studies observing lower PE species in both placental tissue and cord blood from GDM pregnancies compared to healthy pregnancies (46,47). Interestingly, PISD expression was downregulated in placental villi from unexplained miscarriages and in gestational hypertension compared to healthy pregnancies (48,49). Collectively, these findings suggest that suppression of the PSD pathway may be a feature of pathological pregnancies, whereas heightened levels in active pregnancies may serve as a protective mechanism.

Of the 17 identified DHA-containing lipid species in this dataset, seven met statistical significance and FC thresholds, all of which were higher in the active group. DHA is crucial to fetal brain development, especially in the third trimester when neural growth accelerates (50). Lower levels of fetal DHA have been associated with increased problem behaviour and higher risk

of allergy development in offspring, whereas sufficient DHA is linked to improved cognitive abilities and problem-solving skills, outcomes which persist several years into childhood (20,21,51,52). These findings are supported by studies in DHA deficient dams demonstrating slowed neural transmission times in offspring, and lower visual acuity in monkeys deprived of DHA during fetal development (53,54). Several studies have shown that maternal supplementation of DHA during pregnancy increases placental and infant DHA accumulation (55–57), though to our knowledge the relationship between placental DHA and gestational PA has not previously been examined. In nonpregnant populations, DHA remains an important molecule for brain function, and murine studies suggest that PA may enhance DHA transport across the blood-brain barrier. For example, rodents exposed to both PA and DHA supplementation demonstrated improved DHA-related brain performance compared to supplementation alone (58). Another study demonstrated that hypertension-induced reduction in the expression of MFSD2a was rescued by PA (26). Given that dietary intake did not differ significantly between our active and inactive cohort, we propose that PA may act as a physiological stimulus to promote placental DHA accumulation, independent of maternal consumption.

Unexpectedly, placental MFSD2a expression did not reflect the differences observed in DHA composition between active and inactive cohorts. MFSD2a is recognized for its role in transporting DHA in its LPC form across the blood-brain barrier (59) and is proposed to serve a similar function in the placenta (60). However, LPC containing DHA was not annotated in our final lipid dataset, potentially due to low abundance or technical limitations, preventing direct comparison between placental MFSD2a expression and LPC-DHA abundance. Examination of other known long-chain PUFA transporters such as FATP1 and FATP4 may offer additional insight into DHA delivery to the fetus.

Despite the lack of group differences in MFSD2a expression, we did observe a significant negative correlation between MFSD2a and infant weight-to-length percentiles (r = -0.40; p = 0.019). Moreover, when adjusting for MFSD2a, a significant interaction between activity status and gestational weight gain emerged (p = 0.032), indicating that infant weight-to-length patterns varied across these categories. Among inactive gesP, infants of gesP with insufficient weight gain had lower weight-to-length ratios compared to those categorized as gaining excessively (p = 0.014). This pattern was not identified in active gesP, suggesting that PA may buffer against the effects of a limited energy availability environment on fetal growth. The inverse association between MFSD2a and weight-to-length percentile may reflect a compensatory placental response to reduced growth, potentially prioritizing transport of DHA. Supporting this hypothesis, work in our lab has documented lipid droplet accumulation on the fetal side of the placenta in cases of active pregnancies with insufficient weight gain (unpublished), presumably to facilitate fetal nutrient delivery.

This study is limited in its ability to infer the lipidome across the entire span of gestation. In addition to the dynamic nature of the lipidome throughout gestation, labour and mode of delivery are known to influence lipid metabolism, and may further alter the lipid profile captured in this model (61). Another limitation is the use of relative quantification as opposed to absolute, which would require the use of internal standards for each species of interest. Given the cost associated with employing such methods, and the fact that this was an exploratory study, we opted to use relative quantification. Future research should employ more targeted approaches with absolute quantification of species to determine precise abundance. Additionally, the absence of cord blood lipid analysis leaves a crucial piece of the transport puzzle blank. It is plausible that certain lipids downregulated in the placenta are being transported more rapidly to the fetal

circulation, and those upregulated are increasingly used or stored within the placenta. Without cord blood data, these interactions remain speculative. Finally, this study did not examine potential differences between the two source studies from which samples were drawn. Although participants from both were included in the active and inactive groups, variations in study design and participant characteristics across the two studies may have influenced the lipid profiles observed.

To our knowledge, this study is the first to examine the placental lipidome in healthy active and inactive pregnancies in humans. Using LC-MS/MS, we identified significant differences in placental lipid composition between active and inactive pregnancies. Differential lipid profiles point toward an enriched PSD pathway in placentas from active pregnancies. Seven of the 17 identified placental lipid species containing the essential fatty acid DHA were found to be more abundant in the active group. This novel, exploratory analysis highlights key lipid species and pathways that may be mechanistically involved in PA. Future research should build on this work using targeted, mechanistic approaches to fully elucidate the role of PA in supporting healthful pregnancy outcomes.

Author Contributions

MM, AY, and KBA conceived and designed the research. MM, JC, KW, JS, CR, and AD performed experiments. MM interpreted results of experiments, prepared figures, and drafted the manuscript. All authors edited and revised the manuscript and approved the final version.

Acknowledgements

The authors sincerely thank all participants in the *PLACENTA* and *Expecting Success* trials. We also thank the placenta collection teams and the labour and delivery staff at all institutions involved in sample collection.

Grants

This study was supported by the Canadian Institutes for Health Research (CIHR) (MOP 142298; PJT 191862) and Natural Sciences and Engineering Council of Canada (NSERC) (RGPIN-2017-05457) awarded to KBA, and the National Institutes of Health (NIH) (U01 DK094418) awarded to LMR. Additionally, MM was supported by the Canada Graduate Scholarship – Master's (CGS-M) awarded by CIHR, and the Ontario Graduate Scholarship (OGS).

Disclosures

The authors have no conflicts of interest, financial or otherwise, to declare.

Data Availability Statement

Data will be made available upon reasonable request.

References

1. Reiner M, Niermann C, Jekauc D, Woll A. Long-term health benefits of physical activity - A systematic review of longitudinal studies. *BMC Public Health*. 2013. https://doi.org/10.1186/1471-2458-13-813
2. Kraus WE, Powell KE, Haskell WL, Janz KF, Campbell WW, Jakicic JM, et al. Physical Activity, All-Cause and Cardiovascular Mortality, and Cardiovascular Disease. *Med Sci Sports Exerc*, 51(6), 1270-1281. 2019. https://doi.org/10.1249/MSS.0000000000001939
3. Marquez DX, Aguinãga S, Vásquez PM, Conroy DE, Erickson KI, Hillman C, et al. A systematic review of physical activity and quality of life and well-being. *Transl Behav Med*. 2020. https://dx.doi.org/10.1093/tbm/ibz198
4. Corson AE, MacDonald M, Tzaneva V, Edwards CM, Adamo KB. Breaking Boundaries: A Chronology with Future Directions of Women in Exercise Physiology Research, Centred on Pregnancy. *Advanced Exercise and Health Science*. 2024. https://linkinghub.elsevier.com/retrieve/pii/S2950273X24000262
5. Davenport MH, Ruchat SM, Poitras VJ, Jaramillo Garcia A, Gray CE, Barrowman N, et al. Prenatal exercise for the prevention of gestational diabetes mellitus and hypertensive disorders of pregnancy: a systematic review and meta-analysis. *Br J Sports Med*, 52(21):1367–75, 2018. https://bjsm.bmj.com/content/52/21/1367
6. Dipietro L, Evenson KR, Bloodgood B, Sprow K, Troiano RP, Piercy KL, et al. Benefits of Physical Activity during Pregnancy and Postpartum: An Umbrella Review. *Med Sci Sports Exerc*. 51(6):1292–302, 2019. https://pubmed.ncbi.nlm.nih.gov/31095086/
7. Xie Q, Li Q, Li J. Effect of Prenatal Exercise on the Rate of Cesarean Section: A Systematic Review and Meta-Analysis. *Clin Exp Obstet Gynecol*. 52(3):26582, 2025. https://www.imrpress.com/journal/CEOG/52/3/10.31083/CEOG26582/htm
8. Davenport MH, Meah VL, Ruchat SM, Davies GA, Skow RJ, Barrowman N, et al. Impact of prenatal exercise on neonatal and childhood outcomes: a systematic review and meta-analysis. *Br J Sports Med.* 52(21):1386–96, 2018. https://bjsm.bmj.com/content/52/21/1386
9. Bhattacharjee J, Mohammad S, Adamo KB. Does exercise during pregnancy impact organs or structures of the maternal-fetal interface? *Tissue Cell*. 72, 2021. https://pubmed.ncbi.nlm.nih.gov/33940567/
10. Avela HF, Sirén H. Advances in lipidomics. *Clinica Chimica Acta*. Vol. 510, 123–41, 2020. https://doi.org/10.1016/j.cca.2020.06.049
11. Hornburg D, Wu S, Moqri M, Zhou X, Contrepois K, Bararpour N, et al. Dynamic lipidome alterations associated with human health, disease and ageing. *Nature Metabolism.* (9):1578–94, 2023. https://www.nature.com/articles/s42255-023-00880-1
12. Yang Y, Wu L, Lv Y, Miao Z, Wang Y, Yan J, et al. LC-MS/MS based untargeted lipidomics uncovers lipid signatures of late-onset preeclampsia. *Biochimie*. 208:46–55, 2023. https://pubmed.ncbi.nlm.nih.gov/36496182/
13. Brown SHJ, Eather SR, Freeman DJ, Meyer BJ, Mitchell TW. A Lipidomic Analysis of Placenta in Preeclampsia: Evidence for Lipid Storage. *PLoS One*. 11(9), 2016. https://pubmed.ncbi.nlm.nih.gov/27685997/
14. Zhang L, Bi S, Liang Y, Huang L, Li Y, Huang M, et al. Integrated Metabolomic and Lipidomic Analysis in the Placenta of Preeclampsia. *Front Physiol*. 2022. https://pubmed.ncbi.nlm.nih.gov/35185616/

15. Wong G, Weir JM, Mishra P, Huynh K, Nijagal B, Gupta V, et al. The placental lipidome of maternal antenatal depression predicts socio-emotional problems in the offspring. *Transl Psychiatry.* 11(1), 2021. https://pubmed.ncbi.nlm.nih.gov/33542173/
16. Percy P, Vilbergsson G, Percy A, Månsson JE, Wennergren M, Svennerholm L. The fatty acid composition of placenta in intrauterine growth retardation. *Biochim Biophys Acta*. 1084(2):173–7, 1991. https://pubmed.ncbi.nlm.nih.gov/1854802/
17. Duttaroy AK. Transport of fatty acids across the human placenta: A review. *Prog Lipid Res.* 2009;48:52–61. https://doi.org/10.1016/j.plipres.2008.11.001
18. Wadhwani N, Patil V, Pisal H, Joshi A, Mehendale S, Gupte S, et al. Altered maternal proportions of long chain polyunsaturated fatty acids and their transport leads to disturbed fetal stores in preeclampsia. *Prostaglandins Leukot Essent Fatty Acids*. 91(1–2):21–30, 2014. https://pubmed.ncbi.nlm.nih.gov/24928794/
19. Pagán A, Prieto-Sánchez MT, Blanco-Carnero JE, Gil-Sánchez A, Parrilla JJ, Demmelmair H, et al. Materno-fetal transfer of docosahexaenoic acid is impaired by gestational diabetes mellitus. *Am J Physiol Endocrinol Metab*. 305(7):826–33, 2013. https://journals.physiology.org/doi/10.1152/ajpendo.00291.2013
20. Krabbendam L, Bakker E, Hornstra G, van Os J. Relationship between DHA status at birth and child problem behaviour at 7 years of age. *Prostaglandins Leukot Essent Fatty Acids*. 76(1):29–34, 2007. https://pubmed.ncbi.nlm.nih.gov/17074476/
21. Larqué E, Gil-Sánchez A, Prieto-Sánchez MT, Koletzko B. Omega 3 fatty acids, gestation and pregnancy outcomes. *Br J Nutr*. 107, 2012. https://pubmed.ncbi.nlm.nih.gov/22591905/
22. Hutchinson KA, Vuong NH, Mohammad S, Everest C, Leung ML, Bhattacharjee J, et al. Physical Activity During Pregnancy Is Associated with Increased Placental FATP4 Protein Expression. *Reprod Sci*. 27(10):1909–19, 2020. https://pubmed.ncbi.nlm.nih.gov/32519158/
23. Son JS, Liu X, Tian Q, Zhao L, Chen Y, Hu Y, et al. Exercise prevents the adverse effects of maternal obesity on placental vascularization and fetal growth. *J Physiol*. 597(13):3333–47, 2019. https://pubmed.ncbi.nlm.nih.gov/31115053/
24. Ruebel ML, Borengasser SJ, Zhong Y, Kang P, Faske J, Shankar K. Maternal Exercise Prior to and during Gestation Induces Sex-Specific Alterations in the Mouse Placenta. *Int J Mol Sci*. 24(22), 2023. https://pubmed.ncbi.nlm.nih.gov/38003633/
25. Basak S, Mallick R, Duttaroy AK. Maternal Docosahexaenoic Acid Status during Pregnancy and Its Impact on Infant Neurodevelopment. *Nutrients*. 12(12):1–25, 2020. https://doi.org/10.3390/nu12123615
26. Perego SM, Raquel HA, Candido VB, Masson GS, Martins MM, Ceroni A, et al. Hypertension depresses but exercise training restores both Mfsd2a expression and blood-brain barrier function within PVN capillaries. *Am J Physiol Regul Integr Comp Physiol*. 325(3):R299–307, 2023. https://journals.physiology.org/doi/10.1152/ajpregu.00049.2023
27. Redman LM, Gilmore LA, Breaux J, Thomas DM, Elkind-Hirsch K, Stewart T, et al. Effectiveness of SmartMoms, a Novel eHealth Intervention for Management of Gestational Weight Gain: Randomized Controlled Pilot Trial. *JMIR Mhealth Uhealth*. 5(9), 2017.: https://pubmed.ncbi.nlm.nih.gov/28903892/
28. Da Silva DF, Mohammad S, Nagpal TS, Scremin Souza SC, Colley RC, Adamo KB. How Many Valid Days Are Necessary to Assess Physical Activity Data From Accelerometry During Pregnancy? *J Phys Act Health.* 18(3):337–44, 2021. https://pubmed.ncbi.nlm.nih.gov/33592577/

29. Mottola MF, Davenport MH, Ruchat SM, Davies GA, Poitras VJ, Gray CE, et al. 2019 Canadian guideline for physical activity throughout pregnancy. *Br J Sports Med*. 52(21):1339–46, 2018. https://pubmed.ncbi.nlm.nih.gov/30337460/
30. Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. *Can J Biochem Physiol.* 37(8):911–7, 1959. https://pubmed.ncbi.nlm.nih.gov/13671378/
31. Broadhurst D, Goodacre R, Reinke SN, Kuligowski J, Wilson ID, Lewis MR, et al. Guidelines and considerations for the use of system suitability and quality control samples in mass spectrometry assays applied in untargeted clinical metabolomic studies. *Metabolomics*. 14(6):72, 2018. https://doi.org/10.1007/s11306-018-1367-3
32. Smirnov D, Mazin P, Osetrova M, Stekolshchikova E, Khrameeva E. The hitchhiker's guide to untargeted lipidomics analysis: Practical guidelines. *Metabolites*. 11(11):713, 2021. https://pmc.ncbi.nlm.nih.gov/articles/PMC8624948/
33. Carter J V., Pan J, Rai SN, Galandiuk S. ROC-ing along: Evaluation and interpretation of receiver operating characteristic curves. *Surgery*. 159(6):1638–45, 2016. https://doi.org/10.1016/j.surg.2015.12.029
34. Gaud C, Sousa BC, Nguyen A, Fedorova M, Ni Z, O'Donnell VB, et al. BioPAN: a web-based tool to explore mammalian lipidome metabolic pathways on LIPID MAPS. *F1000Research.*10 (4*),* 2021. https://doi.org/10.12688/f1000research.28022
35. Powell TL, Uhlson C, Madi L, Berry KZ, Chassen SS, Jansson T, et al. Fetal sex differences in placental LCPUFA ether and plasmalogen phosphatidylethanolamine and phosphatidylcholine contents in pregnancies complicated by obesity. *Biol Sex Differ*. 14(1):66, 2023. https://pmc.ncbi.nlm.nih.gov/articles/PMC10540428/
36. Joyce CW, Wagner EM, Basso F, Amar MJ, Freeman LA, Shamburek RD, et al. ABCA1 overexpression in the liver of LDLr-KO mice leads to accumulation of pro-atherogenic lipoproteins and enhanced atherosclerosis. *J Biol Chem.* 281(44):33053–65, 2006. https://europepmc.org/article/MED/16928680
37. Takahashi Y, Zhu H, Xu W, Murakami T, Iwasaki T, Hattori H, et al. Selective uptake and efflux of cholesteryl linoleate in LDL by macrophages expressing 12/15-lipoxygenase. *Biochem Biophys Res Commun.* 338(1):128–35, 2005. https://pubmed.ncbi.nlm.nih.gov/16105647/
38. Staff AC, Johnsen GM, Dechend R, Redman CWG. Preeclampsia and uteroplacental acute atherosis: immune and inflammatory factors. *J Reprod Immunol.* 101–102(1):120–6, 2014. https://doi.org/10.1016/j.jri.2013.09.001
39. Ludmir J, Alvarez JG, Mennuti MT, Gabbe SG, Touchstone JC. Cholesteryl palmitate as a predictor of fetal lung maturity. *Am J Obstet Gynecol*. 157(1):84–8, 1987.: https://europepmc.org/article/MED/3605272
40. Ludmir J, Alvarez JG, Landon MB, Gabbe SG, Mennuti MT, Touchstone JC. Amniotic fluid cholesteryl palmitate in pregnancies complicated by diabetes mellitus. *Obstetrics and Gynecology*. ;72(3 Pt 1):360–2, 1988. https://europepmc.org/article/MED/3405552
41. Mamtani M, Kulkarni H, Wong G, Weir JM, Barlow CK, Dyer TD, et al. Lipidomic risk score independently and cost-effectively predicts risk of future type 2 diabetes: Results from diverse cohorts. *Lipids Health Dis*. 15(1):1–12, 2016. https://lipidworld.biomedcentral.com/articles/10.1186/s12944-016-0234-3
42. Voelker D. Glycerolipid Structure, Function, and Synthesis in Eukaryotes. *Encylopedia of Biological Chemistry*. 2:412–8, 2013. DOI:10.1016/B978-0-12-378630-2.00510-7

43. Wong G, Narasimhan K, Cheong WF, Ng S, Aris IM, Loy SL, et al. Umbilical Cord Plasma Lysophospholipids and Triacylglycerols Associated with Birthweight Percentiles. *Nutrients*. 16(2):274, 2024. https://pmc.ncbi.nlm.nih.gov/articles/PMC10820643/
44. St Germain M, Iraji R, Bakovic M. Phosphatidylethanolamine homeostasis under conditions of impaired CDP-ethanolamine pathway or phosphatidylserine decarboxylation. *Front Nutr*. 9:1094273, 2023. https://doi.org/10.3389/fnut.2022.1094273
45. Baig S, Lim JY, Fernandis AZ, Wenk MR, Kale A, Su LL, et al. Lipidomic analysis of human placental Syncytiotrophoblast microvesicles in adverse pregnancy outcomes. *Placenta*. 34(5):436–42, 2013. https://doi.org/10.1016/j.placenta.2013.02.004
46. Li Y, Hao C, Chen W, Meng Q. Analysis of specific lipid metabolites in cord blood of patients with gestational diabetes mellitus. *Biocell*. 46(6):1565–73, 2022. https://www.sciencedirect.com/org/science/article/pii/S0327954522001657
47. Jiang D, He J, Hua S, Zhang J, Liu L, Shan C, et al. A comparative lipidomic study of the human placenta from women with or without gestational diabetes mellitus. *Mol Omics*. 18(6):545–54, 2022. https://doi.org/10.1039/d2mo00083k
48. Lyu SW, Song H, Yoon JA, Chin MU, Sung SR, Kim YS, et al. Transcriptional profiling with a pathway-oriented analysis in the placental villi of unexplained miscarriage. *Placenta*. 34(2):133–40, 2013. https://doi.org/10.1016/j.placenta.2012.12.003
49. Varghese B, Babu S, Jala A, Das P, Raju R, Borkar RM, et al. Integrative Placental Multi-Omics Analysis Reveals Perturbed Pathways and Potential Prognostic Biomarkers in Gestational Hypertension. *Arch Med Res*. 55(1):102909, 2024. https://doi.org/10.1016/j.arcmed.2023.102909
50. Haggarty P. Fatty acid supply to the human fetus. *Annu Rev Nutr*. 30:237–55, 2010. https://pubmed.ncbi.nlm.nih.gov/20438366/
51. Jensen CL, Voigt RG, Llorente AM, Peters SU, Prager TC, Zou YL, et al. Effects of early maternal docosahexaenoic acid intake on neuropsychological status and visual acuity at five years of age of breast-fed term infants. *J Pediatr*. 157(6):900–5, 2010. https://pubmed.ncbi.nlm.nih.gov/20655543/
52. Braarud HC, Markhus MW, Skotheim S, Stormark KM, Frøyland L, Graff IE, et al. Maternal DHA Status during Pregnancy Has a Positive Impact on Infant Problem Solving: A Norwegian Prospective Observation Study. *Nutrients*. 10(5), 2018. https://pubmed.ncbi.nlm.nih.gov/29695097/
53. Neuringer M, Connor WE, Van Petten C, Barstad L. Dietary omega-3 fatty acid deficiency and visual loss in infant rhesus monkeys. *J Clin Invest*. 73(1):272–6, 1984. https://doi.org/10.1172/JCI111202
54. Church MW, Jen KLC, Dowhan LM, Adams BR, Hotra JW. Excess and deficient omega-3 fatty acid during pregnancy and lactation cause impaired neural transmission in rat pups. *Neurotoxicol Teratol*. 30(2):107–17, 2008. https://doi.org/10.1016/j.ntt.2007.12.008
55. Krauss-Etschmann S, Shadid R, Campoy C, Hoster E, Demmelmair H, Jiménez M, et al. Effects of fish-oil and folate supplementation of pregnant women on maternal and fetal plasma concentrations of docosahexaenoic acid and eicosapentaenoic acid: A European randomized multicenter trial. *American Journal of Clinical Nutrition*. 85(5):1392–400, 2007. https://pubmed.ncbi.nlm.nih.gov/17490978/
56. Bergmann RL, Haschke-Becher E, Klassen-Wigger P, Bergmann KE, Richter R, Dudenhausen JW, et al. Supplementation with 200 mg/day docosahexaenoic acid from mid-pregnancy through lactation improves the docosahexaenoic acid status of mothers with a habitually low fish intake

and of their infants. *Ann Nutr Metab*. 52(2):157–66, 2008. https://pubmed.ncbi.nlm.nih.gov/18446020/
57. Francis EC, Dumolt JH, Zemski-Berry K, Jansson T, Powell TL. Maternal Plasma Choline Levels Are Positively Correlated with Maternal and Placental Phospholipid-DHA Content in Females with Obesity Who Receive DHA Supplementation. *Journal of Nutrition*. 155(3):880–9, 2025. https://pubmed.ncbi.nlm.nih.gov/39742970/
58. Chytrova G, Ying Z, Gomez-Pinilla F. Exercise contributes to the effects of DHA dietary supplementation by acting on membrane-related synaptic systems. *Brain Res*. 1341:32–40, 2010. https://pubmed.ncbi.nlm.nih.gov/19446534/
59. Nguyen LN, Ma D, Shui G, Wong P, Cazenave-Gassiot A, Zhang X. Mfsd2a is a transporter for the essential omega-3 fatty acid docosahexaenoic acid. *Nature*. 509, 2014. https://doi.org/10.1038/nature13241
60. Prieto-Sánchez MT, Ruiz-Palacios M, Blanco-Carnero JE, Pagan A, Hellmuth C, Uhl O, et al. Placental MFSD2a transporter is related to decreased DHA in cord blood of women with treated gestational diabetes. *Clin Nutr*. 36(2):513–21, 2017. ://pubmed.ncbi.nlm.nih.gov/26869380/
61. Delhaes F, Giza SA, Koreman T, Eastabrook G, Mckenzie CA, Bedell S, et al. Altered maternal and placental lipid metabolism and fetal fat development in obesity: Current knowledge and advances in non-invasive assessment. *Placenta*. 69:118–24, 2018. ://doi.org/10.1016/j.placenta.2018.05.011

Supplemental Materials

Table S1. Significantly different lipids between active and inactive groups, with full lipid names.

Lipid Species	Full Name	Log_2 Fold-Change (Active/Inactive)	FDR-Adjusted P-Value
CE 16:0	Cholesteryl palmitate	2.71292906	< 0.001
CE 18:2	Cholesteryl linoleate	-7.6741255	< 0.001
Cer_NS d18:1_24:0	N-lignoceroyl-sphingosine	3.67958909	< 0.001
Cer_NS d18:2_24:1	N-nervonoyl-sphingadienine	-2.1777691	< 0.001
DG 16:0_18:1	Diacylglycerol (palmitoyl/oleoyl)	-0.2695521	0.003
DG 16:0_20:3	Diacylglycerol (palmitoyl/eicosatrienoyl)	-0.6531549	0.040
DG 16:0_22:6	Diacylglycerol (palmitoyl/docosahexaenoyl)	1.65376834	< 0.001
EtherPE 18:1e_18:1	Plasmenylethanolamine (oleyl/oleoyl)	3.9338798	< 0.001
EtherPE 18:2e_20:4	Plasmenylethanolamine (linoleyl/arachidonoyl)	5.60857306	< 0.001
FA 16:0	Palmitic acid	4.05226606	< 0.001
FA 18:1	Oleic acid	4.29421084	< 0.001
HexCer_NDS d40:1	Hexosyl-dihydroceramide (d40:1)	2.38834267	< 0.001
PC 16:0_16:2	Phosphatidylcholine (palmitoyl/hexadecadienoyl)	5.07094122	< 0.001
PE 18:1_22:5	Phosphatidylethanolamine (oleoyl/docosapentaenoyl)	3.69513435	< 0.001
PG 18:0_22:6	Phosphatidylglycerol (stearoyl/docosahexaenoyl)	0.61047667	0.016
PG 18:2_18:2	Phosphatidylglycerol (dilinoleoyl)	-1.9133438	< 0.001
PG 20:4_22:6	Phosphatidylglycerol (arachidonoyl/docosahexaenoyl)	2.64569939	< 0.001
PG 22:5_22:6	Phosphatidylglycerol (docosapentaenoyl/docosahexaenoyl)	0.62943857	0.016
PG 22:6_22:6	Phosphatidylglycerol (didocosahexaenoyl)	0.77564848	0.015
PI 16:0_16:0	Phosphatidylinositol (dipalmitoyl)	2.06009574	< 0.001
PI 16:0_18:1	Phosphatidylinositol (palmitoyl/oleoyl)	0.9424202	0.037
PI 18:0_20:4	Phosphatidylinositol (stearoyl/arachidonoyl)	0.64377905	0.048
PI 18:0_22:6	Phosphatidylinositol (stearoyl/docosahexaenoyl)	0.60065509	0.011
PI 18:1_20:4	Phosphatidylinositol (oleoyl/arachidonoyl)	0.75196621	0.045
PI 18:2_20:4	Phosphatidylinositol (linoleoyl/arachidonoyl)	0.87358523	0.001
PS 16:0_16:0	Phosphatidylserine (dipalmitoyl)	0.76026867	0.015
PS 16:0_18:2	Phosphatidylserine (palmitoyl/linoleoyl)	0.96276284	0.026
PS 16:0_20:4	Phosphatidylserine (palmitoyl/arachidonoyl)	1.15227868	< 0.001
PS 18:0_20:4	Phosphatidylserine (stearoyl/arachidonoyl)	0.87203314	< 0.001
PS 18:0_22:5	Phosphatidylserine (stearoyl/docosapentaenoyl)	2.51775411	< 0.001

Lipid Species	Full Name	Log_2 Fold-Change (Active/Inactive)	FDR-Adjusted P-Value
PS 18:0_22:6	Phosphatidylserine (stearoyl/docosahexaenoyl)	0.82921231	0.031
SM d36:0	Sphingomyelin (d18:1/18:0 or d18:0/18:0)	-2.3233438	< 0.001
TG 16:0_18:0_18:1	Triacylglycerol (palmitoyl/stearoyl/oleoyl)	3.91491556	< 0.001
TG 18:0_18:1_18:1	Triacylglycerol (stearoyl/dioleoyl)	2.61088537	< 0.001

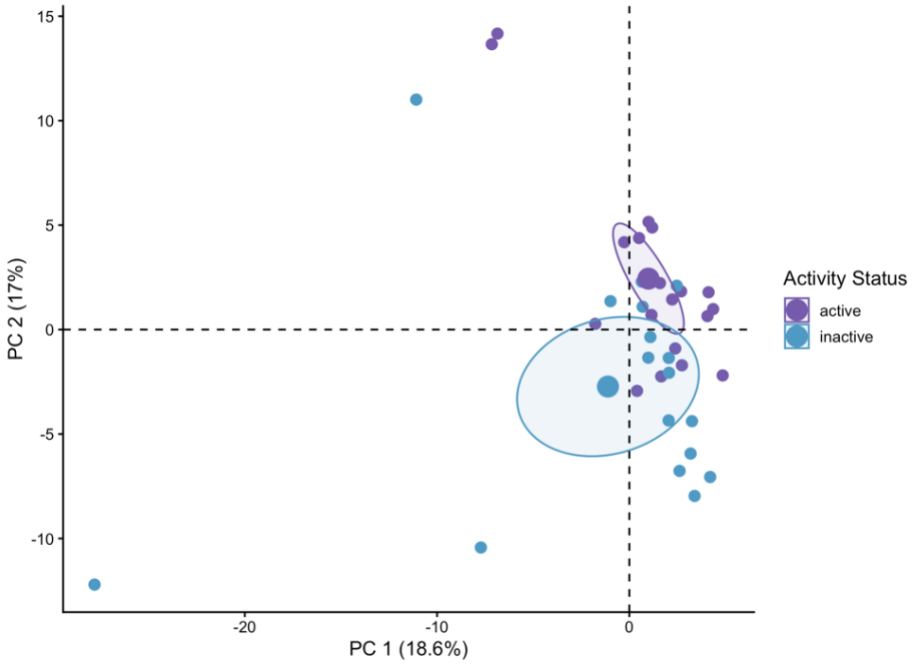


Figure S1. PCA score with 95% confidence of placental lipidome in active vs. inactive pregnancies.

MS Additional Supporting information:

The following source parameters on an Agilent 6546 QToF were used for all analysis, gas temp 200 °C, drying gas 10 L/min, nebulizer 50 psi, sheath gas temp 300 °C, sheath gas flow 12 L/min, VCap 3500 V, fragmentor 150 V, skimmer 75 V, Oct 1 RF Vpp 750 V, mass range of *m*/*z* 40–1700 with an acquisition rate of 3 spectra/s. A data-dependent MS/MS acquisition method previously used [i] was adapted for all experiments and used the following settings: quadrupole isolation width of 1.3 *m*/*z*, 10 precursors/cycle, absolute threshold 5000 counts,

active exclusion enabled after 1 spectrum and released after 0.15 min, abundance dependent accumulation of 25,000 counts/spectrum, purity stringency of 70% and a purity cutoff of 0%. A static exclusion list of 40 to 250 m/z was used for the MS/MS iterative injections.

[1] Roberts, J.A.; Godbout, E.; Menard, J.A.; Boddy, C.N.; Diallo, J-S; Smith, J.C. Comprehensive Untargeted Lipidomic Profiling of Third Generation Lentiviral Vectors and Packaging Cells. Molecular Omics. **October 1, 2024,** 20 (10), 642-653, https://doi.org/10.1039/D4MO00052H

Preamble to Chapter 3:

The manuscript entitled *The Impact of an* in vitro *Model of Physical Activity on Docosahexaenoic Acid Uptake in Human Primary Trophoblast Cells* is formatted for submission to *Placenta*. This manuscript addresses the second aim of this thesis. Throughout, we investigate a novel cell culture model of PA to assess DHA uptake under various conditions.

Contributions: I was responsible for the conceptual design and primary authorship of this study. I conducted benchwork with assistance from Lena Neuper and Michael Gruber, with the exception of mass spectrometer operations, which were performed by the Mass Spectrometry Core Facility at the Medical University of Graz. Additionally, I performed all data processing, statistical analysis, and figure preparation. I drafted the initial manuscript, to which all co-authors contributed revisions.

Chapter 3: The Impact of an *In Vitro* Model of Physical Activity on Docosahexaenoic Acid Uptake in Human Primary Trophoblast Cells

Research Article

Meaghan MacDonald[1], Martin Gauster[2], Lena Neuper[2], Michael Gruber[2], Kristi B. Adamo[1]

[1] School of Human Kinetics, Faculty of Health Sciences, University of Ottawa, Ottawa, ON, Canada

[2]Division of Cell Biology, Histology, and Embryology, Medical University of Graz, Graz, Styria, Austria

Correspondence: Dr. Kristi B. Adamo (kadamo@uottawa.ca)

Highlights

- Oxygen tension and shear stress levels modulate DHA uptake by primary human trophoblasts
- DHA uptake is reduced under intermittent hypoxia and high shear stress conditions
- Intermittent hypoxia and shear stress may not fully model physical activity-related placental exposure

Abstract: Physical activity during pregnancy confers substantial benefits to both gestational parents and offspring, though the mechanisms responsible for imparting these benefits remain unexplained. As the placenta is the primary point of interaction between parent and fetus, it is a likely mediator of physical activity. Docosahexaenoic acid (DHA), an omega-3 fatty acid essential for fetal brain and retinal development, is a key nutrient of interest during pregnancy. This study examined the impact of intermittent hypoxia and high shear stress, used as proxies for physical activity, on placental DHA uptake in a primary human trophoblast cell culture model. Cells isolated from four independent donors were differentiated and exposed to one of six conditions varying in intermittent hypoxia and shear stress levels. After two days of conditioning, DHA-supplemented media was circulated for 1 hour. Cells were lysed and lipids were extracted. DHA content was determined by gas chromatography–mass spectrometry analysis, and normalized to an internal standard and total protein content. Static and normoxic conditions yielded greater intracellular DHA content than intermittent hypoxia and high shear stress (p = 0.008), as well as intermittent hypoxia and low shear stress (p = 0.003). These findings may reflect an inability of the model to accurately reflect placental physical activity conditions in vivo. Future studies should look at DHA transport as well as uptake to create a more comprehensive understanding of these mechanisms and the utility of this model.

Keywords: placenta; docosahexaenoic acid; physical activity; intermittent hypoxia; shear stress

Introduction

During pregnancy, the placenta develops to perform several functions critical to supporting both the mother[a] and the fetus. This impressive organ serves as the maternal-fetal interface, and is responsible for gas, nutrient and waste exchange, as well as endocrine and immune functions [1]. Nutrient transport of energy substrates, such as glucose, fatty acids, and amino acids, is primarily performed by the syncytiotrophoblast (STB), the outermost cell layer of placental villi which are in direct contact with maternal circulation [2]. These nutrients serve as building blocks both for the developing fetus and the placenta itself [3]. Docosahexaenoic acid (DHA), an essential omega-3 fatty acid, is critical for fetal brain, nervous system, and retinal development [4]. Composing up to 50% of phospholipid fatty acids in these tissues, DHA plays an indispensable role in healthy fetal growth [4]. Higher levels of DHA in the fetal and neonatal periods have been linked to positive long-term offspring outcomes such as higher cognitive performance, problem-solving ability, and visual acuity, as well as reduced problem behaviour and lower risk of allergy development [5–9]. Conversely, DHA deficiency is associated with pregnancy complications like gestational diabetes and preeclampsia, with adverse effects that may persist into childhood [10–12]. Thus, proper supply and transport of this essential fatty acid is integral to optimizing pregnancy and child health outcomes, especially during the third trimester when fetal brain growth reaches its peak [13].

Engaging in physical activity (PA) throughout pregnancy is a highly advantageous behaviour. The Canadian guidelines recommend pregnant individuals accumulate a minimum of 150 minutes of moderate PA per week to promote the associated benefits [14]. Meeting these

[a] Maternal and/or mother, or other gender derived terminology referred to with pregnancy denotes all individuals with the biological capacity for pregnancy, including gender-diverse people.

recommendations can significantly reduce the risk of gestational diabetes, preeclampsia, instrumental delivery, and excessive gestational weight gain [15–17], as well as improve psychological well-being [18, 19]. Offspring are also positively impacted, as gestational PA is associated with healthier neonatal adiposity and a decreased risk of metabolic disease [20–22]. Evidently, PA has a net positive effect in pregnancies without contraindications.

What is yet unclear are the physiological mechanisms underlying these benefits. Given the placenta's central role in maternal-fetal communication and supporting the pregnancy, it is theorized that this organ is implicated in transmitting outcomes of gestational PA. As the pregnant body engages in physical activity, blood flow is redirected to the working musculature [23], which may reduce oxygen delivery to the placenta [24]. This hypothesis is supported by evidence of increased uteroplacental vascular resistance assessed via Doppler sonography [25] and reduced uterine blood flow in ewes [26]. Once PA has ceased, blood flow would then rapidly return to the intervillous space, restoring oxygen levels and exposing STB to a transient increase in shear stress [27]. We therefore propose that intermittent hypoxia and high shear stress are defining features of gestational PA, potentially triggering placental alterations that contribute to its wide-ranging benefits.

Sufficient DHA uptake and delivery promote healthy pregnancies and offspring. The literature suggests that PA increases DHA transporter expression and DHA-related functions at the blood-brain barrier [28, 29], though this relationship has yet to be examined in the placenta. It is plausible that greater placental DHA uptake and delivery contribute to the favourable outcomes of gestational PA. To explore this possibility, we utilized a novel *in vitro* model simulating intermittent hypoxia and high shear stress as proxies for gestational PA, enabling functional analysis of DHA uptake in primary STB.

Materials and Methods

Cell Culture

Primary human cytotrophoblasts (CTBs) (CTBPRO2®, Amnion Foundation, USA) harvested from four distinct donors (Table 1) were cultured in CTBPRO2GRO® cell media (Amnion Foundation, USA) with 5% fetal bovine serum (FBS) (HyClone, cat. SV30160.03) and supplemented with 1x penicillin-streptomycin (Gibco, cat. 15140-122) and 0.1mM beta-mercaptoethanol. As cells were commercially available and without identifiers, no further ethics were required. During proliferation, CTBs were cultured at 37°C, 2% O_2, and 5% CO_2 [30]. All culture ware was coated in human collagen type IV (Sigma-Aldrich, cat. C5533).

To differentiate CTBs into STB, CTBs were seeded on glass slides in 6-well plates at a density of $1.2x10^6$ cells per well. Initially, cells were seeded in CTBPRO2GRO® media to promote adherence to the well; after seven hours, the cells were gently washed with PBS, then switched to DMEM (1x) high glucose media (Gibco™, cat. 11995065) with 10% FBS and 1% penicillin-streptomycin. They were incubated for 72 h at 37°C, 10% O_2, and 5% CO_2 to allow for syncytialization [30].

Table 1. Trophoblast Cell Donor Characteristics

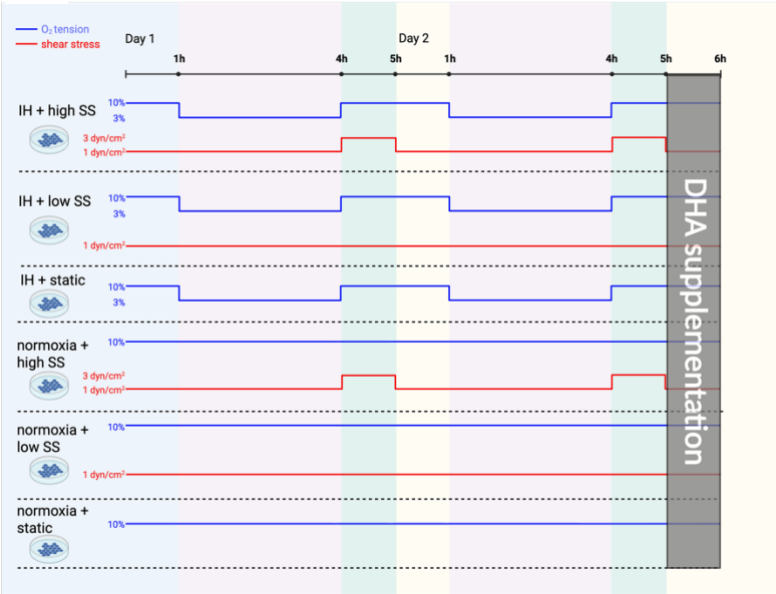
Maternal Age (years)	32.5 (7.9)
Maternal Height (m)	1.6 (0.1)
Maternal Weight (kg)	102.4 (5.1)
Infant Gestational Age (weeks)	38.4 (1.9)
Infant Weight (g)	3465.0 (1047.1)
Infant Sex (M/F)	4/0
Delivery Mode (c-section/vaginal)	1/3

Note. Data are presented as mean (standard deviation). Maternal weight was measured at admittance to hospital for delivery. n = 4.

Fluidic Flow Culture

Following differentiation and formation of STB, glass slides were either transferred from 6-well plates into FlowTiss flow chambers (Flow Cell Innovations, Switzerland) or retained in the original 6-well plates to serve as static controls. Each chamber was connected via tubing (Kirkstall; 2 x 1/16" 7 diameter 22 cm length, 1 x 3/32" diameter 22 cm length) to a 30 mL reservoir bottle, with each chamber-bottle forming its own circuit. Bottles and wells were filled with 8 mL of DMEM (1x) high glucose supplemented with 10% FBS and 1% penicillin-streptomycin. Flow experiments were conducted in a TEB500 flow bioreactor (Ebers Medical Technology SL, Zaragoza, Spain), maintained at 37°C, 10% O_2, and 5% CO_2, with flow rates at 1.2 mL/min (equating to ~1 dyne/cm^2) unless otherwise specified in the experimental conditions. Cells from each biological replicate were exposed to the following six conditions, respectively: (1) normoxia + low shear stress (SS); (2) normoxia + intermittent high SS; (3) normoxia + static conditions; (4) intermittent hypoxia (IH) + low SS; (5) IH + intermittent high SS; (6) IH + static conditions (Figure 1). Normoxia was defined as 10% O_2 and hypoxia as 3% O_2 [31] where bouts of IH lasted three hours. The bioreactor pumps were set to flow rates of 1.2mL/min for low SS and 3.6mL/min (~3 dyne/cm^2) for high SS, both within physiological ranges [32]. Bouts of high SS lasted 1 h before returning to low SS flow rates. In condition 5, SS was increased immediately following the 3 h of IH exposure. To mimic chronic PA rather than an acute bout, cells were exposed to experimental conditions twice over two consecutive days before DHA uptake assays commenced.

a.



b.

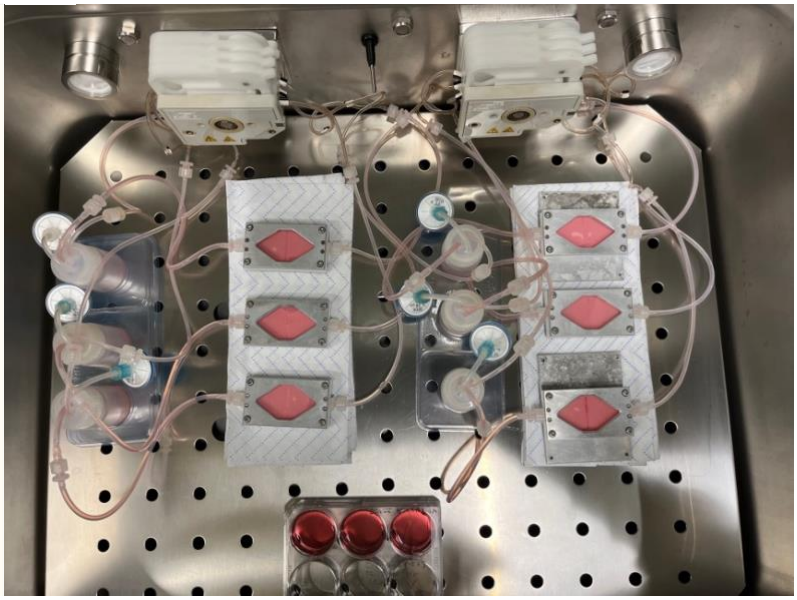


Figure 1. Overview of STB cell culture system. a) Schematic representation of the experimental design, illustrating the timing of bouts of intermittent hypoxia (IH) and application of varying shear stress (SS) levels. Image created with Biorender. b) Image of the experimental setup within the TEB500 flow bioreactor. Each reservoir bottle was connected to an independent cell chamber, generating three separate circuits and technical replicates per pump. The two pumps were operated at distinct flow rates, allowing for concurrent high- and low- SS conditions. Cells maintained under static conditions were housed in the bioreactor (pictured at bottom of image) to maintain consistent oxygen exposure across conditions.

DHA Uptake

Stock solutions were prepared in advance: 6% fatty acid free bovine serum albumin (BSA; Millipore Sigma, USA) in DMEM (1x high glucose, supplemented with 1x penicillin-streptomycin), and 0.5% fatty acid free BSA in PBS. Docosahexaenoic acid (DHA; Sigma Aldrich, USA) was diluted at 100 mg/mL in ethanol, aliquoted, and stored at -20°C until use. Immediately prior to uptake experiments, DHA was prepared according to Tobin *et al* [33]. Briefly, DHA was complexed with 6% BSA in DMEM by incubation in a 37°C water bath for 10 minutes. The

complex was then diluted in DMEM to achieve final concentrations of 100 uM DHA and 150 uM (1%) BSA. The ethanol concentration in the final solution was <0.03%.

After completion of the second day of experimental exposures, media was removed from reservoir bottles and wells and replaced with 8 mL of 0.5% BSA in PBS to wash cells. Following complete circulation of the wash solution, PBS was removed and replaced with 8 mL of the DHA complex solution, which was incubated for 1 h. Cells were then removed from flow chambers and the bioreactor, washed 3x with 0.5% BSA in PBS, and lysed with cold 0.2 M NaOH. Lysates were stored at -80°C until analysis by gas-chromatography mass spectrometry (GC-MS).

To assess potential DHA loss due to adherence to tubing, the DHA complex was circulated for 1 h in the absence of a biological sample under both flow and static conditions. Media samples were subsequently analyzed for DHA content via GC-MS.

Lipid Extraction

Lipids were extracted from 400 µL of cell lysate or 100 µL of cell media. Each sample was spiked with 50 µL of a 1mM C17:0 internal standard diluted in $CHCl_3$/MeOH. Next, 1.5mL MeOH and 5 mL of methyl tert-butyl ether (MTBE) were added, samples were vortexed for 10 seconds, then placed on ice in an ultrasonic bath for 10 min. Samples were placed in an overhead shaker for 10 min, then centrifuged for 10 min (2300 rpm; room temperature). The upper phase was collected into fresh glassware, while the lower phase was re-extracted with 2 mL of MTBE/MeOH/A.dest (10:3:2.5 v/v/v), followed by centrifugation (10 min, 2300 rpm; room temperature). The upper phase from the second extraction was combined with the first and dried overnight in a vacuum centrifuge.

For GC-MS analysis, methyl ester derivatization was performed. Dried extracts were treated with 1mL of methanolic NaOH, vortexed, then placed at -80°C for 10 min, followed by 5

min on ice. This process was repeated, substituting 1 mL of boron trifluoride instead of NaOH. Next, 1 mL saturated NaCl and 2 mL cyclohexane were added, vortexed, and placed in an overhead shaker for 5 min. Samples were centrifuged for 4 min (2500rpm; room temperature), and 1800 µL of the upper phase was transferred to fresh glassware. The remaining sample was re-extracted beginning with the addition of 2 mL cyclohexane step. The resultant upper phase was combined with the previous upper phase and evaporated under a constant stream of N_2 gas. Finally, 350 µL cyclohexane was added to the dried sample, which was transferred to vials for mass spectrometry analysis.

GC-MS

DHA was analyzed using a single quadrupole GC-MSD5977 system (Agilent Technologies, USA) operating in electron ionization mode. Separation was performed on a TRACE TR-FAME capillary column (30 m x 0.25 mm 0.25µm; Thermo Fisher Scientific, USA) with helium as the carrier gas at a constant flow rate of 1 mL/min. Samples were injected at a volume of 1 µL in 10:1 split mode. The inlet was maintained at 250°C and the transfer line at 200°C. The oven temperature program was as follows: 150°C (0.5min); ramped at 10°C/min to 180°C; ramped at 0.5°C/min to 190°C; then ramped at 40°C/min to 250°C with a 3 min hold.

Spectra were acquired in both full scan (m/z 40-800) and selected-ion monitoring modes (50 ms/ion). DHA was identified based on retention time and characteristic fragment ions (m/z 342). Quantification was performed by normalizing DHA peak area to that of the internal standard (C17:0). Data acquisition and processing were carried out using Agilent MassHunter software. Results were normalized to protein content per sample, as determined by BCA assay, to account for variation in cell number.

Immunofluorescence Staining

To assess syncytialization, CTBs cultured under the pre-flow protocol described above were compared to CTBs cultured in parallel in CTBPRO2GRO® media at 2% O_2 (negative control; no syncytialization). Glass slides were washed with PBS and fixed in ice-cold acetate for 5 minutes, then rehydrated with PBS. Ultra V Block (Thermo Scientific Labvision, TA-125-PBQ) supplemented with 10% human AB-serum was applied for 7 minutes to block nonspecific background staining.

Half of each slide was double stained with beta-human chorionic gonadotropin (β-hCG) polyclonal rabbit antibody (1:100) and E-cadherin (E-Cad) monoclonal mouse antibody (1:200). The other half was double stained with E-Cad monoclonal rabbit antibody (1:200) and cytokeratin-7 (CK7) monoclonal mouse antibody (1:200) (Table 2). Primary antibodies were diluted in antibody diluent (Agilent, S302283-2) and incubated for 30 minutes at room temperature. After four PBS washes, Alexa Fluor™ goat anti-mouse (1:200) and anti-rabbit (1:200) secondary antibodies were incubated for 30 minutes at room temperature. Slides were then stained with DAPI (Thermo Scientific Invitrogen, D21490) for 5 minutes, dried, and mounted using Prolong™ Gold Antifade Mountant (Thermo Fisher Invitrogen, P36930). Imaging was performed using Olympus SLIDEVIEW VS200 and qualitatively analyzed with OlyVIA software (version 3.4.1).

Table 2. Antibody details and uses for staining CTBs and STB.

Antibody	Product	Purpose	Dilution
	Double Stain 1		
CK7 monoclonal mouse antibody	Thermo Fisher Scientific (cat. MA5-11986)	Pan trophoblast marker	1:200
E-Cad monoclonal rabbit antibody	Cell Signaling (cat. 3195)	Cell membrane marker	1:200
goat anti-mouse IgG (H+L) Alexa Fluor™ 555	Invitrogen (cat. A32727)	Ab2 for CK7	1:200
goat anti-rabbit IgG (H+L) Alexa Fluor™ 633	Invitrogen (cat. A21070)	Ab2 for E-Cad	1:200
	Double Stain 2		
β-hCG polyclonal rabbit antibody	ProteinTech (cat. 11615-1-AP)	Marker of differentiation (secreted by STB)	1:100
E-Cad monoclonal mouse antibody	Cell Signaling (cat. 14472)	Cell membrane marker	1:200
goat anti-rabbit IgG (H+L) Alexa Fluor™ 555	Invitrogen (cat. A21428)	Ab2 for β-hCG	1:200
goat anti-mouse IgG (H+L) Alexa Fluor™ 633	Invitrogen (cat. A21050)	Ab2 for E-Cad	1:200

Note. Ab2 = secondary antibody.

Statistical Analysis

All experiments were performed in triplicate, and results were averaged. Data analysis was conducted using R (version 4.5.0). Normality was assessed with the Shapiro-Wilk test. As the data were non-normal ($p < 0.05$) and right skewed, differences in DHA uptake between experimental conditions were evaluated using a generalized linear mixed effects model with gamma regression and log link. Donor identity was included as a random intercept to account for within-subject correlation. Model diagnostics were performed using simulation-based residuals to confirm appropriate variance and the absence of influential outliers. Post-hoc comparisons were conducted using Tukey's method, and significance was established as $p < 0.05$.

Results

Syncytialization of Primary CTBs in Culture Conditions

Immunofluorescent staining allowed for qualitative analysis of E-Cad, CK7 and β-hCG expression in cells exposed to conditions promoting syncytialization, i.e. formation of multinucleated syncytia (cultured in DMEM at 37°C, 10% O_2, and 5%CO_2 for 72h) compared to undifferentiated mononucleated control cells (cultured in CTBPRO2GRO® media at 37°C, 2% O_2 and 5%CO_2 for 72h). Both undifferentiated and differentiated cells expressed CK7, confirming epithelial-like trophoblast lineage (Figure 3b, e). In controls, E-Cad localized to membrane boundaries of a largely mononucleated cell population (Figure 2a; Figure 3a). In cells encouraged to differentiate, the breakdown of cell boundaries was noted (Figure 2d, Figure 3d). Minimal β-hCG expression was observed in undifferentiated cells (Figure 2b), becoming more evident in differentiated cells, consistent with presence of a functional STB (Figure 2e).

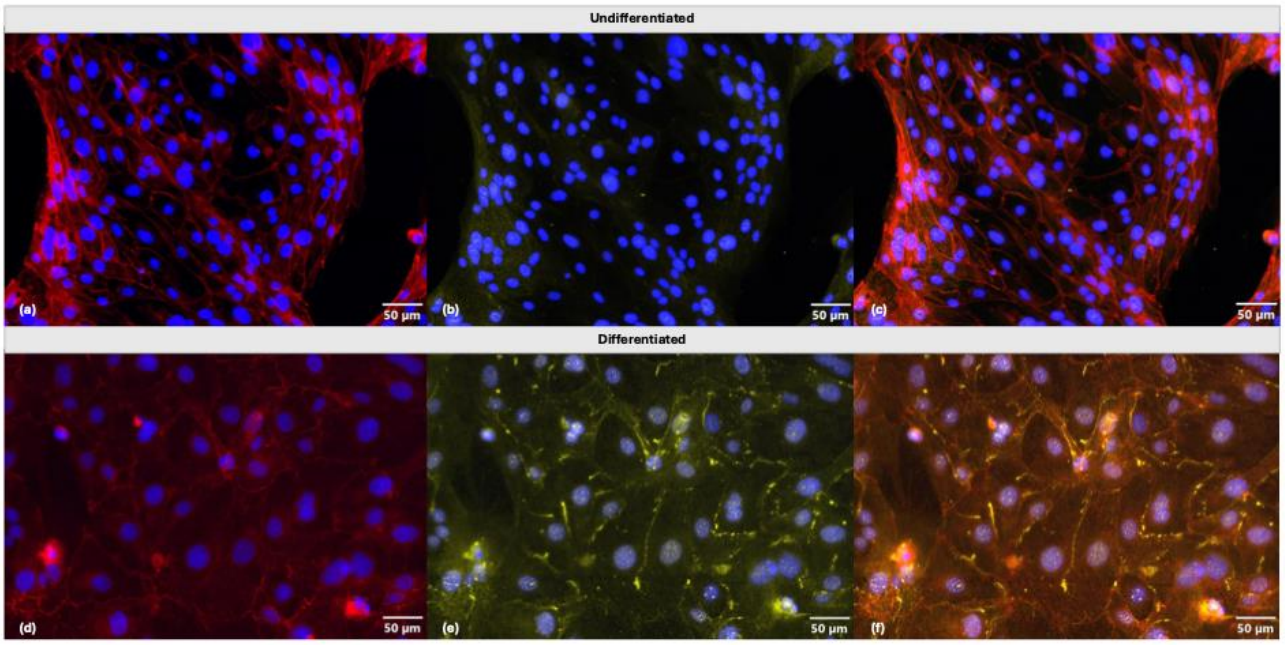


Figure 2. Immunofluorescent staining of E-Cad (a, d; red) and β-hCG (b, e; yellow) on undifferentiated (a-c) and differentiated (d-f) primary trophoblasts isolated from human term placenta. Panels (c) and (f) show merged images. Nuclei are stained with DAPI (blue). Scale bar = 50µm.

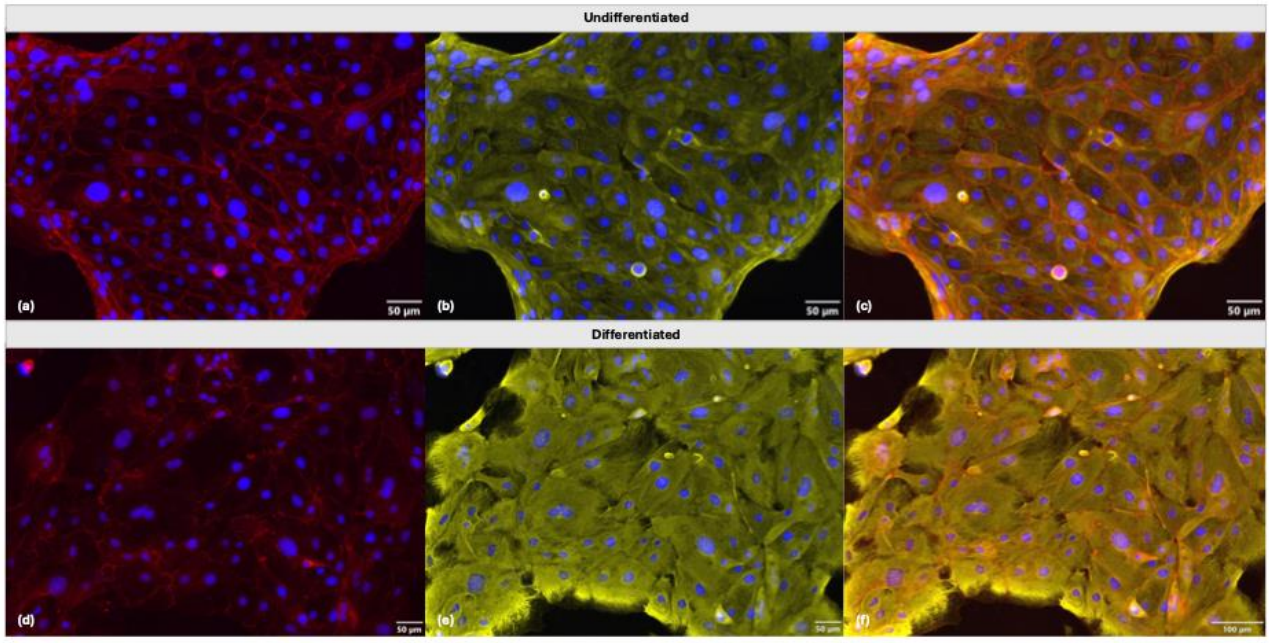


Figure 3. Immunofluorescent staining of E-Cad (a, d; red) and CK7 (b, e; yellow) on undifferentiated (a-c) and differentiated (d-f) primary trophoblasts isolated from human term placenta. Panels (c) and (f) show merged images. Nuclei are stained with DAPI (blue). Scale bar = 50µm.

Uptake of DHA by STB

Using the TEB500 flow bioreactor, we exposed each of the four biologically distinct primary cell populations to six different experimental conditions in triplicate. DHA abundance in cell lysates was quantified via GC-MS. Two outliers were excluded from the dataset due to extreme DHA or internal standard raw values likely reflecting technical failure (one from the normoxia + low SS group, and one from the normoxia + high SS group), and technical replicates were averaged prior to statistical analysis. The model revealed a significant main effect of experimental condition on DHA uptake (p = 0.0018). *Post-hoc* pairwise comparisons indicated that DHA uptake was significantly greater in the normoxia + static condition compared to both IH + high SS (p = 0.008) and IH + low SS conditions (p = 0.003) (Figure 4). DHA uptake in the normoxia + high SS condition was also greater than IH + low SS, although this difference did not reach statistical significance (p = 0.052).

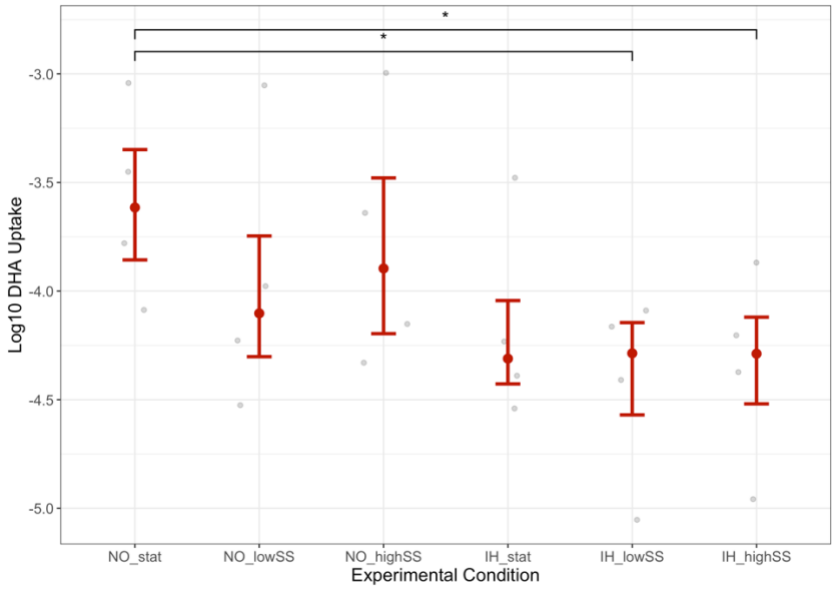


Figure 4. Uptake of DHA by STB in various cell culture conditions following 1 hour of incubation with DHA-supplemented media. The x-axis represents the experimental conditions, and the y-axis shows the abundance of DHA, normalized to C17:0 and protein content, and log transformed. Data are presented as mean DHA abundance ± SD from four biological replicates, each run in triplicate.

* = $p < 0.05$. *NO_stat = normoxia + static; NO_lowSS = normoxia + low shear stress; NO_highSS = normoxia + high shear stress; IH_stat = intermittent hypoxia + static; IH_lowSS = intermittent hypoxia + low shear stress; IH_highSS = intermittent hypoxia + high shear stress*.

DHA Abundance in Media-Only Static vs. Flow Conditions

To assess potential DHA loss under flow conditions, we conducted an experiment in which DHA-supplemented media was incubated for 1 hour under both flow and static conditions without cells, each maintained in normoxia. Lipid extraction was performed on media samples, followed by DHA quantification. The mean DHA/C17:0 value was slightly higher in static vs. flow conditions; however, no significant differences were found (Figure 5).

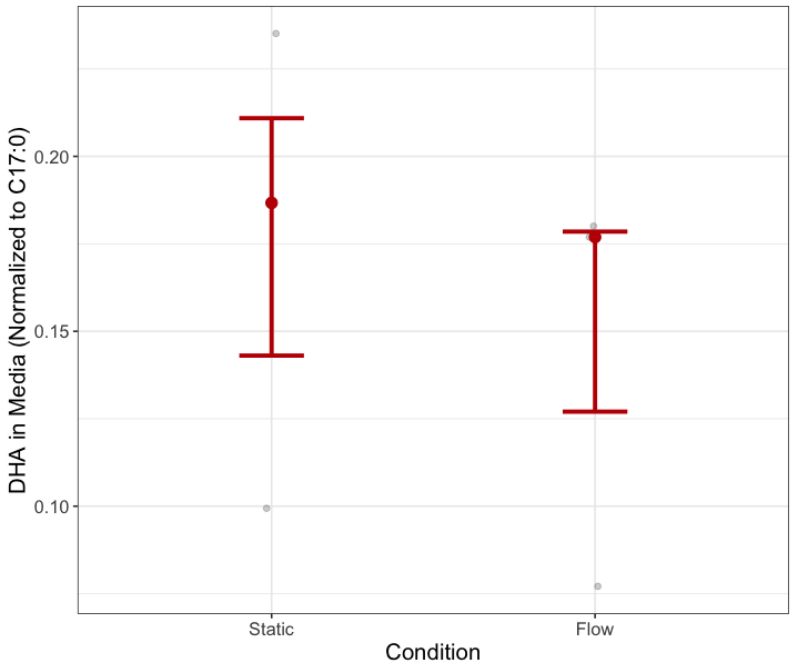


Figure 5. DHA content in media samples from flow and static conditions. The *x*-axis represents the experimental condition, and the *y*-axis represents DHA peak area normalized to C17:0 peak area.

Discussion

This study aimed to simulate PA *in vitro* by applying intermittent hypoxia and high shear stress, to functionally analyze DHA uptake by STB. We utilized primary human CTBs in combination with a flow bioreactor to target a better representation of the *in-vivo* environment, and DHA content was measured using GC-MS. We hypothesized that, given the critical importance of DHA transport to the fetus during pregnancy, and the known benefits of gestational PA, IH and high SS (used here as proxies for gestational PA) would yield the greatest DHA uptake. Contrary to expectations, the normoxia + static condition, which served as a control, showed significantly greater DHA uptake compared to IH + high SS and IH + low SS conditions.

Placental oxygen tension varies throughout pregnancy depending on gestational stage [31]. In early pregnancy, trophoblasts experience much lower oxygen tension, with a pO_2 around < 20 mmHg (~2.5% O_2), which rises to 40-80 mmHg (~8-10% O_2) in the second and third trimester [34, 35]. Estimates suggest that around 8% O_2 may be reasonable cell culture conditions for a fully developed STB, while pathologic hypoxia is closer to 1-2% O_2. As *in vivo* O_2 tension measurements in human pregnancies are complex, these values remain approximations. For this experiment, normoxia was defined as 10% O_2 and hypoxia as 3% O_2, to maximize separation between the two oxygen tensions while remaining within physiologically relevant, non-pathological ranges.

The shear stress values were selected using the same logic. Given that the architecture of the intervillous space inherently produces highly heterogenous shear stress, computational fluid dynamics estimate averages of 0.5±0.2 to 2.3±1.1 dyn/cm^2 in the third trimester [32]. We therefore selected flow rates corresponding to 1dyn/cm^2 for low shear stress, to mimic regular blood flow at

rest, and 3dyn/cm^2 for high shear stress, to mimic the rapid return of blood to the intervillous space following PA.

As we proposed IH and high SS to be mediators of PA, we expected these conditions to enhance DHA uptake. Instead, significant effects were observed in two of the IH conditions, both showing reduced uptake compared to normoxia + static. A third comparison (IH + low SS compared to normoxia + high SS) showed a near-significant reduction. These results imply that IH did not improve DHA uptake. Supporting our original hypothesis that PA may promote fetal DHA availability, unpublished data from our lab indicate that several DHA-containing lipid species have higher abundance in placentas from physically active pregnancies compared to inactive. It is possible that this incongruity lies in the *in vitro* modeling of PA. In alignment with this idea, a recent study from our group investigated whether IH could account for the higher proportion of anti-inflammatory 'type' Hofbauer cells observed in placentas from physically active vs inactive pregnancies [36, 37]. However, IH exposure in a cell culture model did not significantly alter Hofbauer cell polarization, suggesting that IH may not be an appropriate *in vitro* proxy for the physiological adaptations associated with PA. Collectively, these findings warrant a re-examination of the use of IH to simulate PA.

The theory behind IH as a proxy for PA is based on the redistribution of blood flow away from the placenta and toward working musculature, supported by human and ewe models demonstrating increases in uteroplacental vascular resistance and reductions in uterine artery blood flow [25, 26]. What this theory may be failing to account for, however, is the shift in hemodynamics associated with PA. Cardiac output (Q) – the amount of blood the heart pumps per minute – is a combination of heart rate and stroke volume. Both variables, and consequently Q, increase with the onset of PA to ensure sufficient O_2 delivery to working tissue [23]. It is possible

that although the redistribution of blood flow to contracting musculature and the heart may reduce relative blood flow to the intervillous space, the absolute volume may be maintained, thus guarding STB from a hypoxic environment. This protective mechanism is seen in the brain, where absolute cerebral blood flow remains stable or even slightly increased in response to PA [38, 39]. Moreover, acute and transient hemoconcentration occurs during PA, with reductions in plasma volume driving greater red blood cell concentration [40]. Evidence of PA-induced hemoconcentration in pregnant humans and sheep [41, 42], supports the possibility of maintained O_2 delivery to the intervillous space. Direct observation of maternal-fetal hemodynamics during PA in human pregnancy remains technically and ethically challenging.

Given the recent findings of our lab, shear stress, an identified mechanism of exercise-induced improvements in vascular function [43], may be a more plausible stimulus for future PA and placenta-related investigation. Gestational exercise training has been shown to increase placental endothelial nitric oxide synthase and nitric oxide [44], both of which are related to vascular function, supporting a possible link between shear stress and gestational PA that warrants further exploration.

This study combined flow culture, physiological oxygen tension, and primary trophoblasts in a novel model. We were, however, limited to analyzing DHA uptake rather than DHA transport. As DHA is preferentially shuttled to the fetus [45], cellular uptake may not be a linear reflection of the rate at which this essential fatty acid crosses the syncytium. BeWo cell studies show oleic acid induces greater triacylglycerol accumulation, while DHA remains largely in the free fatty acid pool, potentially facilitating efflux to fetal circulation [46]. If the STB is primed to transport DHA as efficiently as possible, perhaps lower intracellular levels reflect less retention due to an improved capacity to transfer it across and out of the cell. If so, our findings may not be

inconsistent with the hypothesis that PA promotes fetal DHA availability. Future work should directly assess DHA transport to clarify this relationship.

The results of the immunofluorescence staining provide evidence of CTB differentiation into STB. The presence of CK7, a general marker of trophoblast lineage, in both undifferentiated and differentiated slides, demonstrates the purity of the primary cell culture [47]. As CTBs fuse to form the STB, multinucleated regions appear (2). E-Cad is localized to cell membranes, highlighting distinct borders in undifferentiated cells and breakdown of borders in differentiated cells. β-hCG, a hormone secreted by STB and identifier of syncytialization [48], was minimally expressed in undifferentiated cells but more pronounced in differentiated. Although not quantified, our findings provide qualitative evidence of successful syncytialization.

This study is limited by a small sample size, with only four biological replicates. *Post-hoc* power analysis using G*Power (version 3.1) revealed that this study was underpowered (Supplemental Table 1). Additionally, all donor cells originated from pregnancies carrying male fetuses, which is noteworthy given the evidence of sex-specific differences in placental lipid profile and metabolism [49]. For example, in pregnancies with obesity, reduced cord blood DHA was noted only with male fetuses [50], and mouse models show sex-dependent effects of PA on mRNA expression of several lipid transporters [51]. It is therefore unclear what impact this model may have on trophoblasts originating from female sex pregnancies. Strengths of this study include the large number of experimental conditions, which allowed for evaluation of flow culture, hypoxia, and shear stress individually and in varying combinations, providing insight into the overall quality of the model. Moreover, GC-MS offers high sensitivity and selectivity for derivatized fatty acids, enabling robust and reliable DHA quantification.

To our knowledge, this work is the first to model PA *in vitro* using IH and high SS. While these conditions seem to influence DHA uptake, the precise biological interpretation remains inconclusive. Future work should incorporate measures of DHA transport and continue refining *in vitro* models to better represent PA physiology *in vivo*. Such progress is essential for advancing our understanding of how PA influences placental function and transport of DHA, a nutrient critical to fetal growth and development.

Author Contributions: MM and KBA conceptualized the project. MM, MG, LN and MG designed the methodology. MM, LN and MG performed data curation. MM performed analysis and prepared the manuscript. All authors reviewed and edited the manuscript.

Funding

This work was supported by the Canadian Institutes of Health Research (CIHR) through the Canada Graduate Scholarship (CGS-M) and Michael Smith Foreign Study Supplement (MSFSS) awarded to MM.

Disclosures

The authors have no conflicts of interest, financial or otherwise, to declare.

Data Availability Statement

Data will be made available upon reasonable request.

References

1. Burton GJ, Jauniaux E. What is the placenta? *Am J Obstet Gynecol* 213: S6.e1-S6.e4, 2015. doi: 10.1016/J.AJOG.2015.07.050.
2. Knöfler M, Haider S, Saleh L, Pollheimer J, Gamage TKJB, James J. Human placenta and trophoblast development: key molecular mechanisms and model systems. *Cell Mol Life Sci* 76: 3479, 2019. doi: 10.1007/S00018-019-03104-6.
3. Hay WW. The Placenta: Not Just a Conduit for Maternal Fuels. *Diabetes* 40: 44–50, 1991. doi: 10.2337/DIAB.40.2.S44.
4. Basak S, Mallick R, Duttaroy AK. Maternal Docosahexaenoic Acid Status during Pregnancy and Its Impact on Infant Neurodevelopment. *Nutrients* 12: 1–25, 2020. doi: 10.3390/NU12123615.
5. Jensen CL, Voigt RG, Llorente AM, Peters SU, Prager TC, Zou YL, Rozelle JC, Turcich MR, Fraley JK, Anderson RE, Heird WC. Effects of early maternal docosahexaenoic acid intake on neuropsychological status and visual acuity at five years of age of breast-fed term infants. *J Pediatr* 157: 900–905, 2010. doi: 10.1016/J.JPEDS.2010.06.006.
6. Braarud HC, Markhus MW, Skotheim S, Stormark KM, Frøyland L, Graff IE, Kjellevold M. Maternal DHA Status during Pregnancy Has a Positive Impact on Infant Problem Solving: A Norwegian Prospective Observation Study. *Nutrients* 10, 2018. doi: 10.3390/NU10050529.
7. Krabbendam L, Bakker E, Hornstra G, van Os J. Relationship between DHA status at birth and child problem behaviour at 7 years of age. *Prostaglandins Leukot Essent Fatty Acids* 76: 29–34, 2007. doi: 10.1016/J.PLEFA.2006.09.004.
8. Larqué E, Gil-Sánchez A, Prieto-Sánchez MT, Koletzko B. Omega 3 fatty acids, gestation and pregnancy outcomes. *Br J Nutr* 107 Suppl 2, 2012. doi: 10.1017/S0007114512001481.
9. Helland IB, Smith L, Saarem K, Saugstad OD, Drevon CA. Maternal supplementation with very-long-chain n-3 fatty acids during pregnancy and lactation augments children's IQ at 4 years of age. *Pediatrics* 111, 2003. doi: 10.1542/PEDS.111.1.E39,.
10. Massari M, Novielli C, Mandò C, Di Francesco S, Porta M Della, Cazzola R, Panteghini M, Savasi V, Maggini S, Schaefer E, Cetin I. Multiple Micronutrients and Docosahexaenoic Acid Supplementation during Pregnancy: A Randomized Controlled Study. *Nutrients 2020, Vol 12, Page 2432* 12: 2432, 2020. doi: 10.3390/NU12082432.
11. Judge MP, Casavant SG, Dias JAM, McGrath JM. Reduced DHA transfer in diabetic pregnancies: mechanistic basis and long-term neurodevelopmental implications. *Nutr Rev* 74: 411–420, 2016. doi: 10.1093/NUTRIT/NUW006.
12. Mauro AK, Rengarajan A, Albright C, Boeldt DS. Fatty acids in normal and pathological pregnancies. *Mol Cell Endocrinol* 539, 2022. doi: 10.1016/J.MCE.2021.111466.
13. Clandinin MT, Chappell JE, Leong S, Heim T, Swyer PR, Chance GW. Intrauterine fatty acid accretion rates in human brain: implications for fatty acid requirements. *Early Hum Dev* 4: 121–129, 1980. doi: 10.1016/0378-3782(80)90015-8.
14. Mottola MF, Davenport MH, Ruchat SM, Davies GA, Poitras VJ, Gray CE, Jaramillo Garcia A, Barrowman N, Adamo KB, Duggan M, Barakat R, Chilibeck P, Fleming K, Forte M, Korolnek J, Nagpal T, Slater LG, Stirling D, Zehr L. 2019 Canadian guideline for physical activity throughout pregnancy. *Br J Sports Med* 52: 1339–1346, 2018. doi: 10.1136/BJSPORTS-2018-100056.
15. Davenport MH, Ruchat SM, Poitras VJ, Jaramillo Garcia A, Gray CE, Barrowman N, Skow RJ, Meah VL, Riske L, Sobierajski F, James M, Kathol AJ, Nuspl M, Marchand AA, Nagpal TS, Slater LG, Weeks A, Adamo KB, Davies GA, Barakat R, Mottola MF. Prenatal exercise for the

prevention of gestational diabetes mellitus and hypertensive disorders of pregnancy: a systematic review and meta-analysis. *Br J Sports Med* 52: 1367–1375, 2018. doi: 10.1136/BJSPORTS-2018-099355.
16. Cid M, González M. Potential benefits of physical activity during pregnancy for the reduction of gestational diabetes prevalence and oxidative stress. *Early Hum Dev* 94: 57–62, 2016. doi: 10.1016/J.EARLHUMDEV.2016.01.007.
17. Davenport MH, Ruchat SM, Sobierajski F, Poitras VJ, Gray CE, Yoo C, Skow RJ, Jaramillo Garcia A, Barrowman N, Meah VL, Nagpal TS, Riske L, James M, Nuspl M, Weeks A, Marchand AA, Slater LG, Adamo KB, Davies GA, Barakat R, Mottola MF. Impact of prenatal exercise on maternal harms, labour and delivery outcomes: a systematic review and meta-analysis. *Br J Sports Med* 53: 99–107, 2019. doi: 10.1136/BJSPORTS-2018-099821.
18. Davenport MH, McCurdy AP, Mottola MF, Skow RJ, Meah VL, Poitras VJ, Jaramillo Garcia A, Gray CE, Barrowman N, Riske L, Sobierajski F, James M, Nagpal T, Marchand AA, Nuspl M, Slater LG, Barakat R, Adamo KB, Davies GA, Ruchat SM. Impact of prenatal exercise on both prenatal and postnatal anxiety and depressive symptoms: a systematic review and meta-analysis. *Br J Sports Med* 52: 1376–1385, 2018. doi: 10.1136/BJSPORTS-2018-099697.
19. Cai C, Busch S, Wang R, Sivak A, Davenport MH. Physical activity before and during pregnancy and maternal mental health: A systematic review and meta-analysis of observational studies. *J Affect Disord* 309: 393–403, 2022. doi: 10.1016/J.JAD.2022.04.143.
20. Di Mascio D, Magro-Malosso ER, Saccone G, Marhefka GD, Berghella V. Exercise during pregnancy in normal-weight women and risk of preterm birth: a systematic review and meta-analysis of randomized controlled trials. *Am J Obstet Gynecol* 215: 561–571, 2016. doi: 10.1016/J.AJOG.2016.06.014.
21. Davenport MH, Meah VL, Ruchat SM, Davies GA, Skow RJ, Barrowman N, Adamo KB, Poitras VJ, Gray CE, Jaramillo Garcia A, Sobierajski F, Riske L, James M, Kathol AJ, Nuspl M, Marchand AA, Nagpal TS, Slater LG, Weeks A, Barakat R, Mottola MF. Impact of prenatal exercise on neonatal and childhood outcomes: a systematic review and meta-analysis. *Br J Sports Med* 52: 1386–1396, 2018. doi: 10.1136/BJSPORTS-2018-099836.
22. Blaize AN, Pearson KJ, Newcomer SC. Impact of Maternal Exercise during Pregnancy on Offspring Chronic Disease Susceptibility. *Exerc Sport Sci Rev* 43: 198–203, 2015. doi: 10.1249/JES.0000000000000058.
23. Joyner MJ, Casey DP. Regulation of increased blood flow (hyperemia) to muscles during exercise: a hierarchy of competing physiological needs. *Physiol Rev* 95: 549–601, 2015. doi: 10.1152/PHYSREV.00035.2013.
24. Clapp JF. The effects of maternal exercise on fetal oxygenation and feto-placental growth. *European Journal of Obstetrics and Gynecology and Reproductive Biology* 110, 2003. doi: 10.1016/S0301-2115(03)00176-3.
25. Morrow RJ, Ritchie JWK, Bull SB. Fetal and maternal hemodynamic responses to exercise in pregnancy assessed by Doppler ultrasonography. *Am J Obstet Gynecol* 160: 138–140, 1989. doi: 10.1016/0002-9378(89)90106-3.
26. Chandler KD, Leury BJ, Bird AR, Bell AW. Effects of undernutrition and exercise during late pregnancy on uterine, fetal and uteroplacental metabolism in the ewe. *Br J Nutr* 53: 625–635, 1985. doi: 10.1079/BJN19850072.
27. Bhattacharjee J, Mohammad S, Adamo KB. Does exercise during pregnancy impact organs or structures of the maternal-fetal interface? *Tissue Cell* 72, 2021. doi: 10.1016/J.TICE.2021.101543.

28. Perego SM, Raquel HA, Candido VB, Masson GS, Martins MM, Ceroni A, Michelini LC. Hypertension depresses but exercise training restores both Mfsd2a expression and blood-brain barrier function within PVN capillaries. *Am J Physiol Regul Integr Comp Physiol* 325: R299–R307, 2023. doi: https://doi.org/10.1152/ajpregu.00049.2023
29. Chytrova G, Ying Z, Gomez-Pinilla F. Exercise contributes to the effects of DHA dietary supplementation by acting on membrane-related synaptic systems. *Brain Res* 1341: 32–40, 2010. doi: 10.1016/J.BRAINRES.2009.05.018.
30. Li L, Schust DJ. Isolation, purification and in vitro differentiation of cytotrophoblast cells from human term placenta. *Reprod Biol Endocrinol* 13, 2015. doi: 10.1186/S12958-015-0070-8.
31. Chang CW, Wakeland AK, Parast MM. Trophoblast lineage specification, differentiation, and their regulation by oxygen tension. *J Endocrinol* 236: R43, 2018. doi: 10.1530/JOE-17-0402.
32. Lecarpentier E, Bhatt M, Bertin GI, Deloison B, Salomon LJ, Deloron P, Fournier T, Barakat AI, Tsatsaris V. Computational Fluid Dynamic Simulations of Maternal Circulation: Wall Shear Stress in the Human Placenta and Its Biological Implications. *PLoS One* 11, 2016. doi: 10.1371/JOURNAL.PONE.0147262.
33. Tobin KAR, Johnsen GM, Staff AC, Duttaroy AK. Long-chain polyunsaturated fatty acid transport across human placental choriocarcinoma (BeWo) cells. *Placenta* 30: 41–47, 2009. doi: 10.1016/J.PLACENTA.2008.10.007.
34. Tuuli MG, Longtine MS, Nelson DM. Review: Oxygen and trophoblast biology — A source of controversy. *Placenta* 32: S109, 2011. doi: 10.1016/J.PLACENTA.2010.12.013.
35. Burton GJ, Cindrova-Davies T, Yung HW, Jauniaux E. Hypoxia and reproductive health: Oxygen and development of the human placenta. *Reproduction* 161: F53–F65, 2021. doi: 10.1530/REP-20-0153.
36. Corson A. Characterizing the Effect of Intermittent Hypoxia on Hofbauer Cell Polarization and Placenta Vascularization, in vitro. University of Ottawa: 2024.
37. Goudreau AD, Tanara L, Tzaneva V, Adamo KB. Examining the Effects of Gestational Physical Activity and Hofbauer Cell Polarization on Angiogenic Factors. *Int J Environ Res Public Health* 20: 6298, 2023. doi: 10.3390/IJERPH20136298.
38. González-Alonso J, Dalsgaard MK, Osada T, Volianitis S, Dawson EA, Yoshiga CC, Secher NH. Brain and central haemodynamics and oxygenation during maximal exercise in humans. *J Physiol* 557: 331–342, 2004. doi: 10.1113/JPHYSIOL.2004.060574.
39. Bada AA, Svendsen JH, Secher NH, Saltin B, Mortensen SP. Peripheral vasodilatation determines cardiac output in exercising humans: insight from atrial pacing. *J Physiol* 590: 2051–2060, 2012. doi: 10.1113/JPHYSIOL.2011.225334.
40. Lundvall J, Mellander S, Westling H, White T. Fluid Transfer between Blood and Tissues during Exercise. *Acta Physiol Scand* 85: 258–269, 1972. https://doi.org/10.1111/j.1748-1716.1972.tb05259.x
41. Heenan AP, Wolfe LA, Davies GAL, McGratha MJ. Effects of human pregnancy on fluid regulation responses to short-term exercise. 95: 2321–2327, 2003. doi: 10.1152/JAPPLPHYSIOL.00984.2002.
42. Lotgering FK, Gilbert RD, Longo LD. Exercise responses in pregnant sheep: Oxygen consumption, uterine blood flow, and blood volume. *J Appl Physiol Respir Environ Exerc Physiol* 55: 834–841, 1983. doi: 10.1152/JAPPL.1983.55.3.834.
43. Niebauer J, Cooke JP. Cardiovascular effects of exercise: Role of endothelial shear stress. *J Am Coll Cardiol* 28: 1652–1660, 1996. doi: 10.1016/S0735-1097(96)00393-2.

44. Ramírez-Vélez R, Bustamante J, Czerniczyniec A, Aguilar De Plata AC, Lores-Arnaiz S. Effect of Exercise Training on Enos Expression, NO Production and Oxygen Metabolism in Human Placenta. *PLoS One* 8: e80225, 2013. doi: 10.1371/JOURNAL.PONE.0080225.
45. Haggarty P, Page K, Abramovich DR, Ashton J, Brown D. Long-chain polyunsaturated fatty acid transport across the perfused human placenta. *Placenta* 18: 635–642, 1997. doi: 10.1016/S0143-4004(97)90004-7.
46. Duttaroy AK. Transport of fatty acids across the human placenta: A review. *Prog Lipid Res* 48: 52–61, 2009. doi: 10.1016/j.plipres.2008.11.001.
47. Maldonado-Estrada J, Menu E, Roques P, Barré-Sinoussi F, Chaouat G. Evaluation of Cytokeratin 7 as an accurate intracellular marker with which to assess the purity of human placental villous trophoblast cells by flow cytometry. *J Immunol Methods* 286: 21–34, 2004. doi: 10.1016/J.JIM.2003.03.001.
48. Kliman HJ, Nestler JE, Sermasi E, Sanger JM, Strauss III JF. Purification, characterization and in vitro differentiation of cytotrophoblasts from human term placentae *Endocrinology*, *118*(4), 1567–1582. https://doi.org/10.1210/endo-118-4-1567
49. Kadam L, Veličković M, Stratton K, Nicora CD, Kyle JE, Wang E, Monroe ME, Bramer LM, Myatt L, Burnum-Johnson KE. Sexual dimorphism in lipidomic changes in maternal blood and placenta associated with obesity and gestational diabetes: A discovery study. *Placenta* 159: 76–83, 2025. doi: 10.1016/J.PLACENTA.2024.12.002.
50. Powell TL, Barner K, Madi L, Armstrong M, Manke J, Uhlson C, Jansson T, Ferchaud-Roucher V. Sex-specific responses in placental fatty acid oxidation, esterification and transfer capacity to maternal obesity. *Biochim Biophys Acta Mol Cell Biol Lipids* 1866, 2021. doi: 10.1016/J.BBALIP.2020.158861.
51. Ruebel ML, Borengasser SJ, Zhong Y, Kang P, Faske J, Shankar K. Maternal Exercise Prior to and during Gestation Induces Sex-Specific Alterations in the Mouse Placenta. *Int J Mol Sci* 24, 2023. doi: 10.3390/IJMS242216441.

Supplemental Materials

Table S1. G*Power parameters and output for *post-hoc* power analysis.

Variable	Value
Statistical test	ANOVA: Fixed effects, omnibus, one-way
Effect size *f*	0.56
α error probability	0.05
Total sample size	24
Number of groups	6
Achieved power (1-β error probability)	0.41

Chapter 4: Discussion and General Conclusions

4.1 Key Findings

This thesis investigated the relationship between PA and the placental lipidome in uncomplicated pregnancies and further examined how PA may impact DHA uptake through the implementation of a novel *in vitro* model. The findings demonstrated significant differences in lipid profiles of placentas originating from active compared to inactive pregnancies. Moreover, DHA uptake was influenced by our model, albeit not in the anticipated direction.

Chapter 2, presented as the manuscript entitled *Exploring the Placental Lipidome in Physically Active vs. Inactive Pregnancies*, reports results from an exploratory, untargeted mass spectrometry analysis. We evaluated a total of 36 term placenta samples with participants stratified according to objectively measured levels of activity, using the *2019 Canadian Guideline for Physical Activity During Pregnancy* as our threshold for 'active' status (6). We identified 34 distinct lipid species that differed significantly between active and inactive cohorts, and multivariate analyses demonstrated moderate group separation. A focused investigation of DHA revealed that seven of 17 DHA-containing species were higher in the active cohort, although MFSD2a expression did not differ significantly between groups. Pathway analysis of significant lipid species highlighted enrichment of the PSD pathway in placentas from physically active pregnancies, which is responsible for conversion of PS to PE.

Chapter 3 features the manuscript titled *The Impact of an In Vitro Model of Physical Activity on Docosahexaenoic Acid Uptake in Human Primary Trophoblast Cells*, which describes the implementation of a functional model of PA by manipulating oxygen tension and flow, followed by assessment of DHA uptake in STB. Cells from four distinct donors were exposed to six different conditions combining normoxia, IH, high shear stress, low shear stress and static. We

hypothesized that IH + high shear stress, our proxy for PA, would maximize DHA uptake. Contrary to expectations, we found the normoxia + static condition yielded significantly greater DHA uptake than both IH + high shear stress and IH + low shear stress conditions. Analysis of DHA-supplemented media (without cells) found no significant differences between flow and static conditions, suggesting that adherence to tubing was not a major confound in flow conditions.

4.2 Physical Activity and its Relations to the Placental Lipidome and Lipid Uptake

The placenta is a vital organ that facilitates nutrient, gas, and waste exchange, while also performing endocrine and immune functions. Increasing evidence indicates that gestational lifestyle behaviours and environmental exposures can shape placental anatomy and physiology. Gestational PA is one such behaviour, consistently associated with favourable maternal and fetal outcomes, though the mechanisms underlying these benefits remain unclear. Collectively, these observations support the position that the placenta plays a central role in mediating the influence of PA on pregnancy and maternal-fetal health.

Among the placenta's many functions, the uptake and transport of key nutrients such as lipids is critical for supporting growth and development. Lipids serve as a major fuel source for both placenta and fetus, provide structural components for cell membranes, and contribute to hormone production and cellular signalling (20). Accordingly, this project sought to examine the relationship between PA and placental lipids, with particular emphasis on the essential omega-3 DHA.

The first study of this thesis presented an exploratory lipidomic analysis of placentas from active and inactive pregnancies. As all participants experienced uncomplicated pregnancies, we were not surprised by the modest separation in lipidome between groups. Extreme differences in lipid profiles are more prominent in pathology; in contrast, this work focused on PA as a health-

promoting behaviour within a nonpathological baseline. While our cohort spanned a range of BMI values, no confounding health complications were present, and BMI did not differ between activity groups. Importantly, the BMI distribution within our sample reflects that of pregnant populations in North America (67, 69), supporting the external relevance of these findings. This perspective is novel, as the majority of research in this area has focused on disease states. For example, Gázquez *et al*. investigated placental lipid droplet content in response to a combined diet and PA intervention in participants exclusively with obesity, but found minimal significant differences between groups (70), suggesting that in cohorts defined by obesity, the influence of PA on placental lipid stores may be less readily detectable.

The higher abundance of DHA-containing lipid species in the active cohort is notable, as DHA is essential in supporting offspring neurodevelopment and long-term cognitive function (45). Fetal DHA demand peaks in the third trimester to support the rapid acceleration in brain growth during this period. Given that placenta samples were collected at term, our findings likely reflect the *in vivo* lipidome of late gestation, suggesting that PA may enhance DHA availability precisely when fetal requirements are greatest. Insufficient DHA during late gestation and the early neonatal period has been linked to adverse neurodevelopmental outcomes (56, 62), underscoring the potential significance of these observations. Nonetheless, delivery mode and labour can influence placental lipid composition, which should be considered when interpreting post-delivery profiles (68).

We anticipated that MFSD2a expression would parallel the observed differences in DHA-containing lipid species, with higher expression in the active cohort. However, no differences between groups were detected. MFSD2a has been positively correlated with cord blood DHA at the placental level (46, 70), and with brain DHA at the level of the blood- brain barrier (71), making

our current findings unexpected. It is possible that PA-related enhancement of placental DHA transport is not uniquely attributable to MFSD2a. FATP-1 and FATP-4 also facilitates DHA transport across the STB, and recent work in our lab has identified greater FATP-4 in placentas from active pregnancies (52). Subsequent cord blood and/or neonatal serum lipid analysis is required to determine whether higher levels of DHA in the placenta translate to fetal availability. A more comprehensive evaluation of LCPUFA transporters in placental tissue would provide a greater understanding of the relationship between PA and fetal transport.

To complement the observational findings of Aim 1, Aim 2 implemented a functional model to interrogate DHA handling at the cellular level. Whereas Aim 1 quantified DHA abundance in tissue, Aim 2 evaluated uptake in STB, thereby addressing related but distinct aspects of placental DHA biology. Based on Aim 1, we hypothesized that DHA uptake would be greatest under the PA proxy condition. Interestingly, we observed the opposite. These findings may be reconciled by considering the distinction between cellular uptake and transport. As discussed in chapter 3, efficient fetal transport of DHA by STB may not be evident by cellular DHA content alone, as cells may streamline this process by shuttling DHA rather than retaining it intracellularly (44). We postulate that the greater DHA content observed under normoxia + static conditions may be a reflection of less efficient transfer to fetal circulation.

It is also plausible that our results reflect the viability of the model itself. The use of IH and shear stress was based on a theoretical framework of blood and oxygen redistribution in the intrauterine space during PA. Unfortunately, direct evidence is lacking in humans due to technical and ethical challenges. Our group previously attempted Doppler ultrasound at the uterine artery during PA to assess the validity of this theory (unpublished), however, participant movement interfered with imaging. To date, no human data exist regarding placental blood flow or oxygen

availability during PA, though ultrasound measurements immediately post-exercise have shown increased uteroplacental vascular resistance (17). While some animal data corroborate this redistribution theory (16, 72), interspecies differences in pregnancy, such as gestational length, placentation, and litter size limit translation. Murine models of PA and pregnancy often diverge from human findings, as demonstrated in a scoping review from our group examining circulating myokines (73). Extrapolating between species warrants careful consideration of biological differences and is perhaps not entirely logical in the context of gestation.

This was not the first attempt by our group to apply IH as a proxy for PA. Recently, Corson *et al.* sought to replicate the observed higher proportions of anti-inflammatory Hofbauer cells in placentas from active pregnancies in an *in vitro* model by exposing cells to IH (74, 75). In parallel with the findings of this thesis, Corson *et al.* were unable to reproduce the *in vivo* findings. More work is necessary to investigate the pragmatism of IH in assessing PA effects.

In Aim 2, two bouts of IH were applied to mimic chronic as opposed to acute PA exposure. Each bout lasted three hours, a duration selected to ensure sufficient time for oxygen tension in media to drop to desired levels. This was optimized before experimentation by attaching an O_2 sensor to the tubing system in flow and monitoring media O_2 in the bioreactor. One hour after adjusting oxygen tension from 10% to 3%, O_2 levels in media had decreased and stabilized. Three hours was therefore selected to ensure cells were sufficiently exposed to reduced O_2 levels. We acknowledge that while these decisions were made using the best available knowledge, there is currently no consensus on physiological normoxia and hypoxia in primary CTBs. Many studies have used atmospheric oxygen tension (21% O_2), which does not mirror *in vivo* conditions, which range from ~2-10% O_2 depending on the gestational timepoint and anatomical location (76). Moreover, both the timing and duration of IH can differentially influence the placental response

(77), highlighting the necessity for standardized approaches to improve comparability and generalizability across studies.

4.3 Future Directions

The findings of this thesis provide the foundations for a broad range of future investigations related to PA and placental lipids. The differentially expressed lipids identified between placentas from active and inactive pregnancies offer a multitude of targets for deeper analysis. Specifically, the five lipids that achieved perfect separation based on AUC analysis may represent potential biomarkers of physically active pregnancies. A targeted lipidomic approach focusing on these lipids in a larger sample could offer valuable insight into their utility. With targeted mass spectrometry, internal standards could feasibly be incorporated for each lipid of interest, allowing absolute quantification. Confirming such biomarkers would not only strengthen mechanistic understanding but also inform evaluation of the pathways and processes involved, which may eventually assist in assessing risk or health status of the pregnancy. The PSD pathway, enriched in the active cohort, is another promising avenue for exploration. Future projects should analyze PISD gene expression in placentas from active and inactive pregnancies to validate potential upregulation of this pathway.

Subsequent work should also extend these PA-associated lipid differences to fetal circulation. Untargeted lipidomic analysis of cord blood samples paired with the placenta samples used in this study would create a more comprehensive view of fetal lipid availability. While placental lipid abundance may hint at downstream fetal transport, cord blood lipidomics would allow for the confirmation of such inferences, clarifying how PA might influence fetal growth and development. Although untargeted cord blood lipidomics would provide the most extensive

profile, targeted analysis of lipid species of interest offers a more practical approach that still yields valuable insight into maternal-fetal lipid transport.

Recent innovations enable cellular barrier modeling in flow culture, permitting the assessment of STB uptake and transport of nutrients while maintaining physiological relevance. Replicating Aim 2 of this thesis using such tools would provide a better understanding of DHA interactions across, and not just within, the STB. Other lipids of interest identified in Aim 1 may likewise benefit from functional *in vitro* analysis. However, questions remain regarding the physiological relevance of the *in vitro* model applied here. Going forward, applications of this model should incorporate measures of cell viability (e.g., LDH release into media) to ensure that IH exposure is not inducing cytotoxic stress confounding uptake results. Future work should endeavour to elucidate the precise *in utero* conditions that occur during PA. Placental perfusion studies offer an additional, complementary approach that could cross-validate cell culture and tissue findings by providing an *ex vivo* perspective.

4.4 Significance

This thesis provides a basis for future exploration and contributes to a growing body of literature examining the links between gestational PA and the placenta. These projects represent the first characterization of the placental lipidome of healthy, active pregnancies, offering novel insight into specific lipids and pathways for downstream mechanistic evaluation. The subsequent functional analysis of DHA in a cell culture model provided mechanistic context for the lipidomic findings, advancing understanding of how PA may influence placental lipid transport.

A clearer grasp of these relationships is critical for supporting healthy pregnancies. The ability of PA to improve health above baseline and provide long-term protective effects is substantial. This research helps to build our understanding of gestational PA and the mechanisms

through which it mediates beneficial pregnancy and offspring outcomes, paving the way for more intentional and clinical applications of this highly advantageous lifestyle behaviour.

References

1. Corson AE, MacDonald M, Tzaneva V, Edwards CM, Adamo KB. Breaking Boundaries: A Chronology with Future Directions of Women in Exercise Physiology Research, Centred on Pregnancy. .
2. Colenso-Semple LM, D'Souza AC, Elliott-Sale KJ, Phillips SM. Current evidence shows no influence of women's menstrual cycle phase on acute strength performance or adaptations to resistance exercise training. *Front Sports Act Living* 5: 1054542, 2023. doi: 10.3389/FSPOR.2023.1054542/BIBTEX.
3. Smith ES, McKay AKA, Kuikman M, Ackerman KE, Harris R, Stellingwerff T, Burke LM, Elliott-Sale KJ. Auditing the Representation of Female Versus Male Athletes in Sports Science and Sports Medicine Research: Evidence-Based Performance Supplements. *Nutrients* 14: 953, 2022. doi: 10.3390/NU14050953/S1.
4. Sprague AE. The Evolution of Bed Rest as a Clinical Intervention. *Journal of Obstetric, Gynecologic & Neonatal Nursing* 33: 542–549, 2004. doi: 10.1177/0884217504268523.
5. Silva-Jose C, Mottola MF, Palacio M, Sánchez-Polán M, Zhang D, Refoyo I, Barakat R. Impact of Physical Activity Interventions on High-Risk Pregnancies: A Systematic Review and Meta-Analysis. *Journal of Personalized Medicine 2024, Vol 14, Page 14* 14: 14, 2023. doi: 10.3390/JPM14010014.
6. Mottola MF, Davenport MH, Ruchat SM, Davies GA, Poitras VJ, Gray CE, Jaramillo Garcia A, Barrowman N, Adamo KB, Duggan M, Barakat R, Chilibeck P, Fleming K, Forte M, Korolnek J, Nagpal T, Slater LG, Stirling D, Zehr L. 2019 Canadian guideline for physical activity throughout pregnancy. *Br J Sports Med* 52: 1339–1346, 2018. doi: 10.1136/BJSPORTS-2018-100056.
7. Davenport MH, Ruchat SM, Sobierajski F, Poitras VJ, Gray CE, Yoo C, Skow RJ, Jaramillo Garcia A, Barrowman N, Meah VL, Nagpal TS, Riske L, James M, Nuspl M, Weeks A, Marchand AA, Slater LG, Adamo KB, Davies GA, Barakat R, Mottola MF. Impact of prenatal exercise on maternal harms, labour and delivery outcomes: a systematic review and meta-analysis. *Br J Sports Med* 53: 99–107, 2019. doi: 10.1136/BJSPORTS-2018-099821.
8. Davenport MH, Ruchat SM, Poitras VJ, Jaramillo Garcia A, Gray CE, Barrowman N, Skow RJ, Meah VL, Riske L, Sobierajski F, James M, Kathol AJ, Nuspl M, Marchand AA, Nagpal TS, Slater LG, Weeks A, Adamo KB, Davies GA, Barakat R, Mottola MF. Prenatal exercise for the prevention of gestational diabetes mellitus and hypertensive disorders of pregnancy: a systematic review and meta-analysis. *Br J Sports Med* 52: 1367–1375, 2018. doi: 10.1136/BJSPORTS-2018-099355.
9. Davenport MH, Meah VL, Ruchat SM, Davies GA, Skow RJ, Barrowman N, Adamo KB, Poitras VJ, Gray CE, Jaramillo Garcia A, Sobierajski F, Riske L, James M, Kathol AJ, Nuspl M, Marchand AA, Nagpal TS, Slater LG, Weeks A, Barakat R, Mottola MF. Impact of prenatal exercise on neonatal and childhood outcomes: a systematic review and meta-analysis. *Br J Sports Med* 52: 1386–1396, 2018. doi: 10.1136/BJSPORTS-2018-099836.
10. Dipietro L, Evenson KR, Bloodgood B, Sprow K, Troiano RP, Piercy KL, Vaux-Bjerke A, Powell KE. Benefits of Physical Activity during Pregnancy and Postpartum: An Umbrella Review. *Med Sci Sports Exerc* 51: 1292, 2019. doi: 10.1249/MSS.0000000000001941.
11. Davenport MH, McCurdy AP, Mottola MF, Skow RJ, Meah VL, Poitras VJ, Jaramillo Garcia A, Gray CE, Barrowman N, Riske L, Sobierajski F, James M, Nagpal T, Marchand

AA, Nuspl M, Slater LG, Barakat R, Adamo KB, Davies GA, Ruchat SM. Impact of prenatal exercise on both prenatal and postnatal anxiety and depressive symptoms: a systematic review and meta-analysis. *Br J Sports Med* 52: 1376–1385, 2018. doi: 10.1136/BJSPORTS-2018-099697.
12. Cai C, Busch S, Wang R, Sivak A, Davenport MH. Physical activity before and during pregnancy and maternal mental health: A systematic review and meta-analysis of observational studies. *J Affect Disord* 309: 393–403, 2022. doi: 10.1016/J.JAD.2022.04.143.
13. Bhattacharjee J, Mohammad S, Adamo KB. Does exercise during pregnancy impact organs or structures of the maternal-fetal interface? *Tissue Cell* 72, 2021. doi: 10.1016/J.TICE.2021.101543.
14. Clapp JF. The effects of maternal exercise on fetal oxygenation and feto-placental growth. *European Journal of Obstetrics and Gynecology and Reproductive Biology* 110, 2003. doi: 10.1016/S0301-2115(03)00176-3.
15. Joyner MJ, Casey DP. Regulation of Increased Blood Flow (Hyperemia) to Muscles During Exercise: A Hierarchy of Competing Physiological Needs. *https://doi.org/101152/physrev000352013* 95: 549–601, 2015. doi: 10.1152/PHYSREV.00035.2013.
16. Chandler KD, Leury BJ, Bird AR, Bell AW. Effects of undernutrition and exercise during late pregnancy on uterine, fetal and uteroplacental metabolism in the ewe. *Br J Nutr* 53: 625–635, 1985. doi: 10.1079/BJN19850072.
17. Morrow RJ, Ritchie JWK, Bull SB. Fetal and maternal hemodynamic responses to exercise in pregnancy assessed by Doppler ultrasonography. *Am J Obstet Gynecol* 160: 138–140, 1989. doi: 10.1016/0002-9378(89)90106-3.
18. Clapp JF, Little KD, Appleby-Wineberg SK, Widness JA. The effect of regular maternal exercise on erythropoietin in cord blood and amniotic fluid. *Am J Obstet Gynecol* 172: 1445–1451, 1995. doi: 10.1016/0002-9378(95)90476-X.
19. Ramírez-Vélez R, Bustamante J, Czerniczyniec A, Aguilar De Plata AC, Lores-Arnaiz S. Effect of exercise training on eNOS expression, NO production and oxygen metabolism in human placenta. *PLoS One* 8, 2013. doi: 10.1371/JOURNAL.PONE.0080225.
20. Alves MA, Lamichhane S, Dickens A, McGlinchey A, Ribeiro HC, Sen P, Wei F, Hyötyläinen T, Orešič M. Systems biology approaches to study lipidomes in health and disease. *Biochim Biophys Acta Mol Cell Biol Lipids* 1866, 2021. doi: 10.1016/J.BBALIP.2020.158857.
21. Kenwood BM, Merrill AH. Lipidomics. In: *Encyclopedia of Cell Biology*. Elsevier Inc., 2016, p. 147–159.
22. Calder PC. Functional Roles of Fatty Acids and Their Effects on Human Health. *JPEN J Parenter Enteral Nutr* 39: 18S-32S, 2015. doi: 10.1177/0148607115595980.
23. Voelker D. Glycerolipid Structure, Function, and Synthesis in Eukaryotes. *Encylopedia of Biological Chemistry* 2: 412–418, 2013. doi: 10.1016/B978-0-12-378630-2.00510-7.
24. S D, SK R. Diverse Biological Functions of Sphingolipids in the CNS: Ceramide and Sphingosine Regulate Myelination in Developing Brain but Stimulate Demyelination during Pathogenesis of Multiple Sclerosis. *J Neurol Psychol* 5: 01–07, 2017. doi: 10.13188/2332-3469.1000035.

25. Park J, Choi J, Kim DD, Lee S, Lee B, Lee Y, Kim S, Kwon S, Noh M, Lee MO, Le QV, Oh YK. Bioactive Lipids and Their Derivatives in Biomedical Applications. *Biomol Ther (Seoul)* 29: 465–482, 2021. doi: 10.4062/BIOMOLTHER.2021.107.
26. Hill AM, Staunton J. Type I Modular PKS. *Comprehensive Natural Products II: Chemistry and Biology* 1: 385–452, 2010. doi: 10.1016/B978-008045382-8.00015-0.
27. Dufourc EJ. Sterols and membrane dynamics. *J Chem Biol* 1: 63–77, 2008. doi: 10.1007/S12154-008-0010-6.
28. Donato P, Dugo P, Mondello L. Separation of lipids. .
29. Yang K, Han X. Lipidomics: Techniques, Applications, and Outcomes Related to Biomedical Sciences. *Trends Biochem Sci* 41: 954–969, 2016. doi: 10.1016/J.TIBS.2016.08.010.
30. Zhang L, Bi S, Liang Y, Huang L, Li Y, Huang M, Huang B, Deng W, Liang J, Gu S, Chen J, Du L, Chen D, Wang Z. Integrated Metabolomic and Lipidomic Analysis in the Placenta of Preeclampsia. *Front Physiol* 13, 2022. doi: 10.3389/FPHYS.2022.807583.
31. Lewis RM, Desoye G. Placental Lipid and Fatty Acid Transfer in Maternal Overnutrition. *Ann Nutr Metab* 70: 228–231, 2017. doi: 10.1159/000463397.
32. Herrera E, Ortega-Senovilla H. Maternal lipid metabolism during normal pregnancy and its implications to fetal development. *Clin Lipidol* 5: 899–912, 2010. doi: 10.2217/CLP.10.64.
33. Brown SHJ, Eather SR, Freeman DJ, Meyer BJ, Mitchell TW. A Lipidomic Analysis of Placenta in Preeclampsia: Evidence for Lipid Storage. *PLoS One* 11, 2016. doi: 10.1371/JOURNAL.PONE.0163972.
34. Yang Y, Wu L, Lv Y, Miao Z, Wang Y, Yan J, Li J, Li C, Ding H. LC-MS/MS based untargeted lipidomics uncovers lipid signatures of late-onset preeclampsia. *Biochimie* 208: 46–55, 2023. doi: 10.1016/J.BIOCHI.2022.12.002.
35. Jiang D, He J, Hua S, Zhang J, Liu L, Shan C, Cui X, Ji C. A comparative lipidomic study of the human placenta from women with or without gestational diabetes mellitus. *Mol Omics* 18: 545–554, 2022. doi: 10.1039/D2MO00083K.
36. Kadam L, Veličković M, Stratton K, Nicora CD, Kyle JE, Wang E, Monroe ME, Bramer LM, Myatt L, Burnum-Johnson KE. Sexual dimorphism in lipidomic changes in maternal blood and placenta associated with obesity and gestational diabetes: A discovery study. *Placenta* 159: 76–83, 2025. doi: 10.1016/J.PLACENTA.2024.12.002.
37. Kalapouti E, Bothou A, Harizopoulou V, Vlachou M, Vasilili Zampeli M, Diamanti A. Lipidomic signatures in maternal blood and placenta: Systematic evidence linking lipid profiles to pregnancy outcomes and fetal growth. *Metabol Open* 28: 100398, 2025. doi: 10.1016/J.METOP.2025.100398.
38. Haggarty P. Fatty acid supply to the human fetus. *Annu Rev Nutr* 30: 237–255, 2010. doi: 10.1146/ANNUREV.NUTR.012809.104742.
39. Duttaroy AK, Basak S. Maternal Fatty Acid Metabolism in Pregnancy and Its Consequences in the Feto-Placental Development. *Front Physiol* 12, 2022. doi: 10.3389/FPHYS.2021.787848.
40. Campbell FM, Bush PG, Veerkamp JH, Dutta-Roy AK. Detection and cellular localization of plasma membrane-associated and cytoplasmic fatty acid-binding proteins in human placenta. *Placenta* 19: 409–415, 1998. doi: 10.1016/S0143-4004(98)90081-9.
41. Duttaroy AK. Transport of fatty acids across the human placenta: A review. *Prog Lipid Res* 48: 52–61, 2009. doi: 10.1016/j.plipres.2008.11.001.

42. Larqué E, Krauss-Etschmann S, Campoy C, Hartl D, Linde J, Klingler M, Demmelmair H, Caño A, Gil A, Bondy B, Koletzko B. Docosahexaenoic acid supply in pregnancy affects placental expression of fatty acid transport proteins. *Am J Clin Nutr* 84: 853–861, 2006. doi: 10.1093/AJCN/84.4.853.
43. Campbell FM, Gordon MJ, Dutta-Roy AK. Placental membrane fatty acid-binding protein preferentially binds arachidonic and docosahexaenoic acids. *Life Sci* 63: 235–240, 1998. doi: 10.1016/S0024-3205(98)00267-7.
44. Haggarty P, Page K, Abramovich DR, Ashton J, Brown D. Long-chain polyunsaturated fatty acid transport across the perfused human placenta. *Placenta* 18: 635–642, 1997. doi: 10.1016/S0143-4004(97)90004-7.
45. Basak S, Mallick R, Duttaroy AK. Maternal Docosahexaenoic Acid Status during Pregnancy and Its Impact on Infant Neurodevelopment. *Nutrients* 12: 1–25, 2020. doi: 10.3390/NU12123615.
46. Prieto-Sánchez MT, Ruiz-Palacios M, Blanco-Carnero JE, Pagan A, Hellmuth C, Uhl O, Peissner W, Ruiz-Alcaraz AJ, Parrilla JJ, Koletzko B, Larqué E. Placental MFSD2a transporter is related to decreased DHA in cord blood of women with treated gestational diabetes. *Clin Nutr* 36: 513–521, 2017. doi: 10.1016/J.CLNU.2016.01.014.
47. Wadhwani N, Patil V, Pisal H, Joshi A, Mehendale S, Gupte S, Wagh G, Joshi S. Altered maternal proportions of long chain polyunsaturated fatty acids and their transport leads to disturbed fetal stores in preeclampsia. *Prostaglandins Leukot Essent Fatty Acids* 91: 21–30, 2014. doi: 10.1016/J.PLEFA.2014.05.006.
48. Segura MT, Demmelmair H, Krauss-Etschmann S, Nathan P, Dehmel S, Padilla MC, Rueda R, Koletzko B, Campoy C. Maternal BMI and gestational diabetes alter placental lipid transporters and fatty acid composition. *Placenta* 57: 144–151, 2017. doi: 10.1016/J.PLACENTA.2017.07.001.
49. Jiang Y, Zhou X, He Z, Wei L, Zhu S, Zhang H, Zhang J, Chen Y, Du Y, Ding W, Wang S, Wang Z, Feng L. DHA Improves neurodevelopmental abnormalities in offspring of gestational diabetes mellitus patients via the PPAR-γ/FATP4 pathway. *Biochem Pharmacol* 232: 116726, 2025. doi: 10.1016/J.BCP.2024.116726.
50. 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* 92: 2256–2263, 2007. doi: 10.1210/JC.2006-2403.
51. Brett KE, Ferraro ZM, Yockell-Lelievre J, Gruslin A, Adamo KB. Maternal-fetal nutrient transport in pregnancy pathologies: the role of the placenta. *Int J Mol Sci* 15: 16153–16185, 2014. doi: 10.3390/IJMS150916153.
52. Hutchinson KA, Vuong NH, Mohammad S, Everest C, Leung ML, Bhattacharjee J, Adamo KB. Physical Activity During Pregnancy Is Associated with Increased Placental FATP4 Protein Expression. *Reprod Sci* 27: 1909–1919, 2020. doi: 10.1007/S43032-020-00210-W.
53. Son JS, Liu X, Tian Q, Zhao L, Chen Y, Hu Y, Chae SA, de Avila JM, Zhu MJ, Du M. Exercise prevents the adverse effects of maternal obesity on placental vascularization and fetal growth. *J Physiol* 597: 3333–3347, 2019. doi: 10.1113/JP277698.
54. Bradbury J. Docosahexaenoic Acid (DHA): An Ancient Nutrient for the Modern Human Brain. *Nutrients 2011, Vol 3, Pages 529-554* 3: 529–554, 2011. doi: 10.3390/NU3050529.

55. Kaur N, Chugh V, Gupta AK. Essential fatty acids as functional components of foods- a review. *J Food Sci Technol* 51: 2289–2303, 2014. doi: 10.1007/S13197-012-0677-0.
56. Uauy R, Hoffman DR, Peirano P, Birch DG, Birch EE. Essential fatty acids in visual and brain development. *Lipids* 36: 885–895, 2001. doi: 10.1007/S11745-001-0798-1.
57. Braarud HC, Markhus MW, Skotheim S, Stormark KM, Frøyland L, Graff IE, Kjellevold M. Maternal DHA Status during Pregnancy Has a Positive Impact on Infant Problem Solving: A Norwegian Prospective Observation Study. *Nutrients* 10, 2018. doi: 10.3390/NU10050529.
58. Helland IB, Smith L, Saarem K, Saugstad OD, Drevon CA. Maternal supplementation with very-long-chain n-3 fatty acids during pregnancy and lactation augments children's IQ at 4 years of age. *Pediatrics* 111, 2003. doi: 10.1542/PEDS.111.1.E39,.
59. Jensen CL, Voigt RG, Llorente AM, Peters SU, Prager TC, Zou YL, Rozelle JC, Turcich MR, Fraley JK, Anderson RE, Heird WC. Effects of early maternal docosahexaenoic acid intake on neuropsychological status and visual acuity at five years of age of breast-fed term infants. *J Pediatr* 157: 900–905, 2010. doi: 10.1016/J.JPEDS.2010.06.006.
60. Krabbendam L, Bakker E, Hornstra G, van Os J. Relationship between DHA status at birth and child problem behaviour at 7 years of age. *Prostaglandins Leukot Essent Fatty Acids* 76: 29–34, 2007. doi: 10.1016/J.PLEFA.2006.09.004.
61. Araújo JR, Correia-Branco A, Ramalho C, Keating E, Martel F. Gestational diabetes mellitus decreases placental uptake of long-chain polyunsaturated fatty acids: involvement of long-chain acyl-CoA synthetase. *J Nutr Biochem* 24: 1741–1750, 2013. doi: 10.1016/J.JNUTBIO.2013.03.003.
62. Judge MP, Casavant SG, Dias JAM, McGrath JM. Reduced DHA transfer in diabetic pregnancies: mechanistic basis and long-term neurodevelopmental implications. *Nutr Rev* 74: 411–420, 2016. doi: 10.1093/NUTRIT/NUW006.
63. Wu A, Ying Z, Gomez-Pinilla F. Exercise facilitates the action of dietary DHA on functional recovery after brain trauma. *Neuroscience* 248: 655–663, 2013. doi: 10.1016/J.NEUROSCIENCE.2013.06.041.
64. Wu A, Ying Z, Gomez-Pinilla F. DHA dietary supplementation enhances the effects of exercise on synaptic plasticity and cognition. *Neuroscience* 155: 751, 2008. doi: 10.1016/J.NEUROSCIENCE.2008.05.061.
65. Chytrova G, Ying Z, Gomez-Pinilla F. Exercise contributes to the effects of DHA dietary supplementation by acting on membrane-related synaptic systems. *Brain Res* 1341: 32–40, 2010. doi: 10.1016/J.BRAINRES.2009.05.018.
66. Perego SM, Raquel HA, Candido VB, Masson GS, Martins MM, Ceroni A, Michelini LC. Hypertension depresses but exercise training restores both Mfsd2a expression and blood-brain barrier function within PVN capillaries. *Am J Physiol Regul Integr Comp Physiol* 325: R299–R307, 2023. doi: https://doi.org/10.1152/ajpregu.00049.2023.
67. Public Health Agency of Canada. Effect of maternal weight on pregnancy outcomes [Online]. Government of Canada. 2016. https://www.canada.ca/en/public-health/services/publications/healthy-living/effect-maternal-weight-pregnancy-outcomes.html [December 18, 2025].
68. Delhaes F, Giza SA, Koreman T, Eastabrook G, Mckenzie CA, Bedell S, Regnault TRH, De Vrijer B. Altered maternal and placental lipid metabolism and fetal fat development in obesity: Current knowledge and advances in non-invasive assessment. *Placenta* 69: 118–124, 2018. doi: 10.1016/j.placenta.2018.05.011.

69. Driscoll AK, Gregory ECW. Increases in prepregnancy obesity: United states, 2016-2019 [Online]. Centers for Disease Control and Prevention. 2020. https://www.cdc.gov/nchs/products/databriefs/db392.htm [December 18, 2025].
70. Gázquez A, Uhl O, Ruíz-Palacios M, Gill C, Patel N, Koletzko B, Poston L, Larqué E. Placental lipid droplet composition: Effect of a lifestyle intervention (UPBEAT) in obese pregnant women. *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids* 1863: 998–1005, 2018. doi: 10.1016/J.BBALIP.2018.04.020.
71. Nguyen LN, Ma D, Shui G, Wong P, Cazenave-Gassiot A, Zhang X. Mfsd2a is a transporter for the essential omega-3 fatty acid docosahexaenoic acid. *Nature* 509, 2014. doi: 10.1038/nature13241.
72. Lotgering FK, Gilbert RD, Longo LD. Exercise responses in pregnant sheep: Oxygen consumption, uterine blood flow, and blood volume. *J Appl Physiol Respir Environ Exerc Physiol* 55: 834–841, 1983. doi: 10.1152/JAPPL.1983.55.3.834.
73. Goudreau AD, Everest C, Nagpal TS, Puranda JL, Bhattacharjee J, Vasanthan T, Adamo KB. Elucidating the interaction between maternal physical activity and circulating myokines throughout gestation: A scoping review. *American Journal of Reproductive Immunology* 86, 2021. doi: 10.1111/AJI.13488.
74. Goudreau AD, Tanara L, Tzaneva V, Adamo KB. Examining the Effects of Gestational Physical Activity and Hofbauer Cell Polarization on Angiogenic Factors. *Int J Environ Res Public Health* 20: 6298, 2023. doi: 10.3390/IJERPH20136298.
75. Corson A. Characterizing the Effect of Intermittent Hypoxia on Hofbauer Cell Polarization and Placenta Vascularization, in vitro. University of Ottawa: 2024.
76. Tuuli MG, Longtine MS, Nelson DM. Review: Oxygen and trophoblast biology — A source of controversy. *Placenta* 32: S109, 2011. doi: 10.1016/J.PLACENTA.2010.12.013.
77. Siragher E, Sferruzzi-Perri AN. Placental hypoxia: What have we learnt from small animal models? *Placenta* 113: 29–47, 2021. doi: 10.1016/J.PLACENTA.2021.03.018.

Appendices

Appendix 1: Completed works and awards during master's program.

Awards

Michael Smith Foreign Study Supplement, Canadian Institutes of Health Research (2024-2025)
Canada Graduate Scholarship – Master's, Canadian Institutes of Health Research (2024-2025)
3MT Winner, Minds in Motion Conference (March 2024)
Ontario Graduate Scholarship, University of Ottawa (2023-2024)
Admission Scholarship, University of Ottawa (2023-2024)

Conference Presentations

MacDonald ML., Yadegari A., Chittaro J., Dantas A., Rosales C., Wasslen K., Shearer J., Redman L., Smith J., Adamo KB. Exploring the placental lipidome in physically active vs. inactive pregnancies. *CIHR Institute of Nutrition, Metabolism and Diabetes Meet and Greet 2025.* University of Ottawa, Ottawa, Canada. [Poster]

O'Rourke N., Marderfeld L., Dantas A., Corson A., MacDonald ML., Ferraro Z., Nagpal T., Adamo, KB. Examining the impact of preconception physical activity on offspring related outcomes linked to obesity: A scoping review. *Canadian Society for Exercise Physiology Conference 2025.* University of Western Ontario, London, Canada. [Poster]

MacDonald ML., Puranda JL., Tzaneva V., Adamo KB. Associations between physical activity and circulating brain-derived neurotrophic factor throughout gestation. *International Biochemistry of Exercise Conference 2024.* University of Limerick, Limerick, Ireland. [Poster]

MacDonald ML., Adamo KB. Is there a relationship between gestational physical activity and placental lipid composition and uptake? *Minds in Motion 2024*. McGill University, Montreal, Canada. [Oral]

Publications

O'Rourke N., Marderfeld L., Dantas A., Corson A., MacDonald ML., Ferraro Z., Nagpal T., Adamo, KB. Examining the impact of preconception physical activity on offspring outcomes linked to obesity: A scoping review. *Current obesity reports.* [In press].

Weber, V. M. R., Queiroga, M. R., Puranda, J. L., Macdonald, M. L., Corson, A. E., da Silva, D. F., Semeniuk, K., & Adamo, K. B. (2025). What is the optimal level of cardiorespiratory fitness for cognitive outcomes in females: the inverted "U" theory. *International journal of sport and exercise psychology*, 1–16. https://doi.org/10.1080/1612197X.2025.2542550

Edwards, C. M., Puranda, J. L., Miller, É., MacDonald, M. L., Aboudlal, M., & Adamo, K. B. (2025). Low physical fitness indicates future injury, mental health, menstrual cycle disruptions, and burnout in female emergency service personnel and healthcare providers. *Applied physiology, nutrition, and metabolism, 50*, 1–7. https://doi.org/10.1139/apnm-2024-0266

Ritondo, T., Darroch, F., Montano, A., Mota, P., MacDonald, M., Corson, A., & Adamo, K. (2024). Safe sport training module: Considering pregnant and mothering athletes. Presented by &Mother and Carleton University's Health and Wellness Equity Research Group [pending].

Corson, A.E., MacDonald, M.L., Tzaneva, V., Edwards, C.M., & Adamo, K.B. (2024). Breaking boundaries*:* A chronology with future directions of women in exercise physiology research, centered on pregnancy. *Advanced exercise and health science*. https://doi.org/10.1016/J.AEHS.2024.04.001

Weber V.M.R, Quieroga M.R., Puranda J.L., Semeniuk K., MacDonald M.L., Dantas D.B., da Silva D.F., & Adamo K.B. (2024). Role of cardiovascular fitness, aerobic exercise, and sports participation in female cognition: a scoping review. *Sports medicine – open,* 10(1), 103. https://doi.org/10.1186/s40798-024-00776-8

Edwards, C.M., Puranda, J.L., Miller, É., Aboudlal, M., O'Rourke, N., MacDonald, M.L. and Adamo, K. B. (2024). Musculoskeletal injuries, exercise behaviors, and reproductive health are related to physical fitness of female first responders and health care providers. *Women's health reports. 5*(1), 393–403. https://doi.org/10.1089/WHR.2023.0189

McKendry, J., Lowisz, C.V., Nanthakumar, A., MacDonald, M., Lim, C., Currier, B.S., & Phillips, S.M. (2024). The effects of whey, pea and collagen protein supplementation beyond the recommended dietary allowance on integrated myofibrillar protein synthetic rates in older males: a randomized controlled trial. *The american journal of clinical nutrition.* S0002-9165(24)00472-6. https://doi.org/10.1016/j.ajcnut.2024.05.009

Adamo, K. B., Goudreau, A. D., Corson, A. E., MacDonald, M. L., O'Rourke, N., & Tzaneva, V. (2024). Physically active pregnancies: Insights from the placenta. *Physiological reports*, *12*(11), e16104. https://doi.org/10.14814/phy2.16104