Université de Sherbrooke

Does pre-existing type 1 and type 2 diabetes during pregnancy lower docosahexaenoic acid and arachidonic acid in the umbilical cord blood of newborns and thus interfere with neurodevelopment?

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Thesis presented at the Faculty of medicine and health sciences for the obtention of Master degree diploma maitre ès sciences (M.Sc.) in Physiology,

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RÉSUMÉ

Le diabète de type 1 ou 2 pré-grossesse altère-t-il l'acide docosahexaénoïque et l'acide arachidonique dans le sang de cordon et le neuro-développement du nouveau-né?

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Mémoire présenté à la Faculté de médecine et des sciences de la santé en vue de l'obtention du diplôme de maitre ès sciences (M.Sc.) en Physiologie, Faculté de médecine et des sciences de la santé, Université de Sherbrooke, Sherbrooke, Québec, Canada, J1H 5N4

La prévalence des grossesses avec diabète augmente sans cesse et cela peut engendrer des conséquences pour l'enfant, tel que des scores cognitifs plus faibles. L'acide docosahexaénoïque (DHA) et l'acide arachidonique (AA) sont importants pour le développement cérébral du fœtus. Ces acides gras doivent provenir de l'alimentation puisque l'humain ne peut les fabriquer et leur transfert de la mère au fœtus dans les grossesses avec diabète semble être modifié. D'ailleurs, dans les grossesses compliquées par le diabète gestationnel (GDM), le pourcentage relatif de DHA est plus élevé dans le plasma des mères mais plus faible dans le sang de cordon par rapport aux contrôles sans diabète. Nous pensons que dans chez les mères avec un diabète diagnostiqué avant la grossesse, le transfert de DHA vers le fœtus sera moins efficace puisque ces femmes ont des hypo- et hyperglycémies plus fréquentes. Par conséquent, notre hypothèse est que, pendant la grossesse, les femmes avec un diabète de type 1 et de type 2 (DT1; DT2) diagnostiqué avant la grosses auront un transfert plus faible de DHA et d'AA de la mère au fœtus, ce qui aura pour conséquence un neurodéveloppement plus faible de leur nouveau-né. Sept femmes enceintes avec un DT1 ou un DT2 préexistant et 26 sans diabète ont été recrutées. Le profil en acides gras des lipides totaux du plasma maternel, du sérum et du sang du cordon ombilical, et du placenta maternel et fœtal a été effectué. Une électroencéphalographie a été effectué chez le nouveau-né 24-48 heures après la naissance et 48 semaines post-aménorrhée. Nos résultats montrent que la concentration d'AA, mais non de DHA, était significativement plus faible dans les lipides totaux du sérum du cordon des participantes diabétiques comparativement aux non-diabétiques mais que le niveaux d'AA dans le plasma maternel n'était pas différent entre les groupes. Ce résultat suggère un potentiel dysfonctionnement du transfert de l'AA dans le placenta mais nos résultats n'ont pas montré d'accumulation d'AA ou de DHA dans le placenta maternel ou fœtal. Malgré ce transfert plus faible de l'AA, le neurodéveloppement des nouveau-nés tel qu'évalué par électroencéphalographie était similaire dans les deux groupes. Bien que les résultats démontrent un transfert plus faible d'AA de la mère au fœtus dans les grossesses avec diabète, ils n'expliquent pas le mécanisme. L'étude des niveaux de transporteurs d'acides gras placentaires et des niveaux d'AA dans différents classes lipidiques permettrait de mieux comprendre ce mécanisme.

Mots clés: Diabète de type 1, diabète de type 2, grossesse, acide docosahexaénoïque, acide arachidonique, neuro-développement, électroencéphalographie

SUMMARY

Does pre-existing type 1 and type 2 diabetes during pregnancy lower docosahexaenoic acid and arachidonic acid in the umbilical cord blood of newborns and thus interfere with neurodevelopment?

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The prevalence of diabetic pregnancies has been increasing over the last several decades. Diabetic pregnancies are associated with long-term negative outcomes for the offspring, including lower cognitive scores. Several groups have also investigated the transfer of docosahexaenoic acid (DHA) and arachidonic acid (AA) from mother to fetus in diabetic pregnancies, since these fatty acids are important for fetal brain development. These fatty acids must be obtained through the diet since humans cannot make them. Previous studies have shown that in pregnancies complicated by gestational diabetes mellitus (GDM), relative percentage of DHA is higher in the plasma of GDM mothers but lower in the cord blood of GDM mothers compared to controls. We believe that in pre-existing diabetes, the transfer of DHA and AA to the fetus will be less efficient given the greater frequency of hypo- and hyperglycemia in these women. Therefore, we hypothesize that pre-existing type 1 and type 2 diabetes (T1D and T2D) during pregnancy lowers the transfer of DHA and AA from mother to fetus which results in lower neurodevelopment of the neonate.

Seven pregnant women with pre-existing T1D or T2D and 26 without diabetes were recruited. The fatty acid profile of the total lipids from the maternal plasma, umbilical cord serum and whole blood, and the maternal and fetal placenta were performed. An electroencephalography in the neonate was also performed 24-48 hours after birth and 48 weeks post-amenorrhea.

AA concentration, but not DHA, was significantly lower in the umbilical cord serum total lipids of the diabetic group compared to the non-diabetic group. However, AA levels in the maternal plasma were similar between diabetic and non-diabetic groups. This indicates that there may be a dysfunction at the level of the placenta which results in a lower transfer of AA to the fetus in diabetic pregnancies. However, there was no accumulation of AA or DHA in the maternal or fetal sides placenta. Despite the lower transfer of AA from mother to fetus in the diabetic group, there was no evidence of impaired neurodevelopment in the EEGs of the neonates from the diabetic group at either time point. While the results demonstrate lower transfer of AA from mother to fetus in diabetic pregnancies, they do not explain the mechanism. The investigation of placental fatty acid transporter levels and AA levels in different lipid pools would aid to further clarify this mechanism.

Keywords: Type 1 diabetes, type 2 diabetes, pregnancy, docosahexaenoic acid, arachidonic acid, neurodevelopment, electroencephalography

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LIST OF ABBREVIATIONS

A1C	Glycated hemoglobin
AA	Arachidonic acid
ADHD	Attention deficit hyperactivity disorder
AGA	Appropriately grown-for-gestational-age
ALA	α-linolenic acid
BMI	Body mass index
DHA	Docosahexaenoic acid
EEG	Electroencephalography or electroencephalogram
EL	Endothelial lipase
FABP	Cytosolic Fatty Acid Binding Protein
FAME	Fatty acid methyl ester
FAT/CD36	Fatty Acid Translocase
FATP	Fatty Acid Transporter Protein
FFA	Free fatty acid
GDM	Gestational diabetes mellitus
HDL	High density lipoprotein
IDF	International Diabetes Federation
LA	Linoleic acid
LC-PUFA	Long chain polyunsaturated fatty acid
LDL	Low-density lipoprotein
LGA	Large for gestational age
LPL	Lipoprotein lipase
MFSD2a	Major Facilitator Super Family Domain Containing 2a
MRI	Magnetic resonance imaging
N-3	Omega-3
N-6	Omega-6
NEFA	Non-esterified fatty acid
NICU	Neonatal intensive care unit
pFABPpm	Placental Plasma Membrane Fatty Acid Binding Protein
PSD	Power spectral density
RBC	Red blood cell
T1D	Type 1 diabetes
T2D	Type 2 diabetes
TG	Triglyceride
VLDL	Very-low-density lipoprotein

INTRODUCTION

Diabetes Mellitus

Definition and prevalence

Diabetes mellitus is a chronic metabolic disorder that causes hyperglycemia due to impaired insulin secretion, defective insulin action, or both (Punthakee *et al.*, 2018). Chronic hyperglycemia is associated with a number of complications including cardiovascular disease, neuropathy, diabetic nephropathy, and diabetic retinopathy. According to results from the 9th edition of the International Diabetes Federation (IDF) Diabetes Atlas, the global prevalence of diabetes in 2019 was estimated to be 463 million people, or 9.3% of the world's adult population (Saeedi *et al.*, 2019). Moreover, the IDF Diabetes Atlas results projected that diabetes prevalence is increasing: Worldwide diabetes prevalence will increase to 578 million (10.2% global population) in 2030 and 700 million (10.9% global population) in 2045 (Saeedi *et al.*, 2019). Similar trends are being echoed in Canada. According to Diabetes Canada, the estimated prevalence of diagnosed type 1 and type 2 diabetes cases was 3.655 million (9% of Canadians) in 2019 and this number is expected to increase to 4.785 million (11%) by 2029 (Diabetes Canada, 2019). Moreover, it is likely that many Canadians are living with undiagnosed type 2 diabetes and prediabetes, a condition in which individuals have elevated blood glucose levels which put them at a higher risk of developing diabetes (Punthakee *et al.*, 2018). The prevalence of Canadians with type 1 diabetes, diagnosed and undiagnosed type 2 diabetes, and prediabetes was estimated to be almost 11 million (29%) in 2019 and is expected to increase to over 13.3 million (32%) by 2029 (Diabetes Canada, 2019).

There are three main categories of diabetes mellitus: Type 1, type 2, and gestational diabetes mellitus. Type 1 diabetes mellitus (T1D) accounts for approximately 5-10% of those diagnosed with diabetes (American Diabetes Association, 2008). T1D is caused by the destruction of the beta cells of the pancreas (Punthakee *et al.*, 2018). Pancreatic beta cells produce insulin, and therefore their destruction leads to insulin deficiency. Beta cell destruction may be immune-mediated or idiopathic (American Diabetes Association, 2015 #2). Individuals with T1D are typically diagnosed earlier in life (age of diagnosis < 25 years),

do not usually have a family history of diabetes, and are prone to diabetic ketoacidosis (Punthakee *et al.*, 2018). Type 2 diabetes mellitus (T2D) accounts for 90-95% of total diabetes cases (American Diabetes Association, 2008). T2D is characterized by insulin resistance with relative insulin deficiency (American Diabetes Association, 2015). Unlike T1D, T2D is not associated with auto-immune destruction of pancreatic beta cells or diabetic ketoacidosis (American Diabetes Association, 2015). Individuals with T2D are typically diagnosed later in life (age of diagnosis > 25 years), frequently have a family history of diabetes, and tend to present obesity (Punthakee *et al.*, 2018). Lastly, gestational diabetes mellitus (GDM) is defined as glucose intolerance with first onset during pregnancy (Baz *et al.*, 2016). GDM is typically diagnosed using a 50 g glucose challenge test and/or a 75 g oral glucose tolerance test at 24-28 weeks of gestation (Feig *et al.*, 2018).

As previously mentioned, the number of diabetes cases is increasing (Saeedi *et al.*, 2019 and Diabetes Canada, 2019). The increase in T2D cases, which make up approximately 90% of total diabetes cases, is associated with aging, urbanization, and the obesity epidemic (Saeedi *et al.*, 2019). Similarly, T1D incidence is also increasing, but the cause of this is unclear (Patterson *et al.*, 2009). In line with this trend, the prevalence of diabetes in women of childbearing age is also increasing. According to a population-based cohort study of 1,109,605 women in Ontario who gave birth between 1996 and 2010, the rate of GDM and pre-existing diabetes (T1D and T2D) during pregnancy doubled during this time period (GDM prevalence increased from 2.7% to 5.6% and T1D and T2D prevalence increased from 0.7% to 1.5%) (Feig *et al.*, 2014). In addition, the authors found that the rate of both GDM and pre-existing T1D and T2D during pregnancy was consistently higher in women aged ≥ 30 years compared to women aged 15-29 years (Feig *et al.*, 2014). By the year 2010, almost 1 in every 10 pregnant woman ≥ 30 years of age had GDM (7.4%) or pre-existing T1D or T2D (1.9%) (Feig *et al.*, 2014).

Diabetes during pregnancy and its associated outcomes

Diabetic pregnancies can be divided into two main categories: GDM and pre-existing diabetes. In the case of GDM pregnancies, the glucose intolerance first develops or is first recognized during the pregnancy (Baz *et al.*, 2016). On the other hand, pre-existing diabetes during pregnancy consists of the pregnant woman having T1D or T2D diagnosed prior to the

pregnancy. This means that in the case of pre-existing diabetes, the difficulties in blood glucose control are present from the beginning of the pregnancy. Therefore, in the case of pre-existing T1D and T2D during pregnancy, the fetus may be exposed to the impaired glucose control from conception. This is in contrast to GDM pregnancies, where typically the glucose intolerance develops along the course of the pregnancy and thus the fetus is exposed to elevated blood glucose levels for a shorter period of time. Before discussing the potential impact of this difference in the exposure time to hyperglycemia in pregnancies with pre-existing diabetes versus GDM, we will explore the role of maternal glucose levels during pregnancy and how they may impact the fetus.

Glucose metabolism in normal pregnancies

Over the course of pregnancy, the mother's metabolism must adjust in order to meet the increasing needs of the fetus to ensure growth, development, and adequate energy stores. These changes in metabolism are also required to meet the increasing physiological demands of pregnancy as well as to ensure that the mother has enough energy stores for during and after the pregnancy (Hadden and McLaughlin, 2009). Glucose is an important substrate for the human fetus and must be consistently available in sufficient amounts to meet the needs of the growing fetus. There are two main aspects of carbohydrate metabolism that change during pregnancy: decreasing fasting glucose levels and a gradual increase in postprandial glucose and insulin secretion (Hadden and McLaughlin, 2009). During early pregnancy, fasting glucose levels have been known to decrease, likely due to increased maternal plasma volume (Hadden and McLaughlin, 2009). Fasting glucose levels are measured during the fasting state, during which insulin levels are low and the liver produces glucose in order to fuel the brain, red blood cells, and other organs in the absence of food intake. As the pregnancy progresses, fetoplacental glucose utilization increases which also decreases fasting plasma glucose levels (Hadden and McLaughlin, 2009). As previously mentioned, pregnancy is also associated with a gradual increase in postprandial plasma glucose levels and increased insulin secretion. Normally, insulin secretion would trigger uptake of glucose by muscles and adipose tissue and would inhibit the release of glucose by the liver, resulting in a subsequent decrease in glucose levels. However, in the case of pregnancy, there is also a decrease in insulin sensitivity (Butte, 2000 and Hadden and McLaughlin, 2009). A decrease

in insulin sensitivity means that more insulin is produced in response to food consumption and that nutrients such as glucose remain in the blood for longer. Moreover, decreased insulin sensitivity of the liver results in increased hepatic output of glucose (Hadden and McLaughlin, 2009). Taken together, this all results in higher post-prandial glucose levels and therefore increased availability of glucose for the fetus.

Blood glucose control in diabetic pregnancies

It is clear that a tight control of glucose metabolism over the course of pregnancy is necessary in order to provide enough glucose for fetal development. This then poses a problem for diabetic pregnancies wherein glucose metabolism is altered. In the case of T1D, pancreatic beta cells are no longer able to produce insulin. This results in insulin deficiency and therefore elevated blood glucose levels. Normal blood glucose levels must be maintained through the administration of exogenous insulin (American Diabetes Association, 2015). On the other hand, in pregnancies complicated by T2D, uncontrolled blood glucose levels are caused by insulin resistance with relative insulin deficiency (American Diabetes Association, 2015). In addition, T2D is often associated with obesity, hypertension, and other comorbidities which also increase the risk of pregnancy complications (American Diabetes Association, 2015). Proper management of blood glucose levels can be difficult in pregnancies complicated by either T1D or T2D and therefore these individuals are at a greater risk for hyper- and hypo-glycemic episodes (American Diabetes Association, 2015 and Murphy *et al.*, 2007). One study performed continuous glucose monitoring in 40 pregnant women with T1D and 17 pregnant women with T2D throughout their pregnancies to gain insight into how glucose levels change over the course of the day in these populations (Murphy *et al.*, 2007). The authors observed no difference in A1c between T1D and T2D participants over the course of gestation (Murphy *et al.*, 2007). The authors also found that at 8 weeks of gestation, individuals from both the T1D and T2D group experienced hyperglycemia (blood glucose levels > 140 mg/dL) for more than 40% of their day (approximately 10 hours per day) (Murphy *et al.*, 2007). Percentage of time spent per day in hyperglycemia decreased for both groups over the course of gestation; percentage of time spent hyperglycemic decreased from 41% at the end of the first trimester to 33% at the end of the third trimester for T1D participants and 33% at the end of the first trimester to 12% at

the end of the third trimester for T2D participants (Murphy *et al.*, 2007). They also found that proportion of time spent hypoglycemic did not change significantly for either groups throughout pregnancy (Murphy *et al.*, 2007). Moreover, women with T1D tended to spend more time hypoglycemic (blood glucose level <70 mg/dL and <50 mg/dL) than women with T2D (3.3 hours per day *vs.* 2.3 hours per day) (Murphy *et al.*, 2007). Despite spending less time in hypoglycemia during the day, T2D women were found to have an equivalent risk for nocturnal hypoglycemia to that of the T1D participants (Murphy *et al.*, 2007). This study confirms that both T1D and T2D during pregnancy can lead to both hypo- and hyperglycemic episodes during all three trimesters of pregnancy. This means that the fetus is exposed to a hypoglycemic and hyperglycemic environment from the beginning of gestation. This contrasts with GDM, where glucose intolerance develops over the course of the pregnancy and therefore the fetus may not be exposed to these hypo- and hyperglycemic from the beginning of gestation.

Lack of blood glucose control during pregnancy can potentially lead to complications. For instance, a meta-analysis of 25 reports that included data on 207,172 non-diabetic pregnant women who received oral glucose tolerance tests found that there were positive linear associations between maternal glucose concentrations and cesarean section, induction of labor, large for gestational age (LGA), macrosomia, and shoulder dystocia (Farrar *et al.*, 2016). Furthermore, a retrospective case-control study of pregnancies in which the women underwent a second trimester 1-hour oral glucose challenge test found that the hypoglycemic group had an increased rate of admission to special/neonatal intensive care units (Feinberg *et al.*, 2005). Moreover, frequent maternal hypoglycemia can also be associated with intrauterine growth restriction (American Diabetes Association, 2015). Given the increased occurrence of hypoglycemia and hyperglycemia in diabetic pregnancies, it would be logical to think that diabetic pregnancies would result in similar complications found in the Farrar and Feinberg studies. The following sections will discuss the short- and long-term outcomes of diabetic pregnancies.

Short-term negative outcomes of diabetic pregnancies

Unsurprisingly, the same complications found in the Farrar and Feinberg studies discussed in the previous section are also associated with diabetic pregnancies. A population-

based cohort study of 1,109,605 women who gave birth in Ontario between 1996-2010 found that in women with pre-existing T1D and T2D, there was an 86% increased risk of having an infant with congenital anomalies and a 133% increased risk of perinatal death compared to women without diabetes (Feig *et al.,* 2014). That same study reported that women with GDM had a 26% higher risk of having an infant with congenital anomalies and a lower risk of perinatal death (relative risk 0.63) compared to women without diabetes (Feig *et al.*, 2014). A greater risk for congenital anomalies and perinatal death in diabetic pregnancies compared to non-diabetic pregnancies were also found in other cohorts (Lai *et al.*, 2016 and Tennant *et al.*, 2014).

Several studies reported a greater risk for cesarean section in women with diabetic pregnancies compared to non-diabetic women (Buhary *et al.*, 2016, Kothari & Lim, 2014, Catalano *et al.*, 2012, Cundy *et al.* 2013, and Lai *et al.*, 2016). The risk for cesarean section is greatest in pregnant women with T1D and T2D compared to those with GDM (Kathori & Lim, 2014 and Lai *et al.*, 2016). Moreover, the risk of cesarean section is even greater in pregnant women with T1D, T2D, and GDM who have elevated glycated hemoglobin (A1C) levels (>6.5%) compared to those with normal A1C levels (Buhary *et al.* 2016). Pregnant women with pre-existing diabetes and GDM also have a greater risk for other pregnancy and delivery complications such as miscarriage (Buhary *et al.* 2016 and Alessi *et al.*, 2018), polyhydramnios (Kothari & Lim, 2014), preterm delivery (Lai *et al.* 2016 and Alessi *et al.*, 2018), neonatal intensive care unit (NICU) admission (Lai *et al.* 2016 and Alessi *et al.*, 2018), shoulder dystocia (Catalano *et al.*, 2012, Kotari & Lim, 2014 and Lai *et al.*, 2016), and hypertension and pre-eclampsia (Catalano *et al.*, 2012, Lai *et al.* 2016 and Alessi *et al.*, 2018).

Diabetic pregnancies often result in infants with higher birth weight and increased adiposity. The risk of fetal macrosomia, a condition in which the neonate weighs more than 4000 grams, is greatest in pregnancies complicated by pre-existing T1D and T2D, followed by those complicated by GDM, compared to non-diabetic pregnancies (Lai *et al.*, 2016). One study found that the risk for fetal macrosomia was higher in pregnant women with T1D, T2D and GDM who have elevated A1C levels compared to those with normal A1C levels (Buhary *et al.*, 2016). Another study found that pregnancies complicated by both GDM and obesity had an increased odds ratio of neonates with birth weight and body fat percentage greater

than the 90th percentile compared to those complicated by GDM or obesity alone (Catalano *et al.*, 2012). These complications are thought to be the result of fetal hyperinsulinemia as a response to elevated levels of maternal blood glucose levels in diabetic pregnancies (Burlina *et al.*, 2017).

Long-term negative outcomes for offspring of diabetic pregnancies

There are several long-term consequences of diabetic pregnancies that can negatively impact the offspring throughout their lives. Diabetic pregnancies are thought to be associated with two main categories of long-term complications. The first is related to obesity and metabolic problems and the second category is related to cognitive function. The following sections will discuss these long-term complications in more detail.

Metabolic syndrome, increased body mass index, and Type 2 Diabetes

Offspring of diabetic pregnancies are at a greater risk of developing metabolic syndrome, a group of conditions (abdominal obesity, high blood triglyceride (TG) levels, low levels of high-density lipoprotein (HDL) cholesterol, high blood pressure, and high fasting blood glucose) which increase an individual's risk for heart disease, stroke, and T2D (Clausen *et al.*, 2009). A follow-up study examined the risk of overweight and metabolic syndrome in adult offspring of mothers with diet-treated GDM, T1D, and with risk indicators for GDM. The participants with GDM had a higher fasting (5.2 *vs.* 4.7 mmol/liter) and 2-hour blood glucose (7.8 *vs.* 5.2 mmol/liter) compared to the participants with risk indicators for GDM. The mean blood glucose of T1D participants was 8.9 mmol/liter and 6.8 mmol/liter in the first and third trimesters, respectively. With regard to the offspring, the authors observed that all three groups of offspring had a significantly higher risk for metabolic syndrome than offspring from the background population (Clausen *et al.*, 2009). Similarly, another study found that offspring of mothers with T1D had a higher prevalence of metabolic syndrome compared to offspring of non-diabetic mothers (Vlachová *et al.*, 2015).

Several studies have also found a link between diabetes during pregnancy and higher offspring body mass index (BMI) and obesity risk. One study found that by eight years of age, approximately half of the offspring from the diabetic mothers group weighed greater than the 90th percentile for weight (Silverman *et al.*, 1991). This higher weight in the

offspring of diabetic mothers was positively correlated with the mothers' weight before pregnancy and with amniotic fluid insulin at 32-38 weeks of gestation (Silverman *et al.*, 1991). In that same cohort, the mean BMI of offspring of diabetic mothers was 24.6 ± 5.8 kg/m^2 compared to 20.9 ± 3.4 kg/m^2 in the offspring of control mothers by 14-17 years of age (Silverman *et al.*, 1998). The authors observed an association between childhood obesity adolescence and offspring sex, maternal weight, and amniotic fluid insulin concentration (Silverman *et al.*, 1998). Another study found that the BMI standard deviation score between 1-14 years of age was highest in LGA and non-LGA offspring of T2D mothers, compared to LGA and non-LGA offspring of T1D and GDM mothers (Hammoud *et al.*, 2018). They also examined several covariates (maternal age at pregnancy, parity, maternal education level and employment, maternal and paternal ethnicity, maternal pre-pregnancy BMI, and current paternal BMI) and found that none of them had a statistically significant impact on the slope of the BMI models (Hammoud *et al.*, 2018). In addition, another study found that offspring of mothers with diet-treated GDM and T1D had double the risk of being overweight (BMI ≥ 25 kg/m^2) at 18-27 years of age compared to offspring from the background population (Clausen *et al.*, 2009). Moreover, this risk remained statistically significant after adjustment for maternal age at delivery, maternal pre-pregnancy BMI, offspring age, family occupational social class, and maternal hypertension at first visit (Clausen *et al.*, 2009). Similarly, another study found that adolescent offspring of mothers with T1D had a higher BMI standard deviation score compared to offspring of non-diabetic mothers after adjustment for maternal pre-pregnancy BMI and pubertal development (Vlachová *et al.*, 2015). Finally, another study that compared BMI between siblings who were born before and after their mother was diagnosed with T2D revealed that the mean BMI was 2.6 kg/m^2 higher in siblings born after their mother developed T2D compared to those born prior to the diagnosis (while controlling for sibship) (Dabelea *et al.*, 2000).

Offspring of diabetic pregnancies are also at a greater risk of developing prediabetes and T2D. In a follow-up study of 18-27 years old offspring of mothers with GDM or T1D, the adjusted odds ratio for offspring pre-diabetes/T2D (adjusted for maternal family history of diabetes, the mother's weight, and the age of the offspring) was 7.76 in offspring of GDM mothers and 4.02 in offspring of T1D mothers compared to offspring of the background population (Clausen *et al.*, 2008). In the previously mentioned study which compared

siblings who were born before and after their mother was diagnosed with T2D, the authors reported that the siblings who were born after the mother developed T2D were at a significantly higher risk of developing T2D than their siblings who were born before their mother's diagnosis (Dabelea *et al.*, 2000).

Cognitive abilities

While the link between maternal diabetes during pregnancy and higher offspring BMI and risk for metabolic syndrome and T2D is relatively intuitive, the connection between maternal diabetes and the offspring's cognitive abilities may not be as evident. In short, glucose is transported to the fetus via the placenta based on the maternal-to-fetal glucose concentration gradient (Burlina *et al.*, 2017). This means that if maternal blood glucose levels are elevated, then more glucose is transferred to the fetus. Maternal insulin, however, cannot cross the placenta (Burlina *et al.*, 2017). Therefore, the fetus must produce its own insulin in response to the elevated level of glucose influx. Maternal hyperglycemia often seen in diabetic pregnancies can lead to hyperplasia of the pancreatic beta cells in the fetus which accelerates the fetus's ability to produce insulin in utero (Burlina *et al.*, 2017). Once born, the neonate is no longer exposed to its mother's hyperglycemia via the placenta and therefore experiences hypoglycemia. Neonatal hypoglycemia is associated with increased maternal blood glucose levels during pregnancy (Burlina *et al.*, 2017, Buhary *et al.*, 2016, and Farrar *et al.*, 2016). It seems that both maternal hyperglycemia during pregnancy and neonate hypoglycemia can be detrimental for the developing brain. For instance, one study in rats found that maternal hyperglycemia was associated with the retardation of dendritic development in the fetal brain (Jing *et al.*, 2014). Another study in rats revealed that maternal diabetes-induced hyperglycemia and intracerebral hyperinsulinism decrease fetal brain concentrations of neuropeptide Y, a neurotransmitter that mediates several biological functions including cell neurogenesis (Singh *et al.*, 1997). In addition, another group reported that severe uncontrolled hyperglycemia in pregnant rats resulted in neurodevelopmental delay in the pups as well as a deregulation of the expression of proteins involved in apoptosis, cellular survival, and neuroinflammation in the hippocampus of the pups (Piazza *et al.*, 2019). In terms of neonatal hypoglycemia, a systematic review of eighteen studies that assessed the effect of neonatal hypoglycemia on neurodevelopment in humans found that the

results were mixed, with some finding no differences and others reporting serious brain damage following neonatal hypoglycemic episodes (Boluyt *et al.*, 2006).

Given the potential link between maternal hyperglycemia during pregnancy, neonatal hypoglycemia and disturbances in neurodevelopment, several studies have compared cognitive measures in offspring of diabetic and non-diabetic mothers (Brinciotti *et al.*, 2009, de Regnier *et al.*, 2000, Silverman *et al.*, 1998, Fraser *et al.*, 2012, Clausen *et al.*, 2013, Temple *et al.*, 2011, Bytoft *et al.*, 2016, Clausen *et al.*, 2011, Xiang *et al.*, 2018, and Bytoft *et al.*, 2017). One study reported that average IQ scores at ages 3-5 years were inversely correlated with maternal blood β-hydroxybutyrate and free fatty acid levels in the third trimester (Silverman *et al.*, 1998). Similarly, the authors also reported that average IQ scores of offspring of diabetic mothers at ages 7-11 years were inversely correlated with maternal A1C levels in the 2nd trimester and β-hydroxybutyrate levels in the 3rd trimester (Silverman *et al.*, 1998). Another study found that pre-existing diabetes during pregnancy and GDM were associated with lower average School Entry Assessment scores at age 4, lower age IQ scores at age 8, and lower educational attainment at age 16 in offspring of diabetic mothers compared to offspring of non-diabetic mothers after adjustment for offspring sex, maternal age, pre-pregnancy BMI, smoking, parity, cesarean section, maternal education, and occupational social class (Fraser *et al.*, 2012). It was also reported that adult offspring of mothers with GDM had lower global cognitive scores than offspring from the background population, however the difference was not statistically significant after adjustment for several well-known predictors of cognitive function (Clausen *et al.*, 2013). Studies have also reported that offspring of mothers with T1D had normal overall IQ scores but poorer working memory at ages 6-12 years (Temple *et al.*, 2011), lower scores in composite intelligence, verbal intelligence, nonverbal intelligence, and composite memory during adolescence (Bytoft *et al.*, 2016), and lower global cognitive scores in adulthood (Clausen *et al.*, 2011) compared to offspring of non-diabetic mothers.

It has also been hypothesized that diabetes during pregnancy may also be associated with attention deficits in the offspring. For instance, a recent retrospective birth cohort study of 333,182 individuals born between 1995-2012 found that the hazard ratio for attention deficit hyperactivity disorder (ADHD) in offspring at the 4-year follow-up was 1.57 for offspring of T1D mothers, 1.43 for offspring of T2D mothers, 1.26 for offspring of mother

with GDM requiring anti-diabetes medication, and 0.93 for offspring of mothers with GDM that did not require medication (Xiang *et al.*, 2018). In contrast, another study that assessed attention deficits in 269 adolescent offspring of mothers with T1D did not find any clinically significant differences attention deficit scores in adolescents from T1D mothers compared to those from non-diabetic mothers (Bytoft *et al.*, 2017).

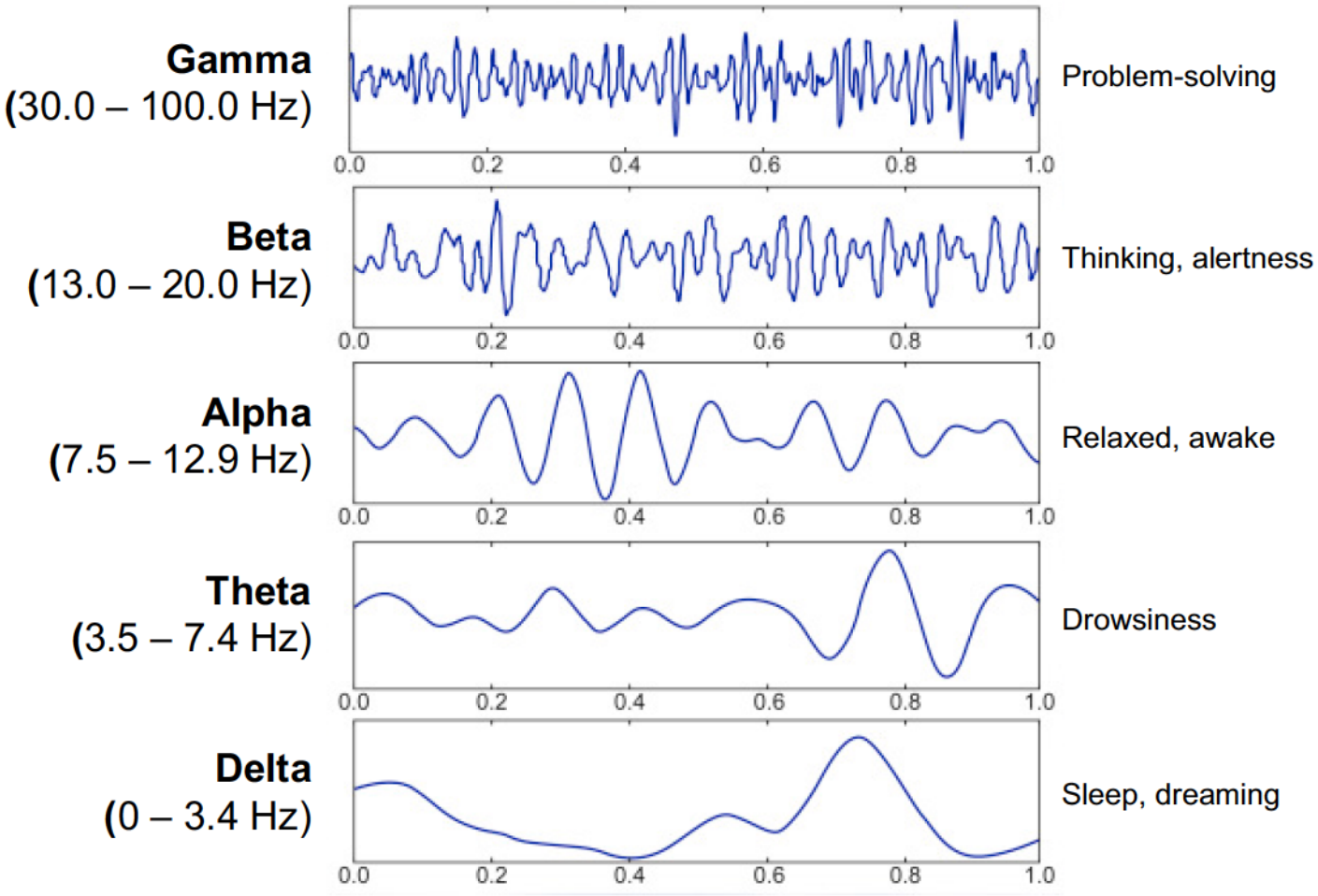

Figure 1. Types of brain waves in electroencephalography (EEG) recording. EEG brain waves are classified based on frequency and correspond to different types of brain activity. Image of EEG waves was modified from Abhang *et al.*, 2016.

Electroencephalography

Since most cognitive tests cannot be performed in humans less than one year of age, there is limited information about the neurodevelopment of neonates from diabetic mothers after birth. While magnetic resonance imaging (MRI) can be used to assess structural brain

development, it might be considered to be too invasive to perform in infants without there being any clinical rationale for the infant's health. Electroencephalography (EEG) is a recording of the brain's electrical activity using electrodes placed along the subject's scalp. EEG is a non-invasive way to detect subtle differences in brain activity and can be performed soon after birth. EEG recordings result in oscillating patterns of brain activity or brain waves which differ in frequency based on the state of the brain or the task it is performing (Marzbani *et al.*, 2016) (Figure 1). Frequency is a measure of the number of waves per second (Hertz (Hz)). Brainwaves can be classified into the following five categories based on their frequency range: Delta (0 – 3.4 Hz), Theta (3.5 – 7.4 Hz), Alpha (7.5 – 12.9 Hz), Beta (13.0 – 20.0 Hz), and Gamma (30 – 100 Hz) (Marzbani *et al.*, 2016 and Léveillé *et al.*, 2018). The exact values of the frequency ranges vary depending on the study. Each type of brain wave corresponds to different states or various tasks performed by the brain. In the mature brain, delta waves (0 – 3.4 Hz) are associated with sleep and dreaming, theta waves (3.5 – 7.4 Hz) are associated with drowsiness, alpha waves (7.5 – 12.9 Hz) are associated with both a relaxed and awake state, beta waves (13.0 – 20.0 Hz) are associated with thinking and alertness, and finally gamma waves (30 – 100 Hz) are associated with problem solving (Marzbani *et al.*, 2016).

In the case of the developing brain, brain activity changes in order to meet the increased demand for computational load. In particular, brain activity increases in both variability and complexity with age (Vakorin *et al.*, 2011, Costa *et al.*, 2002, and Lippé *et al.*, 2009). Vakorin and colleagues assessed the complexity of brain signals in response to auditory and visual stimuli using scalp-recorded EEG measurements in 35 children aged 27 days to 5 years and 5 months (Vakorin *et al.*, 2011). The authors found that developmental changes were explained by a decrease in the local processing of information, with a peak in the alpha frequency range, as well as an increase in synchronization in the delta, theta and alpha rhythms (Vakorin *et al.*, 2011). Moreover, the authors observed an association between brain development and desynchronization effects in the higher beta to lower gamma frequency ranges (Vakorin *et al.*, 2011). To this writer's knowledge, four studies reported using EEG to evaluate neurodevelopment in the offspring of diabetic mothers (Castro Conde *et al.*, 2013, Brinciotti *et al.*, 2009, de Regnier *et al.*, 2000, and Léveillé *et al.*, 2018). One of said studies performed 90-minte video-EEG recordings in 23 full-term neonates of T1D

mothers and 22 from non-diabetic mothers 48 – 72 hours after birth with the goal of recording at least one complete sleep-wake cycle (Castro Conde *et al.*, 2013). The authors found that the video-EEG recordings from newborns from T1D mothers indicated abnormal development and brain function. Notably, the authors observed that infants of diabetic mothers had a higher percentage of indeterminate sleep, higher percentage of discontinuity, higher percentage of δ brushes in the bursts, higher duration of inter-burst intervals, less encoches frontales, less θ/α Rolandic activity, and more transient sharp waves (Castro Conde *et al.*, 2013). In addition, the infants of diabetic mothers with a maternal A1c ≥ 6% in the first and second trimester of pregnancy exhibited a greater percentage of δ brushes in the burst compared to infants of diabetic mothers with maternal A1c < 6% (Castro Conde *et al.*, 2013). Another type of cognitive measure that has been performed in 2-month-old infant offspring of diabetic mothers is visual evoked potential, a test which can be performed in infants to determine changes in brain maturation and how well the visual system is functioning. One study reported that visual evoked potential latencies were significantly longer in infants of diabetic mothers (N = 24 from T1D, 3 from T2D, and 13 from GDM mothers) compared to those of non-diabetic mothers (Brinciotti *et al.*, 2009). Another group assessed recognition memory in 1-year-old infants of diabetic mothers (N = 9 with pre-existing diabetes and 16 with GDM) using event-related potentials; they reported that infants of diabetic mothers seemed to have different event-related potential patterns which indicated lower 1-year cognitive development and subtle recognition memory impairments compared to infants of non-diabetic mothers (de Regnier *et al.*, 2000). More specifically, the infants of diabetic mothers exhibited shorter latencies to the "P2" peak compared to the control group (de Regnier *et al.*, 2000). In addition, the authors observed no difference between the areas under the curve for the mother and stranger event-related potential for the infants of diabetic mothers whereas the infants of control mothers exhibited event-related potential areas under the curve which were more negative in response to the stranger's voice compared to the maternal voice (de Regnier *et al.*, 2000).

Our laboratory performed a pilot study in which they used EEGs to evaluate the neurodevelopment of newborn offspring from mothers with GDM less than 48 hours after birth (Léveillé *et al.*, 2018). The study included 21 neonates from non-diabetic mothers and 25 from GDM mothers. The authors compared coherence (correlation between oscillations

of activity from the same brain region between the two hemispheres) and power spectral density (PSD) (distribution of the signal power over frequency of the signal from each electrode). They found that PSD was significantly lower in the left centro-occipital region in neonates from GDM mothers vs. non-diabetic mothers. However, these differences were no longer statistically significant when adjusted for gestational age. The authors hypothesized that the similarities in coherence and PSD were due to the fact that the GDM mothers in this study had excellent control over their blood glucose levels. This study highlights the importance of maintaining control of blood glucose levels in pregnancies complicated with GDM in order to protect fetal neurodevelopment.

Long-chain polyunsaturated fatty acids

Long-chain polyunsaturated fatty acids and fetal neurodevelopment

This review will focus on the role of long chain polyunsaturated fatty acids (LC-PUFA) in fetal neurodevelopment. Of the LC-PUFAs, there are two that are of particular interest in fetal neurodevelopment: Docosahexaenoic acid (DHA) and arachidonic acid (AA) (Figure 2). DHA is a fatty acid with a 22-carbon chain with 6 double bonds. It is an omega-3 (N-3) LC-PUFA, meaning that the first double bond is located between the third and fourth carbon with respect to the methyl end of the carbon chain. DHA is highly concentrated in brain grey matter and retina phospholipids (Sastry 1985 and Innis 2007). It plays an important role in synaptic plasticity, neurogenesis, and brain growth (Sastry 1985). AA is a fatty acid with a 20-carbon chain and four double bonds. It is an omega-6 (N-6) LC-PUFA, meaning that the first double bond is located between the sixth and seventh carbon with respect to the methyl end of the carbon chain. AA is found in membrane phospholipids throughout the body. It plays a role in growth and is a precursor for the production of eicosanoids which are components of immune and inflammatory pathways (Innis 2007).

Typically, DHA and AA can be obtained either through diet or through the desaturation and elongation of essential fatty acids α-linolenic acid (ALA) and linoleic acid (LA). The main dietary source of DHA is fatty fish whereas AA is primarily found in meat, poultry, and eggs (Friesen & Innis, 2009). DHA and AA can be synthesized from their essential fatty acid precursors ALA and LA, respectively, through a series of desaturation and elongation reactions. However, the activity of the Δ6 and Δ5 desaturase enzymes

involved in the formation of DHA and AA remains very low in utero (Innis 2005). The fetus must therefore rely on the transfer of DHA and AA from the mother through the placenta. During development, the fetus requires large amounts of both DHA and AA, particularly in the third trimester. The third trimester of gestation is an important period of neurodevelopment where the weight of the fetal brain increases approximately 4- to 5-fold (Clandinin *et al.*, 1980). One study estimated that during the last trimester of pregnancy, the fetus accumulates 3660 mg of N-6 and 469 mg N-3 LC-PUFA per week (Clandinin *et al.*, 1981). It was also estimated that the fetal brain accumulates 40.9 mg of N-6 and 21.8 mg of N-3 LC-PUFA per week (Clandinin *et al.*, 1981). A more recent study estimated that the daily rate of fetal accretion was 41.65 mg DHA/day and 95.25 mg AA/day at 35-40 weeks of gestation (Kuipers *et al.*, 2012). Given that the fetus relies on the mother to provide enough DHA and AA for its development, it is imperative that the mother consumes enough dietary DHA and AA in order to meet the needs of both the fetus and herself so as not to have to diminish her own fatty acid reserves.

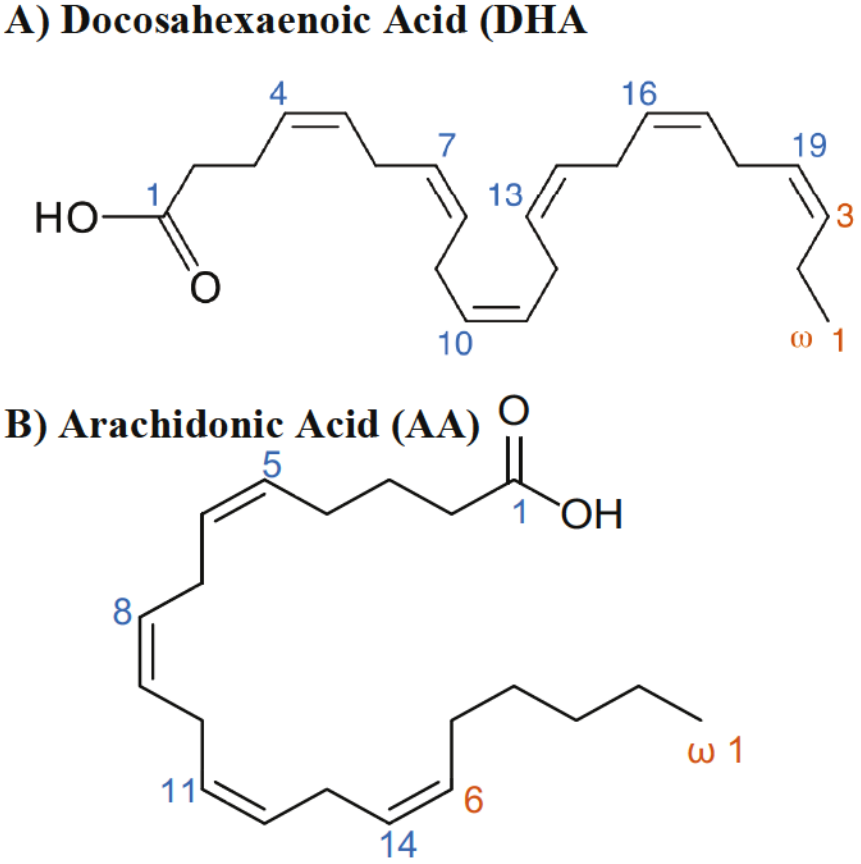

Figure 2. Structures of A) Docosahexaenoic acid (DHA) and B) Arachidonic acid (AA).

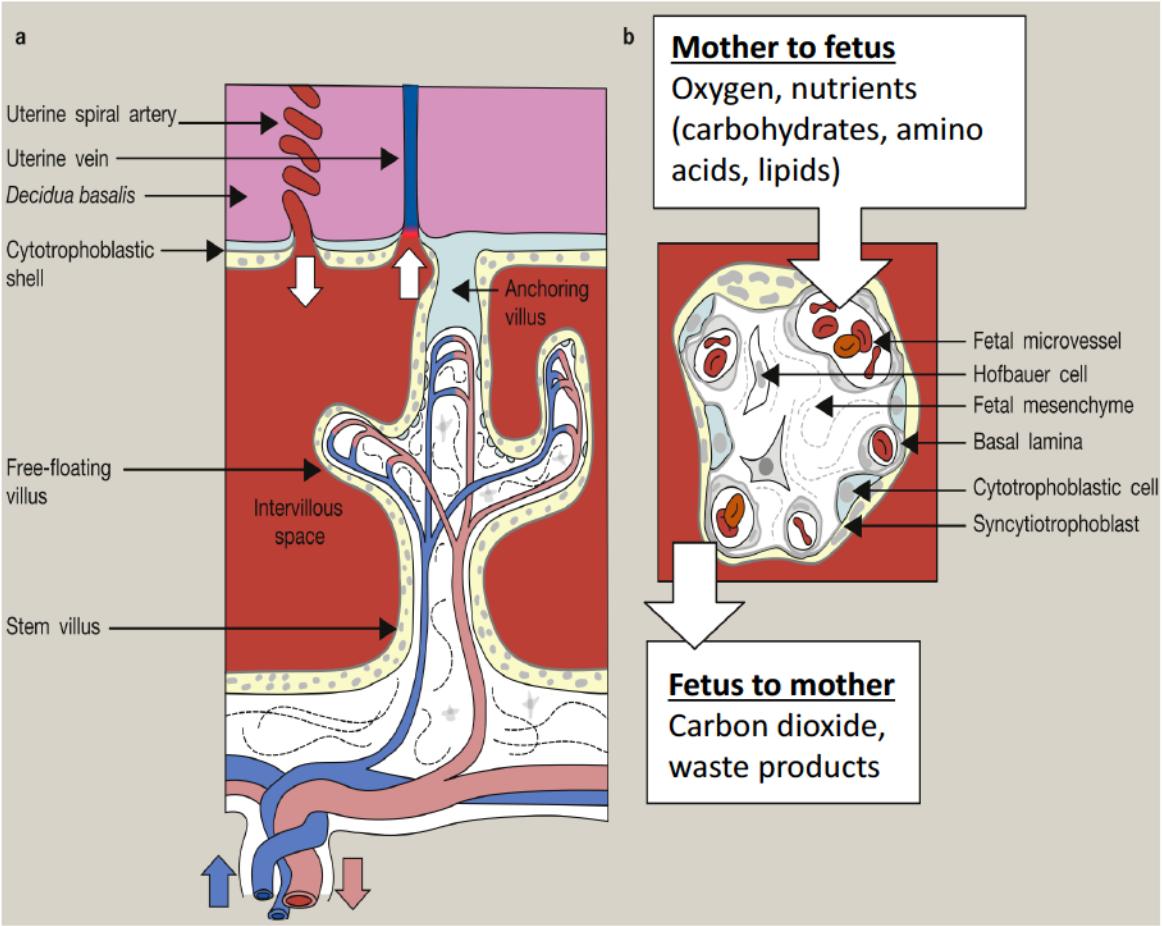

Figure 3. A) Diagram of cross-section of a placental villus. B) Schematic of transfer of molecules between maternal and fetal circulation. Oxygen and nutrients in the maternal blood enter the fetal circulation through the syncytiotrophoblast layer. Carbon dioxide and waste products exit fetal circulation and enter maternal circulation via the syncytiotrophoblast layer. Image is modified from Donelly & Campling, 2016.

Fatty acid transfer from mother to fetus

The transfer of fatty acids such as DHA and AA from mother to fetus takes place at the level of the placenta. The placenta is an organ that develops during pregnancy. It serves as the interface between the maternal and fetal tissues and it is the sight of gas exchange, nutrient uptake, and waste elimination (Donnelly & Campling, 2016). Between the basal (maternal) and chorionic (fetal) plates of the placenta, there is an intervillous space with tree-like villous projections that contain fetal blood vessels (Gude *et al.*, 2004) (Figure 3). The maternal blood floods the intervillous space where it can come into contact with the

villi. The exchange of gases, nutrients and waste between the maternal and fetal blood occurs at the level of the syncytium, a layer of specialized multi-nucleated syncytiotrophoblast cells that line the villi of the placenta (Burton & Fowden, 2015). When lipoproteins in the maternal blood come into contact with the microvillous membrane (the side of the syncytiotrophoblast cells that faces the maternal circulation), they bind to the lipoprotein lipase (LPL) and endothelial lipase (EL) which hydrolyze the TGs into non-esterified fatty acids (NEFA) (Islam *et al.*, 2016). The NEFAs are then transported through the syncytiotrophoblast to the fetal circulation with the help of several fatty acid transporters. While the exact mechanism of the transport of LC-PUFA across the placenta are not exactly known, there are several membrane proteins which are involved in the transport of fatty acids into the syncytiotrophoblast cells, namely Fatty Acid Translocase (FAT or CD36), Placental Plasma Membrane Fatty Acid Binding Protein (pFABPpm), cytosolic Fatty Acid Binding Protein (FABP), Major Facilitator Super Family Domain Containing 2a (MFSD2a), and the Fatty Acid Transporter Protein (FATP) family, (Islam *et al.*, 2016) (Figure 4). Once transported through the syncytiotrophoblast cells, the fatty acids enter the fetal circulation and are transported to the fetus via the umbilical cord vein (Gude *et al.*, 2004).

Fatty acid transfer from mother to fetus in diabetic pregnancies

Changes in maternal lipid metabolism in diabetic pregnancies

Similar to the pregnancy-induced changes in carbohydrate metabolism, lipid metabolism also changes in normal pregnancies compared to the non-pregnant state. The two major changes in maternal lipid metabolism during pregnancy are the increased accumulation of lipids in maternal tissues during early pregnancy and maternal hyperlipidemia in later pregnancy (Herrera & Ortega-Senovilla, 2010). The accumulation of maternal body fat in the first and second trimesters of gestation are largely the result of hyperphagia (an abnormally strong sense of hunger) and an increase in lipid synthesis (Herrera & Ortega-Senovilla, 2010). At this stage, the maternal metabolism is anabolic, meaning that the body is constructing molecules from smaller units. In contrast, maternal metabolism during the third trimester of pregnancy transitions to a catabolic state in which molecules are broken down into smaller units, in order to meet the increasing needs of the

fetus (Barrett *et al.*, 2014). During this period, there is an increase in adipose tissue lipolysis (Herrera & Ortega-Senovilla, 2010 and Barrett *et al.*, 2014). The resulting NEFA and glycerol enter into the maternal circulation and are repackaged as TGs in the liver and re-released into the circulation in very-low-density lipoproteins (VLDL) (Herrera & Ortega-Senovilla, 2010). The combination of increased adipocyte lipolysis and maternal insulin resistance increases TGs in lipoproteins and increases VLDL, HDL and low-density lipoprotein (LDL) concentration (Barrett *et al.*, 2014). Since lipoproteins are unable to cross the placenta, they must bind to LPL or EL on the microvillous membrane of the syncytiotrophoblast cells of the placenta in order to hydrolyze their TGs into NEFAs (Islam *et al.*, 2016). These NEFAs are transported through the syncytiotrophoblast cells to enter the fetal circulation.

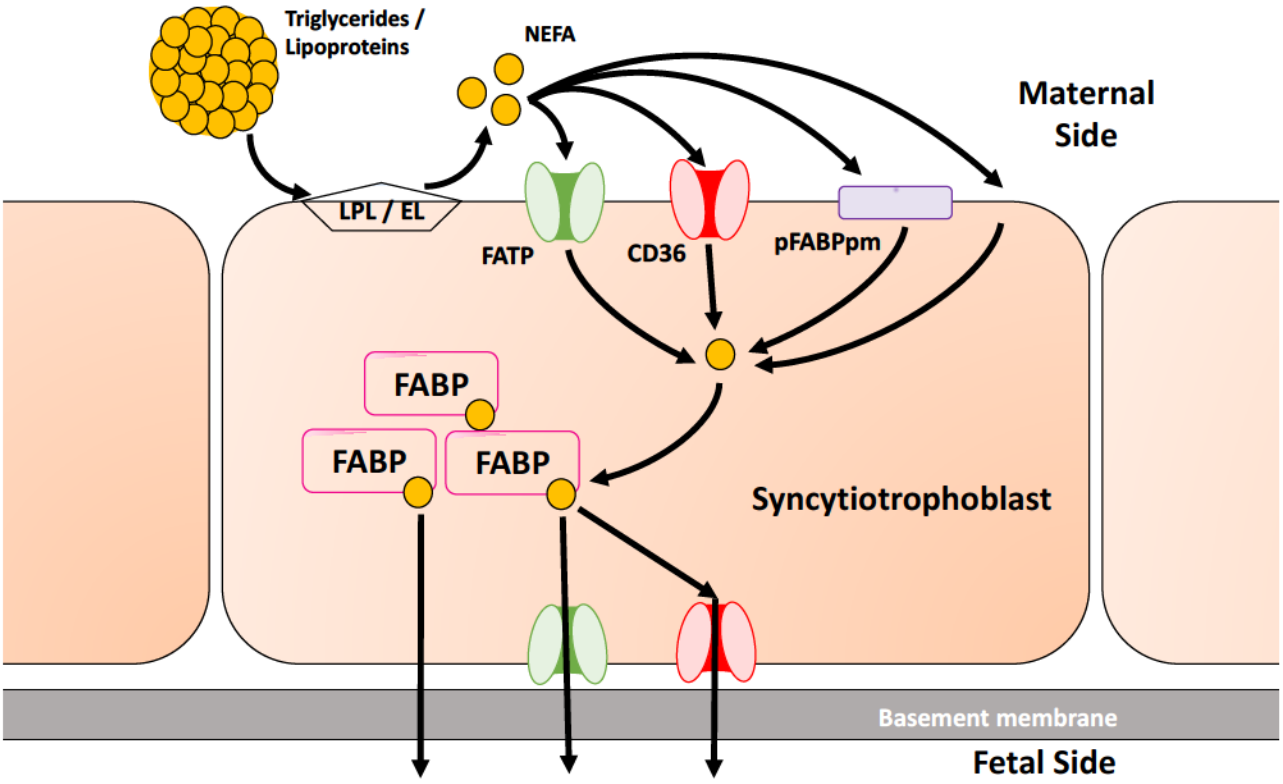

Figure 4. Transport of fatty acids across the syncytiotrophoblast layer of the placenta. Abbreviations: LPL, lipoprotein lipase; EL, endothelial lipase; FATP, fatty acid transporter protein; CD36, fatty acid translocase; pFABPpm, placental plasma membrane fatty acid binding protein; FABP, fatty acid binding protein. Image was inspired by Islam *et al.*, 2016.

According to a review conducted by Barrett and colleagues, the results of studies that examined maternal lipid levels in diabetic pregnancies are mixed, with some studies reporting dyslipidemia and others reporting no differences (Barrett *et al.*, 2014). For pregnant women with T1D, it seems that those who have well-controlled T1D without other maternal factors (such as metabolic syndrome, renal disease, and preeclampsia) have similar lipoprotein levels to non-diabetic pregnant women (Barrett *et al.*, 2014). However, one study reported that T1D pregnant women with poor blood glucose control (A1C = 8.3%) had higher levels of TGs and VLDL and lower levels of HDL_3 compared to T1D pregnant women with good blood glucose control (Merzouk *et al.*, 2000). There are not many studies reporting data on maternal lipid levels in pregnant women with T2D. The existing studies report that pregnant women with T2D may have higher levels of NEFA and TGs and lower levels of HDL-cholesterol, while another reports no differences in maternal lipoprotein levels in T2D compared to T1D, GDM, and non-diabetic pregnant women (Barrett *et al.*, 2014, Montelongo *et al.*, 1992, Göbl *et al.*, 2010, and Toescu *et al.*, 2004). Finally, pregnant women with GDM have been reported to have higher TG levels than non-diabetic pregnant women (Barrett *et al.*, 2014 and Butte *et al.*, 2000). There also seems to be mixed findings on cholesterol levels, with some studies reporting an increase in GDM maternal cholesterol levels and others finding no change (Barrett *et al.*, 2014).

Docosahexaenoic acid and arachidonic acid transfer from mother to fetus in diabetic pregnancies

There are several studies that compare DHA and AA levels in the umbilical cord blood in diabetic vs. non-diabetic women. A review article written by members of our laboratory summarized the findings of 12 studies which compared DHA levels in maternal plasma and in umbilical cord blood in diabetic vs non-diabetic pregnancies (Léveillé *et al.*, 2018). The authors found that DHA levels tended to be higher in the maternal blood but lower in the umbilical cord blood in pregnancies complicated by GDM or pre-existing T1D and T2D compared to non-diabetic pregnancies, but these results were mixed (Léveillé *et al.*, 2018). As for AA levels, of the existing studies that have reported AA levels in the maternal blood of diabetic mothers, 5 reported significantly higher AA (Wijendran *et al.*, 2000, Ortega-Senovilla *et al.*, 2009, Chen *et al.*, 2010, Wijendran *et al.*, 1999, and Thomas

et al., 2004), and 4 reported similar AA levels (Prieto-Sánchez *et al.*, 2017, Zhao *et al.*, 2016, Berberovic *et al.*, 2013 and Min *et al.*, 2005) in diabetic mothers vs. non-diabetic mothers. Of the existing studies that have reported AA levels in the umbilical blood of diabetic mothers, 5 reported significantly lower levels of AA (Wijendran *et al.,* 2000, Ortega-Senovilla *et al.*, 2009, Min *et al.*, 2005, Thomas *et al.*, 2004 and Ghebremeskel *et al.*, 2004) and 4 reported similar AA levels (Zhao *et al.*, 2014, Prieto- Sánchez *et al.*, 2017, Léveillé *et al.*, 2016, and Berberovic *et al.* 2013) in umbilical cord blood from diabetic vs. non-diabetic pregnancies. Our laboratory previously conducted a study in neonates from optimally controlled GDM pregnancies (Léveillé *et al.*, 2016). They compared the fatty acid profile in the umbilical cord plasma phospholipids, NEFA, cholesterol esters, and TGs of 15 GDM and 15 control participants. They found no statistically significant difference in DHA or AA concentrations between the GDM and non-diabetic groups. The authors hypothesized that the similarities between DHA and AA concentrations in the umbilical cord plasma of the groups are the result of the GDM mothers having good control over their blood glucose levels so that their A1C was not significantly different from the A1C of the mothers from the control group. Therefore, this study emphasizes the importance of blood glucose control in diabetic pregnancies.

Since the transport of nutrients across the placenta follow a concentration gradient, one would expect elevated levels of DHA and AA in the maternal blood to result in similarly elevated levels of DHA and AA in the umbilical cord blood. However, in the case of diabetic pregnancies, maternal DHA and AA levels tend to be elevated whereas umbilical cord blood DHA and AA levels tend to be lowered. This discrepancy between maternal and umbilical cord blood DHA and AA levels indicates that there is a problem with the transfer of these fatty acids from mother to fetus in diabetic pregnancies.

Changes in placental fatty acid transporters in diabetic pregnancies

To this writer's knowledge, there are four studies that report data on the level of expression of placental fatty acid transporters in diabetic pregnancies (Magnusson *et al.*, 2004, Radaelli *et al.*, 2009, Prieto-Sánchez *et al.*, 2017, and Segura *et al.*, 2017). The first study (Magnusson *et al.*, 2004) compared placenta samples from 8 T1D mothers and 8 GDM mothers to 8 mothers with infants appropriately grown-for-gestational-age (AGA). The

authors found that the placenta samples from T1D diabetic mothers exhibited significantly higher LPL activity compared to AGA mothers (Magnusson *et al.*, 2004). In addition, they found that both T1D and GDM placenta samples had increased levels of expression of FABP1 protein compared to AGA placenta samples (Magnusson *et al.*, 2004). The second study compared gene regulation in placenta samples from 6 T1D, 9 GDM, and 5 non-diabetic mothers (Radaelli *et al.*, 2009). They found that both T1D and GDM placentas had significantly higher levels of FABP4, FABP5 and LPL mRNA compared to non-diabetic placenta samples (Radaelli *et al.*, 2009). The third study compared the level of protein expression of several placental fatty acid transporters in placenta samples from 23 women with diet-treated GDM, 20 women with insulin-treated GDM, and 25 non-diabetic women (Prieto-Sánchez *et al.*, 2017). The authors found that placenta samples from both the diet- and insulin-treated GDM groups had significantly lower protein expression of LPL compared to placentas from the non-diabetic group (Prieto-Sánchez *et al.*, 2017). Furthermore, placenta samples from both diet- and insulin-treated GDM groups had significantly lower expression of MFSD2a, a transporter that is specific for DHA, compared to the non-diabetic group placentas (Prieto-Sánchez *et al.*, 2017). They also found non-significant increases in FATP1, FABP4, and FAT/CD36 expression and non-significant decreases in FATP4 expression in placentas from diet- and insulin-treated GDM compared to non-diabetic controls (Prieto-Sánchez *et al.*, 2017). Finally, the fourth study compared placenta gene expression of fatty acid transporters in placenta samples from 18 GDM and 37 non-diabetic women (Segura *et al.*, 2017). The authors found that the GDM placenta samples had significantly higher FATP6 and FAT/CD36 mRNA levels compared to non-diabetic placenta samples (Segura *et al.*, 2017). They also found significantly lower FATP1, FATP4, and EL mRNA levels in GDM placenta samples compared to non-diabetic placenta samples (Segura *et al.*, 2017). Taken together, these studies indicate that there may be differences in the expression of transporters involved in fatty acid transfer at the level of the placenta in pregnancies with T1D and GDM.

At this time, there do not seem to be any existing studies that report the level of fatty acid transporters in placentas from T2D women. However, there are two studies that examined placental fatty acid transporters in overweight and obese pregnant women (Lager *et al.*, 2016 and Segura *et al.*, 2017). The first study found that placenta basal plasma

membrane expression of FATP2 was correlated with maternal BMI (Lager *et al.*, 2016). However, they did not find any association between maternal BMI and placenta basal plasma membrane expression of FAT/CD36 or FATP4 (Lager *et al.*, 2016). The second study compared gene expression of several fatty acid transporters in placenta samples from 28 overweight, 30 obese, and 37 normal weight pregnant women (Segura *et al.*, 2017). The authors reported that placenta samples from both the groups who are overweight or have obesity had significantly higher mRNA levels of FAT/CD36 compared to the normal weight group (Segura *et al.*, 2017). In addition, both the groups who are overweight or have obesity had significantly lower mRNA levels of FATP1, FATP4, FABP4, FABP7, and EL compared to the normal weight group (Segura *et al.*, 2017). The authors also reported significantly higher FATP6 mRNA and lower FABP3 mRNA in the placentas from the group with obesity compared to the group with normal weight (Segura *et al.*, 2017).

Although the studies comparing placental fatty acid transporters in diabetic and non-diabetic pregnancies are scarce, they still present evidence of disrupted regulation of fatty acid transporters in diabetic placentas. These findings could provide insight on potential mechanisms for decreased LC-PUFA transfer from mother to fetus in diabetic pregnancies.

Rationale and Hypothesis

Diabetic pregnancies are associated with a lower transfer of DHA and AA relative to other fatty acids from mother to fetus. DHA and AA are both important for fetal neurodevelopment. Offspring of diabetic mothers tend to have poorer long-term cognitive outcomes compared to the background population. In the case of optimally controlled GDM, there is no significant difference in umbilical cord blood levels of DHA and AA between GDM and non-diabetic groups. In that same cohort, there was no difference in coherence and PSD in EEGs of neonates from GDM vs. non-diabetic mothers after adjustment for gestational age. These two studies emphasized the importance of blood glucose control in diabetic pregnancies. In the case of pregnancies complicated by pre-existing T1D or T2D, the diabetic condition is present prior to conception and it may be more difficult to control blood glucose levels compared to GDM patients. Furthermore, pregnant women with T1D and T2D are more prone to hypoglycemic and hyperglycemic episodes throughout gestation. Therefore, we hypothesize that pre-existing T1D and T2D during pregnancy lowers the

transfer of DHA and AA from mother to fetus which results in lower neurodevelopment of the neonate.

Objectives

Objective #1

Evaluate whether the concentration and relative percentage of DHA and AA are different in the maternal plasma, placenta, and umbilical cord serum/whole blood of diabetic and non-diabetic mothers.

Objective #2

Evaluate PSD and coherence in EEGs of newborns from diabetic and non-diabetic mothers at 2 time points (24-48 hours after birth and at 48 weeks post-amenorrhea)

MATERIALS AND METHODS

Population

Two groups of pregnant women (aged 18-40 years, singleton pregnancy) were recruited at the Centre hospitalier universitaire de Sherbrooke between 2017 and 2020. The first group (n = 7) was composed of pregnant women with T1D or T2D which was diagnosed prior pregnancy. The second group (n = 26) was composed of pregnant women without diabetes during pregnancy. The participants were recruited between 24-28 weeks of gestation. Exclusion criteria included twin pregnancy, current or past smoking, current or past alcohol/drug abuse, placental anomalies, premature delivery, uncontrolled endocrine, liver, or renal problems, cancer, consumption of DHA supplements during pregnancy, or any medical condition or medication affecting lipid metabolism.

Ethics Statement

The study was approved by the Ethics Research Committee of the Centre intégré universitaire de santé et de services sociaux de l'Estrie – Centre hospitalier universitaire de Sherbrooke. During recruitment, the participants signed a consent form for both themselves and their baby. Consent was free and informed and participants were free to withdraw from the study or withdraw their baby from the study without explanation at any time of the protocol.

Anthropometric data collection and blood sampling

Maternal weight was measured at hospital admission prior to delivery and BMI was calculated. Maternal blood samples were collected at the hospital admission prior to delivery to measure A1C. An additional 3 mL of blood was collected from the mothers, centrifuged, aliquoted, and stored at -80 °C for fatty acid profiling at a later date. After delivery, the neonate's weight, length, head circumference, and APGAR score were recorded. The APGAR score is a score from 0-10 which indicates the health of the newborn baby. It is comprised of 5 components: colour, heart rate, reflexes, muscle tone, and respiration (American Academy of Pediatrics, 2015). A 5-minute APGAR score of 0-3

is low, 4-6 is moderately abnormal, and 7-10 is reassuring. In addition, 6 mL of venous umbilical cord blood was collected. Half of the venous umbilical cord blood was aliquoted and stored at -80 °C as whole blood for fatty acid profiling at a later date. The other 3 mL of venous umbilical cord blood was left to clot at room temperature and the remaining serum was collected, aliquoted, and stored at -80 °C for fatty acid profiling at a later date. Two placenta samples (one from the maternal/decidual region and the other from the fetal/chorionic region of the placenta) of approximately 1 cm^3 in size were collected and stored at -80 °C for fatty acid profiling at a later date.

Blood and placenta sample analyses

Maternal A1C was measured using high-performance liquid chromatography (Bio-Rad VARIANT) in the Biochemistry laboratory of the Centre Hospitalier Universitaire de Sherbrooke. Fatty acid profiling of the maternal plasma, umbilical cord serum, umbilical cord whole blood, maternal placenta, and fetal placenta were performed as described in the following sections.

Maternal plasma and umbilical cord serum total lipid extraction

Total lipids were extracted from maternal plasma and umbilical cord serum using the Folch extraction method (Folch *et al.* 1957). Briefly, 250 µL plasma or umbilical cord serum were combined with 10 mL 2:1 chloroform-methanol (v/v). A known quantity of internal standard (triheptadecanoin, Nu-Chek Prep, Inc.) was added to the mixture in order to later quantify the concentration of each fatty acid in the sample. The aim was to add an amount of internal standard that corresponded to approximately 10% of the total fatty acids in the sample, but naturally the actual relative percentage of internal standard varied as the total fatty acids varied between each sample. Once the preparation rested for one hour in the dark, 2 mL 0.9% saline was added and the tubes were centrifuged at 1500 rpm for 10 minutes in order to separate the organic and aqueous phases. The organic phase (containing chloroform and lipids) was collected.

Umbilical cord whole blood total lipid extraction

The procedure for total lipid extraction from whole blood was adapted from a previously described method (Rose & Oklander, 1965). Briefly, 250 µL thawed whole blood was combined with 250 µL water to lyse the erythrocytes. While vortexing, 2.5 mL of isopropanol was slowly added to the test tube drop-by-drop in order to avoid clumping of the blood sample. Once the isopropanol was added, the tube was removed from the vortex and internal standard (triheptadecanoin, Nu-Chek Prep, Inc.) and 5 mL of chloroform were added. The tube was briefly shaken and then left to rest in the dark for one hour. After the rest period, 2 mL 0.9% saline was added and the tubes were centrifuged at 1800 rpm for 10 minutes in order to separate the organic and aqueous phases. The organic phase (containing chloroform and lipids) was collected.

Placenta total lipid extraction

Total lipids were extracted using a similar methodology to that of the maternal plasma and umbilical cord serum with some modifications. Since the placenta is a tissue, it first needed to be processed into powder before proceeding with the Folch extraction. After collection, the placenta pieces were frozen at -80 ℃. While on dry ice, the placenta tissue samples were ground into a fine homogenous powder using a BioPulverizer tissue homogenizer (Biospec Products). The placenta powder was collected in 1.5 mL Eppendorf tubes and was subsequently stored at -80 ℃ until extraction. For the extraction, approximately 100 mg of placenta powder, 17:0 PC internal standard (1,2-diheptadecanoyl-sn-glycero-3-phosphocholine, Avanti) and 5 mL 2:1 chloroform-methanol (v/v) were combined in a 7 mL potter tissue homogenizer (Kontes Glass Co.). The tissue homogenizer was then used to combine the mixture of placenta powder with the internal standard and chloroform-methanol until the placenta powder was dissolved into the solution. The mixture was then poured into a 15 mL test tube. An additional 5 mL of 2:1 chloroform-methanol (v/v) was added to the potter in order to recuperate any placenta powder remnants, and then poured into the same 15 mL test tube. The contents of the test tube were briefly shaken and then left to rest in the dark for one hour. After the resting period, 2 mL 0.9% saline was added and the tubes were centrifuged at 1500 rpm for 10

minutes in order to separate the organic and aqueous phases. The organic phase (containing chloroform and lipids) was collected.

Fatty acid profiling

After extraction from the different biological samples, the organic phase, consisting of chloroform and extracted total lipids, was evaporated using a nitrogen evaporator. The extracted lipids then underwent saponification: 3 mL KOH-methanol were added to the tubes which were subsequently capped under nitrogen and heated at 90 °C for one hour. This reaction breaks the ester bonds of the cholesterol esters and TGs and the K^+ from the KOH interacts with the free O^- on the carboxyl end of the fatty acid, resulting in fatty acid salts. This step also serves as a method to remove cholesterol, a contaminant for the gas chromatography column, from the sample. Subsequent to the heating and a brief 15-minute rest period in the dark at room temperature, 2 mL 0.9% saline and 5 mL hexane were added to the tubes. The tubes were shaken and then centrifuged at 1500 rpm for 4 minutes in order to separate the organic and aqueous phase. The organic phase (consisting of hexane and cholesterol) was removed and discarded. The remaining aqueous phase (methanol, saline and fatty acid salts) was combined with 300 µL concentrated hydrochloric acid (37%) and 5 mL hexane. The addition of concentrated hydrochloric acid serves to protonate the fatty acid salts, resulting in free fatty acids (FFAs). The tubes were then shaken and centrifuged at 1500 rpm for 4 minutes to separate the organic and aqueous phases. At this step, the FFAs were located in the organic hexane layer since they were no longer polar. The organic layer (hexane + FFAs) was collected, transferred to new tubes and evaporated using the nitrogen evaporator. The FFAs were trans-methylated through a process called methylation: 3 mL BF_3-methanol were added to the test tubes containing FFAs which were subsequently capped under nitrogen and heated at 90 °C for 30 minutes. During this reaction, a methyl group is added to the carboxyl end of the fatty acids, resulting in fatty acid methyl esters (FAMEs). The addition of a methyl group to the fatty acid increases its volatility and decreases its affinity for the column in gas chromatography. Following a rest period of 15 minutes in the dark at room temperature, 2 mL 0.9% saline and 5 mL hexane were added to the tubes which were then shaken and centrifuged at 1500 rpm for 4 minutes to separate the organic and aqueous phases. The organic hexane layer

(consisting of hexane and FAMEs) was collected and then evaporated using the nitrogen evaporator. Hexane was then added to the FAMEs so that they had a final concentration of approximately 0.3 mg/mL. The samples were then transferred to glass vials to prepare for gas chromatography.

Gas chromatography

FAMEs were analyzed by a gas chromatograph (model 6890, Agilent) equipped with a 50 m fused capillary column (SGE; 0.22 mm inner diameter, 0.25 µm film thickness). Splitless mode injection and flame ionization detection were performed at 250°C and 260°C, respectively. 1 µL injections of FAMEs were made. The oven temperature program began at 50 °C for 2 minutes, increased to 170°C at a rate of 20°C/minute and held for 10 minutes, increased to 195°C at a rate of 10°C/minute and held for 35 minutes, and increased to 220°C at a rate of 20°C/minute and held for 5 minutes. The inlet pressure of the carrier gas (helium) was 107 kPa at 50°C. The total run time was 61.75 minutes. During this process, the FAMEs are vaporized into a gas and are pushed through the column by the carrier gas helium. They then exit the column where they are burned by a hydrogen-fuelled flame. The resulting ions then pass between two charged plates and produce an electric signal which is measured by the detector. The resulting data is a chromatogram, which consists of a series of peaks that correspond to each fatty acid. The area of the internal standard peak is used to calculate the concentration of each fatty acid. Fatty acids are identified based on their retention time, or the time it takes for the FAME to exit the column. Specific retention times are identified using a mix of known FAMEs. There are two factors that influence the time is takes for FAMEs to elute from the column: their boiling point and their affinity for the column. FAMEs with shorter hydrocarbon chains are vaporized before FAMEs with longer hydrocarbon chains due to their lower boiling point. For FAMEs with hydrocarbon chains of the same length, they are further separated in the column due to their differing affinities for the column. Since the column is non-polar, FAMEs with less polarity will have more affinity for the column and therefore will stay in the column for longer. FAMEs with double bonds are less polar, causing them to exit the column at a later time than their saturated counterparts.

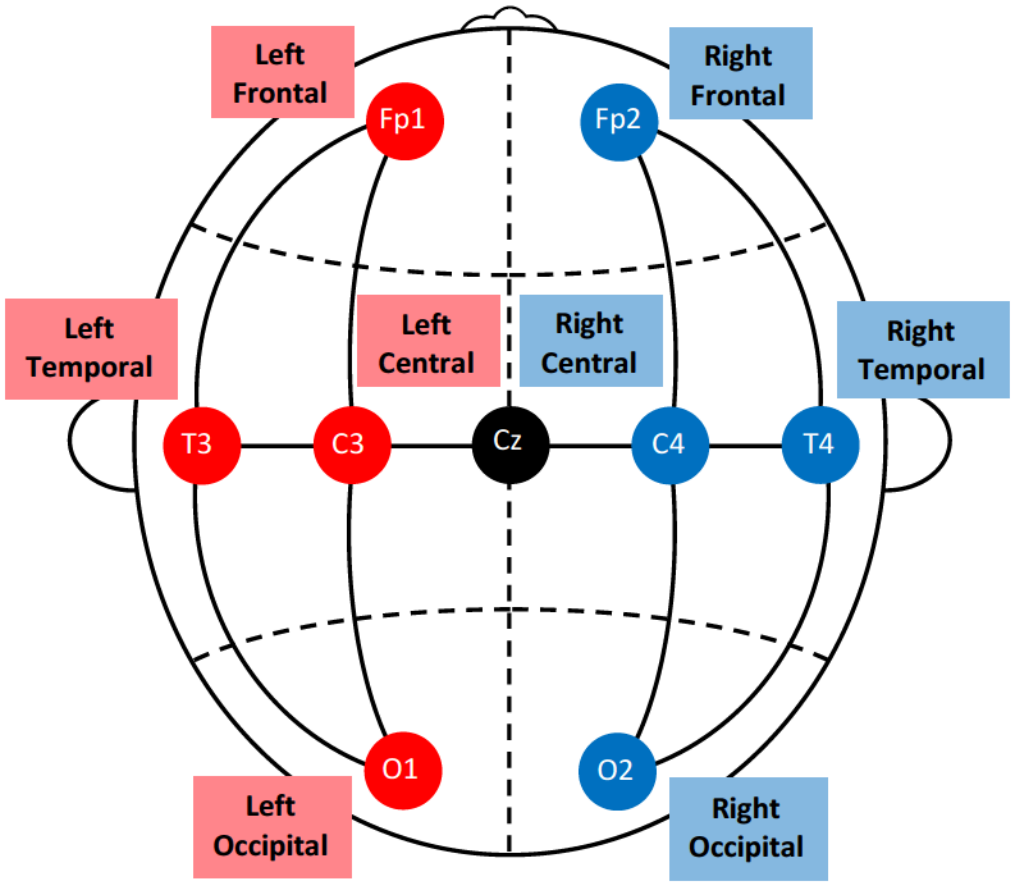

Figure 5. Schematic representation of EEG montage (adapted from Léveillé *et al.*, 2018).

EEG montage and data acquisition

EEGs were performed in newborns at two time points: 24-48 hours after birth and at 48 weeks post-amenorrhea. EEG montage and data acquisition were carried out using the methods described in Léveillé *et al.*, 2018. Briefly, the EEG data was recorded using a 14-channel bi-polar montage based on the 10-20 System (Grass Technologies). The montage, pictured in Figure 5, consisted of the following channels: left and right fronto-temporal channels (Fp_1-T_3; Fp_2-T_4), left and right fronto-central channels (Fp_1-C_3; Fp_2-C_4), left and right central channels (C_3-Cz; C_4-Cz), left and right centro-temporal channels (C_3-T_3; C_4-T_4), left and right temporo-central channels (T_3-Cz; T_4-Cz), left and right centro-occipital channels (C_3-O_1; C_4-O_2), and left and right temporo-occipital channels (T_3-O_1; T_4-

O_2). Silver-silver chloride electrodes were secured onto the infant's scalp using conductive water-soluble fixative paste and adhesive tape. Heart rate and respiratory movements were measured using two electrocardiogram electrodes and a thoracic respiratory band, respectively. EEG signal acquisition was performed for 12.5 minutes at a 200 Hz sampling rate on awake infants. The EEG data was processed using in-house Matlab scripts (Mathworks). The continuous EEG signal was converted into z-scores and divided into non-overlapping 5 second windows. Windows with z-scores greater than 10 were removed because they were considered to be movement artifacts. Power spectral density was calculated for each electrode pair using Welchs' approach on Matlab ('pwelch.m'). Coherence between channels in the left and right hemisphere was computer using 'mscohere.m' in Matlab.

Statistical analysis

Normal distribution was evaluated using the Shapiro-Wilk normality test and variances were compared using the F test. Chi-square tests were used to compare proportions between groups. Mean DHA and AA relative percentage and concentration were compared between groups using either unpaired *t*-tests, unpaired *t*-tests with Welch's correction, or the Mann Whitney test depending on the normality of the distributions and the variances of the groups. Mean coherence and mean PSD were compared between groups using the Mann Whitney test. All tests were performed using GraphPad Prism software, Version 7.0c (GraphPad Software, Inc.). Two-tailed p-value < 0.05 was considered statistically significant

RESULTS

Characteristics of mothers and newborns

Twenty-six control and seven diabetic mothers were recruited. Of the seven diabetic mothers recruited, two had T1D and five had T2D. Age was similar, but body mass index (BMI) and A1C differed between groups (Table 1). The mean ages of the control and diabetic groups were 29.4 ± 2.7 and 30.6 ± 7.8 years, respectively ($P = 0.704$). BMI close to delivery was higher in the diabetic group compared to the control group (34.6 ± 11.3 *vs.* 23.5 ± 2.9 kg/m^2; $P = 0.0004$). All participants in the diabetic group were either overweight (n = 3) or obese (n = 4) according to their BMI status. The mean BMI was 26.2 ± 1.3 kg/m^2 for participants with T1D and 38.0 ± 11.9 kg/m^2 for participants with T2D. The majority of participants in the control group were in the healthy weight range (n = 16) while others were in the overweight (n = 6) and obese ranges (n = 1). A1C was higher in diabetic mothers compared to control mothers (6.1 ± 0.5 *vs.* 5.1 ± 0.3 %; $P < 0.0001$).

The sex ratio, birth weight, length, head circumference, and APGAR score at 1 and 5 minutes of the newborns were similar between the two groups (Table 1). The sex ratio of the neonates was 5 males/2 females and 15 males/11 females in the diabetic and control group, respectively ($P = 0.822$). Birth weight was 3641 ± 488 grams in the diabetic group and 3432 ± 370 grams in the control group ($P = 0.224$). Three infants from the diabetic group were LGA, whereas the control group had one infant who was LGA and one infant who was small for gestational age. Two neonates from the control group and one neonate from the diabetic group weighed ≥ 4000 grams at birth, meaning they fall under the category of fetal macrosomia. Head circumference was similar between the diabetic and control groups (51.0 ± 3.0 cm *vs.* 50.9 ± 2.5 cm; $P = 0.579$). APGAR scores at 1 minute (6.6 ± 3.7 *vs.* 8.5 ± 0.9; $P = 0.542$) and at 5 minutes (8.3 ± 2.1 *vs.* 9.2 ± 0.9; $P = 0.252$) were not statistically different between the diabetic and control groups. All neonates in the control group reached a score between 7 and 10 for the 5-minute APGAR score. For the diabetic group, there was one neonate that had a 5-minute APGAR score of 4, but this neonate's APGAR score increased to 9 by 10 minutes. The only difference in the newborn

characteristics between the two groups was gestational age. The gestational age of the neonates from the diabetic group was significantly lower than the neonates from the control group (38.1 ± 0.9 vs. 40.0 ± 1.1 weeks; $P = 0.0006$). All neonates were born at 37 weeks or later, meaning none of the neonates were born premature.

Table 1
Characteristics of mothers and newborns.

	n	Controls (n = 26)	n	Diabetics (n = 7)	*P* value
Maternal characteristics					
Type of diabetes	26	N/A	7	2 T1D; 5 T2D	
Age (years)	26	29.4 ± 2.7	7	30.6 ± 7.8	0.704
BMI (kg/m^2)	23	23.5 ± 2.9	7	34.6 ± 11.3	**<0.001**
A1C at delivery (%)	25	5.1 ± 0.3	7	6.1 ± 0.5	**<0.001**
Newborn characteristics	26		7		
Sex ratio (male/female), n	26	15/11	7	5/2	0.822
Gestational age (weeks)	26	40.0 ± 1.1	7	38.1 ± 0.9	**<0.001**
Birth weight (g)	26	3432 ± 370	7	3641 ± 488	0.224
Length (cm)	26	50.9 ± 2.5	7	51.0 ± 3.0	0.938
Head circumference (cm)	26	34.4 ± 1.1	7	34.7 ± 1.7	0.579
APGAR score (1 min)	26	8.5 ± 0.9	7	6.6 ± 3.7	0.542
APGAR score (5 min)	26	9.2 ± 0.9	7	8.3 ± 2.1	0.252

Abbreviations: T1D, type 1 diabetes; T2D, type 2 diabetes; BMI, body mass index; Statistical differences between group means were determined using either independent *t*-tests or Mann Whitney tests, as appropriate. Statistical differences between proportions were computed using Chi-square test. Statistical significance is denoted by *P* value in bold. Data is presented as mean ± standard deviation.

Fatty acid profile: Docosahexaenoic acid and arachidonic acid

The DHA and AA relative percentage and concentrations in maternal plasma, umbilical cord serum, umbilical cord whole blood, maternal placenta, and fetal placenta total lipids will be discussed in the following sections (Figure 6). The complete fatty acid profile for each biological sample can be found in the supplementary section (Annex 1-5).

Maternal plasma total lipids

The mean relative percentage of DHA in the maternal plasma did not differ between the diabetic and control groups (1.41 ± 0.09% *vs.* 1.30 ± 0.08%; $P = 0.473$).

Similarly, there was no statistically significant difference in maternal plasma DHA concentration between the diabetic and control groups (6.1 ± 0.6 mg/dL *vs.* 6.1 ± 0.4 mg/dL; $P = 0.932$).

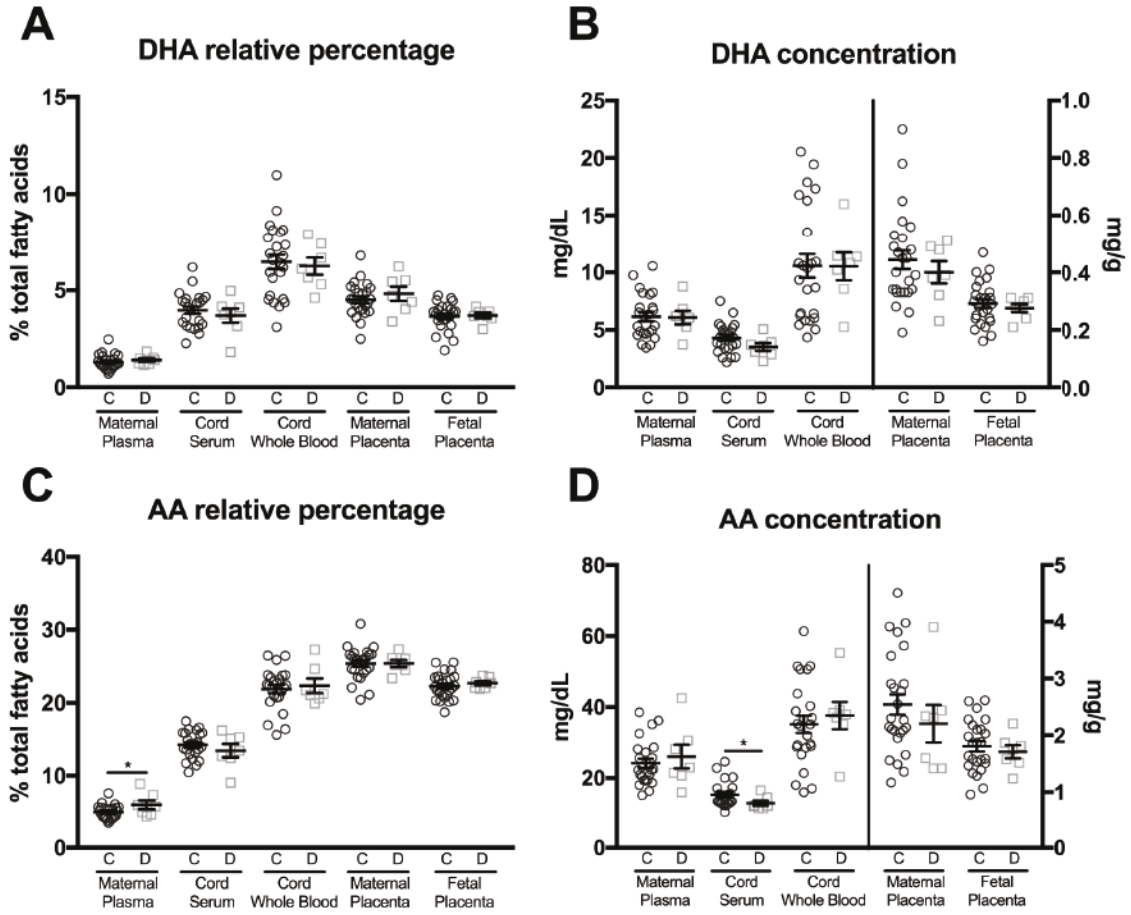

Figure 6. (A) DHA relative percentage, (B) DHA concentration, (C) AA relative percentage, and (D) AA concentration in maternal plasma, umbilical cord serum, umbilical cord whole blood, maternal placenta, and fetal placenta total lipids. Each dark grey circle represents an individual from the control group and each light grey square represents an individual from the diabetic group. The mean and standard error of the mean are denoted by the horizontal lines and the error bars. Statistically significant differences between groups ($P < 0.05$) are denoted by a "*". Abbreviations: DHA, docosahexaenoic acid; AA, arachidonic acid; C, control; D, diabetic.

The mean relative percentage of AA in maternal plasma total lipids was 19% higher in diabetics compared to non-diabetics (6.04 ± 0.61% *vs.* 5.06 ± 0.19%; $P = 0.049$). Maternal plasma AA concentration was not statistically different between the diabetic and control groups (26.0 ± 3.3 mg/dL *vs.* 24.1 ± 1.3 mg/dL; $P = 0.526$)

Umbilical cord serum total lipids

The mean relative percentage of DHA in umbilical cord serum total lipids did not differ significantly between the diabetic and control groups (3.69 ± 0.37% *vs.* 3.97 ± 0.19%; $P = 0.484$). The mean umbilical cord serum concentration of DHA was ~19% lower in the diabetic group compared to the non-diabetic group, but this result was not statistically significant (3.5 ± 0.3 mg/dL *vs.* 4.3 ± 0.3 mg/dL; 0.146; $P = 0.146$).

Umbilical cord serum AA mean relative percentage was not statistically different between the diabetic and control groups (13.47 ± 0.90% *vs.* 14.23 ± 0.37%; $P = 0.368$). However, mean AA concentration was ~16% lower in diabetic compared to non-diabetic cord serum (12.9 ± 0.7 mg/dL *vs.* 15.3 ± 0.7 mg/dL; $P = 0.019$).

Umbilical cord whole blood total lipids

Mean DHA relative percentage in umbilical cord whole blood total lipids was not statistically different between the diabetic and non-diabetic groups (6.24 ± 0.44% *vs.* 6.47 ± 0.38%; $P = 0.763$). Similarly, umbilical cord whole blood mean DHA concentration did not differ between the diabetic and non-diabetic groups (10.5 ± 1.2 mg/dL *vs.* 10.6 ± 1.0 mg/dL; $P = 0.729$).

Mean AA relative percentage in umbilical cord whole blood total lipids was similar between the diabetic and control groups (22.36 ± 1.00% *vs.* 21.90 ± 0.58%; $P = 0.706$). In addition, mean AA concentration was also similar in the umbilical cord whole blood of the diabetic and control groups (37.6 ± 3.8 mg/dL *vs.* 35.1 ± 2.4; $P = 0.622$).

Maternal placenta total lipids

Mean DHA relative percentage was not statistically different between the diabetic and control groups (4.82 ± 0.37% *vs.* 4.51 ± 0.17%; $P = 0.427$). Mean DHA concentration was also not statistically different in the maternal placenta of the diabetic versus the control group (400 ± 39 µg/g *vs.* 444 ± 32 µg/g; $P = 0.696$).

Similarly, there were no significant differences in AA levels between the two groups. Mean AA relative percentage was not statistically different in the maternal placenta

from the diabetic group compared to the non-diabetic group (25.40 ± 0.48% *vs.* 25.40 ± 0.44; $P = 0.999$). Maternal placenta mean AA concentration was also not statistically different between the diabetic and control groups (2206 ± 331 µg/g *vs.* 2543 ± 180 µg/g; $P = 0.386$).

Fetal placenta total lipids

Relative percentage of DHA in fetal placenta tissue was not statistically different between the diabetic and non-diabetic groups (3.69 ± 0.14% ± 3.65 ± 0.14%; $P = 0.886$). Fetal placenta DHA concentrations were also not significantly different between the diabetic and control group (276 ± 14 µg/g *vs.* 292 ± 15 µg/g; $P = 0.602$).

Fetal placenta AA relative percentage was not statistically different between the diabetic and control groups (22.69 ± 0.28% *vs.* 22.28 ± 0.35%; $P = 0.370$). In addition, there was no statistically significant difference in fetal placenta AA concentrations in the diabetic versus the non-diabetic groups (1713 ± 116 µg/g *vs.* 1808 ± 91 µg/g; $P = 0.607$).

Electroencephalography

Of the 26 newborns from the control group, 22 completed the first EEG and 17 completed the second EEG. Of the 7 newborns from the diabetic group, 4 completed the first EEG and 3 completed the second EEG.

Coherence

Coherence represents a correlation coefficient value between the EEG activity of the same brain region in the left and right hemisphere. The mean coherence data between various brain regions at 24-48 hours after birth (T0) and 48 weeks post-amenorrhea (T2) is displayed in full in Supplementary Table 6. For the purpose of brevity and clarity, only the results that differed between groups will be discussed in this section.

Figure 7 shows the mean coherence data in the beta frequency range (13.0 – 20.0 Hz) between various brain regions. There were a number of differences in mean coherence between T0 and T2 within groups. For instance, the control group had a ~5-6% lower mean coherence at T2 compared to T0 in the centro-occipital, temporo-occipital, and temporo-central channels ($P = 0.009$, 0.004, and 0.036, respectively). There was also a trend in the

control group for higher mean coherence at T2 compared T0 in the fronto-temporal channels, but this trend was not statistically significant ($P = 0.091$). The diabetic group also had some trends for differences in mean coherence between T2 and T0; mean coherence was 5.5% higher in the fronto-central channel and 4.5% lower in the temporo-occipital channel at T2 compared to T0, but these trends were not statistically significant ($P = 0.057$ for both). There were also some differences between the diabetic and control group at T0. For example, mean coherence in the fronto-temporal regions at T0 was higher in the diabetic compared to non-diabetic group (0.398 ± 0.010 *vs.* 0.378 ± 0.004; $P = 0.031$). Similarly, there were also some trends towards higher mean coherence in the diabetic group at T0 compared to the control group in the fronto-central and central channels, but these trends were not statistically significant ($P = 0.069$ and 0.082, respectively). There were no differences in mean coherence at T2 between the diabetic and non-diabetic group.

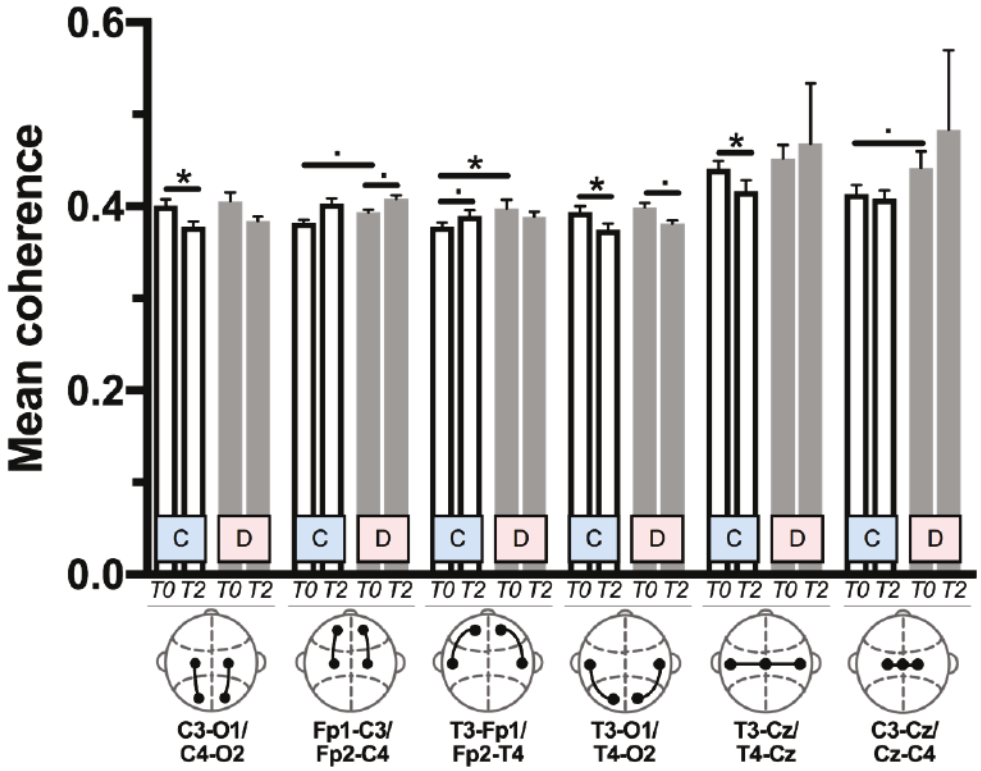

Figure 7. Mean coherence for the beta frequency range (13.0 – 20.0 Hz) in the centro-occipital (C3-O1 / C4-O2), fronto-central (Fp1-C3 / Fp2-C4), fronto-temporal (T3-Fp1 / Fp2-T4), temporo-occipital (T3-O1 / T4-O2), temporo-central (T3-Cz / T2-Cz) and central (C3-Cz / Cz-C4) channels. T0 refers to 24-48 hours after birth and T2 refers to 48 weeks post-amenorrhea. Statistically significant differences ($P < 0.05$) are denoted by "*". P values < 0.1 are denoted represented by ".". Data are presented as mean ± standard error of the mean.

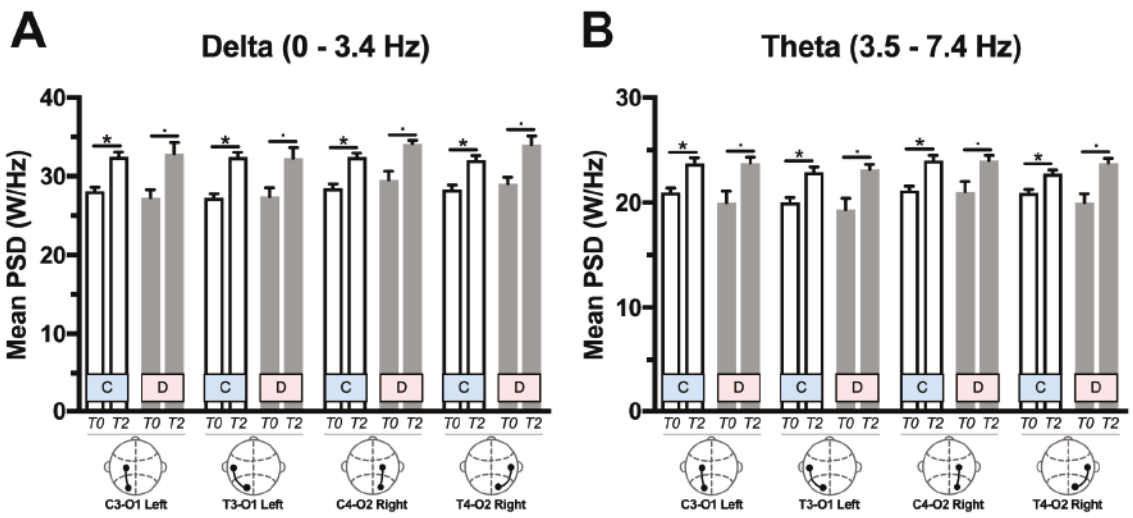

Figure 8. Mean power spectral density (PSD) for the (A) Delta (0 – 3.4 Hz) and (B) Theta (3.5 – 7.4 Hz) frequency ranges in the left centro-occipital (C3-O1), left temporo-occipital (T3-O1), right centro-occipital (C4-O2), and right temporo-occipital (T4-O2) channels. T0 refers to 24-48 hours after birth and T2 refers to 48 weeks post-amenorrhea. Statistically significant differences ($P < 0.05$) are denoted by "*". P values < 0.1 are denoted represented by ".". Data are presented as mean ± standard error of the mean.

Power spectral density

As previously explained, PSD is the power of the EEG signal as a function of frequency, per unit frequency. The mean PSD in all the electrode channels at 24-48 hours after birth and at 48 weeks post-amenorrhea can be found in Supplementary Table 7. Similar to the coherence section, only the PSD data that showed the most differences between timepoints or between groups will be discussed in this section.

Figure 8 summarizes the mean PSD in the delta and theta frequencies in the left and right centro-occipital and temporo-occipital channels at T0 and T2. For the delta frequency (Figure 8.A), both the control and the diabetic groups had a higher mean PSD at T2 compared to T0 in the left and right centro-occipital and temporo-occipital regions. Differences in mean PSD between T2 and T0 were statistically significant for the control group ($P < 0.0001$ for the left and right centro-occipital and left and right temporo-occipital regions) but not statistically significant for the diabetic group ($P = 0.057$ for the left and right centro-occipital and left and right temporo-occipital regions). This same pattern was also echoed in the theta frequency range. The control group had a higher mean PSD at T2 compared to T0 in the left centro-occipital ($P < 0.001$), left temporo-occipital ($P < 0.001$),

right centro-occipital ($P < 0.0001$), and the right temporo-occipital ($P = 0.001$) channels. The diabetic group also had a trend towards higher mean PSD at T2 compared to T0 in the same channels ($P = 0.057$ for all). PSD did not differ significantly between the diabetic and control groups at T0 nor at T2.

DISCUSSION

Characteristics of mothers and newborns

Of the maternal characteristics that were measured, only two differed between the diabetic and non-diabetic groups: BMI and A1C at delivery. As previously stated, BMI was much higher in the diabetic mothers compared to the non-diabetic mothers. This is unsurprising given that approximately 80-90% of individuals with T2D are overweight or obese (Wing, 2000). In addition, obesity is also becoming more prevalent in individuals with T1D (Conway *et al.*, 2010). A1C at delivery was also significantly higher in diabetic mothers compared to non-diabetic mothers. According to the 2018 Diabetes Canada Clinical Practice Guidelines Expert Committee, women with pre-existing T1D and T2D during pregnancy should aim for an A1C level of ≤ 6.5% (ideally ≤ 6.1%) during pregnancy (Feig *et al.*, 2018). In the case of the diabetic participants in this study, four out of seven participants had A1C levels ≤ 6.1% and two out of seven had A1C levels ≤ 6.5%. One of the diabetic participants had an A1C level of 6.7% which is higher than recommended. Overall, it can be said that the diabetic participants in this study had adequate control of their blood glucose levels, however their A1C levels were still significantly higher than the non-diabetic participants. In terms of the newborn characteristics, only gestational age differed significantly between the groups. On average, neonates from diabetic mothers were approximately two weeks younger than neonates from the non-diabetic mothers at birth. This is also unsurprising given the use of induction of labour to reduce the risks associated with fetal macrosomia in diabetic pregnancies (Mozurkewich *et al.*, 2009).

Fatty acid transfer from mother to fetus

Maternal plasma DHA and AA levels

Both the relative percentage and the concentration of DHA did not differ significantly between in the maternal plasma total lipids of the diabetic and the non-diabetic groups. To this writer's knowledge, there are three other studies that have reported

DHA levels in pregnant women with pre-existing T1D and T2D (Berberovic *et al.*, 2013, Min *et al.*, 2005, and Min *et al.*, 2014). The first study (Berberovic *et al.*, 2013) reported a 55% higher concentration of DHA in maternal vein serum total lipids of T1D pregnant women (N = 32) compared to non-diabetic pregnant women (N = 31). However, Berberovic and colleagues did not find any statistically significant differences in relative percentage of DHA in the maternal vein serum total lipids of the T1D compared to the non-diabetic group. The second study (Min *et al.*, 2005) assessed the relative percentage of DHA in the maternal plasma TG and choline phosphoglycerides and in maternal red blood cell (RBC) choline phosphoglycerides and ethanolamine phosphoglycerides of pregnant women with T1D (N = 32), T2D (N = 17), and without diabetes (N = 39). The authors observed no statistically significant difference in plasma TG relative percentage of DHA between the T1D, T2D and non-diabetic groups (Min *et al.*, 2005). However, relative percentage of DHA was approximately 40% lower in the plasma choline phosphoglycerides of the T1D group compared to the non-diabetic group (Min *et al.*, 2005). They also observed a similar trend in the T2D plasma choline phosphoglycerides, but this difference was not statistically significant (Min *et al.*, 2005). They also found significantly lower relative percentage of DHA in the RBC choline phosphoglycerides of both T1D and T2D compared to non-diabetic pregnant women (Min *et al.*, 2005). Relative percentage of DHA was also significantly lower in the RBC ethanolamine phosphoglycerides of the T1D mothers compared to the non-diabetic mothers (Min *et al.*, 2005). The T2D mothers also had a lower percentage of DHA in their RBC ethanolamine phosphoglycerides compared to non-diabetic mothers, but this difference was not statistically significant (Min *et al.*, 2005). The final study (Min *et al.*, 2014) compared DHA relative percentage in the maternal plasma choline phosphoglycerides and in the maternal red RBC choline phosphoglycerides and ethanolamine phosphoglycerides of pregnant women with T2D (N = 30) and without diabetes (N = 30). They found no significant differences in DHA relative percentage in the plasma choline phosphoglycerides, RBC choline phosphoglycerides, or the ethanolamine phosphoglycerides of T2D compared to non-diabetic pregnant women in the third trimester (Min *et al.*, 2014). In summary, these three studies tended to find lower relative percentage of DHA in the maternal plasma choline phosphoglycerides, RBC choline

phosphoglycerides, and RBC ethanolamine phosphoglycerides compared to the non-diabetic groups (Berberovic *et al.*, 2013, Min *et al.*, 2005, and Min *et al.*, 2014). They also reported no difference between relative percentage of DHA in serum total lipids of T1D women and in plasma TG of T1D and T2D women compared to non-diabetic group (Berberovic *et al.*, 2013, Min *et al.*, 2005). Finally, one study reported higher DHA concentration in serum total lipids of T1D mothers compared to non-diabetic mothers (Berberovic *et al.*, 2013).

When comparing findings between these previous studies and the current study, it is important to consider that they do not all report DHA levels in the same blood pool. For instance, both the Berberovic *et al.* study and the current study reported DHA levels in plasma or serum total lipids. Serum and plasma are similar; the main difference between them is that plasma is collected prior to clotting and therefore still contains clotting agents whereas serum is collected after clotting and therefore does not contain clotting agents. Plasma/serum total lipids are composed of approximately 49% TGs, 24% phospholipids, and 16% cholesteryl esters (Brenna *et al.*, 2018). The fatty acid composition of the TG pool in particular is greatly influenced by recent food intake and therefore can vary greatly between individuals (Brenna *et al.*, 2018). Since a large proportion of plasma/serum total lipids are in the TG form, the fatty acids in total lipids can also vary depending on the individual's recent food consumption. Plasma total lipids are logistically advantageous since plasma samples are routinely collected in clinical settings and their fatty acids are relatively stable over time (Brenna *et al.*, 2018). On the other hand, the Min *et al.* 2005 and Min *et al.*, 2014 studies reported DHA levels in plasma choline phosphoglycerides and in RBC choline phosphoglycerides and ethanolamine phosphoglycerides. Plasma choline phosphoglycerides are part of the plasma phospholipid pool. Since this type of sample does not contain TGs, it is not as susceptible to changes in fatty acid levels based on recent food consumption and is instead thought to be more representative of the fatty acid levels in cell membranes (Brenna *et al.*, 2018). Finally, the RBC choline and ethanolamine phosphoglycerides are part of the RBC phospholipid pool. They represent part of the phospholipids that compose the phospholipid bilayer of the RBCs. The average circulatory lifespan of RBCs is approximately 120 days compared to 2 weeks for plasma phospholipids and within hours for TGs. Due to the longer lifespan of RBCs, their fatty

acid composition is thought to be more reflective of long-term dietary fatty acid intake (Brenna *et al.*, 2018). When considering the different blood pools in the previously mentioned studies, it seems that a greater relative percentage of DHA is present in RBC choline and ethanolamine phosphoglycerides and in plasma choline phosphoglycerides compared to plasma TG and plasma/serum total lipids. Previous studies reported significant differences in relative percentage of DHA in the diabetic groups in these RBC choline and ethanolamine phosphoglycerides and in plasma choline phosphoglycerides pools (Min *et al.*, 2005 and Min *et al.*, 2014). However, relative percentage of DHA does not seem to differ in plasma TG or in serum total lipids of diabetic mothers compared to control mothers (Berberovic *et al.*, 2013 and Min *et al.*, 2005). Since plasma and serum total lipids are composed of a greater proportion of TGs relative to phospholipids, perhaps any changes in relative percentage in DHA in the phospholipid fraction of total lipids may be masked by the larger proportion of TGs present. This could potentially explain why we did not see any significant difference in the relative percentage of DHA in the maternal plasma total lipids of diabetic mothers compared to non-diabetic mothers. Moreover, since our study used plasma total lipids, our results could have been influenced by the participants' food intake at the time of sample collection. That being said, our results for relative percentage of DHA in the maternal plasma total lipids are in line with the other studies that reported DHA levels in similar blood pools (Berberovic *et al.*, 2013 and Min *et al.*, 2005). The main difference between the current study and previous studies is in the DHA concentration. Of the previously discussed studies, only the Berberovic *et al.,* 2013 study reported maternal DHA concentrations. As previously mentioned, they found significantly higher concentration of DHA in the plasma total lipids of T1D pregnant women compared to non-diabetic pregnant women (Berberovic *et al.*, 2013). This higher DHA concentration was not present in our diabetic group compared to the non-diabetic group. It seems that in the Berberovic study, the relative percentage of DHA in the plasma total lipids was the same between groups, but the total concentration of fatty acids overall was higher in the T1D group compared to the control group (Berberovic *et al.*, 2013). As a result, they observed a higher concentration of plasma total lipids DHA. In the current study, neither the total concentration of plasma total lipids fatty acids nor the relative

percentage of DHA differed significantly between the diabetic and non-diabetic groups, and therefore we did not see a difference in plasma total lipid DHA concentration.

As for AA, we observed a significantly higher relative percentage of AA in the plasma total lipids of diabetic mothers compared to non-diabetic mothers. However, we did not observe a statistically significant difference in plasma total lipid AA concentration in the diabetic and non-diabetic groups. To this writer's knowledge, there are only two studies reporting AA in pregnant women with pre-existing T1D or T2D during pregnancy (Berberovic *et al.*, 2013 and Min *et al.*, 2005). The first study (Berberovic *et al.*, 2013) reported no statistically significant differences between AA concentration and relative percentage in plasma total lipids of pregnant women with T1D compared to pregnant women without diabetes. The second study (Min *et al.*, 2005) showed no significant differences in AA relative percentage between the diabetic (T1D and T2D) and non-diabetic groups in multiple maternal blood pools (plasma TG, plasma choline phosphoglycerides, and RBC choline and ethanolamine phosphoglycerides). Although our finding of significantly higher AA relative percentage in maternal plasma total lipid of diabetic mothers is not exactly in line with the other two studies, this result is not surprising. In fact, the inconsistency in maternal AA relative percentage across these three studies could potentially be the result of differences in the participants' diets prior to when the samples were collected.

Umbilical cord DHA and AA levels

In the current study we reported DHA and AA levels in the total lipids of umbilical cord serum and whole blood. In the umbilical cord serum total lipids, both DHA relative percentage and concentration were not statistically different between the diabetic and control groups. However, despite not being statistically significant, it is interesting to note that DHA concentration was approximately 19% lower in the diabetic umbilical cord serum total lipids compared to the control group. Similarly, umbilical cord whole blood total lipid DHA relative percentage and concentration were not significantly different between the diabetic and control groups. As for AA, umbilical cord serum total lipid AA concentration was approximately 16% lower in the diabetic group compared to the control group. However, the umbilical cord serum total lipid relative percentage of AA did not

differ significantly between the diabetic and control groups. On the other hand, both AA relative percentage and concentration in the umbilical cord whole blood total lipids did not seem to differ significantly between the diabetic and the control group. In contrast to serum, whole blood samples contain plasma, RBCs, white blood cells, and platelets. Therefore, the fatty acid composition from whole blood samples can be influenced by hematocrit, changes in white blood cell levels in response to infections, as well as recent dietary intake of fatty acids (Brenna *et al.*, 2018). Since whole blood contains RBCs which are mainly composed of phospholipids, whole blood total lipid samples are composed of a relatively larger proportion of phospholipids compared to serum total lipids. When comparing DHA and AA levels between the umbilical cord serum and whole blood total lipids, it appears that both DHA and AA relative percentages and concentrations are higher in whole blood compared to serum total lipids for both the diabetic and the control groups. This indicates that DHA and AA are more concentrated in the phospholipids of the RBC membranes compared to the TGs and cholesteryl esters. Also, when comparing DHA and AA levels between the cord blood samples and the maternal plasma samples, it is evident that both DHA and AA relative percentage is higher in the umbilical cord serum and whole blood compared to the maternal plasma. Since DHA and AA accumulate in RBC membrane phospholipids, it makes sense that there would be relatively lower levels of DHA and AA compared to other fatty acids in the TG- and cholesteryl esters-rich maternal plasma. Despite the maternal plasma having lower relative percentages of DHA and AA, its DHA and AA concentrations are relatively comparable to the umbilical cord samples. This discrepancy is the result of the maternal plasma samples having a higher concentration of total fatty acids compared to the umbilical cord serum.

To this writer's knowledge, there are four other studies that reported DHA levels (Ghebremeskel *et al.*, 2004, Berberovic *et al.*, 2013, Min *et al.*, 2005, and Min *et al.*, 2014) and three studies (Ghebremeskel *et al.*, 2004, Berberovic *et al.*, 2013, Min *et al.*, 2005) that reported AA levels in the umbilical cord blood of pregnant women with pre-existing diabetes. Beginning with the studies that reported DHA results, the first study (Ghebremeskel *et al.*, 2004) compared fatty acid levels in the umbilical cord plasma choline phosphoglycerides, plasma TG, and plasma cholesterol esters in pregnant women with T1D (N = 31) and without diabetes (N = 59). The authors found the relative

percentage of DHA was approximately 27% and 42% lower in the umbilical cord plasma choline phosphoglycerides and cholesterol esters, respectively, of T1D mothers compared to non-diabetic mothers (Ghebremeskel *et al.*, 2004). They also reported no significant difference in DHA relative percentage in the umbilical cord plasma TGs of the diabetic group compared to the non-diabetic group (Ghebremeskel *et al.*, 2004). The Min *et al.*, 2005 also reported significantly lower DHA relative percentage in the umbilical cord plasma choline phosphoglycerides in T1D mothers but not in T2D mothers compared to the non-diabetic group. However, a later study also by Min and colleagues found significantly lower relative percentage of DHA in the umbilical cord plasma of T2D mothers compared to non-diabetic mothers (Min *et al.*, 2014). Similarly, the Min *et al.*, 2005 study also found significantly lower DHA relative percentage in the umbilical cord plasma TG in T1D mothers but not in T2D mothers compared to the non-diabetic group. Relative percentage of DHA also tended to be lower in the umbilical cord RBC choline and ethanolamine phosphoglycerides in diabetic mothers compared to non-diabetic mothers (Min *et al.*, 2005 and Min *et al.*, 2014). Given these results, one could anticipate that relative percentage of DHA would also be lower in our study, but this was not the case. Instead, we found no significant difference in relative percentage of DHA in both umbilical cord serum and whole blood total lipids in the diabetic group compared to the non-diabetic group. The discrepancy between these results could be due to the differences in blood pools used in the studies. Like the maternal blood results discussed in the previous section, most of the differences in DHA levels observed in these studies were in the phospholipid pools whereas results in the TG pool are more mixed. Since our umbilical cord serum and whole blood samples contain the total lipids, it would be difficult to detect changes DHA levels that may be mainly present in the phospholipid class since the total lipid samples contain a greater proportion of TGs. Although the whole blood samples may contain more phospholipids compared to the serum samples, it still contains a large proportion of TGs as it still has the plasma component of the blood as well. Therefore, it is possible that any differences in DHA in the phospholipids of our samples may be masked by the other lipid classes, namely the TGs. Similar to the current study, the Berberovic *et al.*, 2013 study also reported no significant difference in the relative percentage of DHA in the umbilical cord serum total lipids of T1D mothers compared to non-diabetic mothers. However, in contrast

to the current study, Berberovic and colleagues reported significantly higher concentration of DHA in the umbilical cord serum total lipids of T1D mothers compared to non-diabetic mothers (Berberovic *et al.*, 2013). It is unclear as to why Berberovic and colleagues found increased DHA concentration in diabetic cord blood when DHA has generally been thought to be lower in diabetic cord blood compared to control (Léveillé *et al.*, 2018). However, it is difficult to draw any conclusions about DHA concentration in diabetic cord blood as most studies in this area of research seem to report results in relative percentage rather than concentration. Taken altogether, the DHA results of the current study do not confirm the hypothesis of lower levels of DHA in the diabetic cord blood compared to the non-diabetic group nor are they exactly in line with the existing literature. In addition to the possibility of any differences in DHA levels being masked by pooling all the lipid classes together, the small sample size of our study may also play a role in the lack of difference in DHA levels. The number of diabetic participants in our study (N = 7) is comparatively smaller than the other previous mentioned studies (N = 30-32) (Ghebremeskel *et al.*, 2004, Berberovic *et al.*, 2013, Min *et al.*, 2005, and Min *et al.*, 2014). It's possible that our sample size is not large enough and therefore there is not enough statistical power to detect statistically significant differences in DHA levels in our umbilical cord blood samples.

Three of the four studies discussed in the previous paragraph also reported findings of AA levels in the umbilical cord blood of pregnant women with pre-existing diabetes compared those without diabetes (Ghebremeskel *et al.*, 2004, Berberovic *et al.*, 2013, and Min *et al.*, 2005). Generally, the studies reported a lower relative percentage of AA in the umbilical cord plasma choline phosphoglycerides, plasma cholesterol esters, and RBC choline phosphoglycerides of pregnant women with pre-existing diabetes compared to non-diabetic compared to those without diabetes (Ghebremeskel *et al.*, 2004 and Min *et al.*, 2005). These same two studies also found no statistically significant difference in AA relative percentage in the umbilical cord plasma TG of diabetic mothers compared to non-diabetic mothers (Ghebremeskel *et al.*, 2004 and Min *et al.*, 2005). These results suggest that differences in AA levels occur mainly in the phospholipid class rather than the TG, meaning that these differences could be masked if total lipid pools are used. In the case of the current study, we did not find any significant difference in umbilical cord serum total lipid AA relative percentage and whole blood total lipids AA relative percentage and

concentration in the diabetic versus the non-diabetic group. However, we did find significantly lower AA concentration in the umbilical cord serum total lipids of the diabetic group compared to the non-diabetic group. Again, our results differ from the Berberovic *et al.*, 2013 study which found a significantly higher AA concentration and a non-significant difference in AA relative percentage in the umbilical vein serum total lipids of pregnant women with T1D compared to those without diabetes (Berberovic *et al.*, 2013). The increase in AA concentration in the Berberovic study seems to be related to an increase in the overall concentration of fatty acids in the T1D cord serum compared to the non-diabetic group (Berberovic *et al.*, 2013). In contrast, we did not observe any significant difference in the total fatty acid concentration in the umbilical cord serum total lipids between the diabetic and control groups.

In the current study, the significantly lower AA concentration in the umbilical cord serum total lipids of the diabetic group exists despite there being no difference in AA concentration and significantly higher AA relative percentage in the maternal plasma total lipids of the diabetic group compared to the control group. This disconnect between maternal and umbilical cord levels of AA is in line with the idea that there is a disturbance in the transfer of AA from mother to fetus in diabetic pregnancies and this could be related to the placenta storing DHA and AA.

Placenta DHA and AA levels

In the current study, we investigated the placenta fatty acid profile and found no significant differences in DHA and AA relative percentage and concentration in the total lipids of both the maternal and fetal placenta between the diabetic and the non-diabetic groups. There are very few studies that report findings for placental levels of DHA and AA in diabetic pregnancies. To this writer's knowledge, there is one study reporting placental AA levels in T1D pregnancies (Kuhn *et al.*, 1990) and four reporting placental DHA and AA levels in GDM pregnancies (Bitsanis *et al.*, 2006, Prieto-Sánchez *et al.*, 2017, Segura *et al.*, 2017, and Uhl *et al.*, 2015). Kuhn and colleagues found that a greater percentage of AA is incorporated into the placenta TGs in T1D mothers compared to non-diabetic mothers (Kuhn *et al.*, 1990). They also showed that a smaller percent of AA incorporated into the placenta phosphoglycerides and free fatty acid fractions in T1D pregnancies

compared to non-diabetics (Kuhn *et al.*, 1990). In the case of GDM, it seems that the relative percentage of DHA and AA is higher in the placenta phospholipids in GDM compared to non-diabetic pregnancies (Bitsanis *et al.*, 2006 and Uhl *et al.*, 2015). However, one study reported no difference in DHA and AA relative percentage in placenta phospholipids in GDM compared to non-diabetic pregnancies (Segura *et al.*, 2017). In addition, one study found no difference in DHA and a lower AA relative percentage in placenta TG of GDM compared to non-diabetic women (Bitsanis *et al.*, 2006) whereas another study found no significant difference in DHA and AA levels in placenta TGs of GDM versus non-diabetic women (Segura *et al.*, 2017). Another study also reported no significant difference in DHA and AA relative percentage in placenta total lipids of GDM compared to non-diabetic participants (Prieto-Sánchez *et al.*, 2017). To this writer's knowledge, there are no studies reporting placental DHA and AA levels in T2D women. Although placenta DHA and AA levels did not seem to differ between the diabetic and the non-diabetic group in the current study, it could be the case that measuring these fatty acids in the placenta total lipids may mask any differences that are occurring in the individual lipid classes. Since it appears that most of the previously mentioned studies found differences in DHA and AA levels in placenta phospholipids, it would be interesting to determine the fatty acid composition of the placenta phospholipids in the maternal and fetal placenta samples from the current study.

The placenta results from the current study indicate that there is no significant accumulation of neither DHA nor AA in the maternal and fetal placenta in the diabetic group compared to the non-diabetic group. The concept of DHA and AA accumulating in the placenta is one possible hypothesis for the mechanism behind the lower transfer of these fatty acids from mother to fetus in diabetic pregnancies, but our current results do not support this concept.

Another hypothesis for the reduced transfer of fatty acids from mother to fetus in diabetic pregnancies is related to the impaired uptake and transportation of these fatty acids across the placenta. One study reported disturbed placental uptake of ^{13}C-DHA in GDM compared to non-diabetic placentas (Larqué *et al.*, 2014). In addition, placental uptake of ^{13}C-DHA is lower in obese pregnant women compared to non-obese pregnant women (Gázquez *et al.*, 2019). This has implications for T1D and T2D pregnant women since

obesity is common in individuals with diabetes (Punthakee *et al.*, 2018). In theory, the disturbance in placental uptake of fatty acids such as DHA and AA could be the result of differences in the expression of placental fatty acid transporters in diabetic pregnancies, therefore interfering with the transfer of fatty acids from mother to fetus. As previously discussed, there is some evidence for changes in the expression of placental fatty acid transporters in T1D and GDM pregnancies (Magnusson *et al.*, 2004, Radaelli *et al.*, 2009, Prieto-Sánchez *et al.*, 2017, and Segura *et al.*, 2017). However, only one of these studies reports findings in pre-existing T1D during pregnancy (Radaelli *et al.*, 2009). Further, there are no studies reporting the expression of placental fatty acid transporters in T2D. Therefore, it is difficult to draw any firm conclusions about the level of expression of fatty acid transporters in the placentas of women with pre-existing T1D and T2D during pregnancy.

In summary, we found a lower transfer of AA from mother to fetus in diabetic pregnancies. However, the placental fatty acid profile results of the current study do not support the hypothesis of the accumulation of AA and DHA in the placenta of T1D and T2D women compared to non-diabetic women. Given the importance of AA for neurodevelopment, we wanted to assess if this lower transfer from mother to fetus during gestation impacted the neurodevelopment of the neonates from the diabetic group.

Neurodevelopment (EEG)

The first measure of neurodevelopment used in the current study is coherence, which is the correlation between oscillations of activity from the same brain region between the two hemispheres. Most of the statistically significant differences observed were differences between the two time points within the same group. In other words, we mainly observed slight increases in coherence between the first EEG (24 – 48 hours after birth) and the second EEG (at 48 weeks post-amenorrhea) and not between newborns from control vs pre-existing diabetes mothers. The neonates, particularly those from the control group, exhibited increases in coherence in the theta, alpha, and beta frequency bands of the frontal regions. This is thought to be associated with post-natal maturation and with the development of thalamocortical connections of higher and more complex frequencies (Meijer *et al.*, 2014). Coherence in the fronto-temporal region differed slightly between the

neonates from diabetic mothers and the neonates from non-diabetic mothers at the first EEG. However, these are differences of 0.01 – 0.03. Since coherence is a correlation coefficient, it is evident that a difference in correlation of 0.01 – 0.03, while statistically significant, may not necessarily be clinically relevant. The pilot EEG study that was previously performed in our laboratory on neonates from GDM mothers also found no significant difference in coherence between the neonates from GDM mothers compared to neonates from non-diabetic mothers at ≤ 48 hours after birth (Léveillé *et al.*, 2018).

The second measure of neurodevelopment used was PSD, which is the distribution of the signal power over frequency of the signal from each electrode. In line with the coherence results, the statistically significant differences in PSD were within the same group between the first and second EEGs. Neonates from both the diabetic and non-diabetic group exhibited increases in PSD between the first EEG and the second EEG in the delta and theta frequency bands of the right and left temporo-occipital and the right and left centro-occipital electrodes. However, these increases in PSD between the first and second EEG were not statistically significant in the diabetic group. These increases in PSD observed in the delta and theta frequency bands of the occipital regions between the first and second EEG may be related to the establishment of the posterior dominant rhythm, a rhythm established around 2 months of age and typically in the 3 – 4 Hz frequency (Britton *et al.*, 2016). This is a normal part of neurodevelopment and precedes the alpha rhythm, which is the dominant rhythm of activity in the adult brain (Britton *et al.*, 2016). We did not observe any differences in PSD between the neonates from the diabetic and the control groups at either the first EEG or the second EEG. In contrast, Léveillé and colleagues observed lower PSD in the left centro-occipital region in neonates from GDM mothers compared to neonates from non-diabetic mothers at ≤ 48 hours after birth (Léveillé *et al.*, 2018). However, these results were no longer statistically significant after adjustment for gestational age (Léveillé *et al.*, 2018). This indicates that the difference in PSD in the centro-occipital region was likely due to differences in gestational age rather than GDM. The difference in gestational age between the diabetic and the control group is similar in the current study compared to the Léveillé *et al.*, 2018 study. However, unlike Léveillé and colleagues, the current study does not report data on neonatal hypoglycemia, a condition which is common in offspring of diabetic mothers and can negatively impact

neurodevelopment (Boluyt *et al.*, 2006). The current study also demonstrated higher coherence and PSD in the low-frequency range, particularly the delta frequency band (< 4 Hz), compared to other frequency ranges for all brain regions. This makes sense since the low-frequency range is the dominant infants ≤ 1 year of age since they spend a large proportion of their time sleeping. This same pattern was also observed in the neonates from the Léveillé *et al.*, 2018 study and in infants at 0 - 3 months and 4 – 6 months of age (Cornelissen *et al.*, 2015).

Overall, the changes in coherence and PSD between the first and second EEG observed in the neonates of the current study indicate normal neurodevelopment between birth and approximately two months of age. Moreover, we did not observe any abnormalities in the EEG coherence and PSD in the neonates from the diabetic group compared to the neonates from the control group. Therefore, we did not find any evidence of impaired neurodevelopment in the infants of diabetic mothers.

Our study is the first to evaluate neurodevelopment using EEG at birth in neonates of T1D and T2D mothers compared to non-diabetic mothers. As previously described in the introduction, most of the studies that investigate cognitive scores in offspring of diabetic mothers evaluate the children later in life (Brinciotti *et al.*, 2009, de Regnier *et al.*, 2000, Silverman *et al.*, 1998, Fraser *et al.*, 2012, Clausen *et al.*, 2013, Temple *et al.*, 2011, Bytoft *et al.*, 2016, Clausen *et al.*, 2011, Xiang *et al.*, 2018, and Bytoft *et al.*, 2017). It is therefore not clear whether DHA and AA transfer is at the root of the lower cognitive scores observed in offspring of diabetic mothers or if there are other environmental factors that influence these scores. In the current study, we determined that there was a lower transfer of AA but not DHA from mother to fetus in the diabetic group and that the neonates from the diabetic did not appear to have abnormalities in their coherence and PSD compared to neonates from non-diabetic mothers. This absence of evidence for impaired neurodevelopment in the neonates from the diabetic group occurred despite evidence for lower AA transfer from mother to fetus. As previously discussed, AA concentration was approximately 16% lower in the umbilical cord serum total lipids of the diabetic group compared to the control group. In addition, the concentration of DHA in the umbilical cord serum total lipids was approximately 19% lower in the diabetic group compared to the non-diabetic group, although this was not statistically significant. Given the importance of these

two fatty acids for fetal brain development (Sastry 1985 and Innis 2007), it would be logical to assume that a deficiency in those fatty acids would be detrimental for the fetus's neurodevelopment. One study found a positive correlation between neurological optimality score and umbilical vein and artery DHA and umbilical vein AA in 317 infants 10-14 days after birth (Dijck-Brouwer *et al.*, 2005). Another study reported that 3-month-old infants with mildly abnormal general movements had a lower relative percentage of AA in their umbilical artery compared to infants with normal general movements (Bouwstra *et al.*, 2006). Moreover, another study stated that 18-month-old children with minor neurological function had lower umbilical vein DHA compared to neurotypical children (van Goor *et al.*, 2011). The authors also identified weak positive association between umbilical vein AA and the Bayley Scales of Infant Development mental development index (van Goor *et al.*, 2011). It was estimated that the human fetus accumulates 41.65 mg DHA/day and 95.25 mg AA/day during weeks 35-40 of gestation (Kuipers *et al.*, 2012). It is possible that perhaps the infants from diabetic mothers in the current study had a lower transfer of AA compared to the control group, but the amount of AA and DHA transferred was still sufficient to meet the needs of their brain development in utero.

Strengths and limitations

Strengths

One strength of the current study is that the fatty acid results were reported in both relative percentage and concentration. This is unique compared to other studies in the field which usually communicate fatty acid results in relative percentage only. While the relative percentage of a fatty acid is useful knowledge, it does not give the full picture. For instance, the relative percentage of AA in the umbilical cord serum total lipids did not differ significantly between the diabetic and non-diabetic group. However, the umbilical cord serum AA concentration was significantly lower in the diabetic group compared to the non-diabetic group. When only considering the relative percentage of AA in this case, it would seem like there is no difference when that is clearly not the case. Another problem with only reporting relative percentage is that not all studies include the same list of fatty acids (Brenna *et al.*, 2018). For instance, some authors might include 10 fatty acids while others might include 15. This could drastically impact the resulting relative percentages,

thus making it difficult to compare relative percentages between studies. In contrast, fatty acid concentrations are more reflective of the available fatty acids and can be more easily compared between studies (Brenna *et al.*, 2018).

The current study is also the first study in this area of research to report the fatty acid profile of a wide variety of samples. While it may be more common to report findings in the maternal and umbilical cord blood pools, very few studies also include the fatty acid composition of the placenta. In fact, to this writer's knowledge, this is the first study examining the fatty acid profile of the maternal and fetal placentas in T1D and T2D pregnancies.

In addition, the current study is one of the first studies to evaluate both umbilical cord levels of DHA and AA and neurodevelopment at birth in infants from T1D and T2D mothers. This type of study can provide more insight on the direct link between DHA and AA transfer from mother to fetus and whether this has an impact on the neonate's brain development. We know based on previous studies that pregnancies complicated by pre-existing T1D and T2D are associated with negative cognitive outcomes for the offspring long-term (Brinciotti *et al.*, 2009, de Regnier *et al.*, 2000, Silverman *et al.*, 1998, Fraser *et al.*, 2012, Clausen *et al.*, 2013, Temple *et al.*, 2011, Bytoft *et al.*, 2016, Clausen *et al.*, 2011, Xiang *et al.*, 2018, and Bytoft *et al.*, 2017). This type of study allows us to investigate whether these cognitive deficits are present soon after birth and whether they might be related to decreased DHA and AA transfer from mother to fetus.

The final strength of the current study is the use of EEGs at two time points. Since diabetic pregnancies are associated with increased risk of fetal macrosomia, it is common to induce labour so that the infant is born before full term (Mozurkewich *et al.*, 2008). This could result in differences in gestational age between groups. For instance, in the current study, neonates from the diabetic group were an average gestational age of 38.1 ± 0.9 weeks compared to 40.0 ± 1.1 weeks in the control group. During this period of rapid brain growth and development, two weeks of gestational age can make a difference when assessing neurodevelopment. For example, Léveillé and colleagues observed lower PSD in left centro-occipital region the infants from the diabetic group compared to the non-diabetic group, but this difference was no longer significant after adjustment for gestational age. The second EEG in our current study serves as a method of adjusting for gestational

age. The second EEG was performed 48 weeks post-amenorrhea (which equates to approximately two months of age). Since the timing of the second EEG is based on amenorrhea and not the birth date, it does not matter if one group of infants was born on average two weeks earlier than the other group. Therefore, the second EEG was performed in infants of equivalent age despite their differing gestational age at birth. In addition, having two EEGs allows us to compare neurodevelopment between birth and 2 months of age and to determine whether brain maturation occurred as expected.

Limitations

The main limitation of the current study is the small sample size, particularly of the diabetic group. Throughout recruitment during the years 2017 – 2020, we were only able to successfully recruit 7 diabetic participants. Only a small percentage of pregnant women have pre-existing T1D and T2D; Feig *et al.* observed that 1.5% of pregnancies in Ontario, Canada in 2010 were complicated by pre-existing T1D and T2D (Feig *et al.*, 2014). This in combination with the exclusion criteria and the difficulty of collecting samples during unpredictable labour and delivery times contributed to our small sample size. Of the diabetic participants that were recruited, not all of them completed the EEGs. This resulted in the even smaller sample size of N = 4 for the first EEG and N = 3 for the second EEG. This smaller sample size of the diabetic group can impact the power of the study and therefore make it more difficult to detect statistically significant differences, particularly in the case of small effect sizes such as in comparison of DHA levels. Furthermore, the diabetic group was composed of five T2D mothers and two T1D. The rationale for grouping both T1D and T2D mothers together was because T1D and T2D during pregnancy is associated with more hyperglycemic and hypoglycemic episodes compared to GDM pregnancies. Moreover, the hyperglycemia is present from the beginning of gestation in T1D and T2D compared to GDM which develops over the course of the pregnancy. However, it would have been desired to have enough participants of both T1D and T2D so that they may be considered separately as well. This would have been particularly useful when comparing the DHA and AA findings with the previous studies, namely the Berberovic *et al.*, 2013 study which seemed to have conflicting findings compared to the other studies reporting maternal and umbilical cord DHA and AA levels in diabetic

pregnancies (Ghebremeskel *et al.*, 2004, Min *et al.*, 2005, and Min *et al.*, 2014). However, despite the small sample size of the diabetic group, we were still able to detect some significant differences in the fatty acid composition of the maternal plasma and umbilical cord serum total lipids. The fact that we were able to find differences in fatty acid composition despite the small sample size potentially speaks to the strength of the effect. The fatty acid findings were also in line with some of the previous studies, which further confirms the validity of the findings of the current research project.

Another potential limitation of our study is the use of passive EEG instead of an evoked potential EEG. By measuring the brain activity of the infants using passive EEG, we were able to observe their general brain activity at rest. The advantage of this is that it was a practice that had already been previously implemented by our laboratory and collaborators in a previous study (Léveillé *et al.*, 2018) which then allowed us to compare these results directly with the previous pilot EEG study. On the other hand, using evoked potential can allow researchers to target more specific types of brain activity to assess specific functions such as the functioning of the visual system, the functioning of the auditory system, recognition memory, etc. Using evoked potentials could have also been useful in comparing our results to the previous studies that had assessed evoked potentials in infants of diabetic mothers (Brinciotti *et al.*, 2009 and de Regnier *et al.*, 2000).

Another limitation of the current study is the use of multiple comparisons. When performing multiple statistical tests such as t-tests, there is a chance that some of the findings are a false positive. The writer of this document chose to focus the discussion on the primary outcome to limit multiple comparisons. In addition, given the two time-point design of the EEG portion of our study, it would have been ideal to use a two-way repeated measure analysis in order to compare groups. However, because of an error in the handling of the EEG data files, we were not able to identify and match up specific individuals beyond just the group in which they belonged. As a result, we are unable to pair the two time points from the same individual. Because of this constraint, we compared means between groups and time points using the Mann Whitney test.

Future directions

The current study reported fatty acid composition in the total lipids of various sample types. As previously discussed, many of the existing studies that investigated DHA and AA levels in maternal blood and umbilical cord blood in diabetic pregnancies reported findings in specific lipid classes such as phospholipids and TGs (Ghebremeskel *et al.*, 2004, Berberovic *et al.*, 2013, Min *et al.*, 2005, Min *et al.*, 2014, Bitsanis *et al.*, 2006, Prieto-Sánchez *et al.*, 2017, Segura *et al.*, 2017, Uhl *et al.*, 2015, and Kuhn *et al.*, 1990). Moreover, DHA and AA are highly concentrated in phospholipids and many of the aforementioned studies reported differences in DHA and AA levels in the phospholipid class. However, differences in the fatty acid composition of the phospholipid fraction can be masked by other lipids such as TGs when analyzing the fatty acid composition of total lipids (Brenna *et al.*, 2018). Given this, it would be useful to analyze the fatty acid composition of TGs, phospholipids, free fatty acids, cholesterol esters in the maternal plasma, umbilical cord serum, umbilical cord whole blood, and maternal and fetal placenta. This would allow for more direct comparisons with findings in the current literature. In addition, it would reveal whether DHA levels differ in specific lipid classes such as the phospholipids in the umbilical cord serum of the diabetic group compared to the non-diabetic group.

Another potential avenue of research for this project would be to evaluate the level of expression of fatty acid transporters in the maternal and fetal placenta. As previously discussed, some studies have found evidence of changes in placental fatty acid transporter expression in GDM and T1D pregnancies (Magnusson *et al.*, 2004, Radaelli *et al.*, 2009, Prieto-Sánchez *et al.*, 2017, and Segura *et al.*, 2017). However, to this writer's knowledge, there are no studies in the literature that report the level of expression of placental fatty acid transporters in T2D pregnancies. Performing these analyses in our cohort would result in the first report of fatty acid transporter expression in the placenta of T2D women. This would shed light on a potential mechanism for the lower transfer of fatty acids from mother to fetus in T1D and T2D diabetic pregnancies. We had already begun to analyze placental fatty acid transporters (specifically MFSD2A, FABP4, FATP4, and CD36) by Western Blot, but we had encountered many hurdles in the process. Firstly, it was difficult to find antibodies that were reliable and specific to the proteins that we were investigating. Given

this, we began the process of developing pure proteins to use as a positive control in our Western Blot experiments. This process was halted earlier this year because of the coronavirus disease of 2019 (COVID-19). Because of delays associated with laboratories being closed down from the pandemic, we were unable to produce results for the placental fatty acid transporter levels.

Conclusion

The current study demonstrated that there is a significantly lower concentration of AA in the umbilical cord serum total lipids in T1D and T2D women compared to non-diabetic women. On the other hand, the concentration of DHA in the umbilical cord serum total lipids in T1D and T2D women compared to non-diabetic women did not differ significantly. These lower levels of umbilical cord AA are present despite similar levels of these fatty acids in the maternal plasma of both the diabetic and control groups. This indicates that there is a dysfunction at the level of the placenta that results in a decreased transfer of AA from mother to fetus in diabetic pregnancies. The current study does not provide evidence of accumulation of AA or DHA in the placenta of diabetic mothers, so this dysfunction may be at the level of the placental fatty acid transporters. Despite this lower level of cord blood serum AA, we were unable to find evidence of impaired neurodevelopment in the EEGs of infants of diabetic mothers 24-48 hours after birth and again at 48 weeks post-amenorrhea. This implies that these infants have similar neurodevelopment to their counterparts in the control group.

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APPENDICES

Appendix 1 Maternal plasma fatty acid relative percentage and concentration in total lipids from control and diabetic mothers.

	Relative Percentage (%)			Concentration (mg/dL)		
Fatty acid	Controls (n = 24)	Diabetics (n = 7)	*P* value	Controls (n = 24)	Diabetics (n = 7)	*P* value
C14:0	1.01 ± 0.08	0.58 ± 0.07	**0.009**	4.9 ± 0.5	2.5 ± 0.4	**0.010**
C16:0	27.20 ± 0.44	25.89 ± 0.60	0.148	131.1 ± 6.2	112.0 ± 8.9	0.136
C16:1n-7 trans	0.40 ± 0.02	0.35 ± 0.03	0.161	1.9 ± 0.1	1.5 ± 0.2	0.125
C16:1n-7	2.31 ± 0.17	1.54 ± 0.17	**0.028**	11.2 ± 0.9	6.5 ± 0.7	**<0.001**
C18:0	5.29 ± 0.10	5.546 ± 0.34	0.498	25.1 ± 0.8	23.7 ± 1.9	0.445
C18:1n-9	24.17 ± 0.44	25.37 ± 0.93	0.220	116.1 ± 5.4	111.8 ± 12.0	0.716
C18:1n-7	1.79 ± 0.06	1.88 ± 0.06	0.404	8.6 ± 0.5	8.2 ± 0.8	0.689
C18:2n-6	28.47 ± 0.64	28.24 ± 1.03	0.856	135.3 ± 5.1	121.7 ± 9.7	0.417
C18:3n-3	0.90 ± 0.04	0.84 ± 0.07	0.543	4.4 ± 0.3	3.6 ± 0.2	0.370
C20:3n-6	1.63 ± 0.07	1.75 ± 0.16	0.652	7.7 ± 0.4	7.6 ± 1.0	0.911
C20:4n-6	5.06 ± 0.19	6.04 ± 0.61	**0.049**	24.1 ± 1.3	26.0 ± 3.3	0.526
C20:5n-3	0.29 ± 0.03	0.35 ± 0.08	0.539	1.4 ± 0.1	1.4 ± 0.2	0.695
C22:5n-3	0.21 ± 0.01	0.22 ± 0.02	0.769	1.0 ± 0.1	0.9 ± 0.1	0.632
C22:6n-3	1.30 ± 0.08	1.41 ± 0.09	0.473	6.1 ± 0.4	6.1 ± 0.6	0.932
Saturated	33.50 ± 0.48	32.03 ± 0.71	0.136	161.1 ± 7.2	138.3 ± 10.4	0.126
Monounsaturated	28.65 ± 0.44	29.13 ± 0.97	0.624	137.8 ± 6.3	128.0 ± 13.4	0.480
N-3 PUFA	2.69 ± 0.11	2.82 ± 0.18	0.581	12.9 ± 0.7	11.9 ± 0.7	0.509
N-6 PUFA	35.16 ± 0.60	36.03 ± 1.16	0.503	167.2 ± 6.0	155.4 ± 12.1	0.835
PUFA	37.85 ± 0.61	38.84 ± 1.24	0.452	180.0 ± 6.5	167.3 ± 12.6	0.695
Total	100	100		479.0 ± 18.8	433.6 ± 34.7	0.260

Abbreviations: N-3 PUFA, omega-3 polyunsaturated fatty acids; N-6, omega-6 polyunsaturated fatty acids; PUFA, polyunsaturated fatty acids; Statistical differences between group means were determined using either independent *t*-tests or Mann Whitney tests, as appropriate. Statistical significance is denoted by *P* value in bold. Data is presented as mean ± standard error of the mean.

Appendix 2 Umbilical cord serum fatty acid relative percentage and concentration in total lipids from control and diabetic mothers.

Fatty acid	Relative Percentage (%)			Concentration (mg/dL)		
	Controls (n = 24)	Diabetics (n = 7)	*P* value	Controls (n = 24)	Diabetics (n = 7)	*P* value
C14:0	0.65 ± 0.05	0.74 ± 0.26	0.555	0.7 ± 0.1	0.7 ± 0.3	0.465
C16:0	26.04 ± 0.36	24.97 ± 0.78	0.182	28.1 ± 1.2	24.4 ± 2.0	0.115
C16:1n-7 trans	0.70 ± 0.04	0.57 ± 0.08	**0.026**	0.8 ± 0.1	0.6 ± 0.1	**0.007**
C16:1n-7	3.44 ± 0.13	3.58 ± 0.44	0.753	3.8 ± 0.2	3.6 ± 0.7	0.346
C18:0	10.19 ± 0.15	10.55 ± 0.24	0.248	11.0 ± 0.4	10.3 ± 0.5	0.395
C18:1n-9	19.74 ± 0.49	21.40 ± 0.73	0.108	21.7 ± 1.3	21.0 ± 1.8	0.945
C18:1n-7	3.24 ± 0.09	3.96 ± 0.17	**<0.001**	3.5 ± 0.2	3.9 ± 0.4	0.336
C18:2n-6	12.79 ± 0.47	11.34 ± 0.35	**0.021**	13.8 ± 0.8	11.0 ± 0.5	**0.005**
C18:3n-3	0.78 ± 0.16	1.39 ± 0.57	0.636	0.9 ± 0.2	1.4 ± 0.6	0.738
C20:3n-6	3.55 ± 0.11	3.70 ± 0.20	0.494	3.8 ± 0.2	3.6 ± 0.3	0.527
C20:4n-6	14.23 ± 0.37	13.47 ± 0.90	0.368	15.3 ± 0.7	12.9 ± 0.7	**0.019**
C20:5n-3	0.29 ± 0.02	0.31 ± 0.03	0.539	0.3 ± <0.1	0.3 ± <0.1	0.773
C22:5n-3	0.39± 0.03	0.33 ± 0.06	0.355	0.4 ± <0.1	0.3 ± <0.1	0.190
C22:6n-3	3.97 ± 0.19	3.69 ± 0.37	0.484	4.3 ± 0.3	3.5 ± 0.3	0.146
Saturated	36.88 ± 0.37	36.27 ± 0.43	0.412	39.8 ± 1.7	35.4 ± 2.4	0.216
Monounsaturated	27.12 ± 0.65	29.51 ± 1.34	0.097	29.7 ± 1.8	29.0 ± 2.9	0.800
N-3 PUFA	5.42 ± 0.28	5.72 ± 0.77	0.661	5.9 ± 0.4	5.5 ± 0.8	0.636
N-6 PUFA	30.58 ± 0.57	28.51 ± 1.11	0.100	32.9 ± 1.4	27.5 ± 1.1	**0.019**
PUFA	36.00 ± 0.68	34.23 ± 1.68	0.259	38.9 ± 1.8	33.0 ± 1.6	0.061
Total	100	100	0.555	108.4 ± 4.9	97.4 ± 5.8	0.317

Abbreviations: N-3 PUFA, omega-3 polyunsaturated fatty acids; N-6, omega-6 polyunsaturated fatty acids; PUFA, polyunsaturated fatty acids; Statistical differences between group means were determined using either independent *t*-tests or Mann Whitney tests, as appropriate. Statistical significance is denoted by *P* value in bold. Data is presented as mean ± standard error of the mean.

Appendix 3 Umbilical cord whole blood fatty acid relative percentage and concentration in total lipids from control and diabetic mothers.

	Relative Percentage (%)			Concentration (mg/dL)		
Fatty acid	Controls (n = 24)	Diabetics (n = 7)	*P* value	Controls (n = 24)	Diabetics (n = 7)	*P* value
C14:0	0.45± 0.04	0.49 ± 0.11	0.991	0.8 ± 0.1	0.8 ± 0.2	0.755
C16:0	27.14 ± 0.47	26.82 ± 0.48	0.732	42.4 ± 2.0	44.8 ± 3.5	0.563
C16:1n-7 trans	0.60 ± 0.03	0.54 ± 0.08	0.149	1.0 ± 0.1	0.9 ± 0.2	0.742
C16:1n-7	2.16 ± 0.12	2.00 ± 0.34	0.270	3.5 ± 0.3	3.4 ± 0.7	0.738
C18:0	10.06 ± 0.80	11.06 ± 1.49	0.391	14.7 ± 0.6	18.0 ± 2.4	0.234
C18:1n-9	14.52 ± 0.31	14.64 ± 0.52	0.852	22.8 ± 1.3	24.4 ± 1.9	0.554
C18:1n-7	2.47 ± 0.06	2.72 ± 0.12	0.076	3.9 ± 0.2	4.5 ± 0.4	0.124
C18:2n-6	9.43 ± 0.41	7.91 ± 0.37	0.062	15.1 ± 1.2	13.4 ± 1.3	0.460
C18:3n-3	0.16 ± 0.02	0.11 ± 0.02	0.170	0.3 ± <0.1	0.2 ± <0.1	0.524
C20:3n-6	3.66 ± 0.10	4.00 ± 0.26	0.204	5.7 ± 0.3	6.7 ± 0.7	0.203
C20:4n-6	21.90 ± 0.58	22.36 ± 1.00	0.706	35.1 ± 2.4	37.6 ± 3.8	0.622
C20:5n-3	0.30 ± 0.03	0.31 ± 0.03	0.437	0.5 ± 0.1	0.5 ± 0.1	0.555
C22:5n-3	0.71 ± 0.04	0.85 ± 0.15	0.410	1.2 ± 0.1	1.4 ± 0.3	0.258
C22:6n-3	6.47 ± 0.38	6.24 ± 0.44	0.763	10.6 ± 1.0	10.5 ± 1.2	0.729
Saturated	37.65 ± 0.93	38.37 ± 1.45	0.707	57.9 ± 2.0	63.7 ± 4.8	0.207
Monounsaturated	19.74 ± 0.41	19.88 ± 0.97	0.875	31.2 ± 1.8	33.2 ± 3.0	0.585
N-3 PUFA	7.63 ± 0.44	7.52 ± 0.52	0.894	12.5 ± 1.2	12.7 ± 1.5	0.627
N-6 PUFA	34.98 ± 0.74	34.23 ± 1.22	0.625	56.0 ± 3.7	57.6 ± 5.5	0.826
PUFA	42.62 ± 1.00	41.75 ± 1.60	0.674	68.4 ± 4.8	70.3 ± 6.8	0.847
Total	100	100		157.4 ± 8.2	167.2 ± 12.9	0.566

Abbreviations: N-3 PUFA, omega-3 polyunsaturated fatty acids; N-6, omega-6 polyunsaturated fatty acids; PUFA, polyunsaturated fatty acids; Statistical differences between group means were determined using either independent *t*-tests or Mann Whitney tests, as appropriate. Statistical significance is denoted by *P* value in bold. Data is presented as mean ± standard error of the mean.

Appendix 4 Maternal placenta fatty acid relative percentage and concentration in total lipids from control and diabetic mothers.

Fatty acid	Relative Percentage (%)			Concentration (μg/g)		
	Controls (n = 25)	Diabetics (n = 7)	*P* value	Controls (n = 25)	Diabetics (n = 7)	*P* value
C14:0	0.25 ± 0.03	0.15 ± 0.05	0.144	28 ± 4	16 ± 7	0.228
C16:0	24.37 ± 0.63	22.71 ± 0.80	0.201	2478 ± 194	2004 ± 336	0.255
C16:1n-7 trans	0.28 ± 0.02	0.24 ± 0.03	0.295	30 ± 3	22 ± 6	0.296
C16:1n-7	0.63 ± 0.04	0.45 ± 0.07	0.053	67 ± 8	42 ± 13	0.085
C18:0	11.19 ± 0.78	13.18 ± 1.03	0.302	1014 ± 45	1079 ± 97	0.517
C18:1n-9	10.74 ± 0.20	11.59 ± 0.47	**0.044**	1058 ± 60	989 ± 122	0.599
C18:1n-7	1.70 ± 0.04	1.86 ± 0.07	**0.044**	167 ± 9	160 ± 24	0.733
C18:2n-6	13.71 ± 0.48	12.21 ± 0.60	0.094	1399 ± 118	1089 ± 210	0.286
C18:3n-3	0.18 ± 0.01	0.16 ± 0.01	0.503	19 ± 2	15 ± 3	0.440
C20:3n-6	6.25 ± 0.19	6.21 ± 0.61	0.934	610 ± 34	531 ± 80	0.312
C20:4n-6	25.40 ± 0.44	25.40 ± 0.48	0.999	2543 ± 180	2206 ± 331	0.386
C20:5n-3	0.19 ± 0.02	0.19 ± 0.02	0.647	20 ± 2	16 ± 3	0.599
C22:5n-3	0.86 ± 0.04	0.99 ± 0.04	0.077	83 ± 5	83 ± 9	0.985
C22:6n-3	4.51 ± 0.17	4.82 ± 0.37	0.427	444 ± 32	400 ± 39	0.696
Saturated	35.81 ± 0.45	36.05 ± 0.84	0.806	3520 ± 193	3097 ± 399	0.322
Monounsaturated	13.35 ± 0.21	14.13 ± 0.52	0.221	1320 ± 79	1211 ± 163	0.533
N-3 PUFA	5.73 ± 0.19	6.15 ± 0.40	0.329	566 ± 39	514 ± 52	0.517
N-6 PUFA	45.36 ± 0.49	43.82 ± 1.10	0.168	4550 ± 318	3824 ± 610	0.295
PUFA	51.09 ± 0.50	49.97 ± 0.86	0.291	5116 ± 349	4341 ± 651	0.306
Total	100	0.15 ± 0.05	0.144	9957 ± 612	8654 ± 1193	0.330

Abbreviations: N-3 PUFA, omega-3 polyunsaturated fatty acids; N-6, omega-6 polyunsaturated fatty acids; PUFA, polyunsaturated fatty acids; Statistical differences between group means were determined using either independent *t*-tests or Mann Whitney tests, as appropriate. Statistical significance is denoted by *P* value in bold. Data is presented as mean ± standard error of the mean.

Appendix 5 Fetal placenta fatty acid relative percentage and concentration in total lipids from control and diabetic mothers.

Fatty acid	Relative Percentage (%)			Concentration (µg/g)		
	Controls (n = 25)	Diabetics (n = 7)	*P* value	Controls (n = 25)	Diabetics (n = 7)	*P* value
C14:0	0.38 ± 0.03	0.31 ± 0.02	0.102	32 ± 3	24 ± 3	0.265
C16:0	26.00 ± 0.42	25.89 ± 0.35	0.650	2119 ± 114	1960 ± 144	0.493
C16:1n-7 trans	0.34 ± 0.02	0.31 ± 0.03	0.467	28 ± 2	24 ± 3	0.318
C16:1n-7	0.74 ± 0.04	0.57 ± 0.05	0.063	60 ± 5	44 ± 5	0.083
C18:0	13.03 ± 0.56	14.20 ± 0.21	0.416	1030 ± 53	1074 ± 74	0.687
C18:1n-9	11.79 ± 0.27	11.82 ± 0.36	0.955	939 ± 33	889 ± 63	0.476
C18:1n-7	1.72 ± 0.04	1.79 ± 0.09	0.386	138 ± 6	134 ± 14	0.761
C18:2n-6	13.45 ± 0.45	11.91 ± 0.26	0.062	1087 ± 66	903 ± 70	0.144
C18:3n-3	0.19 ± 0.01	0.16 ± 0.02	0.385	15 ± 1	12 ± 1	0.350
C20:3n-6	5.45 ± 0.15	5.58 ± 0.51	0.818	440 ± 24	423 ± 55	0.467
C20:4n-6	22.28 ± 0.35	22.69 ± 0.28	0.370	1808 ± 91	1713 ± 116	0.607
C20:5n-3	0.18 ± 0.01	0.19 ± 0.03	0.763	14 ± 1	14 ± 2	0.902
C22:5n-3	0.80 ± 0.03	0.88 ± 0.03	0.246	64 ± 4	64 ± 3	0.559
C22:6n-3	3.65 ± 0.14	3.69 ± 0.14	0.886	292 ± 15	276 ± 14	0.602
Saturated	39.41 ± 0.32	40.40 ± 0.17	**0.010**	3182 ± 139	3059 ± 218	0.670
Monounsaturated	14.59 ± 0.30	14.49 ± 0.43	0.870	1165 ± 43	1093 ± 82	0.440
N-3 PUFA	4.82 ± 0.15	4.92 ± 0.18	0.736	386 ± 19	369 ± 18	0.651
N-6 PUFA	41.19 ± 0.44	40.18 ± 0.49	0.264	3336 ± 162	3043 ± 222	0.382
PUFA	46.00 ± 0.39	45.11 ± 0.48	0.267	3722 ± 175	3410 ± 236	0.387
Total	100	100		8068 ± 345	7560 ± 528	0.481

Abbreviations: N-3 PUFA, omega-3 polyunsaturated fatty acids; N-6, omega-6 polyunsaturated fatty acids; PUFA, polyunsaturated fatty acids; Statistical differences between group means were determined using either independent *t*-tests or Mann Whitney tests, as appropriate. Statistical significance is denoted by *P* value in bold. Data is presented as mean ± standard error of the mean.

Appendix 6 Mean coherence (± SEM) in newborns from control and diabetic mothers at two time points: 24-48 hours after birth (T0) and 48 weeks post-amenorrhea (T2).

		Controls			Diabetics			Diabetics vs Controls	
Electrode Pair	Frequency Range (Hz)	T0 (n = 22)	T2 (n = 17)	*P* value T2 vs. T0	T0 (n = 4)	T2 (n = 3)	*P* value T2 vs. T0	*P* value T0 vs. T0	*P* value T2 vs. T2
C3-O1 / C4-O2	0 – 3.4	0.468 ± 0.008	0.466 ± 0.010	0.812	0.450 ± 0.009	0.449 ± 0.019	>0.999	0.471	>0.999
	3.5 – 7.4	0.396 ± 0.004	0.402 ± 0.011	0.566	0.393 ± 0.008	0.393 ± 0.014	>0.999	0.918	>0.999
	7.4 – 12.9	0.394 ± 0.004	0.398 ± 0.008	0.644	0.394 ± 0.006	0.390 ± 0.004	0.857	0.918	0.921
	13.0 – 20.0	0.401 ± 0.007	0.378 ± 0.005	**0.009**	0.406 ± 0.010	0.385 ± 0.004	0.114	0.707	0.146
Fp1-C3 / Fp2-C4	0 – 3.4	0.548 ± 0.013	0.550 ± 0.014	0.989	0.526 ± 0.022	0.574 ± 0.035	0.229	0.471	0.546
	3.5 – 7.4	0.429 ± 0.007	0.451 ± 0.014	0.221	0.450 ± 0.021	0.455 ± 0.024	>0.999	0.352	>0.999
	7.4 – 12.9	0.398 ± 0.004	0.444 ± 0.009	**0.0003**	0.400 ± 0.010	0.458 ± 0.018	0.057	0.607	0.765
	13.0 – 20.0	0.382 ± 0.003	0.403 ± 0.005	**0.002**	0.394 ± 0.003	0.408 ± 0.004	0.057	0.069	0.616
T3-C3 / C4-T4	0 – 3.4	0.438 ± 0.005	0.445 ± 0.006	0.528	0.426 ± 0.009	0.449 ± 0.020	0.400	0.252	0.616
	3.5 – 7.4	0.381 ± 0.002	0.378 ± 0.005	0.190	0.369 ± 0.009	0.370 ± 0.006	>0.999	0.316	0.616
	7.4 – 12.9	0.371 ± 0.003	0.381 ± 0.005	0.172	0.376 ± 0.001	0.385 ± 0.011	0.857	0.256	0.546
	13.0 – 20.0	0.371 ± 0.003	0.368 ± 0.005	0.479	0.376 ± 0.004	0.370 ± 0.006	>0.999	0.352	0.479
T3-Fp1 / Fp2-T4	0 – 3.4	0.513 ± 0.011	0.510 ± 0.013	0.967	0.523 ± 0.022	0.521 ± 0.020	0.857	0.707	0.479
	3.5 – 7.4	0.416 ± 0.005	0.458 ± 0.014	**0.010**	0.449 ± 0.016	0.451 ± 0.028	>0.999	**0.040**	0.921
	7.4 – 12.9	0.398 ± 0.004	0.438 ± 0.008	**<0.0001**	0.410 ± 0.007	0.451 ± 0.010	0.057	0.197	0.616
	13.0 – 20.0	0.378 ± 0.004	0.390 ± 0.006	0.091	0.398 ± 0.010	0.389 ± 0.005	0.629	**0.031**	0.690
T3-O1 / T4-O2	0 – 3.4	0.425 ± 0.005	0.448 ± 0.007	**0.014**	0.436 ± 0.011	0.435 ± 0.013	0.857	0.352	0.690
	3.5 – 7.4	0.386 ± 0.003	0.389 ± 0.006	0.790	0.384 ± 0.011	0.384 ± 0.012	>0.999	0.607	>0.999
	7.4 – 12.9	0.388 ± 0.004	0.386 ± 0.006	0.528	0.388 ± 0.006	0.389 ± 0.006	0.857	>0.999	0.479
	13.0 – 20.0	0.394 ± 0.006	0.375 ± 0.007	**0.004**	0.399 ± 0.005	0.381 ± 0.004	0.057	0.389	0.118
T3-Cz / T4-Cz	0 – 3.4	0.556 ± 0.013	0.565 ± 0.015	0.732	0.550 ± 0.010	0.580 ± 0.018	0.229	0.864	0.765
	3.5 – 7.4	0.506 ± 0.011	0.508 ± 0.015	0.900	0.508 ± 0.031	0.516 ± 0.037	>0.9999	0.973	0.842
	7.4 – 12.9	0.485 ± 0.009	0.508 ± 0.013	0.232	0.482 ± 0.024	0.547 ± 0.043	0.400	0.973	0.479
	13.0 – 20.0	0.441 ± 0.009	0.417 ± 0.012	**0.036**	0.452 ± 0.015	0.468 ± 0.066	0.857	0.550	0.479
C3-Cz / Cz-C4	0 – 3.4	0.470 ± 0.012	0.509 ± 0.015	**0.045**	0.481 ± 0.027	0.515 ± 0.032	0.629	0.707	0.842
	3.5 – 7.4	0.433 ± 0.009	0.411 ± 0.008	0.110	0.444 ± 0.020	0.447 ± 0.062	0.629	0.656	>0.999
	7.4 – 12.9	0.428 ± 0.010	0.442 ± 0.009	0.104	0.440 ± 0.022	0.517 ± 0.060	0.400	0.560	0.146
	13.0 – 20.0	0.414 ± 0.009	0.408 ± 0.009	0.748	0.442 ± 0.018	0.483 ± 0.086	0.629	0.082	0.479

Statistical differences between group means were determined using Mann Whitney tests. Statistical significance is denoted by *P* value in bold. Data is presented as mean ± standard error of the mean.

Appendix 7 Mean power spectral density (± SEM) in newborns from control and diabetic mothers at two time points: 24-48 hours after birth (T0) and 48 weeks post-amenorrhea (T2).

		Controls			Diabetics			Diabetics vs Controls	
Area	Frequency Range (Hz)	T0 (n = 22)	T2 (n = 17)	*P* value T2 vs. T0	T0 (n = 4)	T2 (n = 3)	*P* value T2 vs. T0	*P* value T0 vs. T0	*P* value T2 vs. T2
Fp1 – C3	0 – 3.4	28.8 ± 0.6	29.3 ± 0.6	0.664	28.6 ± 0.3	29.1 ± 0.3	0.229	0.973	0.842
Left	3.5 – 7.4	21.2 ± 0.4	21.6 ± 0.6	0.705	21.3 ± 0.4	20.9 ± 0.4	0.629	0.973	0.765
	7.4 – 12.9	14.8 ± 0.4	15.0 ± 0.7	0.900	15.5 ± 0.3	15.6 ± 0.5	0.857	0.607	0.616
	13.0 – 20.0	11.6 ± 0.5	11.9 ± 1.0	>0.999	12.4 ± 0.7	14.4 ± 0.3	0.057	0.758	0.479
	0 – 20.0	17.4 ± 0.4	17.8 ± 0.7	0.748	17.9 ± 0.2	18.6 ± 0.1	0.057	0.707	0.690
C3 – O1	0 – 3.4	28.1 ± 0.5	32.5 ± 0.6	**<0.001**	27.3 ± 1.0	32.9 ± 1.5	0.057	0.656	0.616
Left	3.5 – 7.4	21.0 ± 0.4	23.7 ± 0.6	**<0.001**	20.0 ± 1.1	23.8 ± 0.5	0.057	0.560	0.690
	7.4 – 12.9	15.2 ± 0.4	14.8 ± 0.5	0.172	15.0 ± 1.0	15.7 ± 1.4	>0.999	0.758	0.690
	13.0 – 20.0	11.7 ± 0.4	10.4 ± 0.6	**0.021**	11.3 ± 0.6	11.9 ± 3.0	0.629	0.607	0.921
	0 – 20.0	17.4 ± 0.4	18.2 ± 0.5	0.510	16.9 ± 0.9	19.1 ± 1.8	0.629	0.656	0.921
Fp1 – T3	0 – 3.4	29.1 ± 0.6	29.9 ± 0.6	0.408	29.4 ± 0.7	29.4 ±<0.1	>0.999	0.918	0.921
Left	3.5 – 7.4	21.2 ± 0.4	21.8 ± 0.5	0.457	21.6 ± 0.3	21.2 ± 0.3	0.629	0.607	0.921
	7.4 – 12.9	15.0 ± 0.5	15.7 ± 0.6	0.408	15.9 ± 0.2	15.4 ± 0.7	>0.999	0.172	0.690
	13.0 – 20.0	12.3 ± 0.6	13.8 ± 0.9	0.232	13.2 ± 0.6	15.0 ± 0.4	0.114	0.607	0.358
	0 – 20.0	17.8 ± 0.5	18.8 ± 0.6	0.255	18.5 ± 0.3	18.9 ± 0.4	0.400	0.389	0.546
T3 – O1	0 – 3.4	27.3 ± 0.5	32.4 ± 0.6	**<0.001**	27.5 ± 1.1	32.3 ± 1.3	0.057	0.918	>0.999
Left	3.5 – 7.4	20.0 ± 0.5	22.9 ± 0.5	**0.001**	19.4 ± 1.0	23.2 ± 0.4	0.057	0.656	0.416
	7.4 – 12.9	14.6 ± 0.5	15.1 ± 0.5	0.475	14.5 ± 1.0	15.8 ± 1.2	0.629	0.973	0.616
	13.0 – 20.0	11.9 ± 0.6	12.6 ± 0.6	0.547	11.8 ± 0.7	13.9 ± 2.7	0.629	>0.999	0.616
	0 – 20.0	17.0 ± 0.5	18.9 ± 0.5	**0.009**	16.9 ± 0.9	19.5 ± 1.6	0.229	0.973	0.546
Fp2 – C4	0 – 3.4	28.8 ± 0.5	29.2 ± 0.5	0.424	29.3 ± 0.5	29.5 ± 0.9	>0.999	0.560	0.842
Right	3.5 – 7.4	21.2 ± 0.3	21.9 ± 0.6	0.475	21.2 ± 0.5	21.6 ± 1.0	>0.999	0.973	0.842
	7.4 – 12.9	15.1 ± 0.3	15.5 ± 0.7	0.566	15.3 ± 0.5	16.3 ± 0.5	0.229	0.656	0.765
	13.0 – 20.0	12.0 ± 0.5	12.6 ± 1.0	0.604	12.2 ± 0.6	15.3 ± 1.0	0.114	0.973	0.258
	0 – 20.0	17.6 ± 0.4	18.2 ± 0.6	0.475	17.9 ± 0.1	19.3 ± 0.8	0.400	0.811	0.479
C4 – O2	0 – 3.4	28.5 ± 0.5	32.5 ± 0.5	**<0.001**	29.6 ± 1.0	34.1 ± 0.4	0.057	0.352	0.216
Right	3.5 – 7.4	21.2 ± 0.4	24.0 ± 0.5	**<0.001**	21.0 ± 1.0	24.0 ± 0.5	0.057	0.973	0.765
	7.4 – 12.9	15.5 ± 0.4	15.2 ± 0.4	0.644	15.7 ± 0.9	15.2 ± 0.4	0.629	0.758	0.765
	13.0 – 20.0	11.8 ± 0.4	10.7 ± 0.6	**0.039**	12.2 ± 0.4	9.7 ± 0.2	0.057	0.811	0.842
	0 – 20.0	17.7 ± 0.4	18.4 ± 0.4	0.408	18.0 ± 0.8	18.4 ± 0.2	>0.999	0.707	0.616
Fp2 – T4	0 – 3.4	29.5 ± 0.6	30.0 ± 0.6	0.362	29.9 ± 0.8	29.8 ± 0.7	>0.999	0.471	0.765
Right	3.5 – 7.4	21.5 ± 0.4	22.0 ± 0.4	0.392	21.4 ± 0.4	21.6 ± 0.1	0.629	0.973	0.765
	7.4 – 12.9	15.5 ± 0.4	15.8 ± 0.6	0.812	15.6 ± 0.4	16.5 ± 0.5	0.229	>0.999	0.479
	13.0 – 20.0	12.9 ± 0.6	13.9 ± 0.9	0.492	13.0 ± 0.7	16.5 ± 0.4	0.057	>0.999	0.179
	0 – 20.0	18.3 ± 0.5	18.9 ± 0.6	0.528	18.4 ± 0.4	19.9 ± 0.4	0.114	0.864	0.305
T4 – O2	0 – 3.4	28.4 ± 0.5	32.1 ± 0.6	**<0.001**	29.0 ± 0.8	34.0 ± 1.1	0.057	0.515	0.179
Right	3.5 – 7.4	20.9 ± 0.4	22.7 ± 0.4	**0.001**	20.0 ± 0.9	23.8 ± 0.5	0.057	0.471	0.146
	7.4 – 12.9	15.3 ± 0.3	14.9 ± 0.4	0.333	14.8 ± 0.8	15.7 ± 0.8	0.629	0.707	0.546
	13.0 – 20.0	12.4 ± 0.4	12.3 ± 0.7	0.748	11.8 ± 0.7	12.6 ± 1.9	>0.999	0.515	>0.999
	0 – 20.0	17.7 ± 0.4	18.6 ± 0.5	0.172	17.3 ± 0.8	19.5 ± 1.0	0.229	0.707	0.305
T3 – C3	0 – 3.4	27.3 ± 0.6	28.4 ± 0.7	0.566	25.3 ± 1.2	27.6 ± 1.0	0.400	0.172	0.690
Left	3.5 – 7.4	20.7 ± 0.5	20.5 ± 0.6	0.392	19.4 ± 1.3	19.5 ± 0.8	0.857	0.471	0.546
	7.4 – 12.9	14.8 ± 0.6	13.1 ± 0.4	**0.025**	14.6 ± 1.1	12.7 ± 0.6	0.400	0.918	0.842
	13.0 – 20.0	11.0 ± 0.6	10.6 ± 0.6	0.624	11.4 ± 0.5	10.3 ± 1.0	0.400	0.707	>0.999
	0 – 20.0	16.9 ± 0.5	16.4 ± 0.4	0.566	16.5 ± 0.9	15.8 ± 0.7	0.629	0.656	0.842

C3 – Cz	0 – 3.4	25.9 ± 0.6	26.8 ± 0.7	0.441	26.4 ± 0.8	27.9 ± 0.7	0.229	0.811	0.546
Left	3.5 – 7.4	20.4 ± 0.5	20.2 ± 0.7	0.664	20.2 ± 1.2	20.4 ± 0.7	0.857	0.864	0.690
	7.4 – 12.9	14.1 ± 0.5	12.5 ± 0.6	0.082	14.9 ± 0.8	13.7 ± 1.2	0.629	0.429	0.479
	13.0 – 20.0	9.1 ± 0.6	6.4 ± 0.6	**0.003**	10.4 ± 0.2	7.5 ± 2.3	0.629	0.283	0.921
	0 – 20.0	15.7 ± 0.5	14.5 ± 0.6	0.172	16.5 ± 0.6	15.4 ± 1.3	0.629	0.607	0.765
Cz – C4	0 – 3.4	26.0 ± 0.6	27.1 ± 0.5	0.492	27.3 ± 0.7	27.8 ± 1.3	0.857	0.560	0.615
Right	3.5 – 7.4	20.5 ± 0.5	20.7 ± 0.6	0.989	20.5 ± 1.4	20.5 ± 1.3	>0.999	>0.999	>0.999
	7.4 – 12.9	14.4 ± 0.5	13.0 ± 0.6	0.092	14.9 ± 1.3	13.7 ± 0.8	0.400	0.560	0.690
	13.0 – 20.0	9.3 ± 0.5	7.2 ± 0.6	**0.023**	10.5 ± 0.7	7.6 ± 1.8	0.400	0.252	0.921
	0 – 20.0	15.9 ± 0.5	15.1 ± 0.6	0.221	16.7 ± 1.0	15.5 ± 1.1	0.629	0.471	0.690
C4 – T4	0 – 3.4	28.0 ± 0.5	28.8 ± 0.5	0.221	27.0 ± 1.2	29.3 ± 0.3	0.114	0.471	0.616
Right	3.5 – 7.4	21.3 ± 0.4	21.1 ± 0.5	0.457	19.1 ± 1.3	20.2 ± 1.1	0.857	0.130	0.690
	7.4 – 12.9	15.5 ± 0.4	14.0 ± 0.4	**0.009**	14.0 ± 1.4	13.6 ± 1.0	0.857	0.471	0.921
	13.0 – 20.0	11.9 ± 0.4	11.1 ± 0.7	0.117	10.8 ± 1.0	10.7 ± 2.2	>0.999	0.352	0.842
	0 – 20.0	17.7 ± 0.4	17.0 ± 0.4	0.292	16.2 ± 1.2	16.7 ± 0.9	>0.999	0.471	>0.999
T3 – Cz	0 – 3.4	29.6 ± 0.5	30.4 ± 0.6	0.408	29.4 ± 0.9	30.9 ± 0.5	0.229	0.918	0.690
Left	3.5 – 7.4	22.8 ± 0.4	22.6 ± 0.6	0.492	22.2 ± 1.1	22.7 ± 0.8	0.857	0.811	0.921
	7.4 – 12.9	16.4 ± 0.5	15.5 ± 0.4	0.292	16.7 ± 0.8	15.8 ± 0.7	0.629	0.471	0.842
	13.0 – 20.0	12.1 ± 0.5	11.8 ± 0.6	0.769	13.0 ± 0.3	12.2 ± 1.1	0.629	0.223	0.842
	0 – 20.0	18.5 ± 0.4	18.3 ± 0.5	0.790	18.8 ± 0.7	18.6 ± 0.6	>0.999	0.429	0.765
T4 – Cz	0 – 3.4	30.0 ± 0.5	30.6 ± 0.5	0.377	29.7 ± 0.8	31.0 ± 0.7	0.629	0.918	0.616
Right	3.5 – 7.4	23.1 ± 0.4	22.8 ± 0.5	0.305	22.0 ± 0.9	22.8 ± 0.8	0.629	0.352	0.842
	7.4 – 12.9	16.7 ± 0.4	15.5 ± 0.4	0.052	16.3 ± 0.9	16.3 ± 0.5	0.629	0.973	0.479
	13.0 – 20.0	12.7 ± 0.4	11.8 ± 0.6	0.087	12.5 ± 0.6	12.7 ± 1.9	0.857	0.973	0.616
	0 – 20.0	19.0 ± 0.4	18.3 ± 0.4	0.305	18.5 ± 0.7	19.0 ± 0.6	0.629	0.811	0.616

Statistical differences between group means were determined using Mann Whitney tests. Statistical significance is denoted by *P* value in bold. Data is presented as mean ± standard error of the mean.