Long-chain polyunsaturated fatty acids and iron status in infants of gestational diabetic mothers

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Abstract

Arachidonic acid (AA), docosahexaenoic acid (DHA) and iron are important nutrients for infants and are hypothesized to be compromised in infants of diabetic mothers (IDM). The objective of this study was to compare AA, DHA and iron status in IDM with those measured in infants of non-diabetic mothers born both appropriate for gestational age (AGA) and large for gestational age (LGA); and to determine if infants that have compromised AA and DHA status also have a compromised iron status. Analyses of covariance between AA, DHA and iron revealed that AA in infants was positively correlated with iron status overall, but this was not the case for DHA. In IDM, the relationship between iron and AA status was more pronounced. These data suggest iron is linked to AA status in all infants and that IDM are at higher risk for low AA status and consequently impaired development.
Résumé

L’acide arachidonique (AA), l’acide docosahexaénoïque (DHA) et le fer sont d’importants nutriments pour le nouveau-né. Il semblerait que leurs niveaux sont compromis chez les enfants de mères diabétiques (EMD). L’objectif de cette étude est de comparer les niveaux d’AA, de DHA et de fer chez les EMD avec ceux mesurés chez les enfants de mères non diabétiques qui sont nés avec un poids moyen ou gros pour l’âge gestationnelle pour déterminer si les niveaux de gras insaturés à longues chaînes et de fer sont tous les deux amoindris chez l’enfant. L’analyse de covariance entre le fer et les gras insaturés a montré que le niveau de fer est positivement corrélé avec le niveau d’AA mais pas avec celui de DHA. Chez les EDM, le lien entre le fer et l’AA est encore plus marquant. Ces résultats suggèrent que le niveau de fer est relié avec le niveau d’AA chez tous les nouveau-nés et qu’il y a un plus grand risque chez les EDM d’avoir un niveau d’AA amoindri et par conséquent de souffrir de problèmes de croissance.
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<tr>
<td>AGA</td>
<td>Appropriate for gestational age</td>
</tr>
<tr>
<td>AA</td>
<td>Arachidonic acid</td>
</tr>
<tr>
<td>DHA</td>
<td>Docosahexaenoic acid</td>
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<tr>
<td>FABPpm</td>
<td>Fatty acid binding protein</td>
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<td>FAT</td>
<td>Fatty acid translocase</td>
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<td>FATP</td>
<td>Fatty acid transport protein</td>
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<tr>
<td>GDM</td>
<td>Gestational diabetes mellitus</td>
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<tr>
<td>Hb</td>
<td>Hemoglobin</td>
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<td>HbA1c</td>
<td>Glycated hemoglobin</td>
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<td>HDL</td>
<td>High-density lipoprotein</td>
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<tr>
<td>IDM</td>
<td>Infants of diabetic mothers</td>
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<tr>
<td>IGF-1</td>
<td>Insulin-like growth factor-1</td>
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<tr>
<td>LGA</td>
<td>Large for gestational age</td>
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<tr>
<td>LC-PUFA</td>
<td>Long-chain polyunsaturated fatty acid</td>
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<tr>
<td>LDL</td>
<td>Low-density lipoprotein</td>
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<tr>
<td>PC</td>
<td>Phosphatidylcholine</td>
</tr>
<tr>
<td>PE</td>
<td>Phosphatidylethanolamine</td>
</tr>
<tr>
<td>p-FABPpm</td>
<td>Placental plasma membrane fatty acid binding protein</td>
</tr>
<tr>
<td>RBC</td>
<td>Red blood cells</td>
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<tr>
<td>sTfR</td>
<td>Serum transferrin receptor</td>
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<tr>
<td>SEM</td>
<td>Standard error of the mean</td>
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<tr>
<td>TfR</td>
<td>Transferrin receptor</td>
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Chapter 1. Literature Review
1. Introduction

Prevalence of diabetes is increasing in both men and women. The percentage of Canadians aged 12 years old and older that has been diagnosed with diabetes was reported to be 5.4% for males and 4.4% for females in 2005 [1] while it was only reported to be 3.4% for males and 3.3% for females in the 1994-1995 National Population Health Survey [2]. Having diabetes has consequences to overall health and longevity and in women of childbearing age the consequences extend to the developing fetus. The prevalence of diabetes in women between 18 and 44 years old was estimated in 2003 to be 1.3% in Canada excluding territories [2]. A new report from Statistics Canada from the 2005 Canadian Community Health Survey (CCHS) reported 0.9% of women between 18 and 34 years old and 2.0% of women between 35 and 44 years old were diagnosed with diabetes by a physician [1]. This is surely an underestimation of the real prevalence of diabetes in Canada as only women diagnosed by a physician were counted. If the prevalence of diabetes in women of childbearing age has been increasing over the years, the prevalence of infants born from a mother who was diabetic during the pregnancy has also risen. The latest report in 2003 indicated that prevalence of gestational diabetes mellitus (GDM) in Canada varies between 3.5% and 3.8% in the non-Aboriginal population to 18.0% in Aboriginal population [3].

It has recently been postulated that several diseases such as type 2 diabetes and hypertension originate from fetal malnutrition and altered endocrine status [4] such as that arising from a pregnancy complicated by GDM. This has been called
the “fetal origins” hypothesis [4] and implies that a critical window exists where growth and development can be affected by lack of or by a high dose of a nutrient [5, 6]. Based on separate reports, infants of diabetic mothers (IDM) indeed have compromised nutritional status at birth [7-10]. Iron and long-chain polyunsaturated fatty acids (LC-PUFA) are of particular interest as they are both required for the development of the brain from the last trimester of gestation to one year postnatal [5]. Therefore, they are possible candidates as contributing factors to the developmental impairment in IDM.

The objective of this thesis research is to examine if infants with low iron status also have low LC-PUFA status. It is hypothesized that both nutrients will be low in IDM who do not achieve adequate glucose control marked by a high random glucose and/or high hemoglobin A1c (HbA1c). To postulate the mechanisms behind this objective and hypothesis, the remainder of this literature review will address metabolism in pregnant women with GDM and its impact on iron and LC-PUFA status in the infant.

2. Carbohydrate metabolism

2.1. Basal metabolism

2.1.1. In pregnant women

One of the primary functions of the liver is endogenous glucose production. In non-pregnant women, the average plasma fasting glucose
concentration is about 5.0 mmol/L and the rates of glucose production and utilization are approximately the same as a result of glucose homeostasis [11].

In healthy pregnancy, fasting glucose concentration decreases progressively with advancing gestation. In late pregnancy, the fasting glucose concentration decreases 0.9% compared to pre-pregnancy state [11]. The mechanism by which the fasting glucose decreases is complex and yet to be clarified, but it has been suggested that this decrease could result from increased utilization (either increased feto-placental transfer in late pregnancy or increased beta-cell function) and/or, reduced production (limitation of hepatic glucose production relative to circulating glucose concentrations) [11]. The former is most likely true since despite a decrease in fasting glucose, hepatic glucose production is increased in tracer studies. Basal hepatic glucose production estimated by using [6,6-2H2] glucose does not differ between values measured pre-pregnancy and at 12-14 weeks of gestation. However, at the third trimester, the production increased by 16%-30% to meet the increasing needs of the placenta and fetus [12].

In addition to the elevated synthesis, insulin also increases during pregnancy. Hepatic glucose production is normally suppressed by insulin. In contrast during pregnancy, hepatic glucose production increases despite increasing fasting insulin concentration supporting the idea that insulin sensitivity is suppressed. The decrease in fasting glucose is further exacerbated with prolonged fasting suggesting an incomplete compensation (mainly from the liver)
or some restraint on endogenous production compared with non-pregnant conditions [11].

2.1.2. In Gestational Diabetes Mellitus (GDM)

The definition of GDM is a carbohydrate intolerance of variable severity with onset or first recognition during pregnancy. It is a heterogeneous disorder in which age, obesity and genetic background contribute to the severity and incidence of the disease [12]. In addition to elevated fasting glucose, GDM is accompanied by alteration in fasting, post-prandial and integrated 24 h-plasma concentration of amino acids and lipids such as a 3-fold increase in plasma triacylglyceride concentration during the third trimester of pregnancy, an elevation of plasma free fatty acids, a delayed post-prandial clearance of fatty acids and an elevation of the branched-chain amino acids. The metabolic changes observed in women with GDM will be covered in the next sections followed by a discussion of the impact these changes have on the infant.

2.2 Insulin sensitivity

One of the most common metabolic abnormalities in pregnancy is a reduction in insulin sensitivity. Insulin sensitivity is defined as the glucose infusion rate during the euglycemic-hyperinsulinemic clamp to maintain euglycemia. A high glucose infusion rate indicates that the person is insulin sensitive while a very low level of glucose infusion rate indicates an insulin resistance. Early in pregnancy, maternal insulin sensitivity in lean women
decreases meaning that glucose infusion rate decreases (i.e. clearance is lower). The effects of insulin on peripheral glucose utilization and hepatic production can be assessed separately if labelled glucose is infused during clamps. When glucose turnover is expressed relative to steady state insulin levels there is a 10% decrease in insulin sensitivity from pre-pregnancy to early gestation in lean subjects [11].

Peripheral insulin sensitivity decreases even more in the later part of gestation [11]. The hyperinsulinemic-euglycemic glucose clamp technique and computer-assisted intravenous glucose tolerance test indicate that insulin action in late normal pregnancy is 50%-70% lower than that of normal non-pregnant women [12]. Using glucose clamp methodology in GDM, it was demonstrated that a 40% further decrease in whole body insulin sensitivity in women with severe GDM happens in late pregnancy compared to controls. This results in a high glucose load delivered transplacentally to the fetus [12].

2.3. Insulin secretion

2.3.1. In normal pregnancy

There are progressive increases in insulin secretion in response to an intravenous glucose challenge with advancing gestation. The normal response of beta-cells to insulin resistance is to increase insulin secretion in order to minimize the impact of insulin resistance on circulating glucose levels. The mechanism which leads to enhanced insulin secretion in pregnancy is not clear however. It could be compensatory to progressive insulin resistance or the hormonal milieu of
pregnancy could exert a primary effect to increase insulin secretion independent of insulin resistance [11].

Insulin response to a glucose challenge occurs in two phases. The first phase refers to change in insulin concentration relative to the increase of glucose concentrations from 0 to 5 minutes while the second phase consists of the rate of insulin release relative to glucose concentrations 5 to 60 minutes after glucose administration. Using the clamp technique, a 120% increase was observed in the first phase of insulin response at week 12 to 14 compared to values before pregnancy. However, there is no difference in the second phase of insulin response between the pre-pregnancy state and early in the pregnancy [12]. As pregnancy progresses, an increase in basal and postprandial insulin concentration occurs. By the third trimester, basal and 24 hour mean insulin concentration may double and both phases of insulin response are 3 to 3.5 fold greater in later pregnancy compared to early in pregnancy [12].

2.3.2. In GDM

Most cases of GDM result from inadequate insulin secretion that arises in women with chronic insulin resistance and therefore seem to be related to type 2 diabetes. Studies reveal beta-cell function in GDM women is decreased 30% to 70% compared to women who maintain normal glucose tolerance during pregnancy. Longitudinal studies reveal that women with GDM follow a pattern of change in insulin sensitivity that is similar to controls (slight increase in early gestation, then large reductions by late gestation). Beta-cell function assessed as
acute insulin response to intravenous glucose follows a pattern in GDM similar to controls (slight increase at the beginning of gestation before sensitivity declines in late gestation when insulin sensitivity falls), but a lower insulin secretion overall relative to the decreased insulin sensitivity. Therefore, women with GDM increase their insulin secretion during pregnancy, but not to the same extent seen in healthy pregnancy, resulting in elevated blood glucose [11].

The pathophysiology of GDM remains controversial. According to some, it may reflect predisposition to type 2 diabetes accentuated under the metabolic conditions of pregnancy or may be the extreme manifestation of metabolic alterations that normally occur in pregnancy [12]. According to Lain et al., GDM is not simply a fixed limitation in insulin secretory reserve that becomes manifest as hyperglycemia when insulin needs increase during pregnancy as suggested by others [13]. Instead GDM represents the detection during pregnancy of chronic metabolic abnormalities that antedate pregnancy, but are detected when pregnancy leads to the first evaluation of glucose tolerance in otherwise healthy young women [11].

In summary, even though the pathophysiology of GDM is unclear, hyperglycemia in women GDM has definite consequence to the developing fetus and infant as discussed in section 5.3.
3. Lipid metabolism

Metabolic changes during pregnancy do not only pertain to carbohydrates, but also to lipids. There is a significant increase in adipose tissue stores in healthy pregnant women during pregnancy. The subcutaneous stores are a ready source of energy for the mother and fetus, particularly in late pregnancy and during lactation [11]. During the first part of the gestation, increased estrogen, progesterone and insulin favour lipid accumulation and inhibit lipolysis. During late pregnancy, human chorionic somatomammotropin promotes lipolysis and fat mobilization. This shift from an anabolic to a catabolic state promotes the use of lipids as maternal energy source while preserving glucose and amino acids for the fetus [12].

Changes in adipose metabolism affect the concentrations of triacylglycerides, fatty acids, cholesterol as well as phospholipids. After an initial decrease in the first 8 weeks of pregnancy, there is a steady increase in triacylglycerides, free fatty acids, cholesterol, lipoproteins, and phospholipids [12]. Total triacylglyceride concentrations increase 2 to 4-fold and total cholesterol concentrations increase 25% to 50% during normal human pregnancy [11]. The higher concentration of estrogen and insulin resistance are thought to be responsible for hypertriglyceridemia of pregnancy [12]. Furthermore there is a 50% increase in low-density lipoprotein (LDL) cholesterol and a 30% increase in high-density lipoprotein (HDL) cholesterol in response to estrogen by mid gestation followed by a slight increase in HDL at term [11].
GDM induces a state of dyslipidemia consistent with insulin resistance. During pregnancy, women with GDM have higher serum triacylglyceride concentration, but lower LDL-cholesterol concentration than do healthy pregnant women. Total cholesterol, HDL-cholesterol and apolipoprotein concentrations are not significantly different between GDM patients and control subjects [12].

4. Screening and diagnosis of GDM

Early in pregnancy, women are evaluated for risk of GDM. The risk factors include previous diagnosis of GDM, previous delivery of a macrosomic infant, member of a high-risk population such as women from Aboriginal, Hispanic, Asian, African descent, being older than 35 years of age and obesity [3]. If the woman has one or multiple risk factors, she should be screened during the first trimester and if the test turns out to be negative she should still be reassessed later on. The Canadian Diabetes Association Clinical Practice Guidelines Expert Committee recommends that all pregnant women must be screened for GDM between 24 and 28 weeks of gestation [3].

The screening test for GDM consists in an oral 50 g glucose load given at any time during the day and a measurement of the plasma glucose after one hour [3, 13]. If the plasma glucose is higher than 10.3 mmol/L, then GDM is confirmed. If the plasma glucose is between 7.8 and 10.2 mmol/L, then a 75 g oral glucose tolerance test (OGTT) must be performed [3, 13].
Tight glycemic control during pregnancy is needed to prevent neonatal complications. Some of the short-term complications are hypoglycaemia, hypocalcemia, hypomagnesemia, hyperbilirubinemia and polycythemia and are mainly related to fetal hyperinsulinemia, hypoxia and prematurity [14]. Some experimental studies have suggested the involvement of reactive oxidative species in neonatal complications [15]. In a hyperglycemic environment, a disturbance in the balance between the production of reactive oxidative species and anti-oxidative defence referred as oxidative stress may lead to adverse consequences on the fetus [16]. Nowadays, with the set-up of modern care units, most of these complications are diagnosed and are readily treated.

Examples of long-term complications for infants include obesity, impaired glucose tolerance, diabetes mellitus and subtle neuropsychological dysfunctions [14, 17]. These long-term complications are related to the severity of the maternal hyperglycemia during pregnancy, the consequent fetal hyperinsulinemia [14] and alteration of fetal iron metabolism [8], as well as disturbances in maternal lipid metabolism.

5. Iron

5.1. The role of iron in early development

Iron plays an important role in early development as it is part of several proteins involved in critical cellular processes such as tissue oxygenation and energy metabolism [18]. Iron is primarily needed for hemoglobin (Hb) synthesis,
but is also an important component of enzymes involved in cell replication, metabolism, neurotransmitter synthesis and myelination [9, 18-20].

The developing brain undergoes rapid structural and functional changes during the perinatal period and requires a regulated supply of iron across the blood–brain barrier. Failure to meet iron requirements leads to inadequate protein synthesis and function that ultimately compromises brain development [18].

5.2. Maternal-fetal iron metabolism

To support the increased need of iron during pregnancy, the recommended daily allowance (RDA) increases from 18 mg/day to 27 mg/day for pregnant women. The third trimester of gestation is a critical period where the fetus is growing rapidly and it is during that period that the majority of fetal iron accretion occurs [18]. Human placental iron transport is mediated by the transferrin receptor (TfR) located on the syncytiotrophoblast, a cell of fetal origin [21]. The iron bound to maternal transferrin binds to TfR located on the syncytiotrophoblastic membrane. Once bound, the whole complex gets endocytosed. Then with the help of several iron transporters such as divalent metal transporter-1 and ferroportin, the iron is released into the fetal circulation where it is either utilised or stored bound to fetal ferritin [18].

Placental iron concentrations seem to be determined by fetal iron needs rather than maternal iron availability. Indeed it was demonstrated that the fetus is able to maintain normal concentrations of iron despite the mother being mildly iron deficient [21]. Conversely, the fetus can become iron deficient even if the
maternal concentrations of iron are sufficient [21]. In the case where iron becomes deficient, the binding activity of syncytiotrophoblastic iron regulatory protein-1 (IRP-1) increases. The role of IRP-1 is to bind to the iron responsive element on TfR mRNA which will stabilize the mRNA and consequently increase the translation of TfR in the prospect of increasing iron uptake [18].

5.3. GDM and its consequences on fetal iron status

When the mother becomes hyperglycemic due to increased insulin resistance, metabolic changes occur in the fetus as a result of the higher rate of glucose transfer across the placenta. Therefore, the fetus also becomes hyperglycaemic. After 20 weeks of gestation, the fetal pancreas is functional, leading to a hyperinsulinemic response in order to control the hyperglycemia [18].

Hyperinsulinemia in the fetus affects growth through insulin-like growth factor (IGF-1). IGF-1 concentrations are modulated by nutrition, an effect believed to reflect direct influences of glucose and insulin. Maternal diabetes during pregnancy results in an increased action of IGF-1 and an enhanced fetal glucose utilization. Increased biological action of IGF-1 is thought to contribute to the increased birth weight in diabetic pregnancies [22]. The combination of rapid cellular growth, leading to rapid cell proliferation, with the higher rate of glucose oxidation leads to intrauterine hypoxia. In order to compensate for this lack of oxygen, fetal metabolism induces polycythemia, a condition in which the number of red blood cells (RBC) increases as a function of hyper-stimulated erythropoiesis [8, 10, 18, 23]. This results in an increased synthesis of Hb, thereby
increasing iron utilization by up to 40% [21]. Over 70% of fetal iron is in the red cell mass bound to Hb. For each additional gram of Hb that is synthesized, an additional 3.5 mg of iron is required. In diabetic pregnancies, there is a 40% up-regulation of TfR expression on the syncytiotrophoblast in an attempt to meet the excess fetal iron demand for erythropoiesis. However, this up-regulation is not sufficient to normalize fetal iron status, in part because TfR structure and function are altered by hyperglycosylation as a manifestation of diabetes [18, 21]. In conclusion, the combination of increased demand of iron and the compromised TfR placental function lead to depletion of fetal iron stores.

5.4. Iron status in IDM

Sixty five percent of newborn IDM have reduced cord serum iron and ferritin concentrations, increased total iron-binding capacity values, elevated free erythrocyte protoporphyrin concentration associated with elevated erythropoietin and haemoglobin concentrations [18, 23]; confirming the fact that IDM are at high risk to become iron deficient.

It is commonly observed that iron is prioritized to RBC even over vital organ development such as liver, brain and heart [8]. In infants (n=7 IDM, n=7 age-matched control infants) who had died before 7 days of age, autopsied liver, heart, and brain tissue had iron concentrations 6.6%, 43.9% and 60.6% of control values, respectively. Based on this small study, hepatic iron was greater than 75% depleted before reductions in heart and brain iron were observed [10]. Therefore it seems that to support the increased RBC production, iron is reduced first in the
liver then in the heart and the brain. Depending on the length of deficiency, low iron status, especially at this age where the brain is developing, can have various consequences on the infants’ cognitive development.

5.5. Short-term and long-term effects of iron deficiency in IDM

As iron is a critical component of several proteins involved in many different cellular processes, iron deficiency can have various adverse effects on the developing fetus and infant. It seems that abnormal myelination occurs in 6 month old infants with iron deficiency [18]. A study conducted by Siddappa et al. looked at the effect of low iron on the hippocampus and particularly how that would affect recognition memory using event-related potentials [18]. It was shown that IDM who were iron deficient had impaired neonatal auditory recognition memory and lower psychomotor developmental scores at 1 year of age compared to iron sufficient IDM [24]. Similarly, Lozoff et al. reported several cases where infants that were iron deficient, had problems in visual recognition memory and scored lower on the motor tests.

The longest follow-up reported was conducted in Costa Rica where children (11-14 years old) seemed to also exhibit a different social-emotional behaviour when compared to iron sufficient children. They were more wary, hesitant, unhappy and did not seem to show any ability to interact socially [19]. The same article reported long-term studies that looked at preschool age children that were iron deficient at birth. There was a correlation in several studies between Hb levels measured soon after birth and mental, motor and socio-
emotional test scores at preschool age after controlling for background variables such as maternal IQ, socio economical background and lead levels [19].

In summary, iron deficiency in infants early in life results in impairment in the development of the infant, but iron is not the only nutrient of interest when one is concerned with infant growth and development. LC-PUFA status is also primordial in the child’s development [25] and can also be comprised due to maternal diabetes [7].

6. Fatty acids

6.1. Function of LC-PUFA in early brain development

The n-6 and n-3 long-chain polyunsaturated fatty acids, especially arachidonic acid (AA, C20:4 n-6) and docosahexaenoic acid (DHA, C22:6 n-3) have a direct impact on optimal fetal growth and development [26]. The n-6 and n-3 fatty acids also regulate carbohydrate and lipid metabolism through the effects on gene expression involving steroid regulatory element binding proteins and peroxisome proliferators-activated receptors [5].

The liver is the major site for the metabolism of LC-PUFA for synthesis of hepatic membrane phospholipids. Both AA and DHA are formed by desaturation and elongation of the essential fatty acids, linoleic acid (LA, C18:2 n-6) and α-linolenic acid (ALA, C18:3 n-3), respectively. The synthesis of AA and DHA requires the same enzyme resulting in a competition between n-3 and n-6 fatty acids [27]. Even though endogenous synthesis occurs in infants, accumulation of
preformed DHA and AA in the brain is observed to be far more efficient than the desaturation and elongation of the precursors [27].

In developing neural cells, AA and DHA are found in high concentration and modulate the structure, fluidity and function of brain membranes [27]; and are also essential to the structure and the function of organs such as blood vessels, pancreatic beta-cells and retina. DHA is selectively accumulated in specific tissues including retina and brain grey matter, which makes it a major component of the developing central nervous system [26]. Depletion of DHA from the brain and retina results in reduced visual function, cognitive and behavioral abnormalities, altered monaminergic neurotransmitter metabolism such as serotonin and dopamine and decreased membrane protein and receptor activities [5, 6, 26, 27].

In all tissues and cells, AA is mainly found in plasma membranes and is a precursor of eicosanoids [5]. It can be oxygenated by three different enzymatic systems: cyclooxygenases forming prostaglandins and thromboxane, lipoxygenases forming leukotrienes and cytochrome P450 monooxygenases forming 19- and 20- HETE [25]. The biological activities of eicosanoids are extensive. For instance, prostaglandin E₂ (PGE₂) affects the vasculature, airways, stomach, kidney, neutrophils and neurotransmitter synthesis [25]. AA is particularly important as a second messenger in synaptic signal transduction [5, 6]. Thus it has ubiquitous function as well as brain specific activity.
6.2. Sources of AA and DHA during the pregnancy

The enzymes, delta 6 and delta 5 desaturases, that are involved in the production of AA and DHA from LA and ALA respectively have been shown to have some activity in liver as early as 17 weeks of gestation [28], but the activity appears to be low before birth [25]. Therefore, the fetus relies mostly on AA and DHA through placental transfer. The exact mechanism by which transfer occurs is still not fully understood. The placenta lacks desaturase activity, and is thought to be primarily dependent on maternal circulation for supply of these vital fatty acids [29]. Therefore it seems that maternal triacylglycerol are first hydrolyzed by lipoprotein lipase before being transferred by the placenta. Transplacental fatty acid transfer involves several proteins such as plasma membrane fatty acid binding protein (FABPpm), fatty acid translocase (FAT), fatty acid transport protein (FATP), and placental plasma membrane fatty acid binding protein (p-FABPpm). FABPpm, FAT, and FATP have not shown any specificity for particular types of free fatty acids. However, p-FABPpm may be involved in the preferential uptake of LC-PUFA by these cells [27].

6.3. Fatty acid transfer under GDM conditions

It has long been accepted that maternal dietary fatty acid status dictates transfer of LC-PUFA to the fetus [30]. As placental transfer of AA and DHA relies completely on maternal plasma [29], transplacental fatty acid transport appears selective for AA and DHA [6].
Fatty acid composition of placenta of women with GDM has a higher concentration of AA and DHA compared to placenta of control subjects, suggesting an enhanced uptake of these two fatty acids in GDM (as the primary source of AA and DHA is the maternal circulation) [31]. However, AA and DHA concentrations in the fetus were lower than normal, while fatty acids concentrations in the placenta were high compared to tissue from healthy pregnancy [29]. It was therefore hypothesized that the two fatty acids were taken up by the placenta and retained as phosphoglycerides instead of being transferred to the fetus [29]. In one of the few studies that measured AA and DHA concentration in phospholipids of RBC of IDM, it was confirmed that AA and DHA concentrations were lower in IDM than in controls [32].

6.4. LC-PUFA transfer during lactation

AA and DHA are particularly important from the last trimester of gestation to the first year of age. DHA intake of at least 200 mg/day during pregnancy and/or lactation is recommended to enable adequate maternal-fetal transfer [25]. However, AA has currently no recommended intake for humans owing to few studies demonstrating it as a conditional essential fatty acid for reproduction. During pregnancy, these are provided by the mother through placental transfer. After birth, these fatty acids can be provided through breastfeeding. Indeed, human milk contains multiple different fatty acids including the n-6 and n-3 such as LA, ALA, AA and DHA that make up 15% to 20% of all the fatty acids [28]. AA is relatively constant worldwide ranging
between 0.35-0.7% of total fatty acids [25]. On the contrary, DHA in human milk is more variable and depends on maternal diet ranging from 0.17% to 1.0% of total fatty acids [25].

7. Relationship between AA, DHA and iron and their impact on brain function

Iron is a cofactor in many enzymes and neurotransmitters in the brain. It also is a cofactor in the delta-6 desaturase enzyme required for synthesis of AA and DHA [33]. Thus despite the brains ability to synthesize AA and DHA de novo, capacity might be limited due to low iron status in brain tissue. It is possible that low iron status would reduce AA and DHA concentrations in the brain causing negative effects on neurobehavior and memory. A deficiency in AA will cause reduced production of PGE₂ via the cyclooxygenase-1 and –2 enzymes which contain iron [34]. Low iron and AA would reduce cyclooxygenase-2 production and inhibition of cyclooxygenase-2 reduces PGE₂ signalling the hippocampus [35]. Release of neurotransmitters in neurons and astrocytes is dependent on PGE₂ [36-38]. It is plausible that less neurotransmitter is released due to insufficient iron for DHA and AA synthesis and low cyclooxygenase-2 due to low iron and AA, all leading to less PGE₂. Thus the combined iron and AA and DHA deficiencies in brain will likely affect neurotransmitter synthesis, storage and function.
8. Conclusion

It is clear that the prevalence of IDM is increasing and that in numerous studies [5, 7, 8, 10, 23, 26, 32, 39] involving separate assessment of iron and LC-PUFA status in IDM, their status are compromised. Because of iron and LC-PUFA interrelationships and impacts on brain function, it is important to consider these two nutrients together.

Therefore, the primary objective of this thesis research is for the first time to study combined dietary assessment of iron and LC-PUFA status in term born IDM. This study is important since despite advanced obstetric care, hyperglycemia during pregnancy continues to exist. If both iron and LC-PUFA are compromised in the majority of IDM, subsequent studies can examine if intervention with these nutrients might improve developmental outcomes in IDM.
Chapter 2. Manuscript

Long-chain polyunsaturated fatty acids and iron status in infants of mothers with gestational diabetes mothers

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Key words: Iron, Long-chain polyunsaturated fatty acids, Arachidonic acid, Docosahexaenoic acid, Infant of diabetic mother, Gestational diabetes mellitus.
2.1. Abstract

Introduction: Iron and long-chain polyunsaturated fatty acids such as arachidonic acid (AA) and docosahexaenoic acid (DHA) are important nutrients for the development and growth of the fetus during the last trimester of gestation and the first year of life. Gestational diabetes mellitus (GDM) compromises these nutrients in infants of diabetic mothers (IDM). The objective was to study nutritional status of these nutrients in newborn IDM as they are interrelated with iron being a cofactor in fatty acid synthesis.

Methods: Thirty-one appropriate for gestational age (AGA) infants, 20 large for gestational age infants (LGA) and 20 IDM were recruited. Iron status was assessed using an iron index (sTfR/log ferritin) and AA and DHA were measured in phosphatidylcholine (PC) and phosphotidylethanolamine (PE) of plasma from the cord. Iron and fatty acid status was also measured in the mothers.

Results: In IDM, AA measured in PE of plasma was significantly reduced by 38% compared to AA in the AGA group. DHA in IDM was significantly reduced by 42% compared to DHA in AGA and significantly reduced by 46% compared to DHA in LGA. DHA measured in PE of plasma was significantly reduced by 52% compared to DHA in AGA group. Ferritin and sTfR-F index levels were not significantly different among the 3 groups. In IDM, sTfR concentrations were significantly higher by 11% compared to sTfR concentrations measured in the AGA group. sTfR-F index was negatively correlated with AA of both PC (r=-0.31, p=0.0129) and PE (r=-0.36, p=0.0047).
Conclusion: Whether the mother had diabetes or not, and regardless of the infant’s weight, if the infant is born with low iron status, it seems that AA status would also tend to be low. However, GDM seems to further lower AA in the newborn infant.

2.2. Introduction

During the last term of gestation, the brain undergoes rapid transformation. This is a crucial phase of development where correct amounts of nutrients are required for neuronal cell growth and development. Important neurodevelopment processes include the onset of myelination, organization of neurotransmitter systems and synapse formation. These happen in regions such as the auditory and visual cortices and the hippocampus which is central for recognition memory processing [40].

Many nutrients are important for the brain, but some are more critical determinants of brain development. Deficiencies of these nutrients could result in long-lasting effects on the central nervous system development. Among these nutrients, iron and long-chain polyunsaturated fatty acids (LC-PUFA) are of particular interest because of their function in brain development [5].

Iron is rapidly accreted by the fetus during the last trimester and is necessary for basic neuronal processes such as myelination, neurotransmitter production, and energy metabolism [20, 40]. Deficiency of iron during the last trimester of gestation results in altered auditory, visual, motor and social-emotional behaviour [19, 41]. Arachidonic acid (AA, C20:4 n-6) and
docosahexaenoic acid (DHA, C22:6 n-3) are LC-PUFA that are essential in the membranes of the central nervous system [28]. DHA is a major structural lipid of cell membranes, especially in the brain and retina [6, 25]. AA is important as it is a membrane component and it is also involved in cell signalling [25, 28]. AA is also a precursor to eicosanoids and leukotrienes which are important signalling molecules [28]. Depletion of DHA in brain and retina results in reduced visual function, cognitive and behavioral abnormalities [5].

Gestational diabetes mellitus (GDM) may compromise the status of iron and LC-PUFA such as AA and DHA in both mother and infant. Maternal diabetes during pregnancy results in an increased action of insulin-like growth factor-1 (IGF-1) and an enhanced fetal glucose utilization. Increased biological action of IGF-1 is thought to contribute to increased birth weight in diabetic pregnancies [22]. The combination of rapid cellular growth, leading to rapid cell proliferation, with the higher rate of glucose oxidation leads to intrauterine hypoxia with consequences to fetal iron demand. To increase oxygen-carrying capacity, the fetus synthesizes more hemoglobin, thereby increasing iron utilization by up to 40% [21]. In diabetic pregnancies, there is a 40% up-regulation of transferrin receptor (TfR) expression on the synctiotrophoblast in an attempt to meet the excess fetal iron demand for erythropoiesis. However, this up-regulation is not sufficient to normalize fetal iron status, in part because TfR structure and function are altered by hyperglycosylation during GDM [18, 21].

It has long been accepted that maternal dietary fatty acid status dictates transfer of LC-PUFA to the fetus [30]. The placenta lacks desaturase activity
therefore placental transfer of AA and DHA relies completely on maternal plasma [29]. The placenta has transplacental fatty acid transport that appears to be selective for AA and DHA [6]. However, AA and DHA concentrations in infants of diabetic mothers (IDM) are lower compared to the concentrations measured in infants of mothers without GDM. It seems that GDM compromises the placental transfer of AA and DHA by retaining them as phosphoglycerides instead of transferring them to the fetus [32].

Iron, AA and DHA are nutrients that are important for brain growth and proper cognitive development. Iron is a cofactor in the delta-6 desaturase enzyme required for synthesis of AA and DHA [33]. It is possible that low iron status would reduce AA and DHA concentrations in the brain causing negative effects on neurobehavior and memory. Previous studies have examined neonatal status of these nutrient in IDM, but separately. Therefore, the primary objective of this article is for the first time to study combined dietary assessment of iron and LC-PUFA status in term born IDM. If both iron and LC-PUFA are compromised in the majority of IDM, subsequent studies can examine if intervention with these combined nutrients might protect IDM from impaired developmental outcomes [31].

**2.3. Methods and experimentation**

**2.3.1. Recruitment and study sample**

The participants were recruited from a large prospective cohort study of pregnant women and their newborn infants conducted at Laval University,
Quebec City, where 6000 of women were studied from early conception with blood samples collected as well as demographic information, weight gain, nutrition and dietary supplement use, and information on diagnosis of complications such as glucose intolerance and GDM. Some participants with GDM (n=5) were also recruited at the Royal Victoria Hospital, Montreal, Quebec, in order to complete the cases group. In total, 20 women with GDM, 31 control women whose infants were appropriate for gestational age (AGA which is defined as weight between 10th and 90th percentiles for sex and gestational age) and 20 control women whose infants were large for gestational age (LGA which is defined as weight higher than 90th percentiles for sex and gestational age) were recruited from October 2007 and March 2009.

Inclusion criteria for this study were: women free of any disease or medical condition (including alcohol use in pregnancy, asthma, medically treated diseases, maternal infection, preeclampsia/eclampsia, Hemolytic anemia Elevated Liver enzymes and Low Platelet count and smoking) other than GDM and they must have given birth to a singleton infant whose weight must not be less than 10th percentiles for sex and gestational age (SGA).

2.3.2. Study protocol

If the women and infants met the inclusion criteria, the maternal blood taken just before delivery and the cord blood were sent to McGill’s School of Dietetics and human Nutrition laboratories for fatty acid and iron assessment.
Maternal characteristics and infant anthropometry were taken from the hospital charts. The participants were also asked to fill out a self-administered modified Willett food frequency questionnaire (mWFFQ) at delivery to assess their food intake in their last trimester of gestation. Food frequency intake data was transformed into nutrient intake and expressed as intake/day using Nutritionist Pro™ (Axxya Systems, USA) and the Canadian Nutrient File. The mWFFQ was selected as it is simple and can be administered by the participant then checked by the researcher for completeness. Furthermore, it has been validated for estimation of fatty acid intake in pregnancy [42, 43].

2.3.3. Experimental

2.3.3.1. Reagents and standards

Methanol, chloroform, hexane, petroleum ether, diethyl ether and acetic acid, 2-propanol were purchased from Fisher (Thermo Fisher Scientific, Canada). Trifluoride-methanol reagent was purchased from Sigma (Sigma-Aldrich®, Canada). The standards, 1,2-dipentadecanoyl-sn-glycero-3-phosphocholine (C15) and 1,2-diheptadecanoyl-sn-glycero-3-phosphatidylethanolamine (C17), were purchased from Avanti Lipids (Avanti Polar Lipids Incorporation, USA). Solid-phase extraction cartridges containing 100 mg of Bond Elut silica gel (SI) or aminopropyl-bonded silica gel (NH₂) were purchased from Varian (Varian Inc, Canada).
2.3.3.2. Fatty acid assessment

Lipid extraction was carried out as explained by Bondia-Pons et al. [44] with a 100 μL sample of plasma. Two internal standards were added in the sample in order to calculate fatty acid concentrations, C15 and C17. After extraction of lipids, separation of plasma membranes phospholipids into phosphatidylcholine (PC) and phosphatidylethanolamine (PE) was accomplished using the method described by Suzuki et al. [45] that required a solid-phase extraction. Once separated into PC and PE portions, methylation of the fatty acids was conducted in each portion according to the method of Bondia-Pons et al. [44]. Fatty acid methyl esters were analyzed using a gas chromatogram (Varian CP-3800, Varian Inc, Canada) equipped with a flame ionisation detector and autosampler (CP-8400, Varian Inc, Canada). AA and DHA were expressed as percent of fatty acids in phospholipids.

2.3.3.3. Iron assessment

Iron status was assessed by measuring ferritin in 10 μl of plasma using a chemiluminescence assay (REF 313551 Liaison, Diasorin Inc, Italy). Replicate analyses of standards yielded a coefficient of variation of < 5% for this assay. Serum transferrin receptor (sTfR) was measured in 20 μl of plasma in duplicate using the quantikine IVD soluble transferrin receptor ELISA (REF DTFR1, Research & Diagnostic Systems, USA). A coefficient of variation < 15% was acceptable according to manufacturer instructions. For any higher coefficient of
variation, the sample was re-analyzed. The ratio of sTfR to log of ferritin (sTfR-F index) was used to reflect tissue iron status where high values indicate low iron status.

### 2.3.4. Statistical analyses

The sample size was estimated based on a study with IDM where AA was reduced by 28% and DHA 37% when compared to infants of women without GDM [32]. To detect differences in RBC phospholipids using this magnitude of change, a sample of 34 per group is required given a power of 0.80, alpha of 0.05 using AA values.

Differences between groups were tested using an analysis of variance (ANOVA) (SAS version 9.1, SAS Incorporated, USA). Post-hoc test for significance was assessed using a Tukey’s test with p<0.05.

Pearson correlations between variables were used to select possible covariates to be included in the model to explain variation in fatty acid proportions. In order to test effects of covariates on fatty acid values, an analysis of covariance (ANCOVA) was employed in two ways and data were graphed for each infant group. First, when no interaction effects were present, the data would exist in parallel lines, meaning that for example differences among the groups were the same at any value of sTfR-F index. Second, when there was an interaction between effects of infant group with covariates (meaning for example that differences among the groups were not the same at any value of sTfR-F), then the values of the sTfR-F index was divided in tertiles and the mean of each tertile was
calculated. Then the estimated fatty acid value for each sTfR-F mean was calculated and compared among the three groups using ANOVA.

When the data set was not normally distributed, transformations such as log, inverse, square root were used to normalize the datasets. Results in tables are expressed as means ± standard error of the mean (SEM).

Recruitment of women was conducted in two different sites, through the cohort study in Quebec City and in the Royal Victoria Hospital in Montreal. Recruitment site was explored as a possible covariate when looking for differences among the groups but was non-significant. Therefore results for women from both sites were reported altogether.
2.4. Results

2.4.1. Mothers’ characteristics

The characteristics of mothers that participated in the study are summarized in Table 1. Women who had diabetes during pregnancy were significantly older than control women that gave birth to infants AGA (p=0.022), but were not significantly older than women that gave birth to infants LGA. The age of the women in the two control groups were not significantly different from one another. The woman who gave birth to LGA infants were significantly taller than the two other groups (vs AGA p=0.0007, vs IDM p=0.0013) and gained significantly more weight during their pregnancies than the GDM group (p=0.0086). The remaining characteristics such as pre-pregnancy weight, pre-pregnancy BMI, Hb, hematocrit were not significantly different among the three groups. The women recruited were mostly (98.6%) Caucasian.

2.4.2. Infants’ characteristics

Infants’ anthropology is summarized in Table 2. According to this study design, infants that were born LGA were significantly longer (p<0.001), heavier (p<0.001) and had a wider head circumference than the two other groups (vs. AGA p=0.0003, vs. IDM p=0.0172). The placental weight of the three groups were all significantly different from one group to another, the placenta of the LGA group being the heaviest and the placenta of the AGA group the lightest. All infants were term born, but the gestational age of the LGA group was
significantly higher than the IDM group. For Hb and hematocrit, there were no significant differences among the three groups.

2.4.3. Mothers' dietary intake at the third trimester of gestation

Energy, carbohydrates, fat and protein intakes as well as amounts of linoleic acid, alpha-linolenic acid, EPA, AA, DHA consumed by the mothers are reported in Table 3. There were no significant differences among the three groups.

Values for iron were broken down in iron obtained from diet and iron obtained from supplements. These values and total iron consumed by the mothers were also reported in Table 3. No difference among the three groups was observed.

2.4.4. Fatty acid and iron assessment

Fatty acid, ferritin, sTfR and sTfR-F index values measured in infants are reported in Table 4. There were no significant differences in AA proportions measured in PC of plasma among the three groups. In IDM, AA measured in PE of plasma was significantly 38% lower compared to AA proportions in AGA group. DHA measured in PC of plasma was significantly different among the three groups. DHA in IDM was 42% lower compared to DHA in AGA and 46% lower compared to DHA in LGA. DHA measured in PE of plasma of IDM was significantly lower by 52% compared to DHA in AGA. Ferritin and sTfR-F index levels were not significantly different among the three groups. In IDM, sTfR
levels were significantly higher by 11% compared to sTfR levels measured in the AGA group.

Fatty acids, ferritin, sTfR and sTfR-F index values of mothers are reported in Table 5. No significant difference was noted among the three groups for any of the fatty acids or iron markers measured.

Pearson correlations are reported in Table 6 for the relationships among AA and DHA in PC and PE of plasma with sTfR-F index. sTfR-F index was negatively correlated with AA of both PC (r=-0.31, p=0.0129) and PE (r=-0.37, p=0.0047). DHA proportions measured in both PC and PE were not correlated with the sTfR-F index. Maternal status of the corresponding fatty acid correlated with AA measured in both PC (r=0.54, p<0.001) and PE (r=0.30, p=0.034) and with DHA measured in PC (r=0.54, p<0.0001), but did not correlate with DHA measured in PE. Infants’ birth weight only correlated with DHA measured in PC (r=0.25, p=0.0466). Placental weight did not correlate with any of the fatty acids.

2.4.5. Covariate Analysis of Fatty Acid Status

For AA measured in PC of plasma (Figure 1), infant sTfR-F index and maternal concentrations of AA were covariates in the model and an interaction effect among group effect, sTfR-F index and maternal AA was present. At the mean of the 1st tertile, AA in IDM was significantly lower than AA in the AGA group. At the mean of 2nd tertile, AA in IDM was significantly lower than AA in the 2 control groups. At the mean of the 3rd tertile, AA in the 3 groups was not significantly different from one another even though AA in the IDM was the
lowest among the 3 groups. For the AA measured in PE of plasma (Figure 2), sTfR-F index was found to be a significant covariate in the model. The lower the iron status, the lower the AA was. There was no significant difference among the 3 groups, but AA status in IDM was the lowest. Because DHA and sTfR-F index were not found to be correlated, the analysis of covariance between DHA and sTfR-F index was not necessary.
2.5. Discussion

Iron and LC-PUFA are important nutrients for the brain development of the infant during the last trimester of gestation and the first year of age and have been reported as low in separate analyses. The objective of the study was to look at these nutrients and to determine if infants that had low AA or DHA status also had low iron status.

Concerning iron, no significant differences in ferritin and sTfR-F index levels were detected among the groups. However, sTfR levels were significantly 11% higher in IDM than in AGA infants. These results are very different from other studies in the literature. Most studies reported that iron status of IDM is lower than infants of healthy mothers. In a study conducted by Georgieff [23], serum ferritin was 60% to 78% lower in IDM. Verner et al. reported similar results to Georgieff: serum ferritin was 72% lower in IDM, sTfR was 35% higher in IDM, and sTfR-F index was 79% higher in IDM [39]. Thus this study did not show to the same magnitude of reduction in iron status in IDM. Maternal iron intake was above the RDA in the three groups. Therefore, adequate iron intake may have contributed to the lack of differences among the groups.

For AA measured in PC of plasma, there were no differences among infants of diabetic and non-diabetic mothers (Table 4). In two other studies [7, 46], in which the researchers measured AA in PC of plasma as well, no significant differences were detected. DHA measured in plasma PC was reduced by 42% to 46% in IDM which is much more than what other studies have found (14% and 17% of reduction in DHA proportions). No other study has reported AA or DHA
proportion in PE of plasma in IDM. In the present study, PE AA was reduced by 38% in IDM compared to the AGA group and DHA in plasma PE was more than 50% reduced in IDM compared to the AGA group.

Dietary DHA intake was similar to the amount recommended by Koletzko et al. [25] in both LGA and IDM groups, but not in the AGA group (<200 mg/day). Nonetheless, DHA status was the lowest in IDM. Because fetal DHA strictly relies on maternal DHA, low DHA can either be due to reduced supply or impaired placental transfer or both. Adequate maternal DHA intake in this study suggests an impaired placental transport caused by GDM which concurs with Bitsanis’ findings [29].

Fatty acid composition in PE seems to be more greatly impacted by GDM of the mothers. A possible explanation is the difference in incorporation of LC-PUFA in PC and PE. Williams and Maunder looked at the impact of different diets on the fatty acid composition of PC and PE. They concluded that LC-PUFA in PE showed a greater response than LC-PUFA in PC. This is due to the fact that PC contains more saturated fatty acids that appear to be more resistant to dietary changes which means that changes in relative proportions of LC-PUFA are less likely to happen in PC than in PE [47]. If LC-PUFA proportions in PE can be affected by dietary changes, it can also be more affected when it comes to impaired transfer of LC-PUFA to the fetus which would explain why DHA and AA proportions in PE are more reduced than in PC.

Fatty acid status can be influenced by several factors such as gestational age, placental weight, birth weight or fatty acid status of the mothers. AA and
DHA accumulate at the end of pregnancy, therefore the longer the gestational age is, the higher AA and DHA are. It was reported in several studies that placental weight and birth weight are positively correlated with AA and DHA [48]. In this study, placental weight and AA and DHA proportions were not significantly correlated (Table 6). Birth weight was positively related with DHA measured in PC. Maternal fatty acid status is typically positively correlated with the infant fatty acid status [48, 49]. In this study, maternal AA was indeed positively correlated with infant AA measured in PC and PE and maternal DHA with infant DHA measured in PC and not with DHA measured in PE.

sTfR-F index was negatively correlated with fatty acid proportion, but it was only significant with AA measured in both PC and PE. The analysis of covariance enabled examination in the differences among the 3 groups while controlling for covariates. For AA measured in PC, both sTfR-F index and maternal AA proportion were significant covariates (Table 6). Furthermore, interactions between these 2 covariates were significant indicating that differences among the groups were different depending on the value of sTfR-F index. Therefore, sTfR-F index values for the infants were divided into tertiles and mean sTfR-F of each of the tertile was calculated. At the means of both the 1\textsuperscript{st} and 2\textsuperscript{nd} tertile, AA in IDM was significantly lower than AA in AGA (p=0.411 at the mean of the 1\textsuperscript{st} tertile and p=0.024 at the mean of the 2\textsuperscript{nd} tertile) (Figure 1). In other words, if two women, one with GDM and one without GDM would give birth to two infants with the same iron status, IDM would have significantly lower proportion of AA in PC than infant of non-diabetic woman. When comparing
with the LGA group, IDM’s AA proportions were significantly lower at the mean of 2\textsuperscript{nd} tertile (p=0.0487). At the mean of the last tertile, which means at the lowest concentration of iron, AA proportions are not different among the 3 groups.

For AA measured in PE of plasma, only sTfR-F index was a significant covariate (p=0.005) (Table 6). Even though maternal AA measured in PE was correlated with infant AA measured in PE, maternal AA was not significant in the ANCOVA. There was no interaction between the group effect and the sTfR-F index effect, which indicated that at any value of sTfR-F index, the differences of AA among the 3 groups would be the same. When graphing sTfR-F index to AA (Figure 2), AA would decrease in the 3 groups as sTfR-F index would increase (ie. as iron stores would decrease). At any level of sTfR-F index, AA measured in plasma of IDM was the lowest and AA measured in AGA the highest, but it was not significantly different.

While this study was limited by the lack of values concerning maternal glucose control as well as the small sample size, the data support a relationship between iron and AA status at the end of term gestation. In conclusion, AA and DHA proportions in IDM tended to be the lowest compared to AGA and LGA groups, which is in agreement with previous studies. Our study was the first to also look at iron status in the same individuals. It was found that proportions of AA (both in plasma PC and PE) and iron status were correlated. IDM AA proportions in plasma PC are the most compromised among the 3 groups. For AA measured in plasma PE, even though it wasn’t significant, a similar trend was observed. Whether the infant’s mother had diabetes or not, and regardless of the
infants weight, if the infant is born with low iron status, it seems that it would also tend to have low AA status. GDM would further compromise AA status in the infants. Future studies are now warranted using multi-nutrient interventions either during pregnancy or lactation and infancy to enhance infant nutritional status and to support normal development.

2.6. Acknowledgement

This work was supported by the Canadian Diabetes Association. The biochemical analyses of fatty acid and iron were conducted at the McGill facilities made possible by a Canadian Foundation for Innovation Grant and Canada Research Chair (HW) and Walter M. Stewart Postgraduate Scholarship (HV). This study was conducted in collaboration with Dr. Jean-Claude Forest and Dr. Yves Giguere at Universite Laval in Quebec City.
2.7. Tables and Figures

Table 1: Mothers’ characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>AGA n=31</th>
<th>LGA n=20</th>
<th>GDM n=20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>28.0 ± 0.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>29.3 ± 0.9&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>31.8 ± 1.2&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>Height (m)</td>
<td>1.63 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.70 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.63 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pre-pregnancy weight (kg)</td>
<td>66.2 ± 2.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>67.2 ± 2.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>71.6 ± 3.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pre-pregnancy BMI (kg/m&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>25.1 ± 0.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>23.6 ± 0.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27.2 ± 1.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Weight gain (kg)</td>
<td>15.5 ± 1.0&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>19.5 ± 1.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.8 ± 1.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hemoglobin (g/L)</td>
<td>125.7 ± 1.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>121.8 ± 2.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>129.3 ± 1.8&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>36.7 ± 0.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>36.8 ± 1.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>35.6 ± 01.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Caucasian (%)</td>
<td>100</td>
<td>100</td>
<td>95</td>
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</tbody>
</table>

Data are mean±SEM.

Values with different superscripts in rows denote significant differences (p<0.05) using post-hoc analysis with Tukey’s test.
Table 2: Infants’ anthropometric indices at birth

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>AGA n=31</th>
<th>LGA n=20</th>
<th>IDM n=20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age (wk)</td>
<td>39.9 ± 0.2&lt;sup&gt;a&lt;/sup&gt;b</td>
<td>40.5 ± 0.9&lt;sup&gt;a&lt;/sup&gt;b</td>
<td>39.3 ± 0.2&lt;sup&gt;a&lt;/sup&gt;b</td>
</tr>
<tr>
<td>Birth length (cm)</td>
<td>51.6 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>55.0 ± 0.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>50.3 ± 1.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>3432.5 ± 53.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4447.1 ± 57.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3447.7 ± 114.9&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Head circumference (cm)</td>
<td>34.3 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>36.2 ± 0.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>34.7 ± 0.4&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>Placental weight (g)</td>
<td>465.3 ± 18.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>683.5 ± 20.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>558.2 ± 31.6&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hemoglobin (g/L)</td>
<td>158.7 ± 3.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>155.9 ± 2.96&lt;sup&gt;a&lt;/sup&gt;</td>
<td>164.6 ± 3.85&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hematocrit (L/L)</td>
<td>46.7 ± 0.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>46.3 ± 0.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>48.5 ± 1.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Male (%)</td>
<td>58</td>
<td>45</td>
<td>40</td>
</tr>
</tbody>
</table>

Data are mean±SEM.

Values with different superscripts in rows denote significant differences (p<0.05) using post-hoc analysis with Tukey’s test.
Table 3: Maternal daily nutrient intake during the last trimester of gestation

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>AGA</th>
<th>LGA</th>
<th>GDM</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=31</td>
<td>n=20</td>
<td>n=20</td>
<td></td>
</tr>
<tr>
<td>Energy (kJ/day)</td>
<td>2431.9 ± 150.2a</td>
<td>2416.1 ± 130.0a</td>
<td>2354.8 ± 187.8a</td>
</tr>
<tr>
<td>Carbohydrates (g/day)</td>
<td>318.7 ± 21.6a</td>
<td>325.2 ± 17.2a</td>
<td>288.4 ± 20.7a</td>
</tr>
<tr>
<td>Carbohydrates (%kcal)</td>
<td>51.0 ± 0.8a</td>
<td>52.8 ± 1.2a</td>
<td>49.1 ± 1.7a</td>
</tr>
<tr>
<td>Fat (g/day)</td>
<td>85.6 ± 5.0a</td>
<td>83.8 ± 6.6a</td>
<td>90.4 ± 9.8a</td>
</tr>
<tr>
<td>Fat (%kcal)</td>
<td>31.5 ± 0.8a</td>
<td>30.3 ± 1.2a</td>
<td>32.9 ± 1.5a</td>
</tr>
<tr>
<td>Protein (g/day)</td>
<td>109.0 ± 7.5a</td>
<td>104.4 ± 6.7a</td>
<td>109.1 ± 9.9a</td>
</tr>
<tr>
<td>Protein (%kcal)</td>
<td>17.5 ± 0.4a</td>
<td>16.9 ± 0.6a</td>
<td>18.0 ± 0.7a</td>
</tr>
<tr>
<td>LA C18:2 n-6 (g/day)</td>
<td>12.6 ± 0.84a</td>
<td>12.9 ± 1.19a</td>
<td>15.3 ± 2.02a</td>
</tr>
<tr>
<td>ALA C18:3 n-3 (g/day)</td>
<td>1.39 ± 0.11a</td>
<td>1.48 ± 0.15a</td>
<td>1.69 ± 0.18a</td>
</tr>
<tr>
<td>EPA C20:5 n-3 (g/day)</td>
<td>0.062 ± 0.013a</td>
<td>0.221 ± 0.102a</td>
<td>0.129 ± 0.058a</td>
</tr>
<tr>
<td>AA C20:4 n-6 (g/day)</td>
<td>0.179 ± 0.021a</td>
<td>0.157 ± 0.018a</td>
<td>0.173 ± 0.024a</td>
</tr>
<tr>
<td>DHA C22:6 n-3 (g/day)</td>
<td>0.139 ± 0.027a</td>
<td>0.288 ± 0.113a</td>
<td>0.218 ± 0.066a</td>
</tr>
<tr>
<td>Dietary iron (mg/day)</td>
<td>16.3 ± 1.3a</td>
<td>21.2 ± 4.2a</td>
<td>15.5 ± 1.4a</td>
</tr>
<tr>
<td>Supplemental iron (mg/day)</td>
<td>28.4 ± 5.7a</td>
<td>39.0 ± 8.6a</td>
<td>33.9 ± 7.5a</td>
</tr>
<tr>
<td>Total iron (mg/day)</td>
<td>44.7 ± 6.0a</td>
<td>60.2 ± 8.1a</td>
<td>49.4 ± 7.4a</td>
</tr>
</tbody>
</table>

Data are mean±SEM.

Values with different superscripts denote significant differences (p<0.05) using post-hoc analysis with Tukey’s test.
Table 4: Comparison of cord blood fatty acids and iron outcomes among the three study groups

<table>
<thead>
<tr>
<th></th>
<th>AGA n=31</th>
<th>LGA n=20</th>
<th>IDM n=20</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA in PC (%)</td>
<td>11.7 ± 0.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.2 ± 1.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.4 ± 0.7&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>AA in PE (%)</td>
<td>6.8 ± 0.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.8 ± 1.2&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>4.2 ± 0.8&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>DHA in PC (%)</td>
<td>3.3 ± 0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.5 ± 0.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.9 ± 0.3&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>DHA in PE (%)</td>
<td>3.3 ± 0.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.9 ± 0.6&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.6 ± 0.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ferritin (ug/L)</td>
<td>131.7 ± 15.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>150.0 ± 16.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>129.6 ± 14.8&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>sTfR (mg/L)</td>
<td>7.0 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.4 ± 0.4&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>7.8 ± 0.7&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>sTfR-F index</td>
<td>3.5 ± 0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.6 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.0 ± 0.4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Data are mean±SEM.

Values with different superscripts denote significant differences (p<0.05) using post-hoc analysis with Tukey’s test.
Table 5: Comparison of maternal fatty acids and iron outcomes among the three study groups

<table>
<thead>
<tr>
<th></th>
<th>AGA n=31</th>
<th>LGA n=20</th>
<th>GDM n=20</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA in PC (%)</td>
<td>5.9 ± 0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.2 ± 0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.4 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>AA in PE (%)</td>
<td>5.2 ± 0.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.0 ± 0.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.4 ± 0.8&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>DHA in PC (%)</td>
<td>1.4 ± 0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.6 ± 0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.1 ± 0.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>DHA in PE (%)</td>
<td>2.3 ± 0.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.6 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.4 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ferritin (ug/L)</td>
<td>22.2 ± 2.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.5 ± 1.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>24.4 ± 2.9&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>sTfR (mg/L)</td>
<td>4.9 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.9 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.1 ± 0.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>sTfR-F index</td>
<td>4.1 ± 0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.3 ± 0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.3 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Data are mean±SEM.

Values with different superscripts denote significant differences (p<0.05) using post-hoc analysis with Tukey’s test.
Table 6: Pearson correlation coefficients of infant AA and DHA proportions with sTfR-F index, maternal AA and DHA proportions, infant weight and placental weight

<table>
<thead>
<tr>
<th></th>
<th>AA in PC of Plasma (%)</th>
<th>AA in PE of Plasma (%)</th>
<th>DHA in PC of Plasma (%)</th>
<th>DHA in PE of Plasma (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>sTfR-F index</td>
<td>r = -0.31</td>
<td>r = -0.37</td>
<td>r = -0.19</td>
<td>r = -0.23</td>
</tr>
<tr>
<td></td>
<td>p = 0.013</td>
<td>p = 0.005</td>
<td>p = 0.144</td>
<td>p = 0.083</td>
</tr>
<tr>
<td>Corresponding maternal fatty acid (%)</td>
<td>r = 0.54</td>
<td>r = 0.30</td>
<td>r = 0.54</td>
<td>r = 0.05</td>
</tr>
<tr>
<td></td>
<td>p &lt; 0.001</td>
<td>p = 0.034</td>
<td>p &lt; 0.001</td>
<td>p = 0.726</td>
</tr>
<tr>
<td>Infant Weight</td>
<td>r = 0.08</td>
<td>r = -0.045</td>
<td>r = 0.25</td>
<td>r = 0.13</td>
</tr>
<tr>
<td></td>
<td>p = 0.505</td>
<td>p = 0.740</td>
<td>p = 0.047</td>
<td>p = 0.318</td>
</tr>
<tr>
<td>Placental weight</td>
<td>r = 0.05</td>
<td>r = 0.03</td>
<td>r = 0.09</td>
<td>r = 0.17</td>
</tr>
<tr>
<td></td>
<td>p = 0.659</td>
<td>p = 0.813</td>
<td>p = 0.510</td>
<td>p = 0.203</td>
</tr>
</tbody>
</table>

Pearson correlation coefficients with p<0.05 denote significant relationship between the two variables.
Figure 1: Arachidonic acid estimations in phosphotidylcholine of plasma at specific sTfR-F index values
Figure 2: Arachidonic acid in phosphotidylethanolamine of plasma in relation with sTfR-F index by groups

![Graph showing the relationship between sTfR-F index and log of AA% for different groups (LGA, IDM, AGA). The graph includes annotations for p-values: p=0.0831, p=0.7324, and p=0.4493.]
Chapter 3. Extended Discussion

Iron and LC-PUFA have been investigated separately in numerous studies. This study is the first that looked at both and how they behave in IDM. The main objective of this study was to show that infants with low LC-PUFA status also had low iron status and that IDM were the infants with the lowest amount of both nutrients compared to the AGA and LGA groups. The present study confirms differences among the groups for LC-PUFA (except for AA measured in PC of plasma). If one expresses the relative proportion of AA and DHA in IDM compared to AGA infants, the magnitude of reduction in AA is 19.7% for PC and 38.2% for PE whereas for DHA, these are 42.4% and 51.5%. This parallels the reductions in maternal plasma. Furthermore, AA proportions in both PC and PE were significantly correlated with iron status. Therefore, this study is the first to show, that infants born with compromised AA status have a high chance to also have compromised iron status. This however was not the case for DHA. Since the variation in DHA is much greater than for AA and the sample size was small, lack of relationship between DHA and sTfR-F index is likely attributed to low power of analysis. This is further suspected based on the observed negative trend for the correlation of DHA in PC and PE with sTfR-F index.

After performing an ANCOVA to compare AA in the 3 groups, this study failed to detect significant differences for AA measured in plasma PE. On the other hand, IDM AA proportions in plasma PC were significantly lower compared to control AA, but only at the first two tertiles of the control group. While this study has created a better understanding of the variations of iron and LC-PUFA
status in IDM, limitations have diminished the potential of this study to determine mechanisms by which GDM alters infant iron and LC-PUFA.

3.1. Limitations

3.1.1. Maternal metabolic control

Clinical management of GDM does not always include assessment of HbA1c levels and thus data for all participants with GDM was not available, limiting the ability to draw conclusions about degree of glycemic control and infant nutritional status [23]. Nonetheless, data regarding insulin and/or nutritional management was available that suggests the women maintained good glycemic control during pregnancy. In this scenario, the fetus may not have been hyperglycemic and this may have precluded increased insulin production by the fetus. This is important for the interpretation of the study results, as discussed previously, chronic fetal hyperglycemia and hyperinsulinemia increase the fetal basal metabolic rate and oxygen consumption leading to a relative hypoxic state [50]. The fetus responds by increasing RBC mass, which would cause a redistribution of iron from developing organs to RBC [23, 39]. This would cause iron status in IDM to be compromised compared to infants of non-diabetic women with adequate maternal-fetal transfer and lower iron demand. In the case of good glycemic control of the pregnant women, then iron status might not be compromised. In this study when iron status was compared among the 3 groups, significant differences were not detected for ferritin and sTfR-F index while sTfR levels were significantly increased by 11% in IDM compared to AGA denoting an increased need for iron in IDM. Without HbA1c values as indicators of the
women’s glycemic control, good glycemic control cannot be ruled out as an explanation for the similar iron status among the infants of the 3 different groups.

3.1.2. RBC phospholipids vs. plasma phospholipids

Plasma phospholipids are commonly used to assess LC-PUFA status of an individual because fatty acid composition of plasma is reflective of recent fat intake [51]. However, its use has some disadvantages. First, plasma phospholipids are transporters of fatty acid that have not yet been incorporated into cellular membranes. Thus, measuring LC-PUFA content in plasma phospholipids might not reflect the actual fatty acid composition of tissues [52]. On the other hand, it was proposed that RBC phospholipids may be more representative of the LC-PUFA status in individuals. Furthermore, RBC appears to be a better marker for long-term dietary fatty acids intake compared to plasma [51, 53].

One of the limitations of this study is the lack of data on the RBC phospholipids LC-PUFA content. It was planned that both AA and DHA concentrations in RBC and plasma would be measured. However, RBC sample preparation according to published methodology [54] did not co-elute the fatty acids with BHT and thus the samples were variably oxidized rendering the results invalid unless measured immediately.

However, in a study by Vlaardingerbroek that measured fatty acids status in both RBC and plasma phospholipids, it was observed that cord AA and DHA proportions in plasma and RBC phospholipids were significantly positively
correlated. Therefore even though it would have been best to also have RBC fatty acid data, conclusions can be drawn using plasma fatty acids alone.

3.1.3. Sample size

The objective of this study was to measure two important nutrients for infant development, iron and LC-PUFA. The sample size was originally calculated using AA values measured in RBC phospholipids. However, the sample estimate may not have been adequate for DHA or iron. In a study that compared fatty acid status in plasma phosphatidylcholine between IDM and infants of non-diabetic mother [7], DHA in IDM was 14% lower than DHA measured in controls. Given a power of 0.80 and an alpha of 0.05, 45 participants per group would be required to detect differences. Concerning iron, in a study where 43 IDM were studied, 28 IDM were reported to have decreased ferritin concentrations [23]. In another study, levels of ferritin, serum transferrin receptor and sTfR-F index in 49 IDM were significantly lower, higher and higher respectively compared to 48 infants of non-diabetic women[39]. A sample size estimate derived from the findings in this study was not possible owing to the data presented without mean and variation.

For the present study, 20 IDM, 20 LGA and 31 AGA were recruited which is lower than estimated and could account for lack of differences among groups. During the timeframe of recruitment, women that fit the inclusion criteria were asked to participate in this study which a majority of them accepted. Unfortunately, cord blood or maternal blood was sometimes not collected at the
birthing center complicating the recruitment process. Recruitment stopped before the sample size was met because of time constraints.

In order to detect differences especially for the iron, recalculation of sample size was conducted using DHA measured in plasma PC. Given a power of 0.80 and an alpha of 0.05, 45 participants per group are required. Therefore, in order to detect differences in both LC-PUFA and iron in future studies, a sample size of 50 participants per group would be recommended.

3.1.4. Other types of diabetes

At the beginning of recruitment, it was intended to also recruit women with type 1 diabetes and type 2 diabetes along with women with GDM. The objective was to show that regardless of the type of diabetes that occurred during pregnancy, LC-PUFA and iron status were compromised in IDM. Unfortunately, difficulties arose to recruit these women. In order for women to participate in this study, they must be first already participating in the cohort study conducted by Dr. Forest at Laval University. Women were recruited into the cohort early in their pregnancy during their first check-up. Unfortunately, women with pre-gestational diabetes were mostly followed in another clinic in Quebec City so very few were recruited in the cohort study.

Therefore at the end of the recruitment period, babies born from women with pre-gestational diabetes were so few that they were not taken into account in the analyses. For future studies, it would be good to confirm that both LC-PUFA and iron status are compromised in babies of women with any type of diabetes.
3.2. Future research

3.2.1. Milk fatty acids

Breast milk was collected at 4 weeks postpartum. Fatty acids were measured, but still needs to be analyzed in relation to the diet consumed by the lactating women (recorded by a 3-day food records). It would be interesting to compared LC-PUFA content between women that had GDM and women that did not. This can give an idea as to AA and DHA concentrations that can be used for supplementation.

3.2.2. Possible intervention with iron in infants

Iron supplementation in lactating mothers alongside LC-PUFA is not discussed as a possible intervention as previous studies did not find any effect of iron supplementation upon concentrations of iron in human breast milk [55, 56]. Instead, iron should be given to infants in the form of medicinal iron or iron-fortified cereal. A recent study observed that both were equally effective in improving iron status in breast-fed infants when 7.0-7.5 mg ferrous sulfate was provided daily [57].

3.2.3. Possible intervention with AA and DHA in lactating mothers

The placental transfer of LC-PUFA might be compromised during gestational diabetes. Therefore supplementing the mother during pregnancy might not change the LC-PUFA content in the babies. A possible way to increase LC-PUFA in babies would be to increase maternal intakes during nursing.
After birth, the brain grows from 350 g at birth for a term infant to 750 g at 12 months of age. The brain is mainly composed of lipids especially LC-PUFA [58] located in cell membranes where they contribute to membrane fluidity and signal transduction [25, 59]. Thus the demand for LC-PUFA in infancy is very high in support of brain development. To accommodate this, human milk is mainly composed of fatty acids, representing about 88% of total milk lipids [60]. The mean value for DHA was reported to be 0.32 ± 0.22% and AA to be 0.47 ± 0.13% (% of total fatty acids) worldwide. DHA had a higher variability (range: 0.06% -1.4%) compared to AA (range: 0.24% - 1.0%) [61]. The lipid content and fatty acid composition of human milk is influenced by a variety of variables such as stage of lactation, gestational age, maternal genetic characteristics, maternal nutritional status, maternal dietary intakes and metabolic disorders [60].

Of relevance to future supplementation studies, from colostrum to mature milk content of essential fatty acids, which are alpha-linoleic acid and linolenic acid (ALA and LA), increase whereas the percentage of LC-PUFA decreases. Koletzko et al. estimated that AA decreased by about 38% and DHA by about 50% during the first month of lactation [60]. High proportions of LC-PUFA in colostrum might benefit the newborn infant after birth when total amounts of milk consumed are still low, but the infant’s requirements for LC-PUFA are high due to deposition of AA and DHA in rapidly growing and membrane rich-tissue like brain and retina [58]. Koletzko suggested that the decrease could be due the increase in total fat content with the duration of lactation and not to an actual decrease in AA and DHA concentration which actually remained relatively stable.
On the other hand, Van Goor et al. hypothesized that the decline of AA and DHA might also reflect depletion of maternal stores [62].

Breast milk LC-PUFA content is known to change accordingly to the maternal dietary intake and to the maternal stores. Innis et al. concluded that the secretion of DHA in breast milk is dependent on the mother’s intake of DHA [63]. On the other hand, AA seems to be mainly dependent on long-term dietary intake. This is suggested by the fact that LC-PUFA in milk are derived from maternal stores [60] and the inability of short-term AA supplementation to increase milk AA concentrations [62]. Therefore, if mothers were supplemented with AA, change in AA status would require months. Furthermore, even though the mothers are capable of producing AA and DHA endogenously, supplementation of the essential fatty acids LA and ALA is not accepted as it is unknown if endogenously produced AA and DHA significantly contribute to AA and DHA in the human milk. It was reported that increased dietary intake of ALA (either from the breast milk or from the infant formula) did not increase DHA in infant blood [63]. Therefore, in order to effectively increase AA and DHA in infants, it is best to supplement with AA and DHA.

While maternal supplementation with DHA is most common, the potential for reduced AA exists owing to competition in the metabolic pathways between AA and DHA [62], therefore it seems best to supplement with a mixture of both [60, 62]. In a recent study, 220 mg of AA and 220 mg of DHA were given daily to pregnant women for 26 weeks (2 weeks postpartum) or 36 weeks (12 weeks postpartum). At 12 weeks, milk AA and DHA decreased compared to
concentrations at 2 weeks postpartum, but their concentrations were still higher when compared to the placebo groups [62]. Therefore, supplementation to at least 3 months postpartum would provide additional AA and DHA to the infant. However, these studies have not been conducted in women following a pregnancy with GDM. Therefore for future studies, it would be interesting to look at AA and DHA concentrations in 4 different groups. Two groups (1 group composed of women with GDM and 1 group control) will receive supplementation with AA and DHA up until 3 months postpartum. While the 2 others groups composed of women with or without GDM will be the placebo groups. Measurements would include AA and DHA proportions in breast milk, infant blood as well as infants’ anthropometry, visual and cognitive development. Since IDM are born with compromised AA and DHA status, by supplementing the mothers during the lactation period this might increase AA and DHA to the proportions seen in infants of healthy mothers and might have a positive impact on their visual and cognitive development.
References


