University of Alberta

Maternal long chain polyunsaturated fatty acid status during pregnancy and lactation in the APrON cohort.

by

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This thesis is dedicated to my lovely mother *"***Hala Sharief***"*. Who taught me the meaning of life. She gave me a lot of love, encouragement and support to achieve my goal throughout the years especially during maternity. Mom without you I will be never been at this stage. All the merit return to you mother.

Abstract

INTRODUCTION: Docosahexaenoic (DHA) and arachidonic acid (AA) are long chain polyunsaturated fatty acids (LC-PUFA) that are essential for fetal and infant development. The overall aim of the study was to characterize DHA, EPA and AA status during pregnancy and lactation in the first cohort of the APrON study and determine the relationship to breast milk composition. This study also determined the effect of taking a daily supplement containing DHA/EPA on maternal status. METHODS: Blood samples were collected at 3rd trimester (n=500) and 3 months postpartum (n=476). The relative percent and concentration of fatty acids in plasma phospholipids (a biomarker of fatty acid status) and the relative fatty acid composition of breast milk (n=398) were identified by gas liquid chromatography. RESULTS: The %DHA in phospholipids was significantly higher at pregnancy than lactation. However, the percentage of EPA and AA did not differ between pregnancy and lactation. The % DHA, EPA and AA in plasma was positively correlated with %DHA, EPA and AA in breast milk. Taking a daily DHA/EPA supplement (479 ± 415 mg) resulted in a significantly higher ($P<0.05$) %DHA in plasma PL and breast milk. CONCLUSIONS: The maternal status of essential n-3 fatty acids was found to positively correlate with the composition of these fatty acids in breast milk. DHA status was lower during lactation then pregnancy supporting the increased demand for incorporation into breast milk. Mothers who reported taking DHA/EPA supplements each day had higher DHA composition in plasma phospholipids and higher DHA content in breast milk.

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List of Abbreviations

delta (Δ)

omega (ω)

fatty acid (FA)

total fat (TF)

long chain polyunsaturated fatty acid (LC-PUFA)

polyunsaturated fatty acid (PUFA)

monounsaturated fatty acid (MUFA)

saturated fatty acid (SFA)

a-linolenic acid (ALA)

linoleic acid (LA)

arachidonic acid (AA)

eicosapentaenoic acid (EPA)

docosahexaenoic acid (DHA)

body mass index (BMI)

gas chromatography (GC)

Chapter one

Introduction and Literature Review

1.1 Dietary Fats

Dietary fats consist of a group of different fats that include triglycerides, phospholipids, sterols, monoglycerides, diglycerides, cerebrosides, terpenes, fatty alcohols and fatty acids. The vast majority of fat in the diet is in the form of triglycerides. A fatty acid (FA) is a carboxylic acid made up of carbon, hydrogen and oxygen molecules, which are assembled in a linear un-branched carbon chain that is either saturated or unsaturated. FAs are important for energetic, metabolic, and structural activities and can be either saturated fatty acid (SFA), monounsaturated fatty acid (MUFA) or polyunsaturated fatty acid (PUFA) depending on the presence or absence of double bonds in the carbon skeleton (Lobb & Chow, 2008).

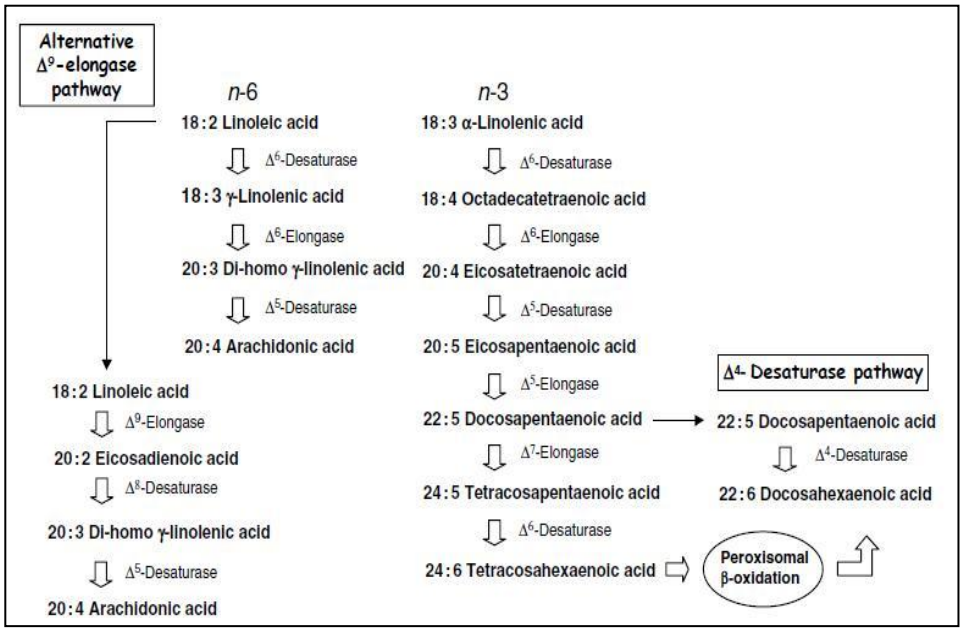
The nomenclature of FAs is often based on a Greek letter system such as omega (ω or n) and delta (Δ). Omega refers to the distance of a double bond from the terminal methyl carbon regardless of the length of the chain. For example, n-6 and n-3 have final double bonds in the n-6 and n-3 positions, from the hydroxyl end of the fatty acid chain, respectively. Conversely, delta naming considers the presence and position of one or more double or triple bonds within the carbon skeleton with respect to a designated carbon atom. For instance, in the α-linolenic

acid (ALA) with a formula of 18:3Δ9c, 12c, 15c means that there are 18 carbon atoms, with three double bonds present in the 9, 12 and 15 carbon position. The most abundant fatty acids in the diet are part of the n-9, n-6 and n-3 FAs (Lands 2005).

1.2 Dietary essential fatty acids

The metabolic conversion of essential fatty acids requires the key enzymes called desaturase and elongase. The precusors, linoleic acid (LA) and α-linolenic (ALA), undergo a series of desaturation reactions and elongation, which convert them to varying lengths of FAs with double bonds in different positions within the carbon skeleton (Figure 1.1).

Figure 1.1 Metabolism n-3 and n-6 essential fatty acid to long chain polyunsaturated fatty acid (Napier J & Sayanova O, 2005).



Both LA and ALA are necessary for the human diet since they cannot be synthesized and one cannot be substituted for the other, as they are not capable of inter-conversion. For example, the essential fatty acid (EFA) arachidonic acid (AA) can be synthesized from dietary or tissue concentrations of LA (Koletzko et al., 2008). Conversely, docosahexaenoic acid (DHA) is an n-3 fatty acid which can be endogenously converted from ALA to eicosapentaenoic acid (EPA) and further to DHA. There is an alternate pathway that involves peroxisomal oxidation for the DHA synthesis from docosapentaenoic acid (DPA) to DHA (figure 1.1).

1.3 Dietary Sources of Essential Fatty Acids

Fatty acids are an integral component of an organism's body and are found in great diversity, ranging from plants to animals. N-3 FAs such as EPA and DHA are abundantly found in fish and other marine food sources, while the n-6 group such as LA and AA are present in vegetable oils and meat food sources, respectively. (Appendix A,B).

1.4 Factors that affect the elongation and desaturation of ALA and LA to DHA and AA

The *de novo* synthesis of DHA and AA are dependent on the presence of the precursor molecules ALA and LA, respectively. In the presence of higher levels of n-6 precursor in the diet, AA production is synthesized by the desaturase-elongase system. A greater dietary intake of n-6 FAs was shown to

increase AA tissue concentration and subsequently reduce DHA concentration (Cetin & Koletzko et al., 2008). The conversion rates of ALA and LA to DHA and AA are influenced by gender, genetics, estrogen and the amount of precursor FAs in the diet (Koletzko et al., 2008). Simopoulos et al. (2010) reported that genetic variants in FADS1 and FADS2 are associated with the metabolism of n-3 and n-6. Delta-5 and delta-6 desaturases, FADS1 and FADS2, correspondingly, influence the serum, plasma and membrane phospholipid levels of LA, ALA and long chain polyunsaturated fatty acids (LC-PUFAs) during pregnancy, lactation, and may well influence an infant's cognitive development.

1.5 Dietary intake of precursor fatty acids and preformed DHA and AA

The presence of EFA precursors such as ALA and LA plays a significant role in the proper growth and development of infants. Low levels of PUFAs in pregnant women have been associated with numerous conditions in children such as asthma, atopic conditions, retinal dysfunction, obesity, diabetes and neurodevelopment disorders (Yu & Björkstén, 1998; Horrobin et al., 2000; Decsi et al., 2002; Uauy et al., 2001; Nguyen et al., 2008).

1.6 Factors Affecting Maternal Essential Fatty Acid Status

The concentration of EFA during pregnancy is higher in the fetus tissue than the mother's circulation. A study had shown that the absolute amount of DHA was highest in the placenta, suggesting a role in the transfer and concentration of EFA into the fetus (Cetin & Koletzko et al., 2008). FAs can cross

the placenta membrane as non-esterified fatty acid (NEFA), which are derived from lipoproteins. The NEFA concentration in maternal plasma is increased during the pregnancy and influences the placental transport of fatty acids since lipid transport occurs in response to the concentration gradient present at the basal binding sites on the placental membranes (Hanebutt et al., 2008, Benassayag et al. 1999). The other sources of lipids that are metabolized by the lipoprotein lipase found in the placenta can also be derived from PUFAs coming from the maternal blood.

The dietary intake of fish was shown to alter the concentration of PUFAs in mothers and their infants. One randomized controlled trial (Helland et al., 2003) and another observational (Whalley et al., 2004)study showed that DHA% in plasma during the period immediately before and after birth may result in better visual and cognitive development of the child. Low consumption of fish, such as in a western or vegetarian diet, was associated with an increased risk of pre-term delivery as well as low birth weight of infants (Olsen & Secher, 2002; Xue F et al., 2007). Furthermore, lower seafood intake , DHA content in the mother's milk and omega-3 index have all been associated with a higher rate of postpartum depression (Hibbeln, 2002; Markhus et al. 2013, De Vriese et al. 2003, Allen et al. 2013).

1.7 Critical Review of the Studies Relevant to this Thesis Research

1.7.1 Relationship between maternal intake of n-3 fatty acids and maternal status (of EPA, DHA and AA)

Four longitudinal cohort studies and three RCT have been published that have examined the relationship between maternal n-3 intake and status of EPA, DHA and AA. A longitudinal observational study published by Rump and Hornstra (2002), reported that higher maternal intake of LA was associated with higher concentrations of LA and lower concentrations of DHA in maternal and umbilical plasma phospholipids in a study conducted in the Netherlands. Another study investigated maternal dietary fatty acid intake using a FFQ and a 24-h recall, and compared intake to plasma concentrations breastfeeding adolescent mothers (n=49) and their infants (da Costa et al., 2011). The intake analysis revealed that the majority of respondents (80%) reported consuming fish less than once per month (da Costa et al., 2011). AA, EPA and DHA were lower in milk compared with maternal and infant plasma, and AA and EPA were not significantly different between the plasma of the mother and infant. DHA, however, was significantly higher in the infants' plasma compared with the mothers', suggesting that this essential fatty acid is selectively transferred to the infant. This is not unexpected, as DHA is well known to be of critical importance for proper infant growth and development (Rogers et al., 2013).

In Bergmann et al. study (2008) reported that the relative percent DHA and EPA in maternal blood was higher in the group that was supplemented with 200mg DHA than the vitamin-mineral supplement group and vitamin-mineral plus 4.5g fructo-oligosaccharide group. However, the relative percent of AA fraction of their red blood cell phospholipids were not different among the 3 groups.

A RCT study carried out by Imhoff-Kunsch B et al. (2010) reported that the daily dietary intakes of PUFA in pregnant Mexican women did not differ between DHA supplemented group (400 mg DHA/ day) and placebo group. Although there was not a significant difference between the groups, the plasma phospholipids DHA level was positively correlated to the milk DHA level. The relative percent of DHA significant differed (20% higher) in the DHA group than placebo group. Another longitudinal study by Marc et al. (2011) reported at day 49 after delivery, the plasma concentration of DHA in mothers in a group of woman who were supplemented with 1200 mg/d of DHA was higher than those in the non-supplemented group (reference group) ($P < 0.001$). Also, another randomized controlled study conducted in Italy reported that plasma composition of DHA, EPA and total n-3 in maternal plasma and infant plasma and umbilical tissue (n=341) was significantly higher when mothers were supplemented with cod liver oil (10 mL daily) throughout pregnancy and to 3 months post-partum compared to those who were not supplemented (Helland et al., 2006). The composition of LC-PUFA was lower at 3 months postpartum in mothers who took supplements in the 3rd trimester compared to those who reported taking supplements in both the 3rd trimester and postpartum. The composition of DHA was higher in women supplemented with DHA and/or or EPA than those that were not supplemented group, however, the composition of AA was not found to differ when DHA was supplemented. Infants from mothers who took cod liver oil supplements had higher plasma DHA and breast milk from supplemented women at 4 weeks and 3 months postpartum also contained higher DHA compared to

milk from corn-oil supplemented women (Helland et al., 2006). While DHA is considered an essential fatty acid, the optimal level of supply to the fetus or infant are not known. However, this study and another have both demonstrated that when breast milk DHA exceeds 0.80% of total fatty acids, no further increase in infant plasma DHA concentration occurs (Gibson et al., 1997, Helland et al., 2006).

1.7.2 Relationship between mother's intake of n-3 fatty acids and breast milk concentration (of EPA, DHA and AA)

Two longitudinal cohort studies (Marangoni et al., 2002; Marc et al., 2011) and four RCT (Boris et al., 2004; Fidler et al., 2000; Imhoff-kunsch et al., 2011) have been published that have examined the relationship between maternal n-3 intake and the concentration of EPA, DHA and AA in breast milk. In a longitudinal observational study published by Marangoni et al. (2002), a direct correlation was observed between maternal plasma and breast milk at 1 month post-partum for LA, ALA and AA. At 3 months postpartum, there was a correlation between plasma and milk observed for LA, ALA and DHA but not for the AA. At 3 month there was a correlation between DHA in plasma and breast milk (Appendix B). In a longitudinal study by Marc et al. (2011), mothers were given 1200 mg of DHA daily for 8 to 12 weeks after birth. This study reported that the composition of DHA of the maternal milk in the DHA group was 12 times higher than in the reference group.

Essential fatty acid status may be more important for preterm infants than full-term infants, as these infants have very limited body stores of PUFAs (Koletzko and Braun, 1991). N-3 content in milk from mothers of preterm infants is positively associated with weight gain, height, BMI and head circumference (Tinoco et al. 2009).

There are a number of studies that have reported the effects of fish oil supplementation during pregnancy and/or during lactation on the FA composition of breast milk. In a RCT carried out by Boris et al. (2004) 36 Danish mothers were divided into 3 groups; the first group (S-PL) was supplemented at 30 weeks pregnancy. The second group was supplemented at 30 weeks pregnancy till delivery (S-P). Third group was a non-supplemented control group (C). The blood sampling was collected at 4, 16 and 30 days postpartum The fish oil supplement contained 1.3 g EPA and 0.9 g DHA. The composition of the average LC-PUFA (n-3) in breast milk was higher (by approximately 67%) in the S-PL group than the two others group. There was no significant difference between the C group and the S-P group. This study suggested that taking supplements in the third trimester only was less effective in altering the composition of LC-PUFA in breast milk than taking supplements that continued from the third trimester into lactation. Moreover, another RCT examined maternal breast milk fatty acid composition in a German population. Fidler et al. (2000) tested the effect of taking supplements rich in DHA (200 mg/d) and no EPA on the composition of LCPUFA. The times of collection of the study 2 weeks before the taking of

supplements and 2 weeks after the supplements. This study did not report the concentration of AA in breast milk. DHA was 43% higher in the supplemented group (0.37% of total FAs) compared to the placebo group (0.21% of total FAs) in mothers plasma. There was no difference in the EPA between the two groups before or after the supplements. A RCT study reported no significant differences in the concentration of EPA between the supplemented group, which took 2 capsules per day (each capsule contained 200mg of DHA) (0.14% of total FAs) and the placebo group (0.16% of total FAs) at 1 month post-partum in Mexican population (Imhoff-kunsch et al., 2011). Also, there was no significant differences in the concentration of AA in breast milk between the supplemented group (0.41% of total FAs) and placebo group (0.43% of total FAs) (Imhoff-kunsch et al., 2011). Alternatively, they found that the concentration of DHA in maternal plasma positively correlated with breast milk DHA in both the placebo (0.20% of total FAs) and supplemented group (0.17 % of total FAs (Imhoff-kunsch et al., 2011).

In summary there appears to be a direct positive correlation observed between maternal plasma and breast milk at 1 and 3 month post-partum for LA and ALA. At 3 months, a correlation has also been observed for DHA, but not for AA concentrations There is considerable research examining the role of dietary EFAs in breast milk and the relationship to infants status and outcomes but less is known as to what is the optimal maternal plasma composition that is associated with an optimal concentration of DHA and AA in breast milk that facilitates infant development. Many studies have suggested that the high consumption of

DHA and a high DHA concentration in plasma might decrease the composition of AA in breast milk but this requires further research. Overall the literature supports the premise that maternal consumption of LC-PUFA is a good predictor for the composition of LC-PUFA in mother's milk.

1.7.3 Relationship between breast milk/infant intake or LCPUFA (n-3 and AA) and status or health of the infant.

Three longitudinal cohort studies (Rump et al., 2002; Marc et al., 2011; Xiang et al .,2000) and two RCT studies (Bergmann et al., 2008; Helland et al. 2006) have been published to study the relationship between breast milk/infant intake or LC-PUFA and status or health of the infant A Netherlands longitudinal observational study by Rump et al. (2002) looked at the concentration of FAs in mothers' plasma and infants' cord plasma. Mothers (n=889) and their infants were examined on the relationship between the concentration of FAs with gestational age and sex. Although the composition of DHA was positively related to gestational age, it was not related to the PUFA concentration at birth for mothers or infants, suggesting a higher DHA status has the potential to lengthen gestational age (Rump et al., 2002). Conversely, the concentration of AA was not significantly correlated to the length of gestation. In a longitudinal study by Marc et al. (2011), mothers of preterm infants were given 1200 mg of DHA daily for 8 to 12 weeks after birth. The amount of DHA provided to the infants via breastmilk was 12 times greater in the DHA group compared with the reference group, which did not receive DHA supplementation during lactation. However, the growth

trends for weight, length, and head circumference over the study period in the DHA group did not differ between the supplemented and reference group.

In a study by Xiang et al. (2000), breast milk samples were collected from 19 mothers at 1st day, 1 month and 3 months postpartum. The aim of this longitudinal observational study was to look at the association between the AA/DHA ratio in breast milk and brain growth in infants (as estimated from occipital head circumference) and investigated the composition of FAs in plasma during lactation. They reported that there was the ratio of AA:DHA was approximately the same in both the breast milk and the infant brain, and was positively correlated with brain size and growth rate at both 1 and 3 months.

Another RCT study carried out by Bergmann et al. (2008) examined the effect of a daily vitamin/mineral supplement with and without 200 mg DHA from mid-pregnancy through maternal lactation and examined infant's red blood cell phospholipids. This research reported that taking a supplement of 200 mg/day of DHA increased the DHA status of RBC their infants 29.7% compared to infants from mothers non-supplemented with DHA at 3 months. In RCT study reported that there were correlation between phospholipid fractions of AA, EPA, n-3, n-6 in breast milk and in infant plasma (Helland et al., 2006). Infants whose mothers were supplemented with cod liver oil (10 mL) did not have higher plasma DHA concentrations. The dose of DHA in cod liver oil was 1183 mg/10 mL of DHA, On the other hand, if the mothers were supplemented with corn oil, the concentration of EPA in their infants increased. Also, when the mother supplemented with corn oil both the composition of EPA and DHA in infants

increased (Helland et al. 2006). However, the specific relationship (mathematical) between DHA status and dietary intake is not well established. It is not clear what is the maternal DHA status associated with optimal infant development.

An older review article concluded that supplementation of women in high-risk pregnancies with n-3 fatty acids did not impact anthropometrics at birth (Horvath et al. 2007). However, more recent studies have reported a significant impact of DHA supplementation on neonatal growth measures. In a randomized, controlled trial, birthweight, length and head circumference were all significantly greater in infants born to mothers who consumed 469 mg/d DHA during pregnancy compared to placebo (Carlson et al. 2013). In a Mexican randomized, controlled trial of women (n=1094) known to have poor n-3 status (mean intake 55 mg/d), supplementation during pregnancy (400 mg/d) resulted in offspring who were significantly heavier and had larger head circumference compared with infants born to women in the placebo group (Ramakrishnan et al. 2010). Clearly maternal DHA status is related to perinatal growth, however, few studies have followed children beyond 12 months of age; it is not clear whether these effects endure through childhood. In one study, 2.5 year old children of mothers who were supplemented with n-3 PUFA during pregancy (4.5 g fish oil/d) had larger waist circumference, head circumference and BMI compared with olive-oil supplemented controls (Lauritzen et al. 2005). Body composition at 4 years of age has been shown to be related to maternal plasma n-3 fatty acid status during pregnancy; children from mothers with higher status had higher lean body mass

(Moon et al. 2013). Further study is required to fully evaluate the long-term effects of maternal n-3 fatty acid status on growth and development.

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Chapter 2

Study Rationale

2.1 Rationale

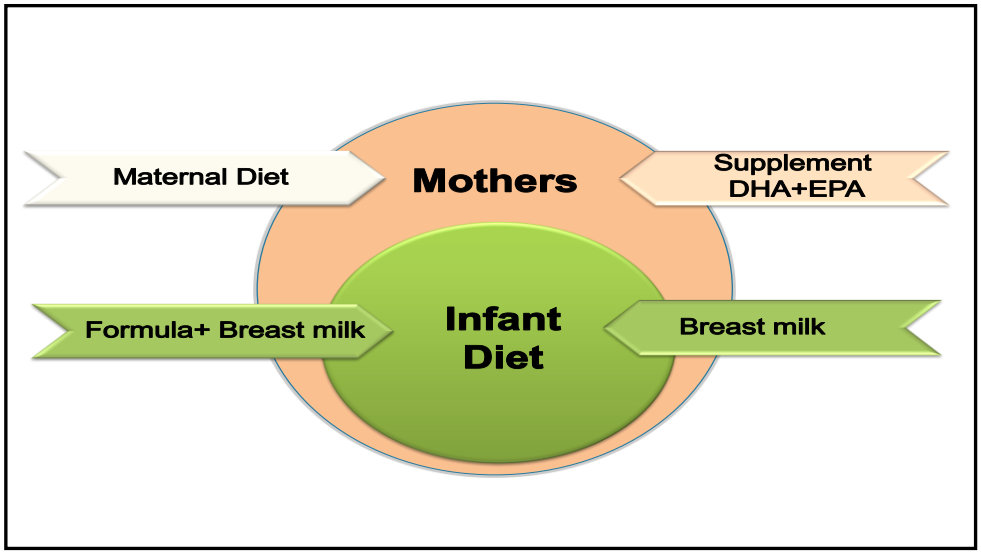
The LC-PUFA n-3 and n-6 fatty acids are very important for infant development during pregnancy and lactation. The most important of the LC-PUFA n-3 and n-6 fatty acids are DHA and AA, respectively. There is considerable evidence demonstrating beneficial effects of supplementing mothers during pregnancy with DHA (with and without EPA) and supplementing the infant with DHA/EPA and AA on the development of the infant (Koletzko et al., 2008, Helland et al. 2005, Marc et al. 2011, Olsen et al. 2002, Tinoco et al. 2009, Van et al. 2005). The requirements of DHA and EPA during pregnancy have not been established yet.

There have been several studies that focused on the relative percent of essential fatty acids (DHA, AA, LC-PUFA ..etc) during pregnancy (Ribeiro et al ., 2012; Al et al., 2000; Smuts et al., 2003), and lactation (Ribeiro et al., 2012; Boris et al., 2004) and their relationship to infant development (Innis, 2007; Huffman et al., 2011; Kilari et al., 2010). Significant correlations have been observed between maternal plasma composition of DHA, EPA and AA and breast milk composition. This is important because optimizing EFA delivery to the infant via breast milk has been associated with improved neonatal and early child development. The current study carried out a comprehensive view to identify maternal FAs status

and their breast milk composition. Furthermore, the correlation between maternal FAs profile and breast milk FAs profile. In addition, the number of subjects came from a large cohort (n=600), which will eventually include 2000 individuals; this will give a comprehensive view of the nutritional status of Albertan women during pregnancy and lactation and on their infant development.

The current study used a provincial longitudinal cohort study, APrON (Alberta Pregnancy Outcomes and Nutrition) that has an overall goal to improve the long-term health of Albertans by understanding the importance of maternal nutrition on maternal, infant and child health. (Kaplan et al., 2012). A key component of this extensive study is to assess the fatty acids status of the mother and the infant early in the post-natal period.

Figure 2.1: A biological framework.



The levels of essential fatty acid greatly vary depending on the maternal lipids status. By determining n-3 and n-6 fatty acids status, we can establish in the

future a link between the maternal status and infant status. A biological framework (figure 2.1) was developed in this thesis to address the main variables that were studied in the current thesis. Understanding status and the relationship between maternal status and breast milk composition is needed to make appropriate recommendations as to how to ensure an optimal concentration of these fatty acids in breast milk, so as to optimize the intake of these two essential fatty acids in infants for their future health.

Determining the relationship between maternal status and the composition of breast milk is essential in understanding the effect of maternal diet on infants' status. This information from this thesis will be used in the future by the APrON team to study the relationship between infants' status and neuro-cognitive development.

2.2 Objective and Hypothesis

The objective of this research was to characterize essential fatty acid status in mothers before pregnancy and at 3 months post-partum when they were breast feeding. The objective was also to determine the relationship between maternal status, pre and post-delivery, on the fatty acid composition of breast milk.

It was hypothesized that:

A. Women who reported taking a daily supplemented with DHA/EPA will have higher relative percentage and concentration of DHA, EPA and n-3 in their plasma than women who did not.

B. Women who reported taking a daily DHA/EPA supplements during pregnancy/lactation will have higher DHA, EPA and optimal AA in their breast milk than women who did not take supplements.

C. Women who did not take a DHA/EPA supplement and were breast feeding will have a lower ALA, DHA and EPA status than women (who were not taking a DHA/EPA supplement) and were feeding their infant with a combination of formula milk and breast milk.

D. Women who have a higher relative percent of DHA and AA in plasma at 3 months post-partum will have a higher relative percent of these fatty acids in their breast milk.

E. Maternal status of DHA, EPA, AA, n-3 and n-6 will be higher in the last trimester of pregnancy compared to 3 months post-partum.

F. Maternal DHA status in the third trimester will be associated with higher gestational age

2.3 Chapter Format

Chapter 3 describes the experimental design and the methods of the thesis.

Chapter 4 presents the results of the thesis that tests the thesis hypothesizes.

Chapter 5 summarizes the findings and provides a general discussion.

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Chapter 3

Experimental Design and Methodology

3.1 Experimental design: Selection of mother and infants in Alberta (APrON)

The primary aims of the APrON study are to examine the relationships between maternal nutrient intake and status, before, during and after gestation, and a) maternal mood, b) birth and obstetric outcomes, and c) infant neurodevelopment. In the APrON study, comprehensive maternal nutrition, anthropometric, biological, and mental health data were collected at multiple points of pregnancy and postpartum, and obstetrical, birth, health and neurodevelopmental outcomes of these pregnancies (Kaplan et al. 2012). The APrON cohort continues to follow the infants up to 36 months of age. Women recruited at <13 weeks gestation were assessed once during each trimester; those recruited at 14 -27 weeks gestation were assessed in the second and third trimesters only. Further assessment points at 3, 6, 12, 24, and 36 months post-partum with the variables monitored are illustrated in Figure 3.1.

This first cohort of APrON consisted of the first 600 women enrolled in APrON. At each pregnancy visit, women were asked to describe the type of supplements (e.g., prenatal multivitamins) consumed in the previous 24-hour period (midnight to midnight), as well as details about average water consumption and supplement intake. In the questionnaire, there were some questions about

infant feeding. These questions were used to identify mothers as exclusively breast feeding, formula feeding or mixed breast and formula feeding at 3 months postpartum. The population demographics were collected, which include age, marital status, education, ethnicity, and family income and these are described in table 3.1. This thesis will report on the fatty acid supplement use and the analysis of the maternal blood collected during the third trimester of pregnancy (time point C) and maternal blood and breast milk samples that were collected at 3 months post-partum (time point E). Also, it will report on infants' weight and length at birth and 3 months of age and the head circumference at birth.

At each visit during pregnancy and after delivery women were asked to describe in detail the quantity and type supplements that they consumed, using open-ended questions. Specifically women were asked for the brand, dose and frequency, “how much do you take?” and “how often do you take it?” (Leung et al., 2013).

Figure 3.1: APrON time line (Leung et al., 2013)

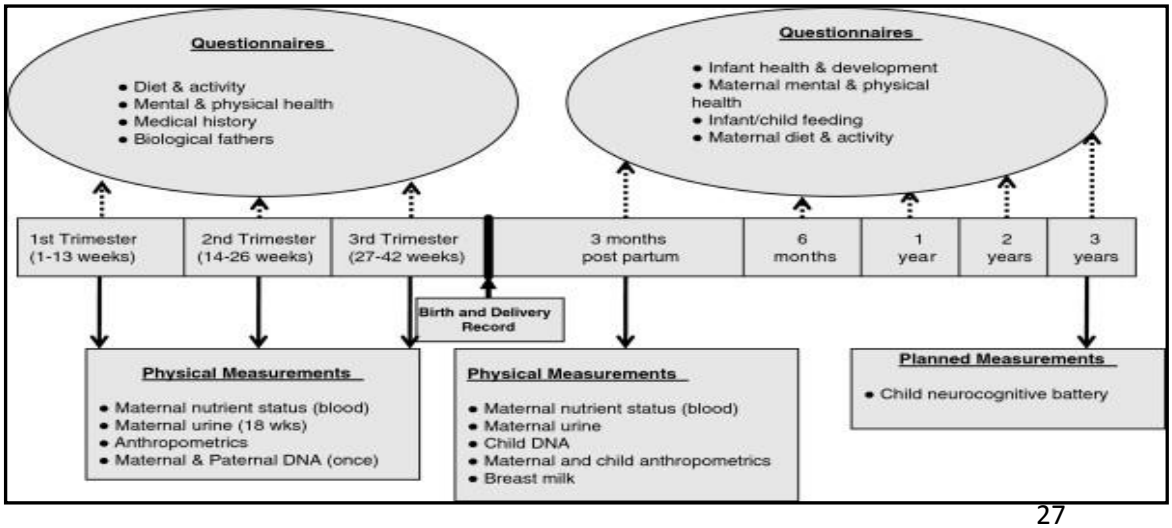


Table 3.1: Maternal Characteristics from APrON cohort 1 (n=600)

Maternal Characteristics (n)	**N (%)**	
Maternal Age (600)	31.6±4.4 (mean±SE)	
Marital Status (562)	Married	480 (85.4%)
	Other	82 (14.6 %)
Maternal Education (559)	≤ High school diploma	55 (9.8%)
	Trade	116 (20.8%)
	University Degree/Post Grad	388 (69.4%)
Ethnicity (558)	Caucasian	486 (87.1 %)
	Other	72 (12.9 %)
Family Income (552)	20-69K	107 (19.4%)
	70-99K	140 (25.4%)
	≥100K	305 (55.2%)

3.2 Data collection

Maternal blood was taken by a trained phlebotomist during the APrON visit at time point C and E and a spot breast milk sample was collected on filter paper by the mother during the 3 month post-natal visit (time point E). The spot sample (breast milk) was air dried and placed in a plastic bag, and the plasma was collected and stored in a freezer at -80°C until analysis. Lipids were extracted from the samples and analyzed using gas liquid chromatography (GLC) (as described in detail below).

3.3 Materials and Methods

3.3.1 Maternal blood-Lipid Extraction

Lipids were extracted from the total lipid component from 0.3 ml maternal plasma using a modified Folch extraction method, followed by separation of phospholipids from neutral lipids by thin layer chromatography. The fatty acids from the PL band were then methylated and identified by gas liquid chromatography (GLC)

3.3.1.1 Modified Folch Extraction for Phospholipids Classes

The following chemicals were incorporated sequentially in the glass vial (Thermo Fisher Scientific, Edmonton, AB, Canada): methanol (0.8 ml); 2.0 mL chloroform:methanol (1:1); 2.7 mL chloroform ; 2.5 mL chloroform:methanol (2:1) and finally, 100 uL of C15-phospholipid standard (Avanti Polar Lipids, Inc, Alabaster, Alabama). The mixture was vortexed and stored at 4°C overnight. The next day, the bottom layer was collected and transferred using a glass pipette into a smaller glass tube. The sample was dried using a heating block (Thermo Fisher Scientific) coupled with nitrogen gas, and stored at -35°C until further analysis.

3.3.1.2 G-Plate

Plasma PL was separated by thin layer chromatography according to the method reported and described in detail by Pratt et al., (2001). Briefly, a G-plate (Thermo Fisher Scientific) was heat activated for an hour at 100°C and the lipid sample (100uL) was spotted on the plate approximately 2 inches from the bottom.

A solvent system was prepared specifically designed to separate lipid classes: 80 mL petroleum ether, 20 mL diethyl ether and 1 mL acetic acid (Thermo Fisher Scientific). This solvent was poured into a tank lined with filter paper and allowed to sit until completely saturated. The spotted plate was placed into the tank for 30 minutes until the solvent was around 1 inch from the top. The G-plate was removed, air dried and sprayed with ANSA (Sigma-Aldrich Canada, Oakville, ON, Canada). Phospholipids (PL) spots were identified by ultraviolet light but comparing to standards and were scraped from the plate into a glass tube and stored at -35 °C until methylation and fatty acid identification.

3.3.1.3 Methylation

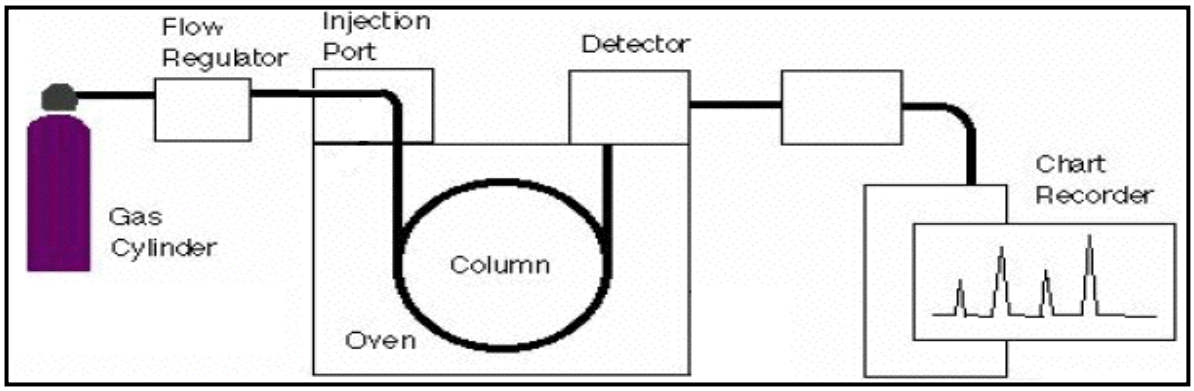
The PL in the scraped silica band were methylated according to the method of Pratt et al.(2001), Briefly, 1.5 mL of boron triflouride (BF3) (Sigma-Aldrich,) and 2.0 mL of hexane was added to each sample and was heated at 100°C for an hour in a heating block (leaks were checked often). One mL of double deionized water (ddH2O) was added into the cooled sample and stored at 4°C overnight. The next day, the top hexane-layer was transferred using a glass pipette into a GLC vial (Chromatographic Specialties, Inc, Brockville, Canada). The sample was then dried down using nitrogen and the lipid was re-suspended in 100uL of hexane. It was then placed in a glass insert and flushed with nitrogen and stored at -35°C in a freezer until it was ready to analyze with gas chromatography.

3.3.1.4 Gas-liquid chromatography (GLC)

The methylated fatty acids (FAME) were then identified by GLC using a 100 meter column (Sil 88) from Agilent (Mississauga, ON). The method and description of the column, gas and oven conditions are detailed in Cruz-Hernandez et al., (2004). This method was specifically designed for identification of long chain fatty acids using a longer column and longer times, which resulted in excellent separation of lipid peaks. Figure 3.2 shows a diagram illustrating steps for the GLC system. Each sample was compared to commercially available standards from Nu-Chek Prep, Inc.(Elysian, MN) in correspondence of retention time and concentration. Concentration of individual fatty acids was calculated using the C15 PC standard.

The concentration of each of the fatty acids in plasma PL was calculated with this formula: [the area peak X 10.6 ug (concentration)] / C15 standard area *3.33 mL.

Figure 3.2: Diagram illustrating a Typical GLC System adapted from Pieper and Rutledge, Laboratory Techniques for Pharmacists, Upjohn 1989, page 20.



3.3.2 Breast milk - Lipid Extraction

Breast milk samples were absorbed in a paper chromatography (PC), which is a useful technique for separating and identifying FAs (from C2 to C24) and determining the amount of the relative percent of each of the FAs (Fratesi et al., 2009). Each sample was stored at -80°C in the freezer until lipid analysis. The chromatography paper was inserted into a glass tube. 2.0 mL of BF3 and 1 mL of hexane was then added into the tube and vortexed. The samples were placed in the heating block at 110 °C for one hour. Samples were cooled down before the addition of 1mL of ddH2O and then vortexed and kept overnight at 4°C. The following day, the top layer was transferred to a small glass vial after which 1.0 mL of hexane was added. Another 1.0 mL hexane was added into the overnight tube, mixed, and the hexane fraction was collected and pulled together with the first top layer. After all the samples were dried under nitrogen gas, 300 uL of hexane was added to the vial and stored at -20.0°C until analysis by GLC, as described above for maternal plasma.

3.4 Statistical Analysis

All results are expressed as mean± SD. All statistical analyses were conducted using SPSS software. The differences between the two groups were analyzed by paired and unpaired T-test with two-tailed distribution. For the linear correlation between two variables, we used Pearson correlation. For all results, $P<0.05$ was considered statistically significant.

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Chapter Four

Result

4.1 Participant demographic information

The average age of the 600 APrON women was 31.6±4.4 y (table 3.1). The majority of the women were married (85.4%) and graduated from college or university (69.4%) (table 3.1). More than half (55.2%) of the participants had a family income higher than $100,000 per year (table 3.1). The majority of the participants were Caucasian (87.1%) (table 3.1).

4.2 Mothers Status During Pregnancy and 3 Months Post-partum

The average pre-pregnant BMI was 24.1 ±4.8 and BMI at 3 months post-partum was 25.7 ±4.8 (table 4.1). In APrON, no women deliver their baby less than 37 weeks. The average of total weight gain of APrON women during pregnancy were 16.0 ±5.6 kilograms (table 4.1). Table 4.2 showed that there is no significant statistical difference between the reported supplement intake of DHA and EPA at 3rd trimester compared with 3 month postpartum. However, there was significant difference between pre-pregnant BMI and BMI at 3 months postpartum ($p = 0.0001$) . In the third trimester during pregnancy, 135 of APrON mothers reported taking a daily fatty acid supplement with DHA averaging 259± 253 mg while EPA is at 310± 234 mg. However, at 3 months postpartum, the number of mothers who reported taking a fatty acid supplement declined to 84

mothers with average of DHA dose 204± 170 mg and EPA at 276±249 mg (Table 4.2).

Table 4.1: Data of APrON mothers' status

Mothers	mean±SD (n)
Pre-pregnant BMI	24.1±4.8 (575)
Gestational age birth (week)	38.9±1.8 (521)
Total pregnancy weight gain (kilo)	16.0±5.6 (475)
BMI at 3 months post-partum	25.7±4.8 (500)

Table 4.2: Average intake of fatty acid (DHA/EPA) supplements in APrON mothers in the third trimester and three months post partum

Supplement dose	3rd trimester	3 months post-partum	P value
Dose of DHA	259± 253 (135)	204± 170 (84)	0.0799
Dose of EPA	310± 234 (135)	276±249 (84)	0.3088

DHA, docosahexaenoic acid ; EPA, eicosapentaenoic acid. All values are given as mean± SD (n). There was no significant statistical difference between 3rd trimester and 3 month postpartum.

4.3 Fatty acids in breast milk (%w/w) at 3 months postpartum

At 3 months postpartum, breast milk was the major food for most infants. Only 9 women did not breast feed. The FA composition of breast milk at 3 months is reported in table 4.3.

Table 4.3: The Relative percent of fatty acids in breast milk at 3 months postpartum.

Fatty acids (breast milk)	**% FAs mean± SD (n)**
∑MCF	11. 7±3.8 (398)
∑SFA	39.1± 5.8 (398)

∑MUFA	42.3± 4.8 (398)
∑PUFA	17.2±4.1 (397)
∑n-6	14.7±3.3 (398)
∑n-3	2.5± 1.7 (397)
n6/n3	6.8±2.6 (396)
ALA/DHA	10. 1± 6.7 (396)
LA/AA	32.1± 11.1 (366)
LA/ALA	8.4± 5.7 (398)
DHA/AA	0.6±0.6 (366)
C10:0	0.8±0.4 (398)
C12:0	4.8±1.8 (398)
C14:0	5.7±1.9 (398)
C15:0	0.4±0.2 (397)
C16:0	20.8± 3.7 (398)
C16:1	3.4±1.0(398)
C17:0	0.2±0.1(397)
C18:0	6.2±1.8 (398)
C18:1 a	1.3±1.1 (62)
C18:1 b	39.0± 4.9 (398)
C18:1 c	1.5±0.9 (316)
C18:2 n-6 (LA)	13.5±3.2 (398)
C20:0	0.2±0.1 (398)
C18:3 n-3 (ALA)	1.9±0.7 (398)
C20:2 n-6	0.3±0.4 (398)
C20:3 n-6	0.4±0.2 (398)
C20:4 n-6 (AA)	0.5±0.2 (367)
C20:5 n-3 (EPA)	0.1±0.1 (395)
C22:4 n-6	0.5±0.2 (367)
C22:5 n-6	0.0±0.1 (388)
C22:5 n-3	0.3±1.5 (393)
C22:6 n-3 (DHA)	0.3±0.3 (398)

AL, Linoleic Acid; ALA, a-linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid, AA, arachidonic acids. All values are given as mean± SD (n).

4.4 Infants status at birth and at 3 months post-partum

Based on APrON data the average of birth weight was 3.4±0.5 kg and 6.1±0.8 kg at 3 months of age (Table 4.4). At birth, the average length was

51.2±3.4 cm and 59.4±3.4 cm at 3 months of age. Birth head circumference was 34.6±2.0 cm in APrON infants (Table 4.4).

4.5 The average of birth weight, length, head circumference and gestational age in supplemented and non-supplemented

Table 4.5 showed a comparison between of birth weight, length and head circumference between infants whom their mothers reported taking a supplement containing DHA/ EPA and infants whom their mothers were non-supplemented. There were no significant differences in birth weight, length, head circumference and gestational age infants whom their mothers reported taking a supplement containing DHA/ EPA compared with who did not.

Table 4.4: Data of APrON infants status at birth and at 3 months post-partum.

Infants	mean± SD (n)
Birth weight (kg)	3.4±0.5 (492)
Birth length (cm)	51.2±3.4 (449)
Birth head circumference (cm)	34.6±2.0 (512)
Weight at 3 months (kg)	6.1±0.8 (490)
Length at 3 months (cm)	59.4±3.4 (300)

Table 4.5: The average of birth weight, length and head circumference between infants whom their mothers reported taking a supplement containing DHA/ EPA and infants whom their mothers did not take supplement containing DHA/ EPA mothers at 3rd trimester

variables	Supplement containing DHA/ EPA	Non-supplemented	P value
Birth weight (kilo)	3.4±0.5 (112)	3.4±0.5 (349)	0.581
Birth length (cm)	51.0±3.0 (104)	51.3±3.4 (319)	0.317
Head circumference (cm)	34.8±1.6 (104)	34.5±1.6 (333)	0.100

Gestational age (week)	39.1±1.6 (117)	39±1.5 (367)	0.537

All values are given as mean± SD (n). Groups comparisons by unpaired T-test.

4.6 In the women not taking a EPA/DHA supplement: Comparison of DHA, EPA and AA concentrations in maternal plasma between women who were exclusively feeding their infants breast milk and those who were feed formula and breast milk

T-test was performed to compare the DHA and EPA between mothers who were exclusively feeding their infants breast milk and those who were feed formula and breast milk (Table 4.6). Mothers not taking supplement containing DHA/EPA and were exclusively breast feeding had the same level of DHA and EPA in their plasma compared to mothers who did not take supplement containing DHA/ EPA and were feeding their infants with combined formula and breast milk (Table 4.6). Although the relative percent of AA tend to be higher in mothers who were feeding their infants breast milk only, the difference did not reach statistical significance (P =0.7078).

Table 4.6: The composition of plasma EPA, AA and DHA in mothers who did not take supplement containing DHA/ EPA were feeding their infants with combined formula and breast milk

	Feeding methods		
Fatty acids (Plasma)	Exclusive Breast milk (n=187)	Formula + Breast milk (n=63)	P value
EPA%	0.9± 0.6	0.9±0.4	1.000
EPA ug/ml	17.4±10.9	16.0±8.5	0.3541
DHA %	2.3±0.8	2.3±0.8	1.000
DHA ug/ml	42.0±17.4	41.7±21.1	0.9109
AA %	9.1±1.9	9.0±1.6	0.7078
AA ug/ml	169.1±50.3	163.2±45.2	0.4100

ALA, a-linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid, AA, arachidonic acids. All values are given as mean± SD (n). Groups comparisons by unpaired T-test.

4.7 The correlation between relative percent of total n-3, AA and DHA with gestational age at 3 months postpartum

No correlation was found between the relative percent of total of n-3, AA and DHA with gestational age at 3 months postpartum (Table 4.7).

Table 4.7: The correlation between relative percent of total n3, AA and DHA with gestational age

Fatty acids	Gestational age	
	P value (n)	r
DHA %	0.949 (406)	-0.003
n-3%	0.671 (435)	-0.020
AA%	0.314 (440)	-0.048

DHA; docosahexaenoic acid, AA, arachidonic acid. Correlation tested by Pearson correlation. No correlation between n-3,DHA and AA with gestational age.

4.8 The association between the dose of DHA and EPA supplement and the composition of DHA and EPA in plasma and breast milk at 3 months postpartum in mothers who taking supplement

The average of DHA content in the supplement was 204± 170mg and the average of EPA was 276 ±249mg. Table 4.8 revealed that there is no correlation between the reported daily intake of DHA supplement and maternal plasma or breast milk DHA %. Furthermore, there was no correlation between the EPA dose supplement with maternal plasma and breast milk EPA %.

Table 4.8: Association between the dose of DHA and EPA supplement and mothers composition of DHA and EPA in plasma and breast milk at 3 months postpartum

Supplement	breast milk	Plasma
	DHA% w/w	DHA% w/w
DHA dose	0.317 (96)	0.246 (128)

	EPA%	EPA%
EPA dose	0.320 (95)	0.759 (124)

EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid . All values are given as p value (n). Tested by Pearson correlation. no association between the dose of DHA and EPA supplement and the composition of DHA and EPA in plasma and breast milk at 3 months postpartum.

4.9 Fatty acids composition of mothers' plasma who reported taking a supplement containing DHA/ EPA compared to mothers who did not take supplement containing DHA/ EPA at 3rd trimester and 3 months post-partum

Table 4.9 and 4.10 compares the plasma FAs composition between mothers who were reported taking supplement containing DHA/EPA and the mothers who did not. Results revealed that mothers who reported taking DHA/EPA supplement had higher relative percent and absolute amount of total n-3, DHA and EPA compared to non-supplemented mothers who were significant at 0.01 levels at 3rd trimester and 3 months postpartum. On the other hand, relative percent of total n-6 and AA were statistically lower in mothers who took a n-3 supplement than those who did not at 3rd trimester and 3 months postpartum. However, the plasma concentration of AA was not significant different between mothers who took a n-3 supplement than those who did not at 3 months postpartum but significant different at 3rd trimester. The concentration of n-6 was not significant different between mothers who took a n-3 supplement than those who did not at 3 months postpartum and 3rd trimester. The relative percent ALA was the same in the mothers who reported taking a fatty acid supplement compared to mothers who did no but the concentration of ALA was higher in

mothers who reported taking a fatty acid supplement in the 2 time points. The relative percent and the concentration of LA were higher in non-supplemented mothers. Statistical analysis did not find significant difference between the measured relative percentage and concentration of LA and ALA.

Table 4.9: Relative percent in mothers' plasma that reported taking a supplement containing DHA/ EPA compared to mothers who did not at 3rd trimester

	3rd trimester		
FAs in plasma	Supplement containing DHA/ EPA (n=132)	Non-supplemented (n=348)	P value
DHA %	4.1±1.2	3.2± 1.0	<0.001*
DHA µg/ml	100.9±38	77.9± 28.7	<0.001*
EPA%	0.9±0.6	0.4± 0.4	<0.001*
EPA µg/ml	21.7±16.3	10.1± 7.9	<0.001*
AA %	7.2±1.5	7.7± 1.7	0.0031*
AA µg/ml	178.2±50.2	189.2± 54.5	0.0443*
n-6%	30.9±3.2	32.11± 3.3	0.0003*
n-6 µg/ml	768.1±165.4	799.71±175.3	0.0740
n-3%	6.0±1.9	4.5± 1.3	<0.001*
n-3 µg/ml	148.6±57.8	110.7± 38.3	<0.001*
ALA %	0.4±0.2	0.4±0.2	1.000
ALA µg/ml	10.6±5	10.3±4.8	0.5459
LA %	19.2±2.7	19.7± 2.6	0.0633

LA µg/ml	472.8±111.1	484.2±115.8	0.3307

LA, linoleic acid; AA, arachidonic acid; ALA, a-linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid. All values are given as mean± SD (n). Groups comparisons by unpaired T-test.* significant at 0.05.

Table 4.10: Relative percent in mothers' plasma who reported taking a supplement containing DHA/ EPA compared to mothers who did not at 3 months post partum.

	3 months postpartum		
FAs in plasma	Supplement containing DHA/ EPA	Non-supplemented	P value
DHA %	2.8±0.9 (60)	2.3±.9 (406)	<0.001*
DHA µg/ml	55.4±19.6 (60)	43.8±19.6 (406)	<0.001*
EPA%	1.3±0.8 (60)	0.9±0.5 (406)	<0.001*
EPA µg/ml	25.1±15.1 (60)	16.8±9.4 (406)	<0.001*
AA %	8.3±1.8 (60)	9.0± 1.8 (406)	0.005*
AA µg/ml	164.0±48.4 (60)	168.3± 48.4 (406)	0.523
n-6%	33.5± 3.2 (60)	34.5±2.8(406)	0.0116
n-6 µg/ml	647.7± 153.0 (60)	643.6±148.3 (406)	0.842
n-3%	5.2±1.5 (60)	4.3±1.3 (406)	<0.001*
n-3 µg/ml	102.9±33.9 (60)	81.2±32 (406)	<0.001*
ALA %	0.4±0.2(59)	0.4±0.2 (405)	0.540
ALA µg/ml	7.2±3.2 (59)	6.4±5.2 (405)	0.260
LA %	21.2± 3.1(60)	21.5± 2.8(406)	0.896
LA µg/ml	420.6± 104.4	394.8± 95.4	0.054

LA, linoleic acid; AA, arachidonic acid; ALA, a-linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid. All values are given as mean± SD (n). Groups comparisons by unpaired T-test.* significant at 0.05.

4.10 Fatty acid composition of DHA, EPA and AA in mothers' breast milk who reported taking a supplement containing DHA/ EPA and the mothers who did not take supplement containing DHA/ EPA at 3 months postpartum

Table 4.11 compares the FAs composition of mothers' breast milk who reported taking a supplement containing DHA/EPA and the mothers who did not at 3 months postpartum. Mothers who reported taking a FAs supplement during lactation had higher relative percent of total n-3, n-6, LA, DHA and EPA than non-supplemented group (P<0.05). On the other hand, the relative percent of AA and ALA was higher but not statistically significant in non-supplemented group. The only FAs that reached statistical significance at the P<0.05 levels were the relative percent of DHA and EPA which were higher in mothers who reported taking a supplement containing DHA/ EPA.

Table 4. 11: Relative percent of FAs in mothers who reported taking a supplement containing DHA/ EPA compared to mothers who did not in breast milk at 3 months post partum

Fatty acids in breast milk	Supplement containing DHA/ EPA	Non-supplemented	P value
DHA %	0.4±0.3 (49)	0.2±0.2 (285)	0.004*
EPA%	0.2±0.2 (49)	0.1±0.1 (282)	0.006*
AA %	0.4±0.1 (45)	0.5±0.2 (262)	0.279
ALA %	1.8±0.7 (49)	1.9±0.8 (285)	0.892
LA %	13.7±2.5 (49)	13.5±3.1 (285)	0.685
n-6%	15.0±2.6 (49)	14.7±3.2 (285)	0.734
n-3%	2.6±1.0 (49)	2.5±2.0 (284)	0.566
PUFA %	38.4± 4.1 (49)	38.3± 4.4 (285)	0.670
SFA%	45.2± 3.4(49)	45.01± 3.2(285)	0.999

LA, linoleic acid; AA, arachidonic acid; ALA, a-linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid. All values are given as mean± SD (n). Groups comparisons by unpaired T-test. * significant at P<0.0.

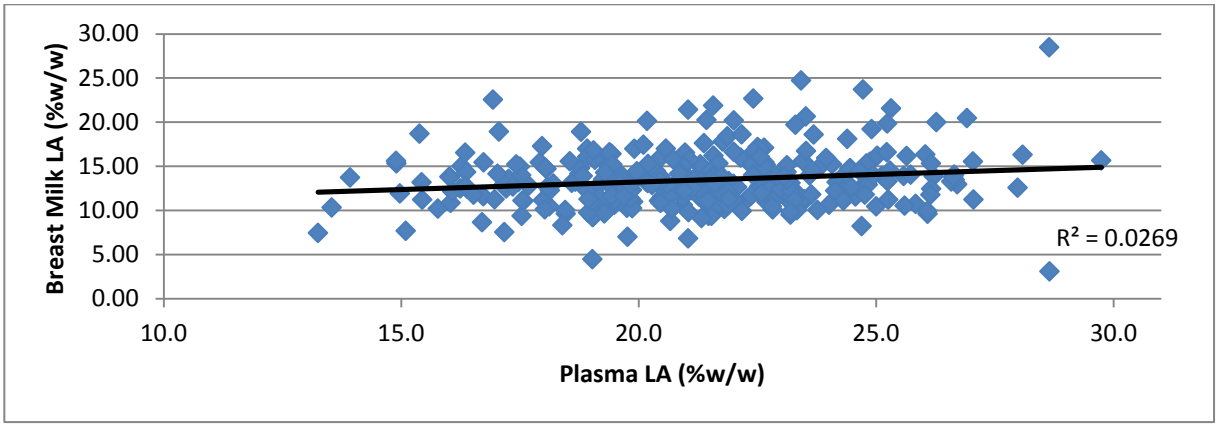
4.11Relationship between n-6 and n-3 fatty acids mothers' plasma and the relative percent in breast milk at 3 months postpartum

Correlation analysis showed that the relative percent of LA found in plasma were positively correlated with relative percent LA in the breast milk (P = 0.003). For every 1% increase in the relative percentage of LA in plasma, LA in milk increased by 0.33% (figure 4.1). Similarly, in figure 4.2, the relative percentage of ALA in plasma found to be positively correlated with the relative percentage of ALA in the breast milk (P< 0.001). A 1% increase in ALA composition in plasma resulted in a 1.29% increase in breast milk ALA composition.

Also, the data reported in figure 4.3 revealed a significant positive correlation between the relative percentage of AA found in the plasma and the relative percentage found in the milk (P= 0.002). For every 1% increase in the composition of AA in plasma, AA in milk increased 0.013%. Figure 4.4 showed positive significant correlation between the relative percentages of DHA in plasma with the relative percentage of DHA in milk (P < 0.001). Also, figure 4.5 showed there is a significant positive correlation between the relative percentage of EPA found in the plasma and the relative percentage found in the milk (P < 0.001). For every 1% increase in the composition of EPA in plasma, EPA in milk increased 0.01%

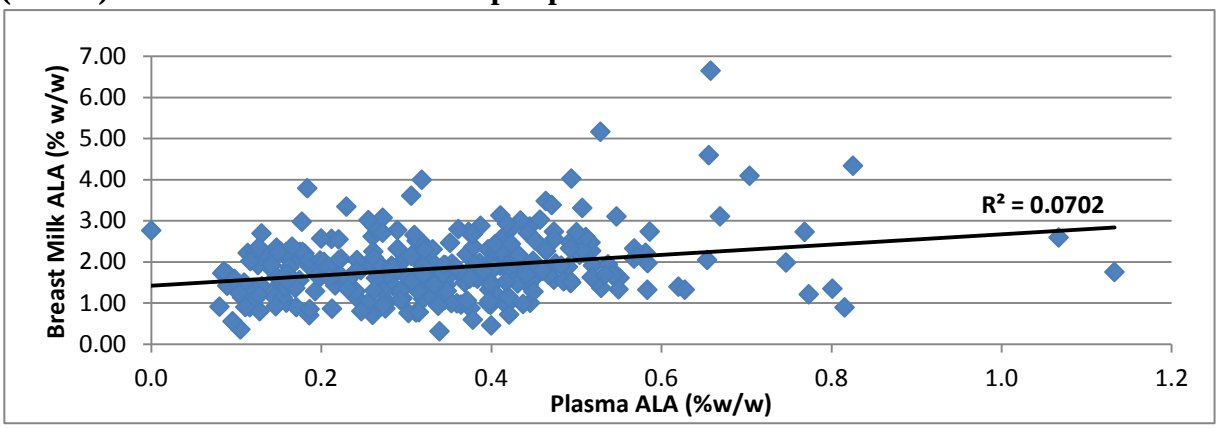
Figure 4.6 reports no correlation between the relative percentage of MUFA found in the plasma and the relative percentage of total MUFA found in the milk (P= 0.098). Figure 4.7 showed significant positive correlation between relative percent of SFA in plasma PL with relative percent SFA levels in the breast milk (P=0.001). In every 1% increase in the composition of SFA in plasma, SFA in milk increased by 0.32%. In addition, the relative percent of PUFA are found to be positively correlated between the plasma relative percent and milk (P< 0.001) (Figure 4.8). In every 1% increase in the composition of PUFA in plasma, PUFA in milk increased by 0.25%. The data reported here showed that there is a significant positive correlation between the relative percentage of total n-6 found in the plasma and the relative percentage of total n-6 found in the milk (P <0.001). In every 1% increase in the composition of LA in plasma, LA in milk will increase by 0.22% (Figure 4.9). Moreover, the relative percent of total n-3 levels found in plasma were significantly and positively correlated with relative percent n-3 levels in the breast milk (P <0.001) (figure 4.10). In every 1% increase in the composition of LA in plasma, LA in milk increased by 0.17% (Figure 4.10).

Figure 4.1: the correlation between LA (%w/w) in plasma with LA (%w/w) in breast milk at 3 months postpartum



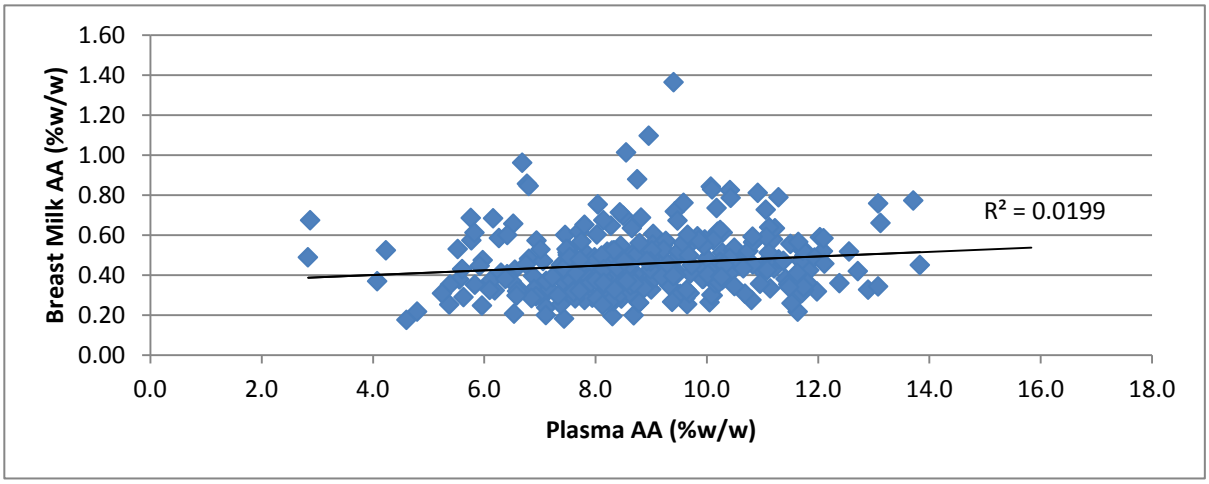
LA, linoleic acid. Correlation co-efficient was significant (P=0.003)

Figure 4.2: The correlation between ALA (%w/w) in plasma with ALA (%w/w) in breast milk at 3 months postpartum



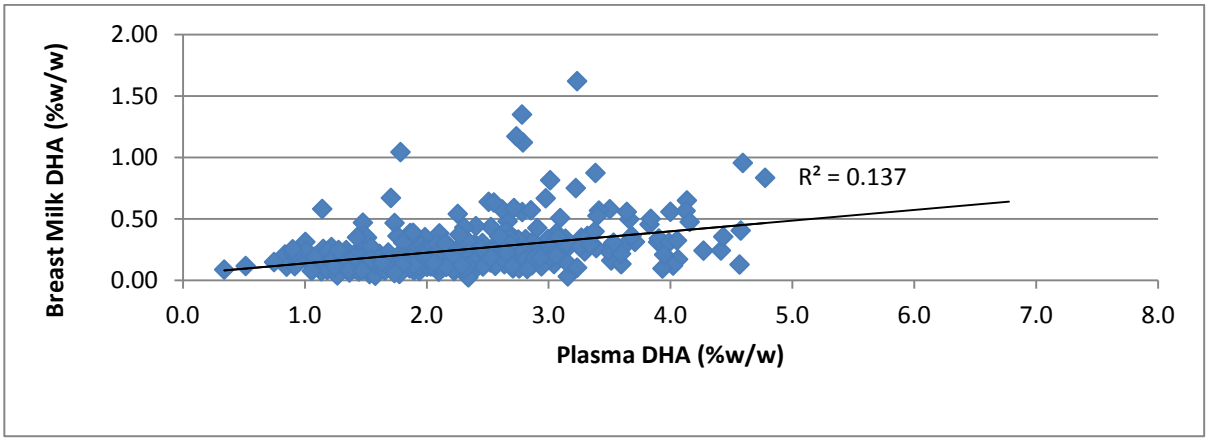
ALA, a-linolenic acid. P . Correlation co-efficient was significant (P=0.001)

Figure 4.3: The correlation between AA (%w/w) in plasma with AA (%w/w) in breast milk at 3 months postpartum



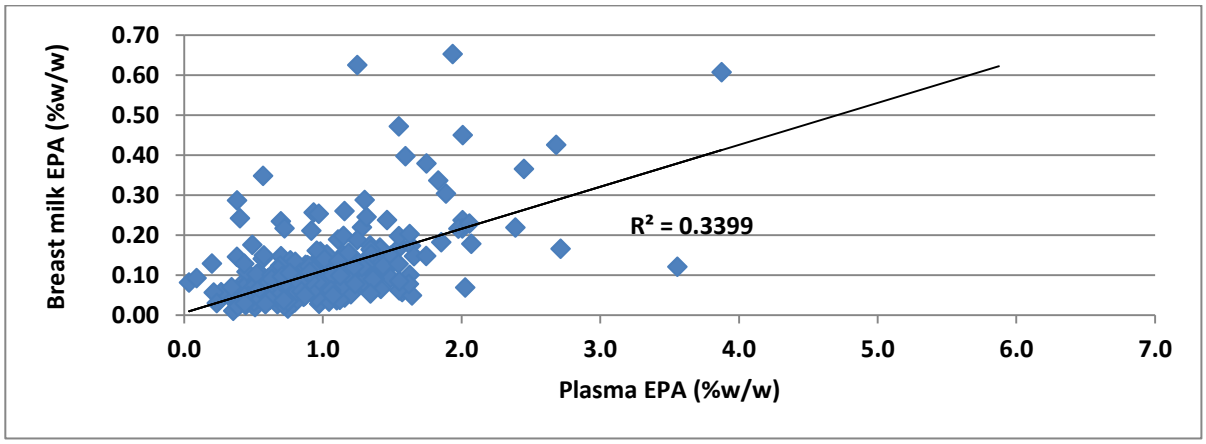
AA, Arachidonic acid. Correlation co-efficient was significant (p=0.011).

Figure 4.4: The correlation between DHA (%w/w) in plasma with DHA (%w/w) in breast milk at 3 months postpartum



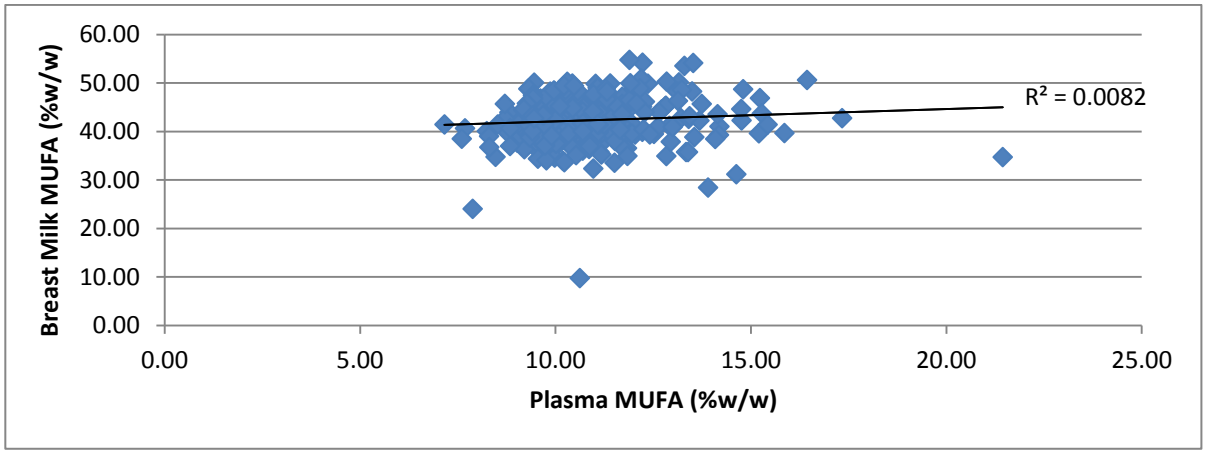
DHA, docosahexaenoic acid. Correlation co-efficient was significant (p<0.001).

Figure 4.5: The correlation between EPA (%w/w) in plasma with EPA (%w/w) in breast milk at 3 months postpartum



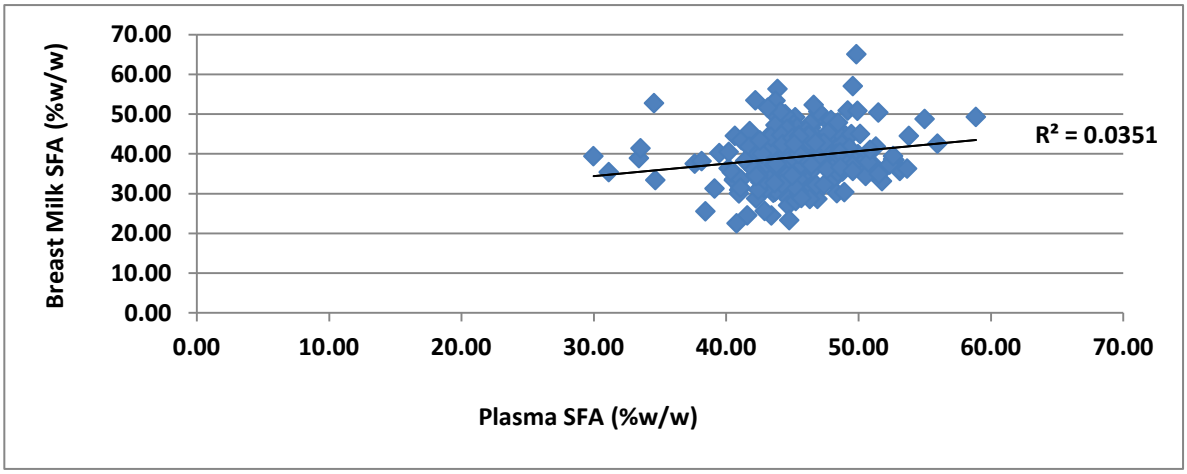
EPA, eicosapentaenoic Correlation co-efficient was significant (p<0.001).

Figure 4.6: The correlation between MUFA (%w/w) in plasma with MUFA (%w/w) in breast milk at 3 months postpartum



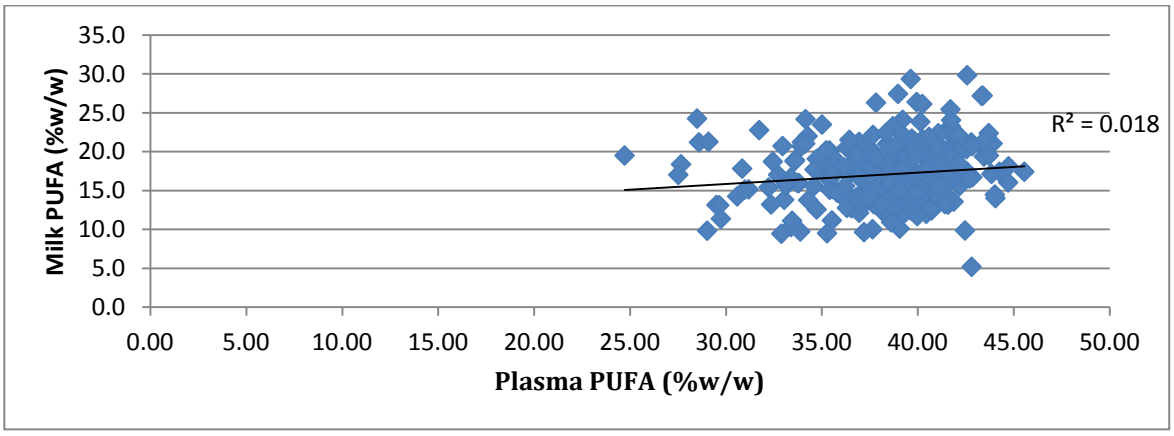
MUFA, Monounsaturated Fatty Acid Correlation co-efficient was significant (P=0.098).

Figure 4.7: The correlation between SFA (%w/w) in plasma with SFA (%w/w) in breast milk at 3 months postpartum



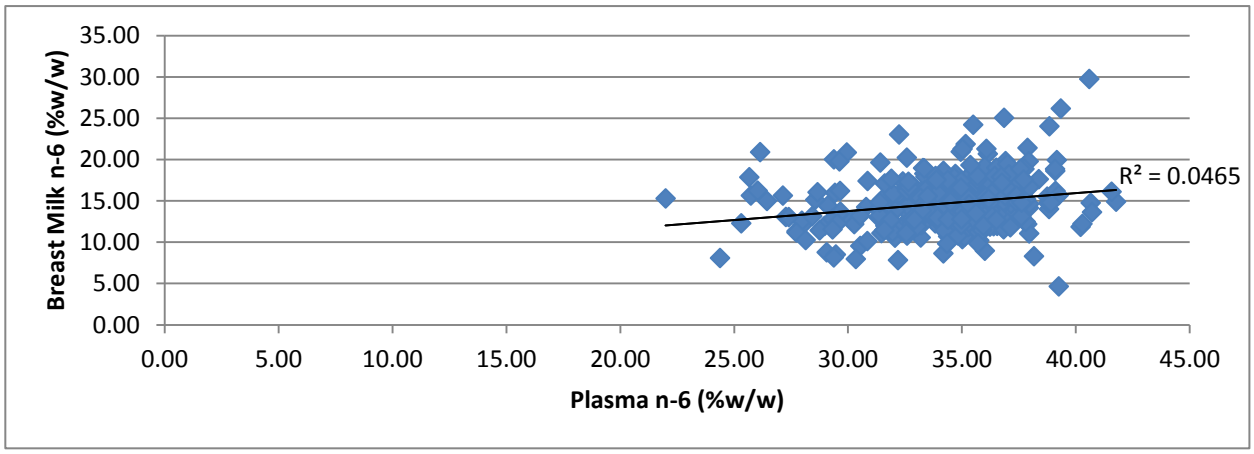
SFA, saturated fatty acids. Correlation co-efficient was significant ($p<0.001$).

Figure 4.8: The correlation between PUFA (%w/w) in plasma with PUFA (%w/w) in breast milk at 3 months postpartum



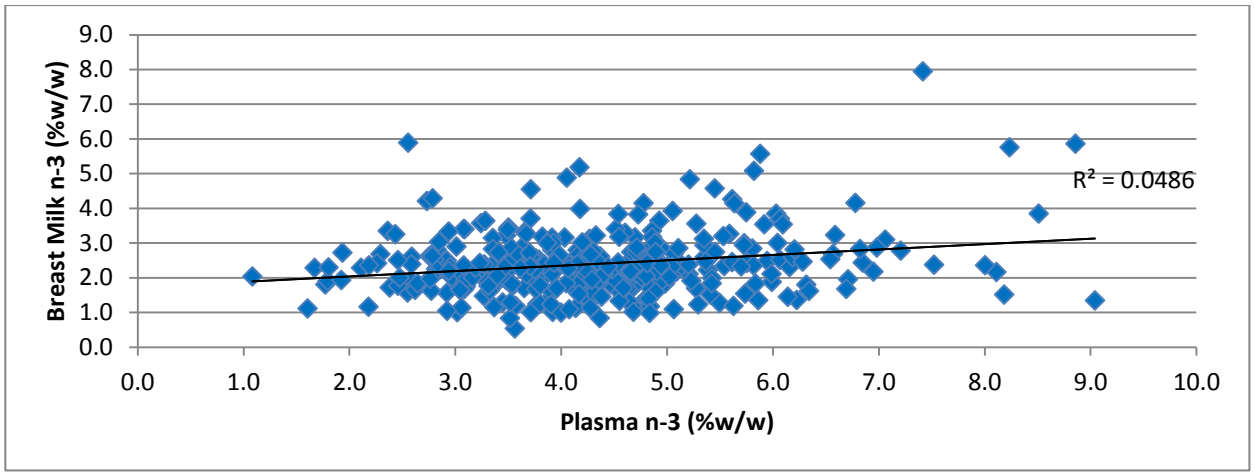
PUFA, Polyunsaturated fatty acids. Correlation co-efficient was significant ($p=0.0136$).

Figure 4.9: The correlation between n-6 (%w/w) in plasma with n-6 (%w/w) in breast milk at 3 months postpartum



Correlation co-efficient was significant (P<0.001)

Figure 4.10: The correlation between n-3 (%w/w) in plasma with n-3 (%w/w) in breast milk at 3 months postpartum

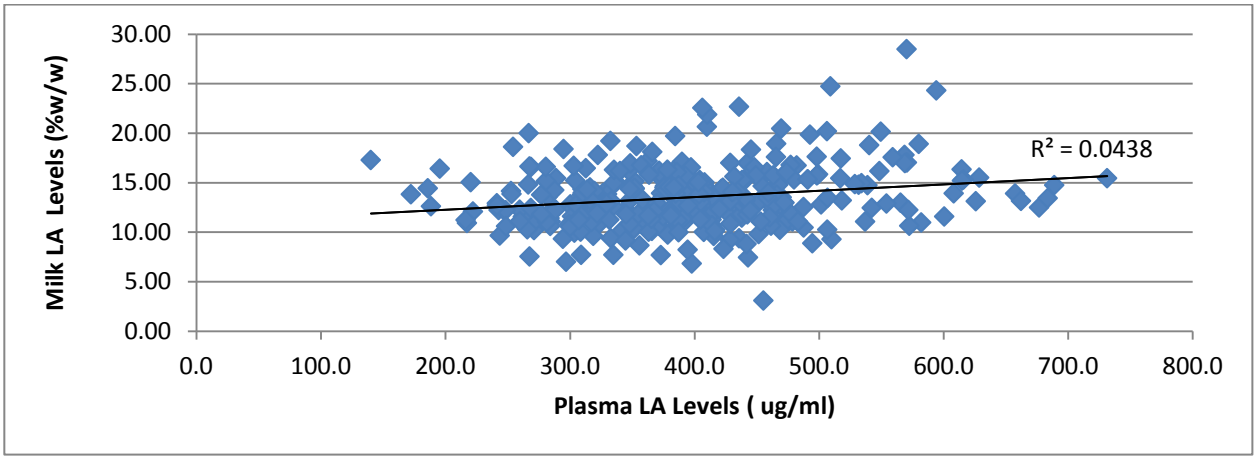


Correlation co-efficient was significant (P<0.001).

4.12 The relationship between concentration of LA, ALA, DHA, AA and EPA mothers' plasma and the relative percent in breast milk at 3 months postpartum

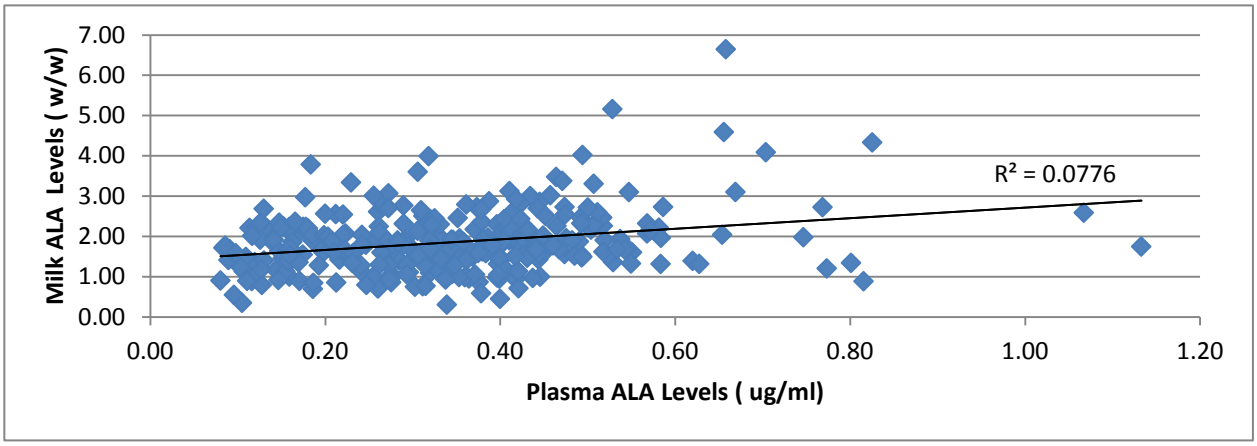
There was a significant positive correlation in LA, ALA, DHA and EPA in plasma with relative percent of LA, ALA, DHA and EPA in milk (P<0.001) (figure 4.11, 4.12, 4.13, 4.14). Conversely, the concentration of AA in plasma did not correlate with relative percent AA in breast milk (p= 0.156) (figure 4.15).

Figure 4.11: The correlation between LA (ug/ml) in plasma with LA (%w/w) in breast milk at 3 months postpartum.



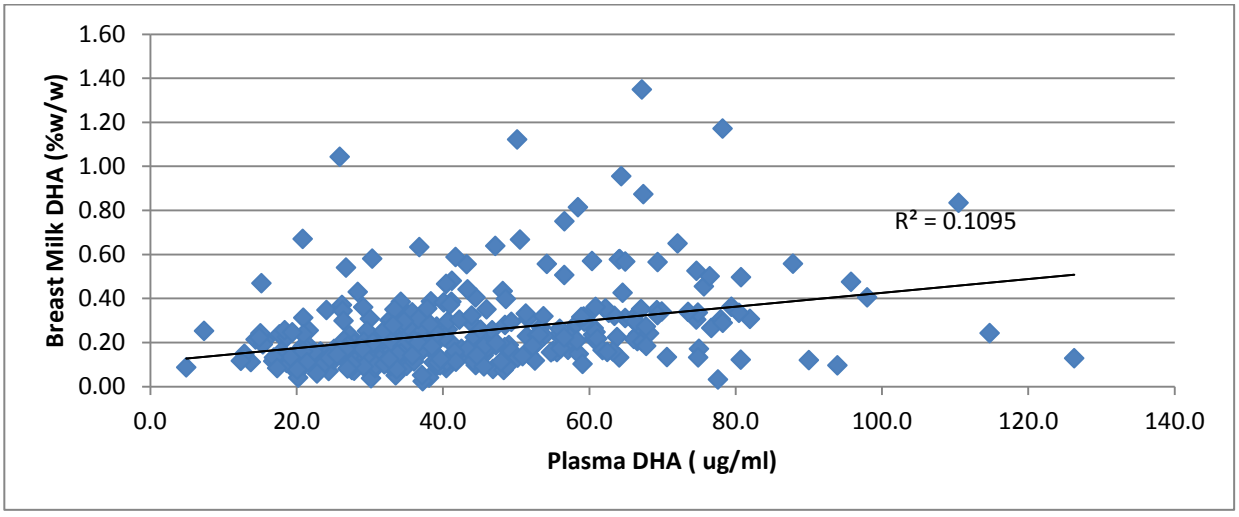
LA, linoleic acid. Correlation co-efficient was significant (p<0.001).

Figure 4.12: The correlation between ALA (ug/ml) in plasma with ALA (%w/w) in breast milk at 3 months postpartum.



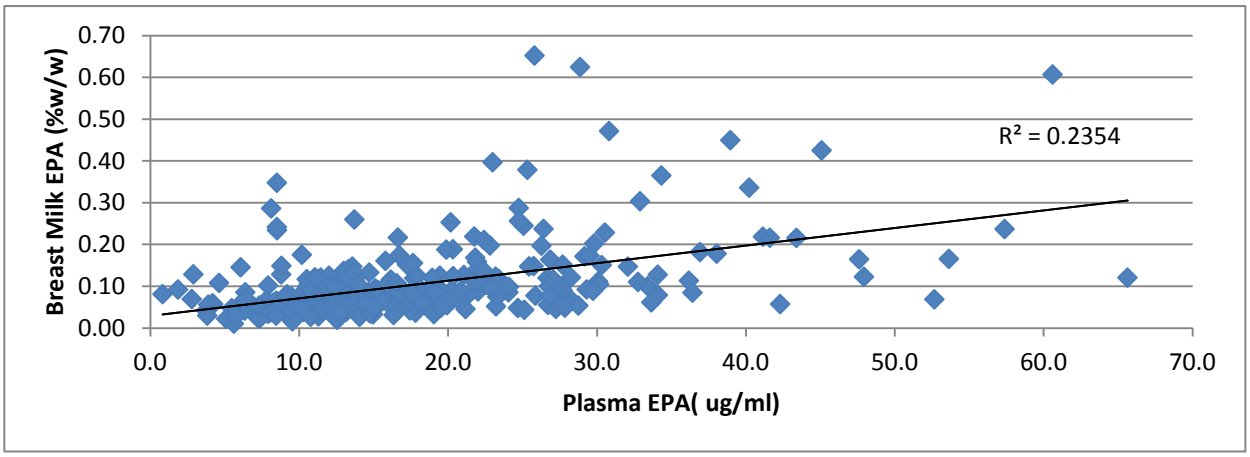
ALA, alpha linoleic acid. Correlation co-efficient was significant ($p<0.001$).

Figure 4.13: The correlation between DHA (ug/ml) in plasma with DHA (%w/w) in breast milk at 3 months postpartum



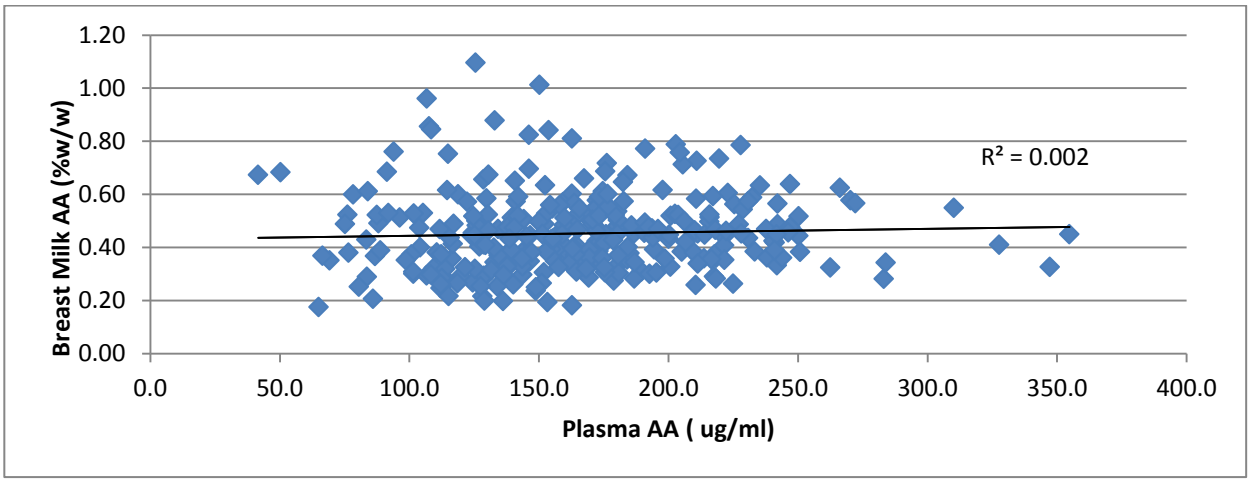
DHA, Docosahexaenoic acid. Correlation co-efficient was significant ($p<0.001$).

Figure 4.14: The correlation between EPA (ug/ml) in plasma with EPA (%w/w) in breast milk at 3 months postpartum



EPA, Eicosapentaenoic acid. Correlation co-efficient was significant ($p<0.001$).

Figure 4.15: The correlation between AA (ug/ml) in plasma with AA (%w/w) in breast milk at 3 months postpartum



AA, Arachidonic acid. There was no significant correlation between plasma concentrations of AA and the relative percent AA in milk ($p=0.156$).

4.13 Correlation between the relative percent of some FAs in plasma and in breast milk at 3 postpartum

A significant negative correlation was found between the relative percent of LA in plasma with relative percent of AA in breast milk (p=0.003) (table 4.12). However, there was no correlation found between the relative percent of ALA in plasma and the relative percent of DHA in breast milk (Table 4.13).

Table 4.12: The correlation between the relieve percent of LA in plasma and AA in breast milk at 3 postpartum

Fatty acids	AA breast milk	
	P value (n)	r
LA plasma	0.003*(406)	-.171

Tested by Pearson correlation. *correlation is significant at the 0.05 level (2 tailed).

Table 4.13: The correlation between the relieve percent of ALA in plasma and DHA in breast milk at 3 postpartum

Fatty acids	DHA in breast	
	P value (n)	r
ALA plasma	0.690(330)	0.022

Tested by Pearson correlation. correlation is not significant.

4.14 Fatty acids in mothers' plasma (%w/w) and (μg/ml) at 3rd trimester of pregnancy and 3 months post partum

Based on APrON maternal plasma composition, the relative percent of SFA, MUFA, n-3, ratio of LA/AA, DHA/AA, C18:1 and DHA were higher in the 3rd trimester of pregnancy than 3 months post-partum (P<0.01). On the other hand, the composition (relative percent) of PUFA, n-6 LA, AA, EPA, the ratio of

n6/n3, LA/ALA and ALA/DHA were higher at 3 months post-partum than the 3rd trimester of pregnancy (P<0.05). The absolute concentration (ug/ml) of most FAs were higher (P<0.05) during the 3rd trimester than 3 months post partum with the exception of LA, EPA, ratio of LA/ALA and ALA/DHA. The relative percent of n-3, ALA and DHA was significantly higher at 3rd trimester than at 3 months postpartum (table 4.14).

Table 4.14: The relative percent and the concentration of fatty acids at 3rd months and at 3 months post-partum.

FAs	Fas % at 3rd trimester	FAs % at 3 months mean± SD (n)	paired t-test	µg/ml at 3rd trimester mean± SD (n)	µg/ml at 3 months mean± SD (n)	P
∑SFA	46.5 ± 3.9 (403)	45.2 ± 3.2 (403)	<0.001*	1143.1 ± 197.4 (403)	837.6 ± 135.2 (403)	<0.001*
∑MUFA	12.5 ± 2.0 (402)	10.9 ± 1.5 (402)	<0.001*	309.8 ± 84.4 (402)	204.6 ± 56.4 (402)	<0.001*
∑PUFA	37.1 ± 3.6(401)	38.6 ± 3.2 (401)	0.002*	921.1 ± 200.4(402)	642.5 ± 149.2 (402)	<0.001*
∑n-6	31.9 ± 3.1 (402)	34.2 ± 3.0 (402)	<0.001*	799.3 ± 175.6 (402)	727 ± 165.23 (402)	<0.001*
∑n-3	4.9 ± 1.6 (402)	4.4 ± 1.4 (402)	<0.001*	121.6 ± 46.1 (402)	82.7 ± 32.3 (402)	<0.001*
n6/n3	7.2 ± 2.6 (402)	8.5 ± 28 (402)	<0.001*	7.3 ± 2.6 (402)	3.2 ± 1.0 (402)	<0.001*
ALA/DHA	0.1 ± 0.1 (402)	0.2 ± 0.1 (402)	0.002*	0.1 ± 0.1 (402)	0.2 ± 0.1 (402)	0.002*
LA/	2.8 ± 1.3	2.5 ± 0.7 (402)	<0.001*	2.7 ± 1.3 (402)	2.5 ± 0.7 (402)	<0.001*

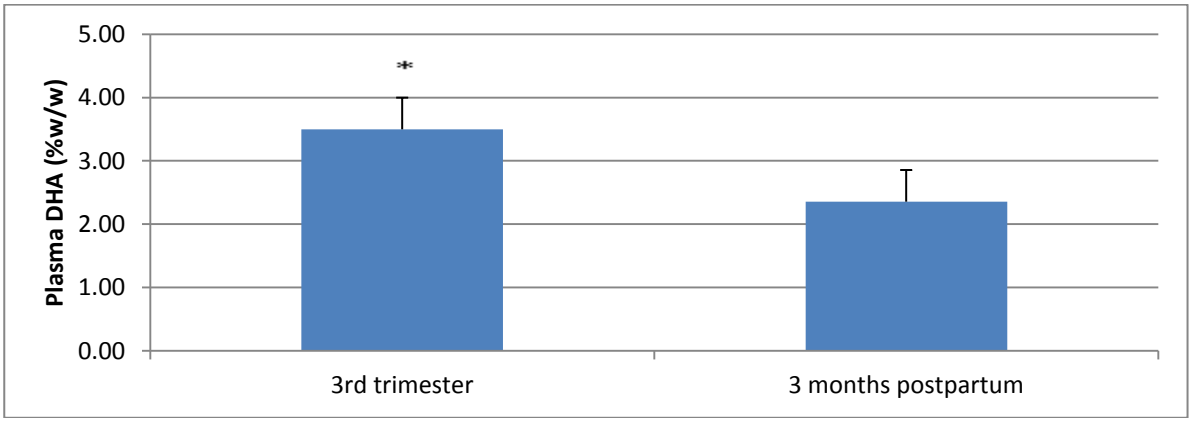
AA	(402)					
LA/ ALA	57.3 ± 42 (400)	78.7 ± 45.6 (400)	0.035*	57.3 ± 42 (402)	78.7 ± 45.6 (402)	0.035*
DHA/AA	0.5 ± 0.2 (402)	0.3 ± 0.1 (402)	<0.001*	0.5 ± 0.2 (402)	0.3 ± 0.1 (402)	<0.001*
C18:1	13.7 ± 2.3 (183)	12.5 ± 2.1 (183)	<0.001*	342.7 ± 96 (183)	239.4 ± 68.5 (183)	<0.001*
C18:2 n-6 (LA)	19.6 ± 2.6 (404)	21.1 ± 2.9 (404)	<0.001*	486.9 ± 117.1 (402)	493.9 ± 98.6 (402)	<0.001*
C18:3 n-3 (ALA)	0.4 ± 0.2 (403)	0.3 ± 0.2 (403)	<0.001*	10.4 ± 4.9 (401)	6.6 ± 5.3(401)	<0.001*
C20:4 n-6 (AA)	7.6 ± 1.6 (404)	8.9 ± 1.9 (404)	<0.001*	187.7 ± 54.2 (402)	166.0 ± 47.9 (402)	<0.001*
C20:5 n-3 (EPA)	0.5 ± 0.5 (404)	1.0 ± 0.5 (404)	<0.001*	13.2 ± 11.4 (402)	17.9 ± 10.7 (402)	<0.001*
C22:6 n-3 (DHA)	3.4 ± 1.1 (402)	2.4 ± 0.9 (402)	<0.001*	84.8 ± 32.9 (402)	44.2 ± 19 (402)	<0.001*
Total FAs	NA	NA		2470.1± 430.5 (402)	1866.4± 376.8 (402)	<0.001*

LA, linoleic acid; AA, arachidonic acid; ALA, a-linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids. All values are given as mean± SD (n). Groups comparisons by paired T-test. * significant at 0.05.

4.15: Fatty acids in mothers' plasma (%w/w) at 3rd trimester compared with 3 months postpartum in non-supplemented mothers

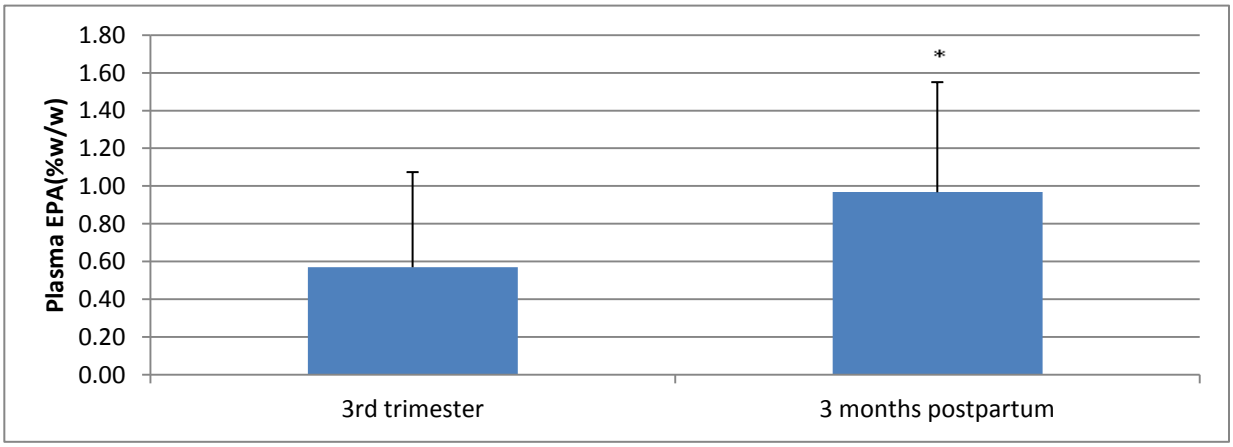
The relative percent of DHA was higher at 3rd trimester than 3 months postpartum (figure 4.16). However, the relative percent of AA and EPA was significant higher at 3 months postpartum than 3rd trimester in non-supplemented mothers (figure 4.17, 4.18).

Figure 4.16: The comparison between DHA (%w/w) in plasma at 3rd trimester with DHA (%w/w) in plasma at 3 months postpartum in non-supplemented mothers.



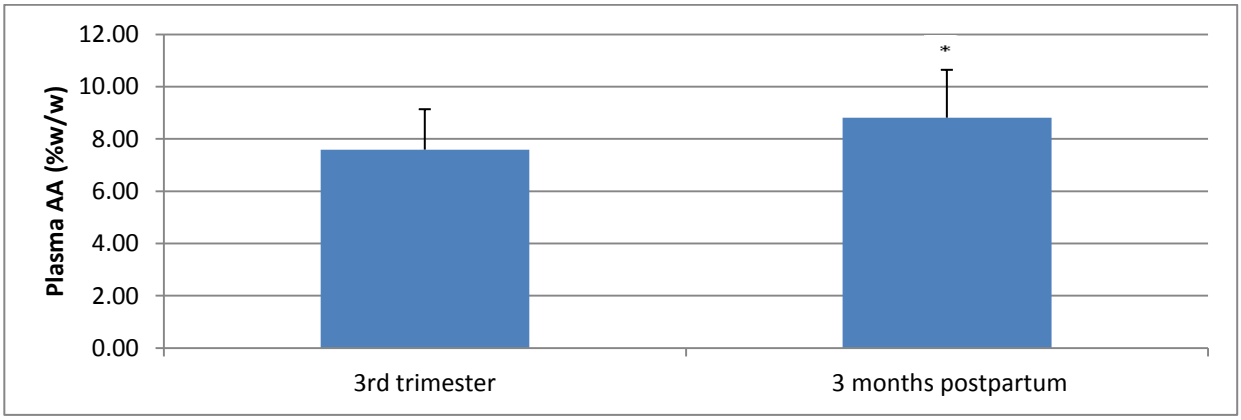
Bars represent mean ± SD for fatty acid consumption; DHA, docosahexaenoic (n=288). *indicates significantly different from 3 months postpartum(P<0.05).

Figure 4.17: The comparison between EPA (%w/w) in plasma at 3rd trimester with EPA (%w/w) in plasma at 3 months postpartum in non-supplemented mothers



Bars represent mean ± SD for fatty acid consumption; (n=288). EPA, eicosapentaenoic *indicates significantly different from 3 months postpartum($P<0.05$).

Figure 4.18: The comparison between AA (%w/w) in plasma at 3rd trimester with AA (%w/w) in plasma at 3 months postpartum in non-supplemented mothers



Bars represent mean ± SD for fatty acid consumption; AA , arachidonic acid (n=288). *indicates significantly different from 3 months postpartum ($P<0.05$).

4.16 Summary of findings

Relative percent of DHA, EPA in mothers who reported taking a supplement containing DHA/ EPA was higher compared to mothers not taking supplement at 3 months post partum. However, the relative percent of AA in mothers' plasma was significant lower in the supplemented group compared with non-supplemented group. APrON infants' birth weight, length and head circumference was within normal ranges, according to World Health Organization (WHO) growth charts and not significantly different between infants whose mothers took a supplement from infants whose mothers are not taking supplement. Data showed no differences in the relative percent of ALA, EPA, DHA in the plasma of mothers who feed their infant with breast milk compared to mothers who feed their infant with combined breast milk and formula. The relative percent of LA, ALA, AA, DHA, PUFA, SFA, n-3 and n-6was correlated with LA, ALA, AA, DHA, PUFA, SFA, n-3 and n-6 in breast milk. In this study, there were statistical significance of correlations of the concentration of LA, ALA, DHA and EPA with the relative percent of LA, ALA, DHA and EPA. The relative percent of LA in plasma correlated AA in breast milk but relative percent of AA in plasma was not correlated with AA in breast milk. There was no correlation between the relative percentage of ALA in plasma and the relative percentage of DHA in breast milk.

Chapter five

Discussion

The chapter discusses the findings reported in chapter 4 of the thesis. In the current study, the vast majority of APrON women (>97%) (Thomas, 2011) were consuming a daily multivitamin supplement during pregnancy which presumably ensured that they met their requirement for micronutrients and that small additional supplement containing DHA/EPA had no further beneficial effect on the length and birth weight of the infant. The proportion of women in the cohort study who took an omega-3 supplement during pregnancy was 27% and 18% during lactation. The average supplemental intake of DHA was 259±253 mg and EPA was 310±234 mg per day during pregnancy and the average supplemental intake of DHA was 204±170 mg and EPA was 276±249 mg per day during lactation.

The purpose of this study was to determine the relationship between maternal status pre- and post-delivery on the fatty acid composition of plasma and breast milk. We also proposed to establish the relationship between maternal status, pre and post-delivery, on the fatty acid composition of breast milk.

A. Mothers who were supplemented with DHA/EPA during pregnancy/lactation have higher relative percentage and concentration of DHA and EPA in their plasma than non-supplemented mothers at 3 months postpartum

APrON data supported this hypothesis. Mothers who were supplemented with DHA/EPA had significant higher relative percentage and concentration of DHA and EPA in their plasma than non-supplemented mothers (table 4.10).

B. Mothers who reported taking DHA/EPA supplements during pregnancy/lactation will have higher DHA, EPA and status and optimal AA in breast milk content than mothers who did not take supplements at 3 months postpartum

The results supported this hypothesis (Chapter 4). Mothers who reported taking DHA/EPA supplements during pregnancy and lactation had significant higher DHA and EPA content without a detrimental effect on the AA content in breast compared to mothers who did not take a supplement (table 4.11).

C. Non-supplemented mothers who were exclusively feeding their infants' breast milk will have less DHA and EPA in their plasma than non-supplemented mothers who were feeding their infant with a combination of formula milk and breast milk.

This hypothesis was not supported by the results reported in chapter 4. Non-supplemented mothers who were feeding their infant exclusively breast milk did not have a significant difference in DHA and EPA in their plasma than non-supplemented mothers who were feeding their infant with a combination of formula milk and breast milk (table 4.6).

D. Higher relative percent of DHA and AA in mothers' plasma at 3 months post-partum will be associated with higher relative percent in breast milk at 3 months.

The results of this thesis supported this hypothesis. At 3 months post-partum, the amount (concentration and relative percent) of DHA and AA in mothers' plasma was positively associated with the amount (relative percent) of these fatty acids in breast milk at 3 months postpartum (figure 4.3, 4. 4, 4.13, 4.15).

E. Lower composition of DHA, EPA, AA, n-3 and n-6 is found in mothers at 3 months post-partum as compared to the last trimester of pregnancy.

Three months post-partum, compared to the third trimester of pregnancy, mothers had a lower concentration of DHA and total n-3 fatty acids and higher concentration of EPA, AA and total n-6 fatty acids (table 4.14).

F. Higher composition of DHA in mothers' plasma at 3 months will be associated with higher gestational age

The data in this thesis did not support this hypothesis. Higher composition of DHA in mothers' plasma at 3 months was not significantly associated with longer gestation (table 4.7)

5.1. Effect of taking DHA/EPA Supplement during pregnancy

5.1.1 Maternal fatty acid status

During pregnancy in APrON women, the relative percent of DHA was 28% higher, EPA was 125% higher and total n-3 was 36.4% higher in plasma PL. However, taking DHA/EPA supplements resulted in a 7% lower relative AA in the plasma PL. The concentration of supplemented mothers resulted in a 28% higher DHA, 112% higher EPA and 33% higher total n-3 in plasma PL.

Similar to our findings, studies reported a higher significant difference between the relative percentage of DHA and EPA (Bergmann et al., 2008; Malcolm et al., 2003) in supplemented group (200mg of DHA/day) in RBC-FA% than in the placebo group. Conversely, the relative percent of AA was lower in the supplemented group than in the placebo group (Bergmann et al., 2008). Comparison between the characteristics of women in Bergmann et al., study (2008) and APrON study, the age, education and pre-pregnant BMI was similar (30.9, 31 years) (66%, 70%) (22.2, 24.2) respectively. Our findings corroborate with the literature, where 200mg of DHA supplements enhanced the DHA status in maternal plasma and decreased AA status. The possible explanation to the decrease of AA is due to higher EPA composition which then inhibit delta-5-desaturase enzyme that is responsible of the production of AA (Sears, 1995). Therefore, the more EPA in the diet or supplements, the less production of AA. The percentage of increase in DHA and EPA depends on the dose of supplement. As APrON and Bergmann et al. study dose was 200mg of DHA/day the

percentage of increase was (21%, 22%) respectively. However, in Helland et al. (2006) the dose of DHA was higher as 1183mg the increase was 44%.

5.1.2 on infants outcome

In the APrON cohort, there were no significant differences in birth weight, length and head circumference of infants whose mothers were reported taking a supplement containing DHA/EPA compared with mothers who did not. Similarly, several studies have also shown that there is no significant difference in birth weight, (Bergmann et al., 2008; Tofail et al., 2006; Stein et al., 2011; D'Vaz et al., 2012) length, (Bergmann et al., 2008; Stein et al., 2011; D'Vaz et al., 2012) and head circumference (Bergmann et al., 2008; Stein et al., 2011; D'Vaz et al., 2012) of infants whose mothers were supplemented with fish oil daily (200-400mg/day) during pregnancy as compared to a placebo or a control group.

Contrary to the studies, Makrides et al. (2010) study resulted in differences in birth weight. In this study the dose of DHA was higher (800mg) and the maternal diet was influenced by DHA status because this study was conducted in an Australian population in which their diet was high in omega-3. Another study reported that Danish women who consumed 2.7g of n-3 LC-PUFA/day from the 30th week of pregnancy until delivery had higher birth weight and length compared to the placebo control group (Olsen et al., 1995). It is hypothesized that DHA and EPA increases the length of gestation which influences birth weight (Makrides et al., 2006). The differences in birth weight may be influenced by premature delivery, smoking, consumption of alcohol, diabetes, pre-pregnancy BMI, exercise and weight gain during gestation (Gillman et al., 2003; Fleten etal.,

2010; Larroque et al., 1993; Andersen, et al., 2009; Ludwig & Currie, 2010). Researchers who excluded those factors from the participants showed more accurate results as Bergmann et al. (2007) excluded mothers with increased risk of premature delivery, diabetes, smoking and consumption of alcohol. Bergmann et al. (2007) reported that birth length was significantly different in supplemented group compared to the control group. The pre-pregnancy BMI and weight gain was not different in both groups. Furthermore, an intervention study in Chile reported increases in birth weight and length, in low-income and underweight women (BMI less than 21.2 at 10 weeks of gestation), after 30 weeks of DHA/EPA supplementation (Mardones et al., 2008).

5.1.3 gestational age

In APrON study, we did not find any significant difference gestational age between the supplemented group compared with non-supplemented group ($p= 0.537$). Similar to our findings, Innis & Friesen 2008; Bergaman et al., 2008; Knudsen et al., 2006; reported DHA/EPA supplementation during pregnancy did not associate with an increase in the length of gestation. Contrarily, Makrides et al., (2010) found that DHA/EPA supplementation increases length of gestation. There are also studies reported that taking fish oil supplements (Grandjean et al., 2001; Olsen et al., 1992; Makrides et al., 2010) and high fish consuming population (Olsen et al., 1986) during pregnancy increases the length of gestation. In addition, studies found association between DHA supplement and the increase of the length of gestation because the dose was higher than APrON (800mg DHA/day) (Makrides et al., 2010). Studies that find differences between

supplemented and non-supplemented group may have other factors in the women that would have contributed to the difference such as pre-pregnancy BMI, exercise, genetic predisposition and smoking (Sasaki et al., 2006; Fleten et al., 2010). Also, the dietary habit is a major factor in the offspring development.

5.2. Effect of DHA/EPA supplement during lactation

5.2.1 Breast milk

Our study found that the composition of EPA and DHA in breast milk was higher in mothers who reported taking DHA/EPA supplements during pregnancy and lactation than in those who did not take supplements. Whereas the relative percent of AA was lower but not significant in mothers who reported taking DHA/EPA supplement during pregnancy and lactation than those who did not take supplements. In the study conducted by Helland et al. (2006), the composition of DHA and EPA was higher and AA was lower in breast milk mothers who were supplemented with cod liver oil than mothers who were not supplemented at 3 months. Furthermore, a Mexican study showed that there was lower but not significant difference in the composition of AA and significant higher composition of DHA between the DHA supplemented group and placebo group in breast milk at 1st month (Mhoff-kunsch et al., 2011).

In conclusion, APrON findings are similar to the literature findings which are the DHA/EPA supplement increase the relative percent of DHA and EPA and lower AA in breast milk. Our results showed that DHA/EPA supplements increased DHA and EPA and decreased the AA status of mothers (Vanet et al.,

1995). The possible reason for the lower AA in the supplemented group may because of the higher EPA that inhibit delta-5-desaturase enzyme that is responsible for the biosynthesis of AA (Sears, 1995). During lactation, a boost of metabolic fuels upholds high rates of lipid and protein synthesis. In the lactating mammary gland, there is a high demand of the LC-PUFAs DHA and AA for incorporation into milk (Rodriguez-Cruz et al., 2006). Some degree of competition between DHA and AA is conceivable since supplementation with DHA have been reported to reduce milk AA, and probably vice versa (Van et al., 2009).

5.2.2 maternal plasma

In APrON maternal plasma, the composition and the of EPA and DHA was higher in mothers who reported taking DHA/EPA supplements during pregnancy and lactation than in those who did not take supplements at 3 months postpartum. Whereas the relative percent of AA was significant in mothers who reported taking DHA/EPA supplement during pregnancy and lactation than those who did not take supplements. The concentration of AA was not significantly different between supplemented and non-supplemented group at 3 months postpartum.

Helland et al. (2006) reported that the composition of DHA, EPA and total n-3 in mothers' plasma was higher between mothers who were supplemented with cod liver oil (10 mL, n-3) than mothers who were not supplemented at 3 months postpartum. In their 2008 study, Bergmann et al. (2008) reported that taking a DHA supplement of 200mg/day from mid-pregnancy through maternal lactation

increased the DHA status of mothers by 20% than non-supplemented mothers in RBC at 3 months. Additionally, fish oil supplements are rich in DHA and EPA, which increase the level in maternal plasma and breast milk and this was found in the current study. Taking a 200mg/day supplement as shown in the study by Bergmann et al. (2008) resulted in the same percentage of increase in DHA status as in the APrON study. It is not clear if the concentrations seen in the current study are the optimal levels. The obtained result is similar to the literature results in plasma PL and breast milk.

5.3 Comparison of the composition of fatty acids in breast milk and plasma in the current study to published values

Mothers around the world want their infants to get all the nutrients that they need during pregnancy and after birth. As such, DHA and AA are really important for infants' development. We looked at the value of APrON breast milk and plasma and compared it with published literature values. Current study found that relative percent of DHA and ALA was higher at 3rd trimester than 3 months postpartum in plasma. Previous study showed that DHA composition was higher in the 2nd trimester than 3 months postpartum in African American women (Stark et al., 2005) and 3rd trimester than 6 months postpartum in Netherlands women (Al et al., 1995). There is an increase in the conversion of DHA from DPA during pregnancy, which could potentially ascribed to the requirement to meet the increased needed for DHA in the fetal brain. The amount of DHA in the plasma PL also depends on the DHA mobilization and/or metabolism of DPA as well. On

the other hand, increasing the relative composition of DHA and EPA reduce plasma AA concentrations (Arterburn, L. M., Hall, E. B., & Oken, H. 2006; Stark et al., 2005).

The relative percent of DHA in APrON mothers in plasma PL at 3rd trimester was 3.4± 1.1% and 2.4±0.9% at 3 months postpartum. The relative percent of ALA was 0.4±0.2 at 3rd trimester and was 0.3±0.2 at 3 month post partum. At 3 months post partum, previous researches reported that the composition of DHA was 1.4± 0.4% in African American women (Stark et al., 2005) and 1.7± 0.3% in Italian women (Marangoni et al., 2000). At 3rd trimester, one Canadian study (British Columbia) reported that the composition of DHA was 5.03± 0.17 % (Elias & Innis et al., (2001). The composition of ALA, (0.37± 0.02%), was similar in Elias & Innis. (2001) to APrON study at 3rd trimester. Also, at 3 months post partum, the composition of ALA was 0.4± 0.2% (Stark et al., 2005) and was 0.3±0.1% (Marangoni et al., 2000).

In the APrON study, the relative percent of EPA and AA was lower at 3rd trimester than 3 months postpartum. In the current study, AA concentration was higher at 3 months postpartum than 3rd trimester in plasma. A previous study showed that AA was lower at 2nd trimester than 3 months postpartum (Stark et al., 2005) and at 3rd trimester than 6 month postpartum (Al et al., 1995). The current study found that the composition of AA was 7.6± 1.6 and EPA was 0.5± 0.5 at 3rd trimester in maternal APrON women. However, at 3 months postpartum, the composition of AA was 8.9± 1.9% and EPA was 1.0± 0.5% in

APrON women. Elias & Innis et al. (2001) reported that AA was 8.6± 0.3% and EPA was 0.53± 0.04% at 3rd trimester. At 3 months post partum, some researchers reported that the composition of AA was 9±1.4% (Stark et al., 2005) and 7.6 ±1.2% (Marangoni et al., 2000) and EPA was 0.4± 0.2% (Stark et al., 2005) and was 0.3±0.1% (Marangoni et al., 2000) . However, at 3 months postpartum, the percentage of EPA is higher in APrON (1.0± 0.5%) than African American (0.4± 0.2) (Stark et al., 2005) and Italian women 0.3± 0.1 % (Marangoni et al., 2000).

In the APrON mothers, the mean relative percent of DHA (0.3± 0.3%) in breast milk at 3 months postpartum was similar to other studies (>0.2%) in German women (Bergmann et al.,2008), Italian women (Agostoni et al., 2003 ; Marangoni et al., 2000), Australasian women (Stoney et al., 2004), Swedish women (Xiang et al., 2000) and Dutch women (Smith et al., 2000). However, in Brazilian and American studies, the relative percent of DHA in breast milk was ≤ 0.2% (Da et al., 2010; Bopp et al., 2005). The lowest DHA contents in breast milk worldwide are in American women due to the changes in the diet (Arterburn et al., 2006). The relative percent of EPA in APrON was 0.1% which is consistent with that reported in other studies (Bergmann et al., 2008; Da et al., 2011; Xiang 2000; Stony et al., 2004). The composition of AA in APrON mothers was 0.5%, which was similar to Agostoni et al., 2003; Da et al.,2011; Bergmann 2008; Xiang et al., 2000 studies. In American and Brazilian studies the composition of AA was reported to be a little lower 0.4% (Costa et al., 2010; Bopp et al., 2005).

Previous studies showed that the high composition of LC-PUFA, especially DHA, in breast milk is influenced by maternal DHA intake and there might also be an influence of LC-PUFA stores from the last trimester of pregnancy (Jensen et al., 1995; Lauritzen et al., 2001; Koletzko et al., 2007). According to data from Demmelmair et al. (1988), it was reported that 30% of milk LA concentration depends on the essential fatty acid intake from diet. The maternal PUFAs status influences the availability of essential fatty acids either for transfer through or conversion within the placenta for supplying human milk for the baby (Dangat et al., 2010).

To summarize, in the APrON study, the composition of ALA and DHA was the same in non-supplemented women who were feeding breast milk only and non-supplemented mothers who were feeding their infant with a combination of formula and breast milk. However, the composition of DHA was found to be higher in mothers RBC who were feeding their infants formula than mothers who were feeding their infant breast milk (Van et al., 2011). In the APrON study, there was a trend for the composition of EPA to be higher in non-supplemented mothers who were feeding their infant breast milk only than non-supplemented women who were feeding breast milk and with a combination of formula milk (p-value 0.1931). A study reported that mothers who were breast-feeding have less EPA in their RBC than those who were feeding their infants formula. This may suggest that the composition of DHA and EPA in breast-fed women will be higher or the same as women who were mix feeding transfer to the breast milk (Xie & Innis, 2008). In APrON plasma, the relative percent of DHA, EPA, ALA and AA

was similar to the literature status during 3rd trimester and 3 months postpartum. Comparing APrON values with literature values is similar to many studies done in Germany, Italy, Australia and Sweden. However, American and Brazilian population have a lower value than the APrON value. The relative percent of DHA, EPA and AA in APrON maternal plasma is in the range of literature values. Women in the British Columbian study had higher DHA composition 5.03± 0.17 % (Elias & Innis et al., 2001) than APrON (3.4±1.1%) at 3rd trimester, which may the dietary intake of fatty fish is higher in that province than in Alberta.

5.4 Limitations and Future Research Directions

This is the first study in Canada with large sample size investigating the effect of the supplement during pregnancy and lactation. This study did not include dietary intake which would have contributed to DHA and EPA status, particularly in the women who were not taking a supplement. Dietary intake evaluation is important to assess nutrition status. Dietary assessment showed food habits, nutrient intake level, available food supplies and cultural and ethnic dietary pattern (Quatromoni & Cook, 1986). Moreover, APrON mothers took different doses of DHA/EPA supplements. The range of the DHA in the supplement was from 7.5 to 1350mg (median= 200) and the range of EPA was from 7.5 to 1200mg (median=300). We did not look at how is the different dose of DHA and EPA affect the maternal FAs status. We just looked at the average of the dose and how its affect FAs status.

Furthermore, we were missing 16% of our participants in the study as some mothers, who were lactating, gave blood samples but not breast milk samples. Very few women who exclusively formula fed so we could not look at the effect of breast feeding compared with not breast feeding on maternal DHA status. Taking a spot sample is an advantage to get breast milk samples from mothers is a limitation as we were not able to look at DHA concentration in milk. As exercise increase mobilization and utilization of fatty acids, having data on maternal exercise would show the influence of the amount of supplement in breast milk and plasma. Bopp et al., study (2005) showed that after half an hour of exercise, there was an increase in LA and ALA concentrations in breast milk. There is a strong evidence for the importance of these FAs in infant. Have infant's status in this study is important to look at the correlation between the fatty acids in breast milk and maternal plasma. Moreover, infants' follow up to later ages is important. DHA accumulates in cerebral cortex during the period of significant brain growth which is between the last trimester of pregnancy and the second year of life. The growth of the brain is completed by 5 to 6 years of age. For future researches, analysis infant and childhood status confirm that they got the fatty acids that they needed. Moreover, one needs to include the analysis of maternal dietary intake of EFAs during pregnancy and lactation.

Finally, future directions of research to understand the role of dietary FAs in breast milk fatty acid composition and the relationship with infant fatty acid status. Determine relationship between infant essential fatty acid and brain development, cognitive skills and behavior.

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Appendix A

Summary of manuscripts used in the critical review of the literature on maternal and infant fatty acid status

Ref	Population	Purpose	Study Design	Dietary Treatment/ Dietary Measures	When were mother's studied	what lipid fraction did they measure?
Marangoni et al., 2002. Prostaglandins, Leukotrienes & Essential Fatty Acids (PLEFA). 66 (5-6), 535-540	95 Italian pregnant mothers average of the age 30y (23-42) mothers who gave blood sample. average of the age 30.5y (20-45) mothers who gave breast milk sample.	To qualitatively and quantitatively evaluate the FA composition of human milk from Italian mothers, throughout extended lactation with particular emphasis on the LC-PUFA	Longitudinal observational study	Not measured.	Day 1 and 3 months postpartum	Total plasma fatty acids
Rump and Hornstra, 2002. Clin Chem Lab Med. 40(1):32-39	889 mother-infant pairs lived in Netherlands. Age 29.3±4.4 y	To provide a description of the distribution of n–3 and n–6 polyunsaturated fatty acids in the plasma phospholipids fraction of pregnant women remaining on a Western-style diet and their neonates.	Longitudinal observational study	Dietary FAs intake was evaluated in a sub-section (288 women) of the population through questionnaires in the 2nd trimester and nutrient intake was estimated by using the Netherlands Nutrient Databank and Netherland Food Table	Plasma sample was collected during the 1st, 2nd, 3rd trimester and after delivery. Umbilical vein blood was also taken right after birth (at birth)	Maternal plasma plasma was separated blood sample was obtained from the umbilical vein.

Helland I et al.,2006. Journal of maternal-fetal and neonatal medicine. 19 (7):397-406	590 pregnant Italian women average age NA. Inclusion criteria were between 19 and 35 years of age,	To study the effect of supplementing pregnant and lactating women with marine n-3 polyunsaturated fatty acids (PUFAs) as compared to n-6 PUFAs related to maternal and infant lipid level	Random clinical trial	FFQ during pregnancy (18 & 35 weeks). Women randomly receive 10 mL/day of cod liver oil or corn oil until 3 months after delivery	At 18 (1st trimester) and 35 (3rd trimester) weeks of pregnancy	Plasma PL
Marc I et al., J Nutr. 141(2):231-6. Epub 2010 Dec 15	23 pregnant : DHA group (n=10). reference group (n=22). Average age of DHA group: 27.2± 4.3y Average age of reference group: 26.9± 4.1y	To evaluate the effect of an immediate DHA supplementation of nursing mothers on the daily DHA intake of their very preterm infants	Longitudinal study	DHA Supplement (1200 mg/d) each capsules 200 (2 cap 3 times a day) from week 8 to 12 after birth. Questionnaire for maternal fish consumption at the 1st month before delivery and at week 7th post birth. Daily consumption of fish meals (90 g) 1 mo before pregnancy did not differ between mothers in the DHA (0.2 ± 0.1) and reference (0.3 ± 0.2) groups, nor did it differ in the month before week 7th in the DHA (0.1 ± 0.1) and reference (0.2 ± 0.3) groups.	Blood samples mothers in the 1st week before taking supplements and at wk (birth to day 49)	Total plasma
Costa et al., 2011. .46:537-543	49 healthy mother aged from 15-19 49 Infants lived in Brazil	To determine the levels of trans-octadecenoic acid (C18:1-trans) and transisomers of linoleic acid (18:2-trans), as well as long-chain polyunsaturated fatty acids (LC-PUFA), in the plasma from infants of adolescent mothers at 3 months of age,	Longitudinal observational study	FFQ + 24 hours recall.	At 3 months postpartum	Free fatty acids (FAA%) of total lipids in maternal plasma and breast milk

		exclusively breastfed, and the relationship with the levels of the same isomers in plasma and milk of the mothers				
Imhoff-kunsch B et al., the J of nutrition. 321-322.2010	1094 pregnant in gestation between 18-22 weeks DHA group age : 26.4± 5.1y placebo group age: 25.8± 4.8y	To determine the effect of DHA supplementation during mid-pregnancy to parturition and its influence on breast milk fatty acid levels at 1 month postpartum in Mexican women	Double-blind randomized clinical trial	Women were randomized to treatment: 485 women in DHA group & 488 in placebo group. Received 400 mg of algal DHA or placebo until delivery. DHA capsules : 2 capsules/d of DHA contain 200 mg Placebo capsules: contained olive oil and were similar to the DHA capsule in appearance and taste. Mom at early gestation : FFQ (110-item) Babies at the age of 12 - 18 months: FFQ (70-item) Estimates of (n-3) PUFA by calculated the grams per day of the various food items that were consumed as an average in this population. Calculations were performed using a special software called SNUT 3.0 (nutriment system), developed at the National Institute of Public Health, Mexico.	Baseline and delivery	Maternal plasma phospholipid

Boris J et al., 2004.Lipids., 39 (12), 1191-1196	36 Danish pregnant women mothers. Age not reported	To investigate the effect of fish oil supplementation in the third trimester of pregnancy and the early lactation period of healthy pregnant Danish women	Randomized control trial	There were 3 groups: fish oil group (FO pregnancy lactation) fish oil group (FO pregnancy) control group: Olive oil (control-1)no oil (control-2) Fish oil supplement(1.3g EPA and 0.9g DHA)-total 2.2g LCPUFA	Supplemented in the third trimester but mothers not studied	Not measured
Bergmann et al 2008, Ann Nutr Metab. 52:157–166	89 pregnant German women and 50 infants average age of vit/min group: 30.0± 4.6y average age of FOS group 31.3± 4.7y average age of DHA-FOS group 30.9±4.6y	To investigate the effect of a daily vitamin/mineral supplement with and without 200 mg DHA from mid-pregnancy through lactation on the DHA concentrations in maternal and infant red blood cell phospholipids (RBC%), and in breast milk FA (%).	Randomized, double-blind clinical trial	1- basic vitamin-mineral supplement (Vit/Min group) 2-Vit/Min plus 4.5 g fructose-oligosaccharide (FOS group) 3-Vit/Min plus 4.5 g FOS plus 200 mg fish oil-derived DHA(DHA-FOS group)	3 months post-partum	Red blood cell fatty acids
Xiang m et al., 2000 Acta Pñdiatr 89: 142±7.	19 Swedish lactating mothers age :29.5 ± 1.0y	To examine the influence of the food composition of lactating women on the concentration of FAs in their milk. Also, to look at the relation between the contents of LCPUFAs in the milk and the rates of increase in weight, length and head circumference after 1 and 3 mo of their exclusively breastfed infants.	Longitudinal observational study	Not measured	Breast milk sample at Colostrums, 1 and 3-months	Not measured

Marangoni et al., 2000. British Journal of Nutrition., 84,103-109	95 women- 10 follow up till 12 months age for breast feeding up to 12 mo group : 29- 36 y age for not breast feeding up to 12 mo group : 21- 42y	to qualitatively and quantitatively evaluate FA composition of breast milk from Italian mothers also, extended lactation with particular emphasis on the LC-PUFA	Longitudinal observational study	FFQ; 1st day, end of the 1st months ,3,6,9,12 months	post partum at day 1 and 3 months	total plasma fatty acids

Appendix B

Summary of manuscripts used in the critical review of the literature on maternal and infant fatty acid status

Continued

Ref	Result of Mothers	Age of infant when were milk's analyzed	Results of mom's milk fatty acid composition	How did they measure essential fatty acid status	Infants status result
Boris J et al., 2004.Lipids., 39 (12), 1191-1196	Not measured	At days 4, 16 and 30 after delivery. Milk sample collected from the beginning and from the end of the feeding.	The composition of LCPUFA and DHA decrease from day 4 - day 30 in the C groups The composition of EPA and DHA increase in FO pregnancy and lactation group than pregnancy only and C group in all time point. % FA FO pregnancy and lactation group at 1 month LA : 11.1± 0.6 ALA : 1±0.1 EPA : 0.7± 0.1 DHA : 1.4± 0.2 % FA FO pregnancy group at 1 month LA : 11.9±0.8 ALA : 1.1±0.2 EPA : 0.2± 0.0 DHA :0.6± 0.1 % FA Control group at 1 month LA : 12.9± 1	Not measured	Not measured

			ALA : 1.2± 0.1 EPA : 0.1±0.0 DHA :0.5±0.0 AA was not reported		
Marangoni et al., 2002. Prostaglandins, Leukotrienes & Essential Fatty Acids (PLEFA). 66 (5-6), 535-540	22 mothers collected plasma blood sample at the 2 time points. total lipids ↓ from 1st day to the 3rd months. The % of FA ↑ from the 1st day compared with the 3rd months in (LA, AA, EPA, DHA) ALA ↓ between the 2 time points. %FA plasma at 3 months. LA : 30.3 ± 0.9 ALA :0.31 ± 0.0 DHA :1.5 ±0.1 EPA : 0.6±0.1 AA : 7.3 ± 0.2	Day 1 and 3 months postpartum	% of LA ALA ↑ and AA, DHA ↓ from the 1st day compared 3 months. EPA and PUFA showed no changes between the 2 time points. Breast milk at 3 months: LA :12.7 ± 0.6 ALA :0.7 ± 0.04 DHA :0.4 ±0.06 EPA : 0.1±0.01 AA : 0.5 ± 0.02	Not measured	Not measured
Fidler N et al.,2000. journal of lipids research. 41,1376-1383	not measured	Milk sample collected at 4 weeks postpartum before taken the supplement and 6 weeks post partum just before 6,12,24,36 and 48 hours after the supplement taken (oral bolus dose of uniformly 13C-labeled DHASCO™).	after 2 weeks of supplement the DHA is higher in the supplemented group than the placebo group. On the other hand no different in the EPA between the two groups before or after the supplements. mean± (median) % wt/wt (study day 0) at 4 weeks postpartum LA : 10.4± (9.5-11.9) ALA : 0.8± (0.7- 0.9) DHA : 0.3± (0.3- 0.42) EPA : 0.1± (0.1-0.09) AA : 0.5± (0.5-0.6)	not measured	not measured

			% wt/wt(study day14) at 4 weeks postpartum LA : 11.6± (10.1-13.1) ALA : 0.8± (0.7-1.4) DHA :0.4± (0.3-0.4) EPA : 0.1± (0.1-0.1) AA : 0.4± (0.4-0.5) % wt/wt placebo (study day 0) at 4 weeks postpartum LA : 8.7± (8-11.7) ALA : 0.6± (0.5-0.6) DHA :0.3± (0.3-0.3) EPA : 0.1± (0.1-0.1) AA : 0.± (0.1 - 0.1) % wt/wt placebo (study day 14) at 4 weeks postpartum LA : 10.2± (8.3-11.7) ALA : 0.7± (0.6-1.1) DHA : 0.2± (0.2-0.2) EPA : 0.1±0.1-0.1) AA : 0.1± (0.1-0.1)		
Costa et al., 2011. .46:537-543	plasma fatty acid % of total fatty acids at 3 months postpartum LA : 37.0±0.8 ALA : 0.1± 0.0 DHA : 0.6±0.1 EPA : 0.5±0.05 AA : 4.1±0.3	at 3 months postpartum	% FA at 3 months postpartum LA : 19.8± 0.7 ALA :0.5±0.04 DHA :0.2±.03 EPA : 0.0±0.0 AA :0.4±0.3	free fatty acids (FAA%) of total lipids in infants plasma	at 3 mo: LA: 3.0 ± 1.0 ALA: 0.2±0.1 AA: 4.5±0.3 EPA:0.3± 0.1 DHA: 1.4± 0.3
Xiang m et al., 2000 Acta Pñdiatr 89: 142±7.	not measured	Colostrums, 1 and 3-months	an increase on LA and ALA and a decrease DHA and AA from 1st day till 3 months %FA at 1 mo LA: 10.3± 0.5 AA: 0.4 ± 0.0 ALA: 1.3±0.1 EPA: 0.1±0.0	not measured	not measured

			DHA: 0.3±0.0 %FA at 3 mo LA:10.9 ± 0.5 AA: 0.4± 0.0 ALA:1.6±0.1 EPA: 0.1± 0.0 DHA: 0.3±0.0		
Rump and Hornstra, 2002. Clin Chem Lab Med. 40(1):32-39	the concentration of DHA was positively related to gestational age but not to the PUFA concentration at birth. the concentration of AA were different between infant born at different gestational ages. higher intake of LA was associated higher concentration of LA and lower concentration of DHA. plasma value at birth: LA 20.7± 2.5 AA 8.6± 1.6 ALA 0.2±0.5 EPA 0.14±0.1 DHA 3.8± 0.8 for umbilical vein value at birth in boys LA 7.6± 1.4 AA 16.7±1.7 ALA NA EPA 0.22± 0.11 DHA 6.12 ± 1.3 for umbilical vein value at birth in girls LA 7.5± 1.4 AA 16.8± 1.6 ALA NA	Not measured	Not measured	umbilical plasma phospholipids	The total concentration of phospholipids associated fatty acid was higher in girls than in boys. The proportion of individual fatty acids wasn't significantly different between boys and girls.

	EPA 0.2± 0.1 DHA 6.1± 1.4				
Helland I et al.,2006. Journal of maternal-fetal and neonatal medicine. 19 (7):397-406	At 35 weeks of pregnancy there were significant differences between the two supplementation groups concerning intake of LA AA EPA DHA. There were no differences between the two maternal groups in plasma at baseline. At the end of pregnancy, the cod liver oil group had significantly higher concentrations of plasma n-3 very long-chain PUFAs and also the ratio of ∑n-3/∑n-6 fatty acids was higher than the corn oil group Cod oil supplemented group at 18 weeks (mg/L) LA : 294.8± 51.1 AA : 114.3± 22.8 ALA: 3.6± 1.1 EPA : 14.7± 11.4 DHA : 94.7± 22.5 Corn oil supplemented group at 18 weeks (mg/L) LA : 291.4± 45.6 AA : 115.3± 22.7 ALA: 3.6± 0.9 EPA : 14.2±10.6 DHA : 94.5± 21.8 Cod oil supplemented group at 35 weeks (mg/L) LA : 321.6± 61.7 AA : 102.8 ±22.7 EPA : 41.5± 17.8 DHA :147.2±34.1	Milk sample collected at 4 weeks and 3 months after delivery The samples were taken from a morning feed (never the first one), 3–5 minutes after the baby started suckling	The level of LA, AA, and ALA didn't change during from 4 weeks to 3 months in both groups. However DHA and EPA decreased after the 3 months in both groups cod oil supplemented group at 3 mo (wt%) LA : 12±3.2 AA : 10.3±0.1 EPA : 0.41±0.2 DHA :1.1±0.6 ALA:1±0.3 Corn oil supplemented group at 4 weeks (wt%) LA : 13.4±3.33 AA : 0.4±0.1 EPA : 0.2±0.1 DHA :0.5±0.4 ALA: 0.9±0.3	concentration of FA in plasma PL	The composition of LA and AA is higher in corn oil group than cod liver oil group in 4 weeks and 3 months Conversly,EPA DHA is higher in cod liver oil than in corn oil group in 4 weeks and 3 months No different in dietary intake, gestational length, birth weight, or head circumference between the 2 groups. Cod oil supplemented group at 4 weeks (mg/L) LA : 194.6± 32.6 AA : 94.0± 18.6 ALA: 1.1±0.3 EPA :22.1±10.3 DHA : 85.0± 17.6 Corn oil supplemented group at 4 weeks (mg/L) LA : 205.3± 34.2 AA : 112.8± 21.4 ALA: 0.9± 0.3 EPA :5.7± 3.5 DHA : 61.7± 14.7 Cod oil supplemented group at 3 mo (mg/L) LA : 206.1 ± 42.6 AA : 87.9± 17.9 ALA: 1.1±0.3 EPA : 25.3±14.4

	ALA: 4.6±1.6 Corn oil supplemented group at 35 weeks (mg/L) LA : 384.1±57.8 AA : 127.7±25.7 ALA: 4.5±1.2 EPA : 11.2± 6.7 DHA : 101.8± 25.9				DHA : 92.3± 22.8 Corn oil supplemented group at 3 mo (mg/L) LA :226.5± 43.3 AA : 109.3± 21.3 ALA: 1.2± 0.3 EPA : 9.7± 7.9 DHA : 72.8± 17
Marc I et al ., J Nutr. 141(2):231-6. Epub 2011	DHA mother had similar BMI before pregnancy and at delivery *At d 49, mothers' plasma DHA concentration in the DHA group was higher than those in the ref group % of DHA, AA, LA, ALA, EPA not reported in the paper	breast milk samples were taken in the 1st week before starting DHA supplementation and at follow-up wk 3 and wk 7.	BM concentration were measured weekly for 7 weeks. however, the formula reported from the company total milk intake (formula + BM) in the DHA group was similar to the ref group The % of total intake of BM didn't differ between the DHA & ref groups At the 7th week, maternal milk DHA in the DHA group was 12 times higher than in ref group The amount of DHA provided to the infants was greater in the DHA group than ref group composition of DHA in the breast milk at week 7th: DHA group 1.92 ±1.10 mmol/L which was 12 times higher than in the reference group (0.15 ± 0.27 mmol/L)	At the 7th week infants' plasma DHA concentration tended to be greater in the DHA group than in ref group and was greater when expressed as a percentage of total fatty acids plasma DHA concentrations in mothers and babies were 2–3 times higher in the DHA group compared with the reference group.	The growth trends for weight, length, and head circumference over the study period in the DHA group did not differ from the ref group Anthropometric measurements and the incidence of neonatal complications in the DHA group did not differ at wk 7 from the ref group

Imhoff-kunsch B et al., the J of nutrition. 321-322.2010	Daily dietary intakes of energy, fat, and PUFA at 18–22 wk gestation did not differ between treatment groups significant different in DHA between baseline and delivery in treatment group supplemented group at delivery: LA :24.7± 3.1 ALA :0.7± 0.2 DHA :1.7±0.5 EPA : 1±0.7 AA : 3.3± 0.9 Placebo group at delivery: LA : 25.2± 3 ALA :0.7± 0.2 DHA : 1.4± 0.4 EPA : 1± 0.6 AA : 3.5± 0.8	one month post-partum Sample were collected at the 1st month: 5 ml of foremilk & 5 ml of hind milk.	Concentrations of both DHA and ALA in breast milk were higher in the DHA group compared with the placebo group. Breast milk concentrations of PUFAs did not differ between treatment groups. supplemented group at 1 mo LA :16.5± 3 ALA :1.4±0.5 DHA : 0.2±0.1 EPA : 0.1±0.1 AA : 0.4±0.5 non-supplemented group at 1 mo LA :15.8± 3.4 ALA :1.3±0.5 DHA : 0.2±0.1 EPA : 0.2± 0.2 AA : 0.4± 0.1	Not measured	Not measured
Bergmann et al 2008, Ann Nutr Metab. 52:157–166	DHA and EPA in maternal blood was higher in the 3rd group than the two other groups. AA were not different between the 3 groups and little lower in the 3rd group % FA of total fat at 3months in DHA -FOS group DHA: 6.5± 1.8 AA: 15.8± 2.1 EPA: 1.6±0.5 % FA of total fat at 3months in FOS group	3 months after delivery	DHA and EPA in breast milk was higher in the 3rd group than in the two other groups. ARA were not different between the 3 groups %FA at 3months DHA:0.5± 0.2 AA: 0.5±0.11 EPA:0.1±0.1	-% fatty acids in RBC	DHA and EPA in infants RBC was higher in the 3rd group than in the two other groups. ARA were similar in the 3 groups %FA of FOS-DHA group at 3 mo: DHA: 9.8± 2.2 EPA: 1±0.3 AA: 16.1±2.0 %FA of FOS at 3 mo: DHA: 6.9± 2.8 EPA: 0.7± 0.4 AA: 17.3± 3.35

	DHA: 5.2± 2.1 AA: 15.8± 2.5 EPA: 1.2±0.5 % FA of total fat at 3months in vit/min group DHA: 5.1±1.5 AA: 15.8±2.1 EPA: 1.2±0.3 LA and ALA not shown				%FA of vit/min at 3 mo: DHA: 7.5± 2.0 EPA: 0.7±0.2 AA: 17.8±2.1
Marangoni et al., 2000. British Journal of Nutrition., 84,103-109	Plasma of total lipids doesn't change significantly from day 1 to 3 months % FA of toal fat at 3 months LA :25.5± 3.2 AA : 7.6± 1.2 ALA : 0.3±0.0 EPA : 0.3±0.1 DHA : 1.7±0.3	Hind milk, colostrums and midstream milk 1st day, end of the 1st months ,3,6,9,12 months	from 1st day throughout 12 months: increase in saturated fatty acids decrease in monosaturated fatty acids no sig change in polyunsaturated fatty acids no sig change in ALA LA EP A but a little decrease in AA DHA total lipids is increased from 1 day till 3months in fore-midstream milk-hind milk %FA at 3months LA :12.1±2.1 AA : 0.5±0.1 ALA : 0.7±0.4 EPA :0.1±0.0 DHA : 0.3±0.1	not measured	not measured

Appendix C

Summary of manuscripts used in the critical review of the literature on maternal and infant fatty acid status

Continued

Ref	Addition Results	Strengths & Limitations	comparison to my results if applicable
Boris J et al., 2004.Lipids., 39 (12), 1191-1196	↑ LCPUFA and DHA in FO pregnancy and lactation group than the 2 other groups.	Strengths : breast milk taken from the beginning of the feed and the end. 3 times collected milk. Limitations: no dietary intake, measured the supplement.	NA
Marangoni et al., 2002. Prostaglandins, Leukotrienes & Essential Fatty Acids (PLEFA). 66 (5-6), 535-540	1st day: Significant correlations between plasma and milk showed in LA, ALA and AA 3 months: Significant correlations between plasma and milk showed in LA, ALA and DHA	strengths: 2 times collected milk. Limitation: small sample size for the 2 time point. Didn't excluded women who deliver their baby less and more than 40 week.	In breast milk at 3 mo AA and EPA was the same in APrON and this study LA and ALA ↑ in APrON DHA was ↓ in APrON IN PLASMA at 3 mo ALA, EPA, DHA, AA ↑ IN APrON than this study LA is lower in APrON
Fidler N et al.,2000. journal of lipids research. 41,1376-1383	The supplement is affecting the % of DHA.	strengths: 2 times collected milk. 7 day weighted dietary records in both groups. Caloric intakes and food composition were calculated with nutrition database which is based on a German nutrient survey with about 12,000 food items. Limitations: small sample size	NA
Rump and Hornstra, 2002. Clin Chem Lab Med. 40(1):32-39	all ∑n-3 and ∑n-6 fatty acids were positively related to the length of gestation.	Strengths : Larger sample population, first report of percentile values for absolute and relative PUFA concentration. excluded multiple pregnancy and preterm delivery. Limitations: The assessment of nutrient intake using the Netherland Food Table does not allow the calculation of individual n-3 and n-6 FAs except LA and total PUFAs.	NA

Helland I et al.,2006. Journal of maternal-fetal and neonatal medicine. 19 (7):397-406	There is colooration between AA, EPA,n-3, n-6 in breast milk and in infant plasma. When infant received cod liver oil supplement;cod liver oil didn't affect plasma concentration of DHA if the mothers were supplemented with cod liver oil. On the other hand , the concentration of EPA increased if the mothers were supplemented with cod liver oil. If the mother supplemented with corn oil both EPA and DHA increased.	Strengths: randomize, double blind, self administrated FFQ, 1st study where mothers supplemented during pregnancy and lactation	% of DHA, EPA,AA and LA was higher in APrON breast milk than this study. However % of ALA was lower in APrON
Marc I et al ., J Nutr. 141(2):231-6. Epub 2011	DHA supplementation during early lactation with low dietary DHA intake increase the plasma DHA status in very preterm infant	Strengths: clinical follow up information for newborn outcomes from the Canadian Neonatal Network Database. Fatty acid measured weekly (BM & plasma) Limitation: small sample size. twins included. Participants required in the same period of time. They were not randomized. Didn't have blood sample for mother and infants for the ref group. The infants who received blood transfusions in both groups may influenced the plasma DHA concentration	In the DHA group, the linear trend over time for DHA concentrations in mothers' plasma was associated with the trend of DHA concentration in milk However, there was no significant association between trends in the plasma DHA concentration of very preterm infants and their DHA intake
Costa et al., 2011..46:537-543	Composition of free fatty acid of total lipids: ALA was higher in milk than maternal and infant plasma. LNA and EPA was higher in maternal plasma than in milk and infant plasma. DHA and AA was higher infant plasma than in milk and maternal plasma negative correlation between AA in milk and LA in infant plasma negative correlation between EPA in milk and LA and AA in infant plasma positive correlation between LA and	Strengths: measured the milk and plasma for maternal and infant at the same time point. used 2 methods for assess dietary intake	% of DHA AA ALA EPA were higher in APrON breast milk than this study. However, LA was lower in APrON

	AA in infant plasma positive correlation between EPA and ALA in infant plasma		
Marangoni et al., 2000. British Journal of Nutrition., 84,103-109	from the 1st day to 12 months: PUFA ALA almost stable AA DHA decrease EPA ALA short-chain FA increase	Strengths: take the dietary intake and breast milk samples 6 times during the year Limitation: small sample size plasma compared in two time points	% of AA, EPA and DHA were the same in APrON breast milk and this study. However LA ana ALA were higher in APrON than this study. % of AA ALA EPA were higher in APrON plasma than this study however, LA and DHA were lower in APrON.
Xiang m et al., 2000 Acta Pñdiatr 89: 142±7.	there is a positively correlated with the brain weight at 1 and 3 months of age. (Brain weight was assessed as a function of the head circumference as described by Cooke et al., 1977)	Strengths: have 3 time points for breast milk. Limitations: small sample size. exclusively breastfed infants are not known,	% of ALA LA AA were higher in APrON breast milk than this study. However, the % the same in EPA and DHA.
Imhoff-kunsch B et al., the J of nutrition. 321-322.2010	Positive correlation between DHA concentrations in maternal plasma and DHA in breast milk at the 1st month in both groups. Daily dietary intake didn't differ between treatment groups.	Strengths: randomized, large sample size, double blind, weekly visit during pregnancy, Follow-up through 18 mo. High items in the FFQ. Reported consumption of DHA in offspring in many time points. Measure of the birth weight, length and Head circumference very accurate 1mm. Limitations: FFQ depends on their memory. Did not assess mother's diet after delivery. Women who had twins were not excluded. Did not take mothers weight during the weekly visit. Gestational diabetes affected the weight which not considered. Did not observe the genes history were really affecting baby's lengths	excluded any women had taken regular fish oil or DHA
Bergmann et al 2008, Ann Nutr Metab. 52:157–166	DHA concentrations of the 3-month-old infants showed a significant correlation with the maternal RBC-DHA concentrations at 3 months after delivery	Strengths: randomized, double-blind clinical trial of 3 dietary supplement groups Limitations: many women didn't give blood sample for their babies	composition of AA and EPA in breast milk was the same in APrON and this study. However, the composition of DHA was higher in this study than APrON at 3 months The composition of AA, DHA and EPA was higher in this study in DHA -FOS group than in APrON at 3 months