Evaluation of the Role of Phospholipids in Fatty Acid Delivery to the Fetus During Pregnancy

by

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AUTHOR'S DECLARATION

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

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Abstract

Docosahexaenoic acid (DHA) is an omega-3 highly unsaturated fatty acid that plays an important role in fetal brain development. The fetal demand for DHA appears to be met by placental transport and various maternal physiological adaptations. Estrogen, which is elevated during pregnancy, is associated with increased DHA biosynthesis, but estrogen is also implicated in the synthesis of phospholipids, specifically the regulation of phosphatidyl ethanolamine methyltransferase (PEMT) that methylates phosphatidyl ethanolamine (PE) to form phosphatidyl choline (PC). PE in various tissues is typically enriched in DHA relative to PC, but PC is the dominant phospholipid in plasma. The conversion of PE to PC by PEMT as a potential mechanism to mobilize maternal DHA to plasma for placental transport was examined in diets that mimic human fatty acid intakes with and without DHA as well as in a standard rat chow diet low in DHA. Rats were examined at baseline, day 15 and day 20 of pregnancy, and 7 days post partum. The accumulation of fatty acids into lipid fractions in maternal plasma and liver were determined with DHA in PC increasing dramatically at day 20, particularly in plasma. PEMT mRNA expression was increased at day 15 and PEMT liver protein tended to be increased at day 20 of pregnancy. In addition, increased dietary DHA appeared to be associated with increased expression of PEMT suggesting DHA hepatic concentrations may be upregulating PEMT by a substrate feed forward mechanism. Given the supporting role of PEMT in mobilizing DHA, and the extent of the increase of DHA in plasma PC during the last stages of pregnancy, other mechanisms are likely involved that remain to be elucidated.

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List of Abbreviations

DHA	Docosahexaenoic acid
DPA n-6	Docosapentaenoic acid n-6
ARA	Arachidonic acid
DAG	Diacylglycerol
TAG	Triacylglycerol
PC	Phosphatidylcholine
PE	Phosphatidylethanolamine
PEMT	Phosphatidylethanolamine Methyltransferase
FFA	Free Fatty Acids
TLC	Thin Layer Chromatography
HPLC	High Performance Liquid Chromatography
GC	Gas Chromatography
qPCR	Quantitative Polymerase Chain Reaction

Chapter 1

Introduction

Docosahexaenoic acid (DHA) is a polyunsaturated fatty acid (PUFA) known to have an important role in fetal neuronal development, and the fetus is dependent on maternal supply (Larque et al., 2006). Evidence indicates that pregnant women in Canada do not meet the dietary recommendations for DHA intake (Denomme et al. 2005). Sex is associated with different fatty acid profiles, including, DHA in various tissues in both humans (Geppert et al., 2009; Metherel et al., 2009) and rat models (Kitson et al., 2012). More specifically, estrogen has been hypothesized to regulate the function of various proteins involved in lipid metabolism, such as elongases and desaturases that are responsible for the *de novo* synthesis of PUFA (Kitson et.al. 2012). Estrogen levels rise during pregnancy (Yoshinaga et al. 1969) and DHA has also been shown to increase in maternal plasma, especially during the third trimester of pregnancy when estrogen levels are at their peak (Stark et.al. 2005). Additionally, evidence suggests that there is preferential deposition of PUFA, especially Arachidonic acid (ARA) and DHA, in fetal tissues during pregnancy (Larque et al. 2006).

Plasma PUFA are generally found in triacylglycerols (TAG) and phospholipids; however, long chain PUFA, such as DHA and ARA, tend to concentrate in the phospholipids (Stark, 2008). Plasma phospholipids are predominantly composed of phosphatidylcholine found in lipoproteins (Christie, 1989). Phosphatidylcholine (PC) is synthesized through the Kennedy pathway, or by phosphatidylethanolamine (PE) methylation through phosphatidylethanolamine methyltransferase (PEMT). Estrogen has been shown to up-regulate the PE methylation pathway (Resseguie et al. 2007). PE

typically has higher DHA content than PC (Kitson et al., 2013), Therefore methylation of PE to form PC could be a mechanism to increase the DHA content of PC. Up-regulating the PE methylation pathway during pregnancy through estrogen action could be a mean to increase maternal plasma DHA levels to make DHA more readily available for uptake by the fetus. Additionally, PEMT activity is affected by the intake of specific fatty acids (Clandinin et al. 1994); therefore, understanding the effect of dietary intake of DHA during pregnancy using a diet with a fatty acid profile that resembles the fat intake of humans on PEMT activity is essential to fully characterize the mechanisms involved in mobilizing maternal DHA stores to plasma during pregnancy.

The aim of this thesis is to understand the role of PEMT during pregnancy on maternal DHA in various lipid pools during different dietary fatty acid intakes. A pregnant rat model was used to determine the effect of pregnancy on PEMT expression and activity. Rats were bred, and upon confirmation of pregnancy, they were placed either on a diet that mimics the typical western diet, with or without additional DHA, or remained on a chow control diet. The expression of PEMT was measured in maternal liver at various stages of pregnancy, and in post partum by determining mRNA and protein levels, as well as enzyme activity. Additionally, plasma and liver phospholipid fatty acid composition was determined to characterize the shift in PC and PE fatty acid composition during pregnancy. Understanding the mechanisms of DHA delivery to the fetus, the role of maternal diet, and maternal adaptations to increase the bioavailability of DHA for fetus delivery, form an important base to determine maternal DHA requirements in order to establish evidence based recommendations to support pregnancy and fetal development.

Chapter 2

Biochemical foundations

2.1 DHA Role in Fetal Development

Several randomized controlled clinical trials have indicated a positive effect of DHA supplementation on brain development as reviewed previously (Innis, 2007), while the effect of low DHA intake on retinal electrophysiology and visual acuity is established in non-human primates (Neuringer et al. 1988). In particular, the third trimester of human pregnancy appears to be a critical phase of DHA incorporation into neural tissues (Martinez 1992). The vulnerability of nervous system to DHA deficiency relates to the physiological structure of the brain; 60% of brain matter is composed of fat (Kurlak et al. 1999), and DHA is one of the most abundant fatty acids in neurons, especially in neuronal membranes and vesicles of the synapse (Breckenridge et al. 1972). Incorporation of DHA into the phospholipids in the bilayer membranes is thought to increase their viscosity, allowing for more efficient signal transduction (Breckenridge 1972). Other physiological effects are produced by metabolites synthesized from DHA. N-docosahexaenoylethanolamide (DEA) is a synaptogenic DHA metabolite that acts as a mediator of DHA induced synaptogenesis and hippocampal neuronal growth (Kim et al. 2011). The effect of DHA and DHA derived synaptogenic mediators has only been demonstrated in neuron cell cultures from 18-day-old mice embryos (Cao et al., 2009)

Pregnancy is generally associated with hyperlipidemia: the significant increase in plasma fatty acids, hypothesized to be an adaptation to facilitate the availability of essential fatty acids for the growing fetus (Hachey, 1994; Warth et.al. 1975).

Additionally, the relative amount of DHA in maternal bloodstream is also shown to increase during pregnancy, especially in the plasma phospholipids fraction (Otto et al. 2001). DHA is shown to increase by almost 50% in plasma phospholipids, while other n-3 fatty acids increase by only 20% (Postle, et al. 1995). DHA is also selectively transported through the placenta, and deposited in fetal tissue through specific placental fatty acid delivery mechanisms (Larque et al. 2011). Increased DHA biosynthesis from 18:3n-3 (Burdge and Calder. 2006) is a possible mechanism to meet fetal DHA demand; however, the mobilization of DHA to the maternal circulation from other tissues is another possibility that is relatively understudied. The effect of dietary intake of DHA and other essential fatty acids during pregnancy has been shown to affect maternal fatty acid metabolism, as well as tissue composition of the fetus (Childs et.al. 2010). Examining maternal intake of essential fatty acids during pregnancy is important, especially since the majority of Canadian women eat low levels of DHA during pregnancy (Denomme et.al. 2005).

2.2 Overview of Phospholipids

Phospholipids are differentiated based on the characteristic polar head group attached to the glycerol molecule. The major phospholipid classes include phosphatidyl-ethanolamine (PE), phosphatidylcholine (PC), phosphatidylinositol (PI), phosphatidylserine (PS), and sphingomyelin. PC followed by PE constitutes the majority of phospholipids in the lipid bilayer in cellular membranes of eukaryotes. PC is predominantly found on the outer layer, while PE is concentrated in the inner layer (Christie 1989). In plasma, phospholipids are mainly located in the monolayer membrane of lipoproteins, and constitute of almost 99% PC and lyso-phosphatidylcholine (lysoPC)

(Christie, 1989). In contrast, erythrocytes in blood have lipid bilayers and therefore contain significant amounts of both PE and PC. PI and PS occur in lower concentrations in lipid membranes. However, PI is a metabolically important phospholipid, which is highly abundant in brain tissue (almost 10% of total phospholipids in the brain) (Holub et.al. 1970)

2.3 De novo synthesis of Phospholipids (Kennedy Pathway; Figure 2.1)

Phospholipids are synthesized in various locations within the cell, including the cytosol, endoplasmic reticulum, and the mitochondrial inner membrane (Vance and Vance, 2004). The first step is the addition of an acyl group from acyl-CoA to sn-glycerol-3-phosphate by sn-glycerol-3-phosphate acyltransferase (GPAT) to form 1-acyl-sn-glycerol-3-phosphate, also known as lyso-phosphatidic acid (lysoPA). Two main isoforms of GPAT have been characterized, which are encoded by different genes, and are localized in different locations within the cell. One GPAT isoform is limited to the mitochondrial outer membrane, while the second isoform is located in the endoplasmic reticulum (Vance and Vance, 2004). The mitochondrial isoform of GPAT is known utilize saturated acyl CoAs, while the endoplasmic reticulum isoform does not have such specificity. Consequently, the mitochondrial GPAT is thought to be responsible for the high proportion of saturated fatty acids in the sn-1 position of the glycerol-3-phosphate backbone. Phosphatidic acid (PA) is then formed through the acylation of the sn-2 position of lysoPA by the action of acyl-CoA:1-acyl-sn-glycerol-3-phsophate acyltransferase (AGPAT), which preferentially utilizes unsaturated fatty acyl CoAs. The resultant PA can then be used as a substrate for the synthesis of phosphatidylglycerol and phosphatidylinositol, but PA can also be dephosphorylated by phosphatidic acid

phosphatase to form diacylglycerol (DAG). DAG can then be used to synthesize PE and PC, in addition to triacylglycerol (TAG).

2.4 PE Synthesis

PE comprises 20-40% of the phospholipids in the membranes of mammalian cells. It is biosynthesized through two main pathways: the CDP-ethanolamine pathway, and the PS decarboxylation pathway, originally described by Kanfer and Kennedy in 1964 (Kanfer and Kennedy, 1964). In the latter pathway, PS is transferred from the ER, where it is synthesized, to the mitochondrial inner membrane, where it is converted to PE by the action of PS decarboxylase (Shiao et al., 1995) However, evidence suggests that the route of PE synthesis is tissue dependent with the CDP-ethanolamine pathway being the main pathway in mammalian hepatocytes (Sundler and Akesson 1975; Tijburg et al. 1989) and heart (Zelinski and Choy 1982). The first step in the CDP-ethanolamine pathway is the phosphorylation of ethanolamine by ethanolamine-kinase in the cytoplasm forming P-ethanolamine. This step is followed by the conversion of P-ethanolamine to CDP-ethanolamine, in a reaction catalyzed by CTP: phosphoethanolamine cytidylyltransferase or ethanolamine phosphate cytidylyltransferase (ET). This is believed to be the rate-limiting step of the CDP-ethanolamine pathway, and is therefore subject to cellular regulation. In the final step, the CDP-ethanolamine is converted to PE by the action of CDP-Ethanolamine phosphotransferase, which is a membrane-bound protein in the endoplasmic reticulum that attaches the phosphoethanolamine to a DAG. CDP-Ethanolamine phosphotransferase appears to prefer DAG containing DHA in the sn-2 position as a substrate (Yamashita et al. 1997). In fact, DHA may have a potential

effect on phospholipids synthesis, since it was shown to elevate PE synthesis in hepatocytes (Sundler et. al. 1974).

2.5 PC Synthesis

PC is biosynthesized through two pathways: the CDP-choline pathway that is parallel to the CDP-ethanolamine pathway for PE synthesis, and the PE methylation pathway

2.5.1 PC synthesis through Kennedy Pathway

The CDP-Choline pathway utilizes choline that is acquired from diet (Best and Huntsman 1932). The first step in the CDP-choline pathway is the rapid phosphorylation of choline upon its entry to the cell by choline kinase in the cytoplasm to form P-choline. The P-choline is then converted to CDP-choline in a reaction catalyzed by CTP:phosphocholine cytidylyltransferase (CT). As in the PE synthesis pathway, the formation of the CDP intermediate is believed to be the rate-limiting step of the CDP-choline pathway, and subject to cellular regulation. For example, the inter-conversion of CT between its soluble, inactive form and its active, membrane-bound form influences PC biosynthesis (Vance and Pelech 1984). CDP-choline is converted to PC by the action of CDP-Choline phosphotransferase: an important membrane-bound protein in the endoplasmic reticulum, which attaches the phosphocholine head group to a DAG molecule.

2.5.2 PC synthesis through PEMT Pathway

While the former pathway tends to dominate, the latter PE methylation pathway has been demonstrated to be responsible for the synthesis of 30%-40% of PC in

hepatocytes (Sundler and Akesson 1975; DeLong et al. 1999: Reo et al. 2002). Choline is essentially a tri-methylated ethanolamine where the three methyl groups are attached to the amine group (Figure 2.1). Consequently, the conversion of PE to PC requires three methylation reactions that are catalyzed by one enzyme, phosphatidylethanolamine-N-methyltransferase (PEMT) (Ridgway and Vance 1988). Evidence shows that phospho-methyl-ethanolamine (PME) and phospho-dimethyl-ethanolamine (PDE), the products of the first and second methylation steps, respectively, have an inhibitory effect on their own formation indicating a negative feedback mechanism. PEMT is found to be active in microsomal membranes and mitochondria-associated membranes (MAM). MAM are a part of the endoplasmic reticulum that is highly rich in enzymes involved in lipid biosynthesis (Cui et al.1993). Each step in the methylation pathway utilizes methyl groups from methionine, and produces S-adenosylhomocysteine as a byproduct that ultimately gets converted to homocysteine. In fact, the activity of PEMT has been hypothesized as a mechanism to regulate homocysteine concentrations in the plasma (Robinson 2001). Since PC synthesized from the PEMT pathway utilizes PE as a substrate, the fatty acyl distribution of PE derived PC species should reflect PE synthesized by the Kennedy pathway rather than PC synthesized by the CDP-choline pathway. Indeed, evidence shows that PC synthesized by the PEMT pathway was predominantly enriched with 16:0 and 18:0 at the sn-1 position, and DHA at the sn-2 position (Pynn et al. 2011).

2.6 Phospholipid Fatty Acid Remodeling (Lands' Cycle)

Eukaryotes maintain specific fatty acid distributions in the phospholipids of cell membranes. Saturated and monounsaturated fatty acids are more likely to be esterified at

the sn-1 position, while polyunsaturated fatty acids are predominantly esterified at the sn-2 position. Glycerophospholipids that are formed through the Kennedy pathway (described above) undergo remodeling by subsequent rounds of de-acylation/acylation reactions, also known as the Lands' cycle (Lands, 1958; reviewed in Shindou et.al. 2009). The composition of fatty acyls at the sn-2 position is altered through the specific and regulated actions of A2 phospholipases (PLA2s) and lyso-phospholipidstransferases (LPLATs). There are numerous forms of LPLATs that are thought to be responsible for the variety in phospholipids species that differ in the polar phosphate head group as well as the fatty acyl attached to their glycerol backbone. The form of active LPLAT is largely tissue dependent. For instance, lyso-phosphatidylcholine-acyl transferase 1 (LPCAT1) is highly expressed in lungs and shows preference for utilizing 18:2-CoA or 18:3-CoA when remodeling PC, while LPLAT3 is ubiquitous in tissues, and shows higher activity towards 20:4-CoA and 18:2-CoA. The same applies for lyso-phosphatidylethanolamine-acyl-transferases (LPEAT), where different forms of LPEAT have different specificities for the fatty acyls utilized during PE remodeling (Reviewed in Kitson et.al. 2012). The end result is that PC synthesized by the Kennedy pathway is enriched in mono (PC 16:0/18:1) and di-unsaturated (PC 16:0/18:2) fatty acids. At the same time, PE synthesized through the same pathway is specifically enriched in 16:0 and 18:0 at the sn-1 position, and 20:4n-6 and 22:6n-3 at the sn-2 position (Pynn et al. 2011). The reason these fatty acids are not incorporated directly in the Kennedy pathway could be related to the essential nature of 18:2 and DHA. If the pathway of phospholipid *de novo* synthesis required DHA or arachidonic acid, the process could be compromised if these fatty acids were not available in the diet.

2.7 DHA, PEMT and Methyl Nutrients

The PEMT pathway and the CDP-choline pathway are the main two pathways known to produce phosphatidylcholine. The majority of the choline the supplies the CDP-choline pathway for PC synthesis is acquired through the diet; however, choline requirements in humans can be partially met through the PEMT pathway for PC biosynthesis (da Costa et al, 2011). Consequently, PEMT activity can be influenced by choline intake, as well as various other methyl nutrients, such as methionine, vitamin B_{12}, and folic acid (Hoffman et al. 1981).

PEMT knockout mice put on choline deficient diet rapidly develop significant liver damage (Walkey et al. 1998). Even when supplied a choline sufficient diet, PEMT knockout mice showed diminished concentrations of DHA and arachidonic acid in plasma PC. In humans, plasma PC content of DHA is directly correlated with PEMT activity, especially when choline dietary intake is low (da Costa et al. 2011). This further supports the physiological importance of PEMT in modulating plasma phospholipid fatty acid composition. Dietary choline intake, however, has no effect on DHA content of PC- in pregnant women during the third trimester of pregnancy (West et al., 2013). While various dietary factors can regulate PEMT, it appears that hormones are also involved in PEMT regulation. In particular, estrogen may influence PEMT activity through direct and indirect mechanisms (Hartz et al. 2006). The effect of different dietary levels of DHA on PEMT activity has not been examined previously.

2.8 Hormonal and Pregnancy Effects on PEMT Activity

Estrogen has been shown to have significant effects on PEMT activity in both human and mouse hepatocytes (Young 1971). Potential estrogen response element

motifs have been identified on the PEMT gene promoter region and treating hepatic cell cultures with estrogen at doses mimicking concentrations in humans (0-100nmol/L) significantly up-regulates mRNA expression and enzyme activity of PEMT (Resseguie et al. 2007). Treatment of rat anterior pituitary membranes with estrogen for 4 days resulted in an increase in V_{max} of PEMT for each methylation step, while the K_m remained consistent, indicating an increased concentration of PEMT protein (Drouva et al. 1986). With elevated estrogen levels during pregnancy, there is increased activity of PEMT and an increase in the rate of methylation of PE to PC (Gwee and Sim, 1979). It is hypothesized that the increased PEMT activity during pregnancy is an evolutionary mechanism developed to protect choline stores and decrease the risk of choline deficiency during pregnancy. However, the conversion of PE (which tends to be higher in DHA) to PC (a principal component of lipoproteins) may also be a mechanism to mobilize maternal DHA for placental transport to the fetus.

In addition to elevations in estrogen, pregnancy is also associated with increased insulin production. Insulin sensitivity remains unchanged during early pregnancy (Catalano et al., 1993) but decreases during the third trimester. While insulin is known to be lipogenic, the direct effect of insulin and insulin resistance on PEMT activity remain unclear, and may be tissue dependent (Hoffman et al.1981; Cabrero et al., 1986; Hartz et al. 2006; Tashiro et al., 1983; Panagia et al., 1990).

2.9 Placental Fatty Acid Transport (figure 2.2)

The placenta plays a major role in delivery fatty acids to the fetus, especially those that are essential for fetal development, such as arachidonic acid (20:4n-6) and

docosahexaenoic acid (DHA). The placental tissue takes up free fatty acids circulating in the maternal plasma by specific fatty acid carriers, as well as passive diffusion (Haggarty, 2002). However, the majority of fatty acids in the plasma are in the form of TAG and phospholipids. To accommodate that, the placenta expresses lipoprotein lipase, which hydrolyzes triacylglycerols into free fatty acids. Phospholipids can be broken down and taken up by placental tissue through the action of extracellular phospholipase A2 type II, which is responsible for 80% of placental phospholipase activity (Rice et al., 1998). In addition, plasma phospholipids can deliver fatty acids to the placenta through the action of endothelial lipase, which has high phospholipase activity (Larque et al., 2010). Endothelial lipases mainly hydrolyze phospholipids in the monolayer of lipoproteins, but they have been also shown to utilize TAG (Lidegaard et al., 2005). The role of these proteins in placental transfer and uptake of fatty acid remains unclear. The free fatty acids released in the maternal plasma are then transported via fatty acid translocase fatty acid transport protein (FATP), and plasma membrane fatty acid binding protein (FABPpm) through the placenta and into the fetal circulation (Hanebutt etl al. 2008). Six FATP genes have been identified in human and mouse genomes, and the placenta is shown to express two forms of this protein: FATP-1 and FATP-4. (Hanebutt et al., 2008). The existence of multiple forms of FATP in placental tissue remain unexplained, but the expression of FATP-1 appears to be affected by DHA intake in maternal diet (Larque et al. 2006).

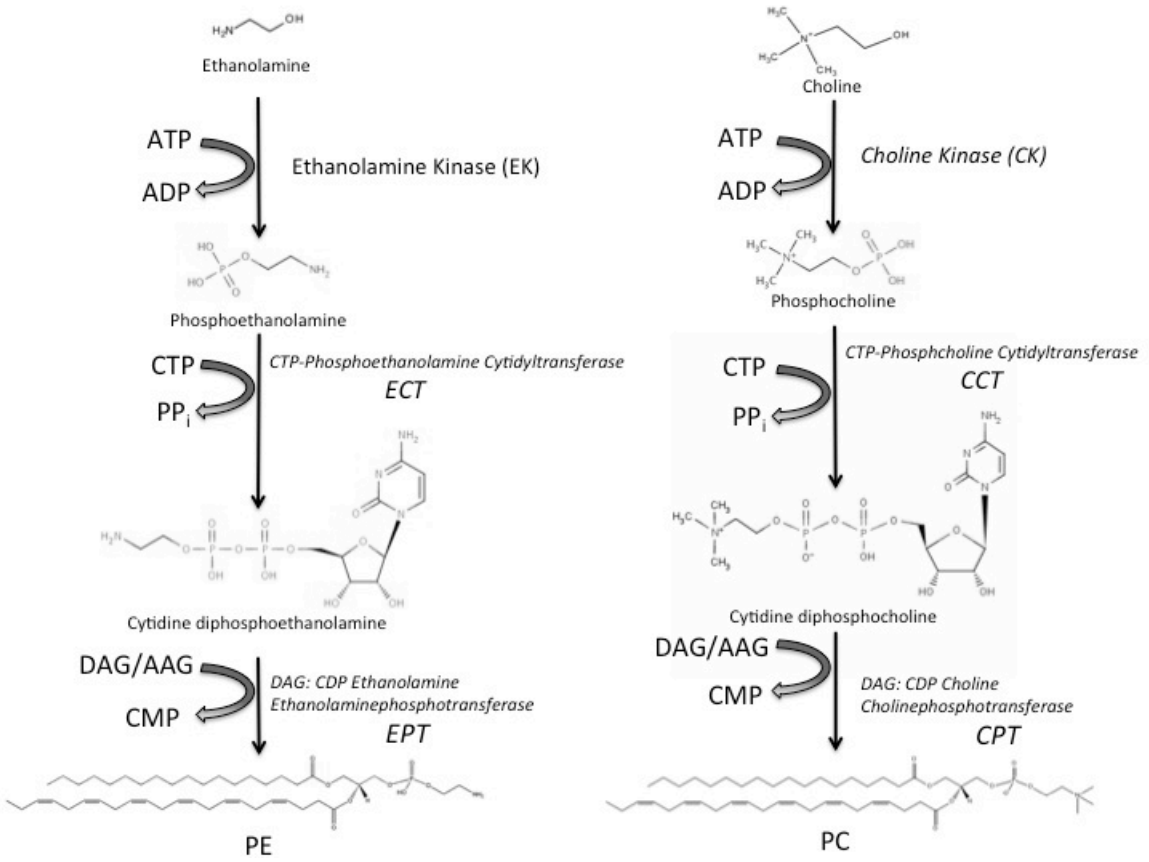

Figure 2.1: The Kennedy pathway for PE and PC synthesis (adapted from by Gibellini and Smith, 2010)

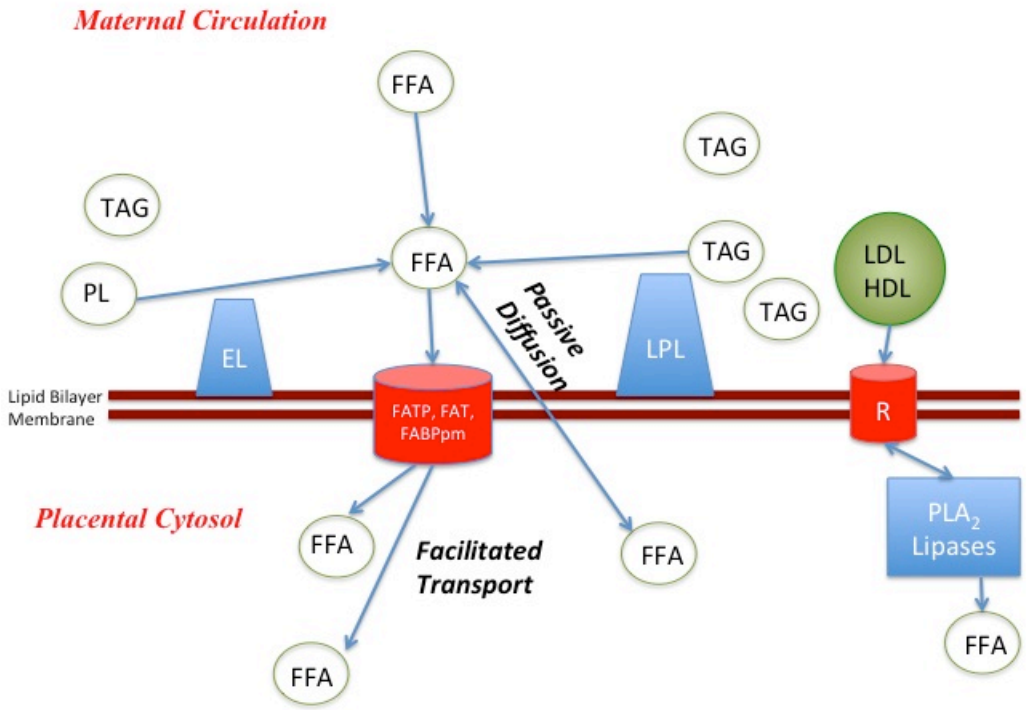

Figure 2.2: Placental lipid fractions breakdown and fatty acids transfer (adapted from Larque et al. 2011). FATP, Fatty acid transport protein; FAT, fatty acid translocase; FABPpm, fatty acid binding protein on plasma membrane; LPL, placental lipoprotein lipase; EL, endothelial lipase; R, lipoprotein receptor; PLA_2, phospholipase A2; FFA, free fatty acids; TAG, triacylglycerols; PL, phospholipids.

Chapter 3

Pilot study to examine the fatty acid composition of phospholipids in blood of women during pregnancy and postpartum

3.1 Methods

As a pilot study, three pregnant women were recruited and blood samples were taken during the third trimester of pregnancy and at 4 months post partum. Diet was neither assessed, nor controlled. Tissue lipids of plasma and erythrocytes were extracted according to Folch et al., using 2:1 chloroform:MeOH (v/v) (Folch et al. 1957). Prior to extraction, 10ug of 17:0 standard was added to each sample in the form of diheptadecanoyl-sn-glyercol-3-phosphocholine and diheptadecanoyl-sn-glyercol-3-phosphoethanolamine (Sigma Aldrich). Individual phospholipids in the lipid extracts were isolated using thin layer chromatography (TLC). In TLC, a silica coated glass plate serves as the stationary phase. The sample is applied to the bottom of the plate, the origin, and placed in a glass tank that contains a liquid mobile phase at a level below the origin. A mixture of liquid solvents is used as the mobile phase, with varying composition depending on the nature of the sample and the separation desired. As the mobile phase is drawn upwards by capillary action, components of the sample move upwards at different rates. The separation of the components is based on polarity, interaction with the stationary phase particles, and their solubility in the mobile phase (Christie, 1989). Samples can be separated into major lipid classes including phospholipids, triacylglycerols, cholesterol esters, and free fatty acids. In addition, the major classes can be separated into subclasses; for example, the phospholipids fraction can be separated

into PC, PE, PI, PS, lyso-phosphatidylcholine, and sphingomyelin fractions. Internal standards, such as 1,2-diheptadecanoyl-sn-glyercol-3-phosphocholine (850360P, Avanti Polar Lipids Inc, Alabaster, AL) and diheptadecanoyl-sn-glyercol-3-phosphoethanolamine are used to allow for quantification. Analysis of the fatty acid composition of isolated lipid classes is then determined by GC as described above.

For this experiment, TLC H-plates (Fisher Scientific) were activated for an hour prior to use. Each plate was scored into 6 equal width lanes, where one of the lanes were reserved for a running standard, containing the phospholipids of interest. Total lipid extract from each sample was dissolved in 50 µl of chloroform and applied to the base of each lane with the use of a syringe. The plate was developed in a solvent mixture containing chloroform:MeOH:propanol:KCl (0.25% w/v):triethylamine (37.5:11.25:31.25:7.5:22.5, vol/vol) in a TLC tank with a filter paper. Once developed and dried, the plate was sprayed with 2,7- dichlorofluorescein in methanol to visualize the bands. Individual phospholipids bands were scraped off the plate, and lipids were extracted using a modified Folch procedure (Folch et al. 1957). TLC extracts were transesterified using 14% BF_3-Methanol for one hour at 95°C to generate fatty acid methyl esters. Finally, the methyl esters were separated and analyzed using GC/FID, where the internal standards were used to quantify individual fatty acids of interest. Data from 10 non-pregnant female controls with low DHA status generated from a previous study were also examined (Patterson, 2012).

3.2 Statistical analysis

A paired samples t-test was used to analyze all fatty acid differences in maternal plasma phospholipids in pregnancy and at 4 months post partum. An independent t test was used to analyze the differences in phospholipid fatty acids profiles when comparing to non-pregnant controls.

3.3 Results

Maternal Plasma Composition

In general, there was a decrease in the relative amount of DHA in PC, but it did not reach statistical significance (3.77 ± 0.85 % during pregnancy compared with 2.65 ± 0.24 % at 4 months post partum and 2.81 ± 0.68 % in non-pregnant controls, Table 3.1). In addition, 18:0 in plasma PC decreased in relative concentration during pregnancy compared with 4 months post partum and non-pregnant controls (11.01 ± 0.64% in pregnancy compared with 14.65 ± 1.51% and 15.85 ± 3.91% for 4 months post partum and non-pregnant controls, respectively). Palmitic acid relative concentration appeared to increase during pregnancy (31.99 ± 0.44 %) compared with 4 months post partum (26.51 ± 2.31 %) and non-pregnant controls (25.07 ± 1.17%).

As for plasma PE, 16:0, 18:0, and 20:4n-6 all appeared to be higher in pregnancy and post-partum as compared with non-pregnant controls (Table 3.2). DHA relative concentration in PE appeared to be elevated in pregnancy compared with 4 months post partum and non-pregnant controls, but the difference was only statistically significant compared to non-pregnant controls (3.8 ± 0.57% in pregnancy compared to 2.65 ± 0.24% and 1.57 ± 0.66 at 4 months post partum and non-pregnant controls, respectively). Based

on these pilot results, assuming the standard deviation is constant, with 80% power, and α=0.5, the number of participants required to achieve statistical significance for DHA content in PC and PE between pregnancy and 4 months postpartum is 10.

The results of this pilot study show a strong trend of increased plasma PC DHA levels during pregnancy. The levels of saturated fatty acids, such as 16:0 and 18:0 also changed during pregnancy, which may indicate a change in phospholipid metabolism during pregnancy. Evidence suggests an increase in overall plasma PC levels, which may be caused by up-regulating the PEMT pathway. The effects of dietary intake of DHA were not accounted for in this study, and need to be considered in follow-up studies.

Table 3.1: Relative percent fatty acid composition of plasma PC during pregnancy and in non-pregnant controls

Name	Control	Pregnant	Post partum
C 14:0	0.52 ± 0.12	0.71 ± 0.09	0.64 ± 0.31
C 16:0	25.07 ± 1.17	31.99 ± 0.44	26.51 ± 2.31
C 18:0	15.85 ± 3.91	11.01 ± 0.64	14.65 ± 1.51
C 20:0	0.18 ± 0.09	0.18 ± 0.05	0.18 ± 0.09
C 22:0	0.20 ± 0.09	0.43 ± 0.16	0.22 ± 0.13
C 23:0	0.32 ± 0.12*	0.15 ± 0.07	0.41 ± 0.15
C 24:0	0.23 ± 0.09	0.36 ± 0.15	0.30 ± 0.13
SFA	43.74 ± 5.45	46.71 ± 1.22	43.69 ± 3.6
C 14:1	0.04 ± 0.04	0.08 ± 0.04	0.05 ± 0.03
C 16:1	0.38 ± 0.16	0.86 ± 0.42	0.40 ± 0.04
C 18:1n-7	1.64 ± 0.41	1.22 ± 0.10	1.73 ± 0.15
C 18:1n-9	12.33 ± 3.8	9.87 ± 0.80	12.74 ± 3.67
C 20:1n-9	0.23 ± 0.08	0.21 ± 0.04	0.22 ± 0.08
C 22:1n-9	0.24 ± 0.17	0.42 ± 0.19	0.29 ± 0.15
C 24:1n-9	0.21 ± 0.1*	0.54 ± 0.19	0.41 ± 0.21
MUFA	15.09 ± 3.84	13.23 ± 1.42	15.85 ± 3.46
C 18:2n-6	19.90 ± 4.10	18.31 ± 1.18	22.54 ± 3.28
C 18:3n-6	0.11 ± 0.05	0.11 ± 0.07	0.09 ± 0.05
C 20:2n-6	0.30 ± 0.08	0.32 ± 0.03	0.58 ± 0.17
C 20:3n-6	2.54 ± 0.82	2.97 ± 0.43	3.36 ± 0.28
C 20:4n-6	9.65 ± 1.82	6.65 ± 1.73	7.50 ± 1.20
C 22:2n-6	0.20 ± 0.15	0.09 ± 0.09	0.20 ± 0.12
C 22:4n-6	0.62 ± 1.31	0.24 ± 0.07	0.25 ± 0.13
C 22:5n-6	0.31 ± 0.13	0.40 ± 0.14	0.38 ± 0.14
N-6	33.57 ± 4.54	29.09 ± 1.8	34.92 ± 2.86
C 18:3n-3	0.25 ± 0.07*	0.46 ± 0.01	0.39 ± 0.14
C 20:3n-3	0.11 ± 0.06	0.15 ± 0.11	0.26 ± 0.18
C 20:5n-3	0.87 ± 0.51	0.80 ± 0.29	0.51 ± 0.07
C 22:5n-3	0.80 ± 0.17*	0.56 ± 0.04	0.57 ± 0.12
C 22:6n-3	2.81 ± 0.68	3.77 ± 0.85	2.65 ± 0.24
N-3	4.84 ± 1.07	5.74 ± 0.90	4.38 ± 0.54
HUFA	17.66 ± 2.00	15.54 ± 2.13	15.49 ± 0.91
PUFA	38.41 ± 4.81	34.83 ± 2.25	39.29 ± 3.32
Total	137.07 ± 20.14	212.22 ± 45.65	168.09 ± 43.55

Mean +/- SD,. *Significantly different compared to pregnancy by paired samples t-test or independent t-test: $p<0.05$.

Table 3.2: Relative percent fatty acid composition of plasma PE during pregnancy and in non-pregnant controls

Name	Control	Pregnant	Post partum
C 14:0	1.90 ± 0.36*	2.66 ± 0.26	1.66 ± 0.42
C 16:0	15.35 ± 4.23*	23.92 ± 1.17	8.22 ± 1.20
C 18:0	19.71 ± 7.20*	24.64 ± 2.11	10.75 ± 2.58
C 20:0	0.62 ± 0.22	0.53 ± 0.09	0.56 ± 0.09
C 22:0	0.94 ± 0.40	1.85 ± 0.62	0.58 ± 0.16
C 23:0	0.87 ± 0.41	1.25 ± 0.50	0.84 ± 0.38
C 24:0	0.99 ± 0.28*	1.60 ± 0.31	0.92 ± 0.49
SFA	42.61 ± 11.54*	62.63 ± 1.52	24.05 ± 3.89
C 14:1	0.12 ± 0.04*	0.37 ± 0.11	0.06 ± 0.01
C 16:1	0.21 ± 0.20	0.42 ± 0.12	0.15 ± 0.05
C 18:1n-7	0.75 ± 0.43	0.61 ± 0.17	0.64 ± 0.06
C 18:1n-9	22.98 ± 23.10	7.73 ± 0.88	58.22 ± 5.02
C 20:1n-9	0.38 ± 0.19	0.37 ± 0.11	0.43 ± 0.08
C 22:1n-9	1.45 ± 0.91	1.81 ± 0.32	0.89 ± 0.65
C 24:1n-9	0.88 ± 0.48	0.80 ± 0.24	0.69 ± 0.12
MUFA	26.86 ± 22.22*	12.36 ± 1.23	61.14 ± 4.31
C 18:2n-6	5.00 ± 2.99	5.02 ± 1.07	6.64 ± 1.58
C 18:3n-6	0.23 ± 0.22	0.21 ± 0.13	0.09 ± 0.01
C 20:2n-6	0.35 ± 0.15	0.41 ± 0.26	0.30 ± 0.14
C 20:3n-6	0.69 ± 0.42*	1.04 ± 0.16	0.53 ± 0.43
C 20:4n-6	4.26 ± 2.72	6.15 ± 2.11	1.77 ± 0.50
C 22:2n-6	0.62 ± 0.25	0.45 ± 0.15	0.76 ± 0.44
C 22:4n-6	1.02 ± 0.89*	0.51 ± 0.05	0.81 ± 0.42
C 22:5n-6	0.72 ± 0.46	0.75 ± 0.12	0.48 ± 0.19
N-6	12.89 ± 6.26	14.54 ± 1.94	11.38 ± 0.07
C 18:3n-3	0.52 ± 0.36	0.44 ± 0.11	0.28 ± 0.12
C 20:3n-3	0.47 ± 0.30	0.56 ± 0.20	0.32 ± 0.25
C 20:5n-3	0.63 ± 0.27	0.65 ± 0.16	0.55 ± 0.19
C 22:5n-3	1.24 ± 0.94	1.06 ± 0.51	1.01 ± 0.16
C 22:6n-3	1.57 ± 0.66*	3.80 ± 0.57	1.00 ± 0.13
N-3	4.43 ± 1.75*	6.50 ± 0.78	3.17 ± 0.63
HUFA	10.60 ± 5.75*	14.51 ± 1.32	6.47 ± 1.54
PUFA	17.32 ± 7.60*	21.04 ± 1.30	14.54 ± 0.70
Total	68.42 ± 18.94	50.16 ± 1.34	42.36 ± 17.83

Mean +/- SD,. *Significantly different compared to pregnancy by paired samples t-test or independent t-test: $p<0.05$

Chapter 4

Rationale hypotheses and objectives

4.1 Rationale

Docosahexaenoic acid (DHA), an omega-3 fatty acid, plays an important role in fetal brain development. Evidence suggests multiple maternal physiological adaptations in order to deliver DHA to the fetus (De Vriese et al 2003). Hormones such as estrogen, which is elevated during pregnancy, can increase DHA biosynthesis, but may also increase lipids that contain DHA, such as phospholipids in maternal plasma.

Estrogen increases PEMT expression and activity in isolated hepatocytes (Resseguie et al. 2007), and in maternal liver in pregnant rats (Gwee and Sim 1979), where activity of PEMT was shown to increase at day 6 of pregnancy. PEMT catalyzes the methylation of PE to form PC in the liver. The PEMT pathway of PC biosynthesis is thought to be a mechanism to regulate choline levels, especially when choline demands are high, such as during pregnancy. However, PEMT may have a potential role in regulating plasma PC fatty acid composition, as PE and the resulting PC synthesized by the PEMT pathway are predominantly enriched with 18:0 and DHA. The dominant phospholipid in plasma is PC located in circulating lipoproteins that can supply fatty acids for fetal transport. Therefore, PEMT may be involved in mobilizing DHA stores to maternal plasma in order to increase its availability to the developing fetus.

The effects of DHA intake on PEMT expression and activity during pregnancy were examined. To our knowledge, the research in this area has focused on the effect of choline intake on the PEMT pathway, whereas the role of DHA intake is not well

understood. Understanding the dynamics and the mechanisms involved in this pathway would provide the basis for dietary recommendations for essential fatty acids such as DHA, and advance our understanding of hormonal regulation of fat metabolism. The use of a dietary manipulation in rat models in combination with the DHA treatment is important considering that 20% of pregnant women in Canada do not consume DHA (Denomme et al. 2005). Given that fatty acids can influence PEMT expression, we used a rodent diet with a fatty acid profile that mimics the intakes of North Americans, known as the Typical Western Diet (TWD). The new TWD was recently developed by Harlan based on the 2007-2008 National Health and Nutrition Examination (Hintze et al., 2012) and the use of the total Western diet (TWD) in a rat pregnancy study is a novel approach in nutritional research.

4.2 Objectives

The objects of this thesis are to elucidate the role of PEMT and associate it with changes in DHA and phospholipid levels in pregnant rats. Rats were bred, and upon confirmation of pregnancy, were placed on either a DHA supplemented, or DHA deficient Total Western Diet. An additional group of dams were fed with a regular chow diet, to serve as a control group. The genetic expression of PEMT in maternal liver was examined at the mRNA level, and liver protein levels of PEMT were measured by immunoblotting. Additionally, we examined the effects of DHA intake on plasma and liver phospholipid fatty acid composition in pregnant rats. Total fatty acids profile of plasma and liver tissue, in addition to the fatty acid composition of PC and PE in maternal plasma and liver, were determined using gas chromatography.

4.3 Hypotheses

1- PEMT mRNA expression, protein levels, and activity will be increased during pregnancy.

2- Feeding total Western diet (TWD) supplemented with DHA will decrease PEMT mRNA expression, protein levels, and enzymatic activity in maternal liver.

3- Concentrations of 18:0 and DHA in plasma and liver PC will be increased during pregnancy relative to baseline and post partum indicating increased PC formed from PE.

4- Providing DHA to pregnant rats will increase DHA and 16:0 in plasma and liver PC indicating a decreased role of PEMT in producing DHA enriched PC.

Chapter 5

Pregnant Rats model study- PEMT analysis

5.1 Study Design

All animal procedures were approved by the University of Waterloo Animal Care Committee and are in accordance with the guidelines of the Canadian Council on Animal Care. A total of 82 Sprague Dawley rats were purchased (72 female rats at 7 weeks of age, and 10 male rats at 6 months of age). The 10 male rats were only used for breeding purposes, and were not involved in the study. Rats were bred on campus, and upon confirmation of pregnancy, pregnant female rats were assigned to either 1) a high DHA Total Western Diet (TWD-DHA+) which consists of 1.3% DHA in total fatty acids 2) a Total Western Diet with no DHA but omega-3 sufficient (TWD-DHA-) or 3) a typical chow rodent diet, throughout 21 days of pregnancy. Diets were purchased from Harlan Laboratories (TD.110424 New Total Western Diet; 8640 Teklad 22/5 Rodent Diet) (Table 6.1). Pregnant rats (n=6 from each of the three diet groups at each time point of pregnancy) were sacrificed at day baseline (non-pregnant rats after 7 days of dietary intervention) 15 and 20 of pregnancy, and 7 days postnatal. Pregnant rats were sacrificed by exsanguination following anesthesia, using isoflurane, after an overnight fast. Maternal blood, liver, heart, brain, and placenta were collected. Fetuses obtained from all time points were also sacrificed immediately after separation from the mother by decapitation using sharp scissors, while pups from 7 days postnatal were sacrificed by exsanguination following anesthesia using isoflurane. Fetal tissues were collected and stored for analyses for fatty acid analysis. The collected brains, livers, and placentas were flash frozen in liquid nitrogen and stored at -80°C for fatty acid composition

analyses, mRNA expression, and Western blots and activity assays for phosphatidylethanolamine-N-methyltransferase (PEMT).

5.1.1 Assessing PEMT Gene Expression

The mRNA expression was determined by quantitative real-time PCR (qRT-PCR). Reverse-transcriptase real-time PCR is a technique used to amplify a specific sequence of RNA. The first step is to isolate RNA from samples, which is then reverse-transcribed to cDNA for more stability. Trizol reagent, containing RNAse inhibitor, was used to isolate RNA from cells. Chloroform was then added in order to separate RNA from other cellular components. Spinning the Trizol layer using a micro-centrifuge pelleted RNA. The RNA pellet was then washed with ethanol and dissolved in deionized water. Following that, agarose gel electrophoresis with ethidium bromide was performed, which insured the integrity of isolated RNA prior to cDNA synthesis. The concentration of the samples was then confirmed using absorbance at 260 nm, while the 260/280 ratios were used to determine sample purity, as determined by a NanoDrop spectrophotometer. Only RNA samples with high purity (260/280 ratio above 1.90) were used for subsequent cDNA synthesis by reverse transcriptase. Primers were designed for *PEMT* through Primer-BLAST program on the NCBI website and ordered from Sigma-Aldrich *(5'-CCCAGCTTTGTGGCGGCTGT-3')*. A mix containing the *PEMT* primer and SYBR green, a special fluorescent dye that binds to double-stranded DNA used to visualize the amplification of a sequence in real time, was added to the cDNA templates. qRT-PCR was performed using the following set of conditions: initial incubation at 95°C for 10 min, followed by 40 cycles of 95°C and 60°C, which resulted in the amplification of the target sequence. The qRT-PCR data was expressed relative to glyceraldehyde-3-

phosphate dehydrogenase gene (GAPDH) (used as a house keeping reference gene during pregnancy) (Rekawiecki, et al. 2012).

PEMT protein levels were determined by immunoblotting as described previously (Kitson et al., 2012). Tissue samples were homogenized in a buffer containing complete protease inhibitor tablets (0.25mol/L sucrose, 0.01mol/L tris-HCl, 0.01mol/L $MgCl_2$, 2.5mmol/L DTT). Protein quantification was completed using a bicinchoninic acid procedure. Twenty µg of the protein was then resolved on a 12.5% SDS-PAGE gel and transferred to a polyvinylidene fluoride membrane. Next, 5% milk was used to block the membranes in TBS with 0.5% (v/v) Tween (TBST) over night at 4°C. The membranes were then incubated with primary antibodies for PEMT (Donated by Professor Dennis Vance, University of Alberta) (1:1000 dilution) for 2h at room temperature. Following incubation, the membranes were washed with TBST, incubated again for 1hr at room temp with horseradish peroxidase-conjugated secondary antibody (rabbit anti-goat, Santa Cruz Biotechnology, 1:8000 dilution), and washed again. The proteins were treated with Enhanced Chemiluminescence Western Blotting Detection Reagents and visualized on a Chemigenius 2 Bioimaging System using Genesnap software v 7.07. Finally, the molecular weights of proteins, equal protein loading, and adequate transfer of protein to membrane were confirmed using ponceau staining and blotting for ß actin.

5.1.2 Determining the Enzyme Activity of PEMT

A liver PEMT activity assay was performed as described by Ridgway and Vance (Ridgway and Vance, 1998). To assess activity, we used 50 µg of protein homogenized in Tris-HCl/L (pH 9.2) and 5 mm DTT buffer/L (Sigma). Samples were incubated with 200 µmol S-adenosyl-L-methionine/L containing 0.5µCi S-adenosyl-L-[methyl-^{3}H] methionine (55.70 Ci/mmol) and 0.4 nmol exogenous phosphatidyl di-methyl-

ethanolamine /L (P2; Avanti Polar Lipids). The reaction was carried out for 60 minutes at 37°C, when it is stopped by adding ice cold $CHCL_3$:MeOH:1 N HCL (100:50:1, vol/vol). Samples vials were vortexed, followed by the addition of 1 ml of 0.1M KCL in 50% MeOH wash solution. Samples were centrifuged, and the top layer was discarded. Finally, samples were dried under nitrogen flow, and resuspended in 60 µl of chloroform. A portion of the chloroform phase (20µl) was applied to a silica gel TLC/ G plate (ANALTECH, cat. 01011), and the plate was developed in $CHCL_3$:MeOH:Acetic acid: H_2O (50:30:5:2,vol:vol). Liquid Scintillation spectro-photometry was used to determine disintegrations per minute from [^{3}H]-PC in TLC bands.

5.1.3 Determining 17β-estradiol concentrations

ELISA kits were ordered from Cayman Chemical (Ann Arbor, MI) (Estradiol EIA Kit, Cayman Chemical Item Number 582251) for the determination of plasma 17β-estradiol concentrations of the baseline non-pregnant as well as the pregnant animals. 17β-estradiol was extracted from 500 µl of plasma with methylene chloride, and reconstituted in 200 µl of EIA buffer. For each assay, 50 µl of samples and the appropriate volume of standards were loaded to the 96-well plate in duplicate. Two wells were designated each for blanks, total activity, non-specific binding and maximum binding. The concentration of estradiol was measured by exposing the plates to 420 nm wavelength, where the wavelength reading of each well was compared to the standard curve.

5.2 Statistical Analysis

Rats were divided among twelve groups based on diet and pregnancy (Three diets × 4 pregnancy time points). A one-way ANOVA test was used to analyze differences in fatty acids, mRNA, and protein at different stages of pregnancy in rat models. Data are

presented as mean ± standard deviation. Statistical significance was determined at $\alpha=0.5$ via a Tukey post hoc test.

5.3 Results

5.3.1 Food Intake and Body Weight

Average caloric intake increased significantly in the 7 days post partum group for all diets ($p < 0.05$) and there were no other differences (Figure 5.1)

Bodyweight increased steadily throughout pregnancy, and reached its highest at day 20 ($p < 0.05$) (Figure 5.2). There was no significant difference in bodyweight across diets at any time point ($p >0.05$).

5.3.2 Plasma Estradiol

There was no significant difference between baseline, 15 days of pregnancy, and 7 days post partum plasma estradiol levels. Hormone levels increased significantly at 20 days of pregnancy compared to baseline, 15 days pregnancy, and 7 days post partum, in all diets ($p < 0.05$) (Figure 5.3). The high fat diet (either TWD+DHA or TWD DHA deficient) had no significant effect on plasma estradiol levels at any of the time points ($p \geq 0.05$).

5.3.3 PEMT Gene mRNA Expression

At baseline, PEMT mRNA expression was lowest in the chow fed group. This was significantly different than the TWD without DHA, but not the TWD with DHA. Day 15 was associated with increases in mRNA expression especially in the animals fed diets without DHA (342-375 % increase from baseline, $p < 0.05$) but muted when DHA was in the diet (172% increase from base line, $p =0.05$). *PEMT* mRNA expression

decreased significantly from day 15 at day 20 of pregnancy (74-90 % decrease), and 7 days post partum (88-91% decrease) in all diet groups, (Figure 5.4-A).

5.3.4 PEMT Protein Levels

PEMT protein expression tended to gradually increase throughout pregnancy peaking at day 20 and then decreasing with postpartum (Figure 5.4-B). In the chow-fed rat the difference between baseline and day 20 was significant, while day 15 levels were intermediate, and PEMT returned to baseline levels at 7d postpartum. In TWD without DHA, the increase in PEMT over time was not significantly higher than baseline, but the day 15 and day 20 PEMT levels were significantly higher than 7d postpartum. In the TWD with DHA, PEMT levels tended to be higher than the other diets with significantly higher levels at baseline and 7 d postpartum. The PEMT increase at d20 in the TWD +DHA was significantly higher than at d15, but was not significantly different than baseline or 7d postpartum. Additionally, the PEMT at d20 of the TWD+DHA was significantly higher than the d20 PEMT in the TWD – DHA group.

5.3.5 PEMT Enz Activity

PEMT enzymatic activity increased significantly during pregnancy in all dams (Figure 5.4-C). However, the time point of pregnancy where PEMT activity reached its peak was different between diet groups. Liver PEMT activity increased significantly at day 15 of pregnancy in both Chow and DHA deficient diet fed dams. PEMT activity decreased significantly at day 20 of pregnancy in chow fed dams, but not in DHA deficient diet fed dams. High DHA diet fed dams had significantly lower liver PEMT

activity at day 15 of pregnancy compared to chow and DHA deficient diet fed dams, but the enzyme activity increased significantly at day 20 of pregnancy. Animals from all diet groups had significantly lower PEMT activity at 7 days post partum.

Table 5.1 Relative Percent fatty acid composition analysis of TWD and chow diets

Name	TWD-DHA+	TWD-DHA-	Chow
C 16:0	19.52 ± 0.03	20.24 ± 0.04	13.63 ± 0.06
C 18:0	9.03 ± 0.01	9.70 ± 0.02	2.18 ± 0.33
SFAs	33.32 ± 0.04	34.31 ± 0.10	17.35 ± 0.26
C 16:1	1.20 ± 0.01	1.17 ± 0.01	0.88 ± 0.01
C 18:1n-9	37.12 ± 0.05	36.94 ± 0.02	21.03 ± 0.05
MUFA	40.79 ± 0.03	40.69 ± 0.04	23.73 ± 0.01
C 18:2n-6	20.85 ± 0.01	20.73 ± 0.01	49.69 ± 0.22
C 22:5n-6	1.58 ± 0.01	1.89 ± 0.01	0.02 ± 0.01
N-6	22.79 ± 0.02	23.04 ± 0.07	50.01 ± 0.18
C 18:3n-3	1.87 ± 0.01	1.88 ± 0.01	5.72 ± 0.09
C 22:6n-3	1.14 ± 0.02	*n.d.*	0.22 ± 0.01
N-3	3.11 ± 0.04	1.96 ± 0.01	6.19 ± 0.07
HUFA	2.96 ± 0.04	2.08 ± 0.08	0.69 ± 0.05
PUFA	25.90 ± 0.06	25.00± 0.06	56.20 ± 0.25

n.d, not detected

Table 5.2 Nutrient composition of TWD and Chow diets

Ingredient (g/kg)	TWD DHA+	TWD DHA-	Chow
L-Cystine	3	3	3
Cellulose	50	50	39
Choline bitartrate	2.5	2.5	2.38
TBHQ, antioxidant	0.014	0.014	0
Kcal/g	3.7	3.7	3.0
TWD Diet Formulation			
Corn starch	378.186	378.186	
Maltodextrin	132	132	
Sucrose	100	100	
Casein	200	200	
Corn oil	70	70	
Mineral mix, AIN-93G-MX (94046)	48	48	
DHASCO oil (40% DHA)	17	0	
Macronutrients Breakdown (% Weight)			
Protein	17.7	17.7	22
Carbohydrate	59.2	59.2	40.6
Fat	7.2	7.2	5.5

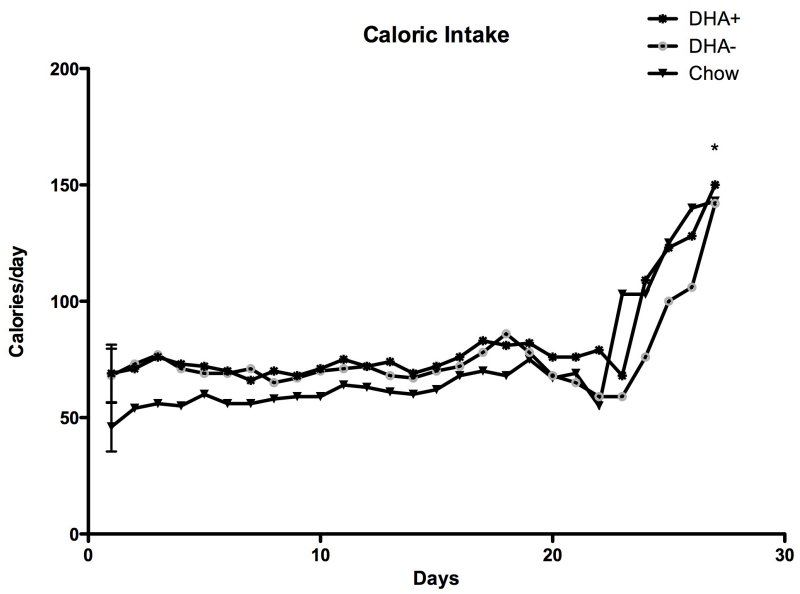

Figure 5.1 Daily average Food intake pattern of dams fed with a 1.3% DHA Total Western Diet (DHA+), DHA deficient Total Western Diet (DHA-) or a chow diet (Chow) during pregnancy. Time points labeled with * are significantly different from baseline, 15 days pregnancy and 7 days postpartum by Tukey's post hoc test ($p<0.05$) following significant F-value by one-way ANOVA.

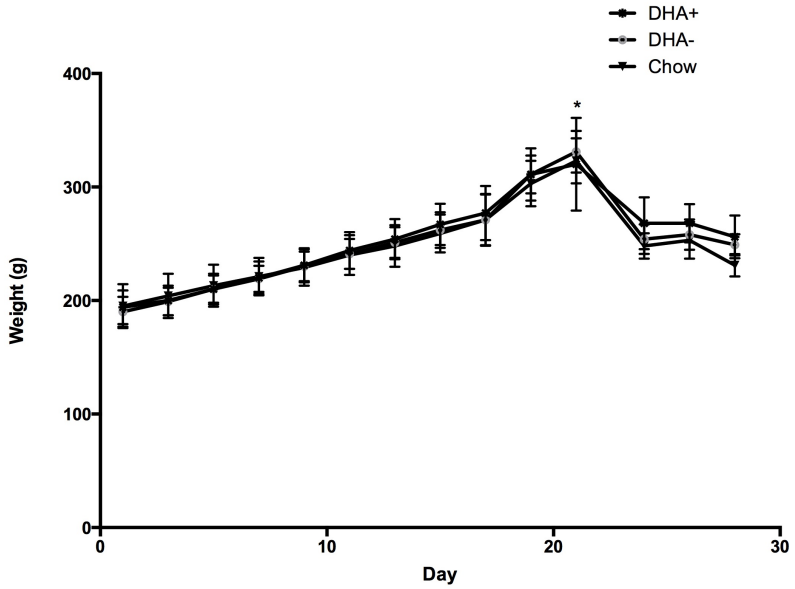

Figure 5.2 Average body weight of dams fed a 1.3% DHA Total Western Diet (DHA+), DHA deficient Total Western Diet (DHA-) or a chow diet (Chow) during pregnancy. Time points labeled with * are significantly different from baseline, 15 days pregnancy, and 7 days postpartum by Tukey's post hoc test ($p<0.05$) following significant F-value by one-way ANOVA.

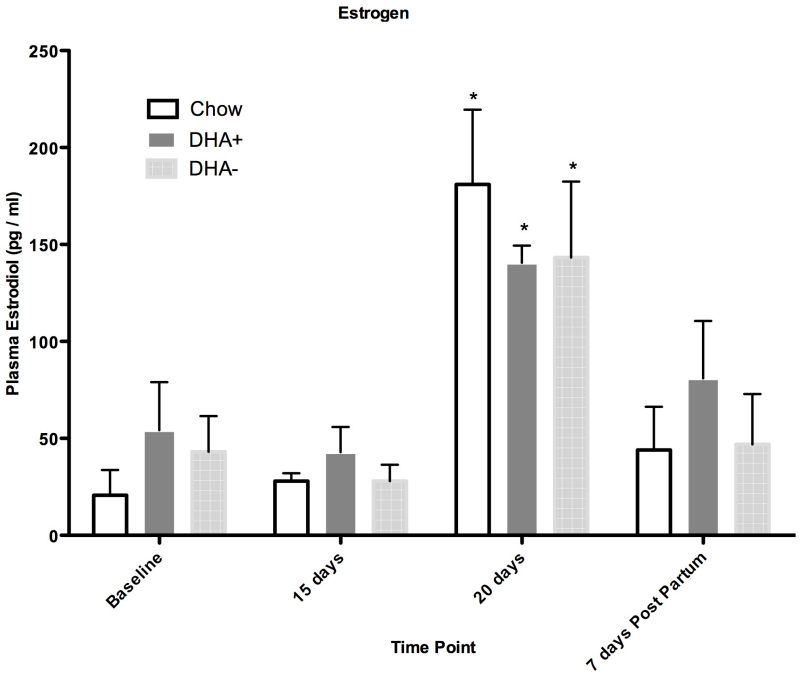

Figure 5.3 Plasma Estradiol concentrations of rats on a 1.3% DHA Total Western Diet (DHA+), DHA deficient Total Western Diet (DHA-) and chow diet (Chow) throughout pregnancy and post-partum. *Significantly different than other time points within a diet by Tukey's post hoc test ($p<0.05$) following significant F-value by one-way ANOVA.

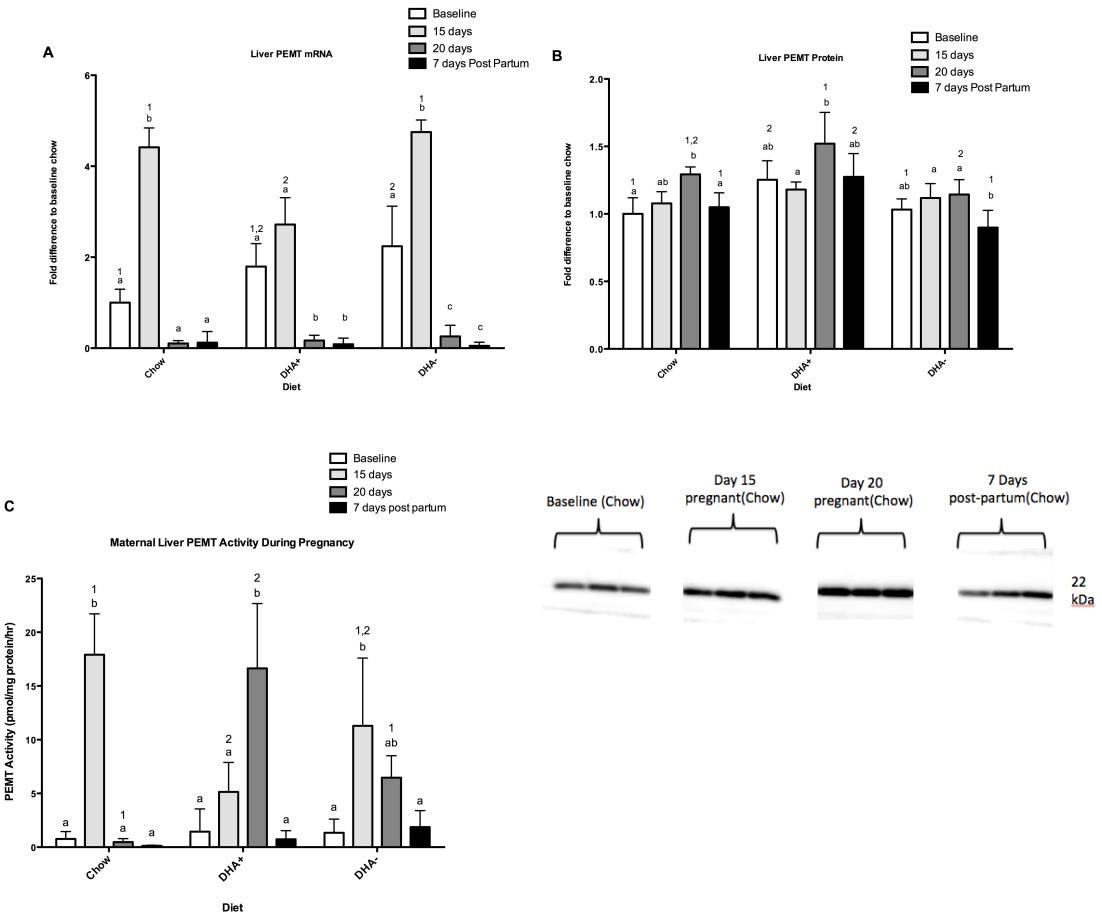

Figure 5.4 Pregnancy Effect on phosphatidylethanolamine methyl transferase (PEMT) expression in maternal liver: (A) Fold difference in mRNA gene expression of PEMT in non-pregnant female rats and dams placed on a 1.3% DHA Total Western Diet (DHA+), DHA deficient Total Western Diet (DHA-) and chow diet (Chow) as identified by qPCR.**(B)** Fold difference in protein expression of PEMT in non-pregnant female rats and dams placed on a 1.3% DHA Total Western Diet (DHA+), DHA deficient Total Western Diet (DHA-) and chow diet (Chow) as identified by densitometric analysis of representative immunoblots (Shown below the figure). **(C)** Fold difference in the enzyme activity of PEMT in non-pregnant female rats and dams placed on a 1.3% DHA Total Western Diet (DHA+), DHA deficient Total Western Diet (DHA-) and chow diet (Chow) as indicated by the radio-enzymatic activity assay. Values with different superscripts are significantly different within the same diet, while values with different numbers are different within the same time point. Significance is determined by Tukey's post hoc test ($p<0.05$) following significant F-value by one-way ANOVA.

Chapter 6

Pregnant Rats model study- fatty acids analysis

6.1 Methods

6.1.1 Extraction of plasma and liver total lipids

Tissue samples, including pups' whole bodies, were pulverized under liquid nitrogen, and stored at -80°C until analyzed. Fatty acids were extracted from pulverized tissues according to Folch's procedure (Folch, 1957), using 2:1 (v/v) chloroform:methanol. The chloroform:methanol solution contained an internal standard (22:3n-3 ethyl ester, Nu-Chek Prep Inc, Elysian, MN) for quantitation of fatty acid concentrations by GC/FID analysis. Sodium phosphate buffer (0.5 ml) was added before centrifuging to separate the mixture into two layers, so the chloroform bottom layer (containing lipids) could be collected. The collected chloroform was dried under nitrogen, and 1ml of BF_3 in methanol and 0.3 ml of hexane were added in preparation for the trans-esterification reaction. The samples were heated for one hour at 90 °C, and then allowed to cool to room temperature. One ml of hexane and 1ml of ultrapure water were added to each sample, briefly vortexed and centrifuged to separated the mixture into two layers. The top organic layer was collected, dried under nitrogen, and reconstituted in an appropriate volume of hexane for final analysis on GC/FID.

6.1.2 Thin layer chromatography of plasma and liver phospholipids

Maternal plasma and liver phospholipids were separated into PC, PE, PI, PS, lyso-phosphatidylcholine, and sphingomyelin fractions. Internal standards, such as 1,2-diheptadecanoyl-sn-glyercol-3-phosphocholine (850360P, Avanti Polar Lipids Inc,

Alabaster, AL) and diheptadecanoyl-sn-glyercol-3-phosphoethanolamine were used to allow for quantification (see Ch4, Section 1 for details)

6.1.3 Fatty acid determination by GC

Fatty acid methyl esters dissolved in hexane were separated by fast gas chromatography (Stark and Salem, Jr., 2005). Sampled were analyzed using a Varian 3900 gas chromatograph coupled with a DB-FFAP 15 m × 0.10 mm injected dose × 0.10 μm film thickness, nitroterephthalic acid modified, polyethylene glycol, capillary column (J&W Scientific from Agilent Technologies, Mississauga, ON, Canada), and used hydrogen as the carrier gas. A volume of 2 μL of each sample was introduced by a Varian CP-8400 auto-sampler into the injector, with a split ratio of 200:1. Initial temperature was maintained at 150°C for 0.25-minute, followed by a 35°C/min ramp to 200°C. This was followed by an 8°C/min ramp to 225°C with a 3.2-minute hold, and then an 80°C/min ramp up to 245°C with a 15-minute hold at the end. The flame ionization detector (FID) temperature was maintained at 300°C with air and nitrogen make-up gas flow rates of 300 and 25 ml/min, respectively. Sampling frequency was set to 50 Hz. Individual fatty acid peaks were identified using Galaxie software (version 1.9.3.2) by comparison to a reference mixture of fatty acids (GLC-462, Nu-Chek Prep Inc), and peaks areas were quantified relative to the internal standard (17:0 in PC or PE, Avanti Polar Lipids, Inc). Fatty acid results are presented qualitatively as relative weight % of total fatty acids.

6.2 Results

6.2.1 Fatty Acid Composition of plasma lipids (Appendices A-C)

The concentration of the sum of fatty acids in plasma total lipids, PC, and PE increased significantly at day 20 of pregnancy, followed by a significant decrease at 7 days post partum. Within the general increase in the concentration of fatty acids, the relative percent (rel%) of HUFA decreased significantly at day 20 in plasma TLE, as well as in plasma PE in rats fed TWD-DHA+. The rel% of HUFA with 22 carbons, especially DHA, increased significantly at day 20 of pregnancy in plasma total lipids in animals fed chow and TWD-DHA+, as well as the PC and PE fractions of animals in all diet groups (figures 6.1, 6.3). The rel% of 22 carbon HUFA, including DHA, decreased significantly at 7 days post partum in all diet groups, in plasma total lipids, plasma PC, and plasma PE of chow fed animals and animals fed TWD-DHA-. To the contrary of DHA, arachidonic acid (20:4n-6) rel% decreased significantly at day 20 pregnancy in plasma total lipids and plasma PC, but not plasma PE. However, arachidonic acid rel% did decrease in plasma PE but only at 7 days post partum.

In both TWD groups, the rel% of PUFA decreased throughout pregnancy and up to 7 days post partum in plasma total lipids and in plasma PC, and at 7 days post partum in plasma PE the TWD-DHA- fed animals. However, this decrease in the PUFA percentage was a result of decreases in n-6 PUFA, and not n-3 PUFA. The percentage of n-3 PUFA in plasma PC increased up to day 20 of pregnancy in plasma total lipids in chow and TWD-DHA+ fed animals, and in plasma PC. N-3 PUFA rel % in plasma total lipids and plasma PC and plasma PE at 7 days post partum decreased compared with day 20 of pregnancy in all diet groups ($p<0.05$).

The percentage of palmitic acid (16:0) increased in relative concentration at day 20 of pregnancy in plasma total lipids and plasma PC in all diet groups ($p < 0.05$). While stearic acid (18:0) increased significantly in absolute concentration in plasma total lipids and plasma PC at day 2, it decreased in percentage in all diet groups ($p < 0.05$). Contrary to plasma total lipids and plasma PC, stearic acid relative percent in plasma PE increased significantly at day 20 pregnancy in chow and TWD-DHA+ fed animals.

6.2.2 Fatty acid composition in liver lipids (Appendices D&E)

Unlike in maternal plasma, maternal liver PC and PE total fatty acids did not change during pregnancy or at 7 days postpartum. PC PUFA relative percent decreased significantly at 7 days post partum in TWD-DHA- fed rats ($p < 0.05$), but did not change in liver PE. HUFA relative percent did not change from baseline and during pregnancy in either liver PC or liver PE, but decreased significantly at 7 days postpartum in liver PC, only in TWD fed animals. Within the HUFA pool, PC and PE 22:5n-3, 22:4n-6, and 22:5n-6 relative percent increased significantly at 20 days of pregnancy in all diet groups. However DHA relative percent in liver PC and PE increased in TWD-DHA+ and chow fed animals ($p < 0.05$) (figures 6.2,6.4), but not in TWD-DHA- fed animals ($p > 0.05$). All 22 carbons HUFA relative percent decreased significantly at 7 days post partum in both TWD fed animals ($p < 0.05$). N-3 fatty acids relative percent increased significantly at 20 days of pregnancy in liver PC, as well as liver PE in TWD-DHA+ and chow fed animals, before returning to baseline levels at 7 days post partum. Contrary to that trend, n-6 fatty acids relative percent decreased significantly at day 20 of pregnancy in liver PC, and liver PE in TWD-DHA+ and chow fed animals, before they increased back to baseline values at 7 days post partum.

Palmitic acid (16:0) relative concentration increased significantly in relative concentration at day 20 of pregnancy in liver PC in all diet groups, and in liver PE in chow fed animals, before returning to baseline levels at 7 days post partum. On the other hand, stearic acid relative concentration decreased significantly at day 20 of pregnancy in liver PC in all diet groups and liver PE in chow and TWD-DHA- fed animals; liver PC 18:0 relative percent returned to normal levels in both TWD-DHA- and chow fed animals, but not in TWD-DHA+ fed animals.

6.2.3 Fatty acid composition in whole-body fetuses (Appendix F)

Total fatty acids in whole body fetuses at day 15 and day 20 gestations were significantly lower than in 7-day-old pups. There was no significant difference in the absolute concentrations of total fatty acids between diets at 15 days and 20 days gestation, but at 7 days post partum, the pups from mothers fed TWD diets had significantly higher total fatty acid concentrations as compared with pups from chow-fed mothers (figure 6.5). Within this increased fatty acid content of the pups from the TWD mothers, MUFA percentages were significantly higher in the 7-day-old pups as compared with 15-d and 20-d fetuses. The 7 day-old pups from TWD fed mothers had a significantly larger body mass than those from chow fed mothers (Figure 6.6).

The percentage of n-3 PUFAin the fetuses increased significantly at day 20 gestation from dams fed both TWD-DHA+ and chow fed groups, but not TWD-DHA- which was largely a reflection of changes in fetal/pup DHA percentages.

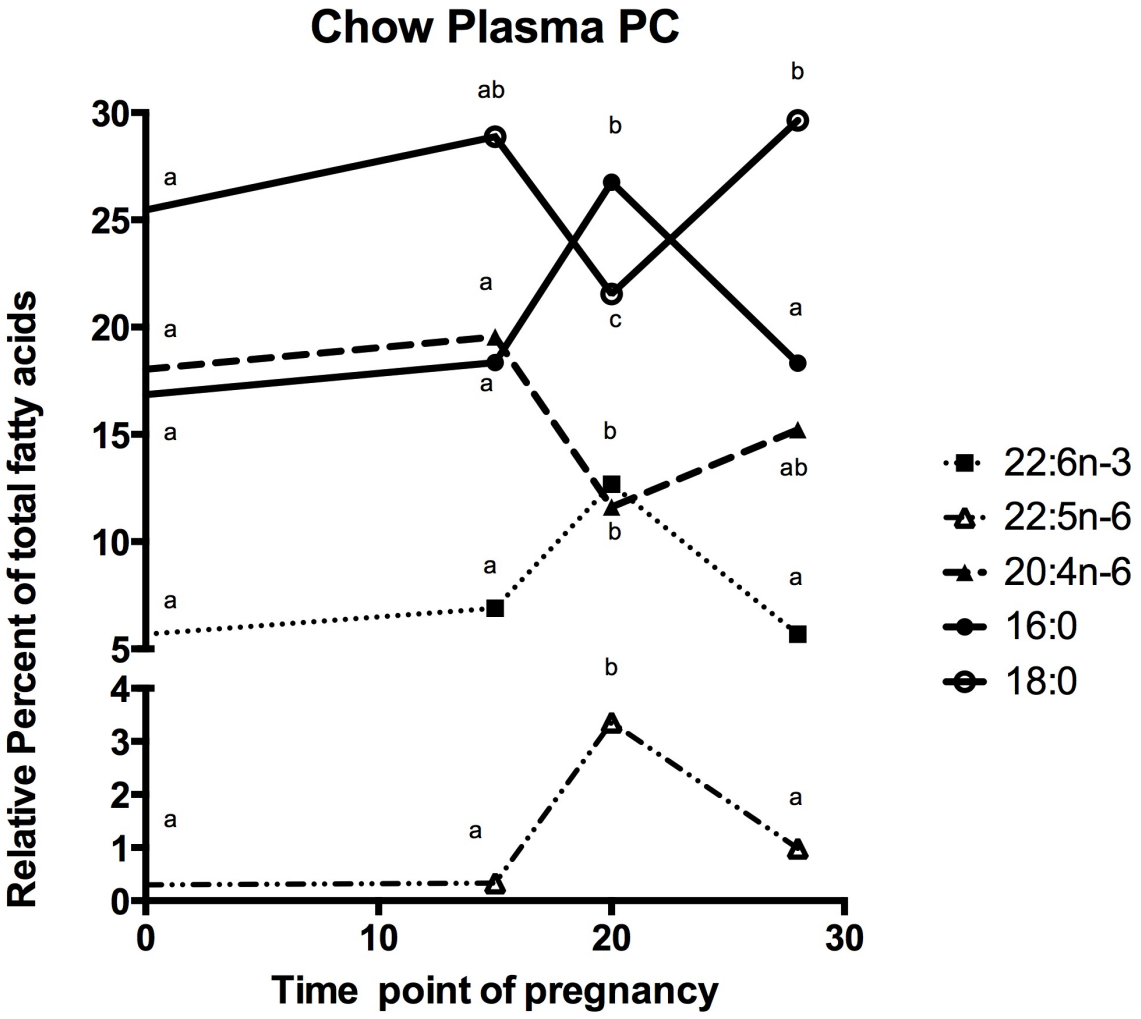

Figure 6.1 Percentage of individual fatty acids in rat plasma PC. Each point represents the mean for plasma samples (n =18 for baseline and 20 days pregnancy; n=17 for 15 days pregnancy and 7 days post partum) at each time point from non-pregnant female rats and dams placed on a chow diet. Values with different superscripts are significantly different by Tukey's post hoc test ($p<0.05$) following significant F-value by one-way ANOVA.

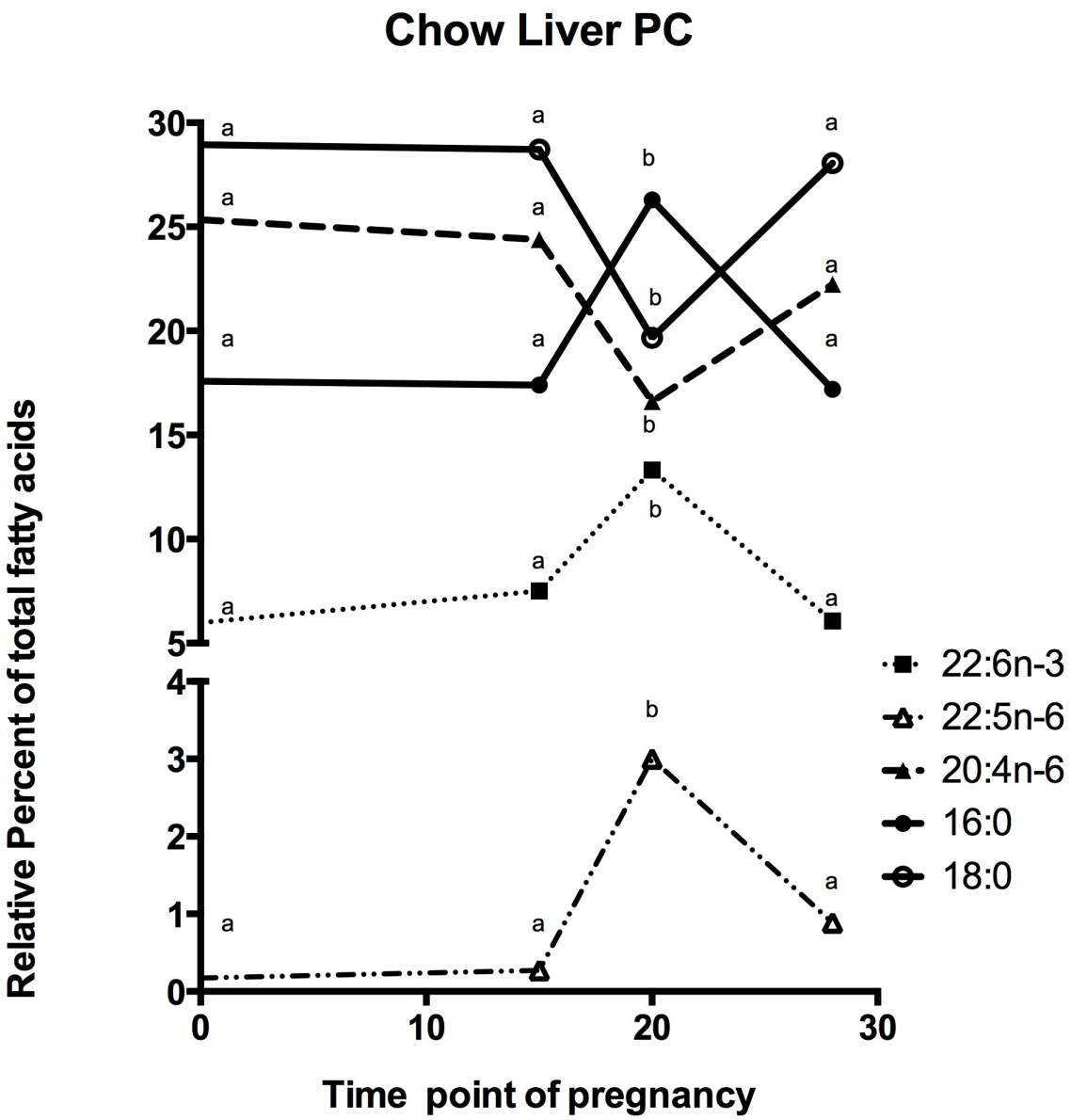

Figure 6.2 Percentage of individual fatty acids in rat liver PC. Each point represents the mean for liver samples (n =18 for baseline and 20 days pregnancy; n=17 for 15 days pregnancy and 7 days post partum) at each time point from non-pregnant female rats and dams placed on a chow diet. Values with different superscripts are significantly different by Tukey's post hoc test ($p<0.05$) following significant F-value by one-way ANOVA.

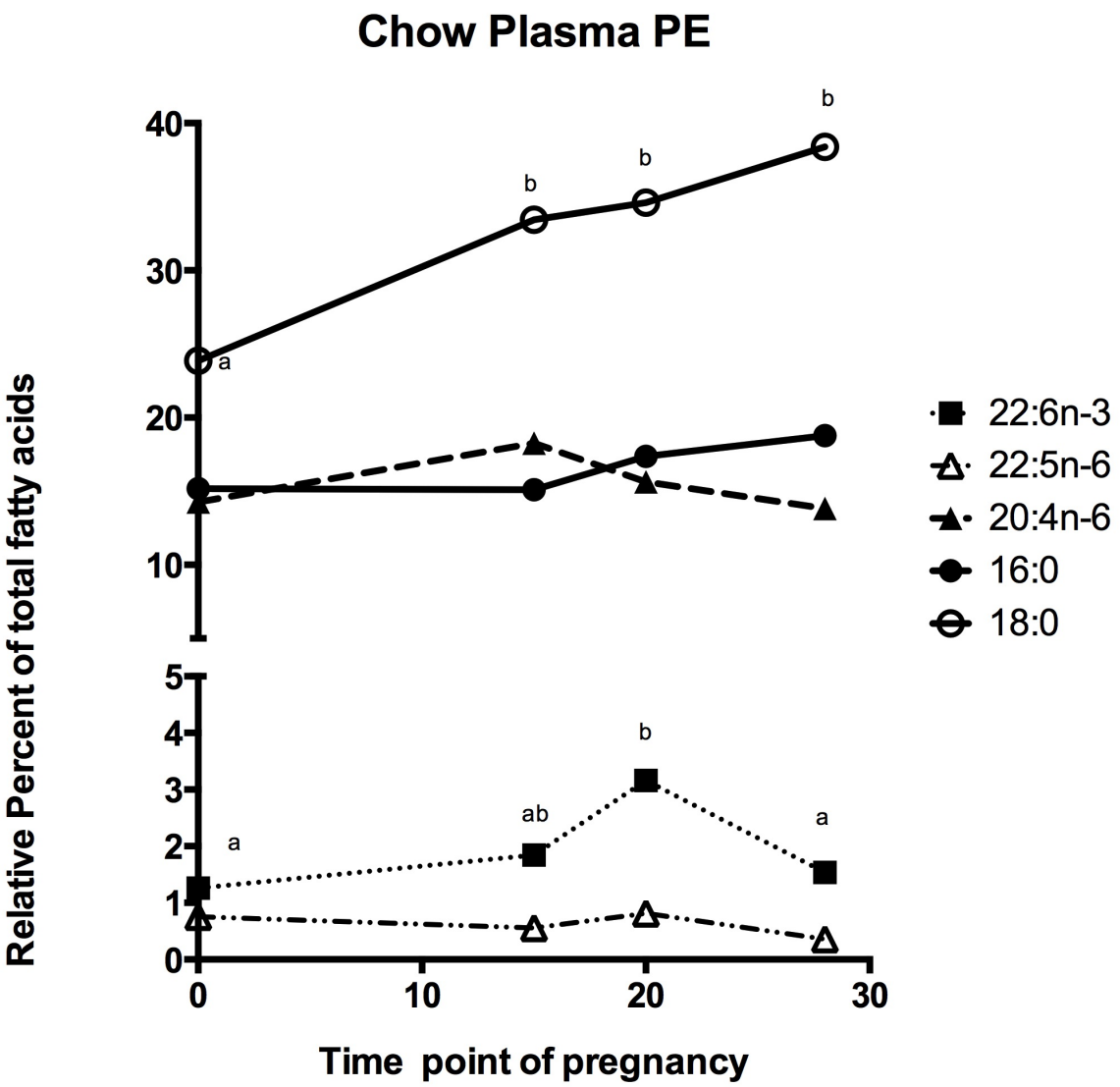

Figure 6.3 Percentage of individual fatty acids in rat plasma PE. Each point represents the mean for plasma samples (n =18 for baseline and 20 days pregnancy; n=17 for 15 days pregnancy and 7 days post partum) at each time point from non-pregnant female rats and dams placed on a chow diet. Values with different superscripts are significantly different by Tukey's post hoc test ($p<0.05$) following significant F-value by one-way ANOVA.

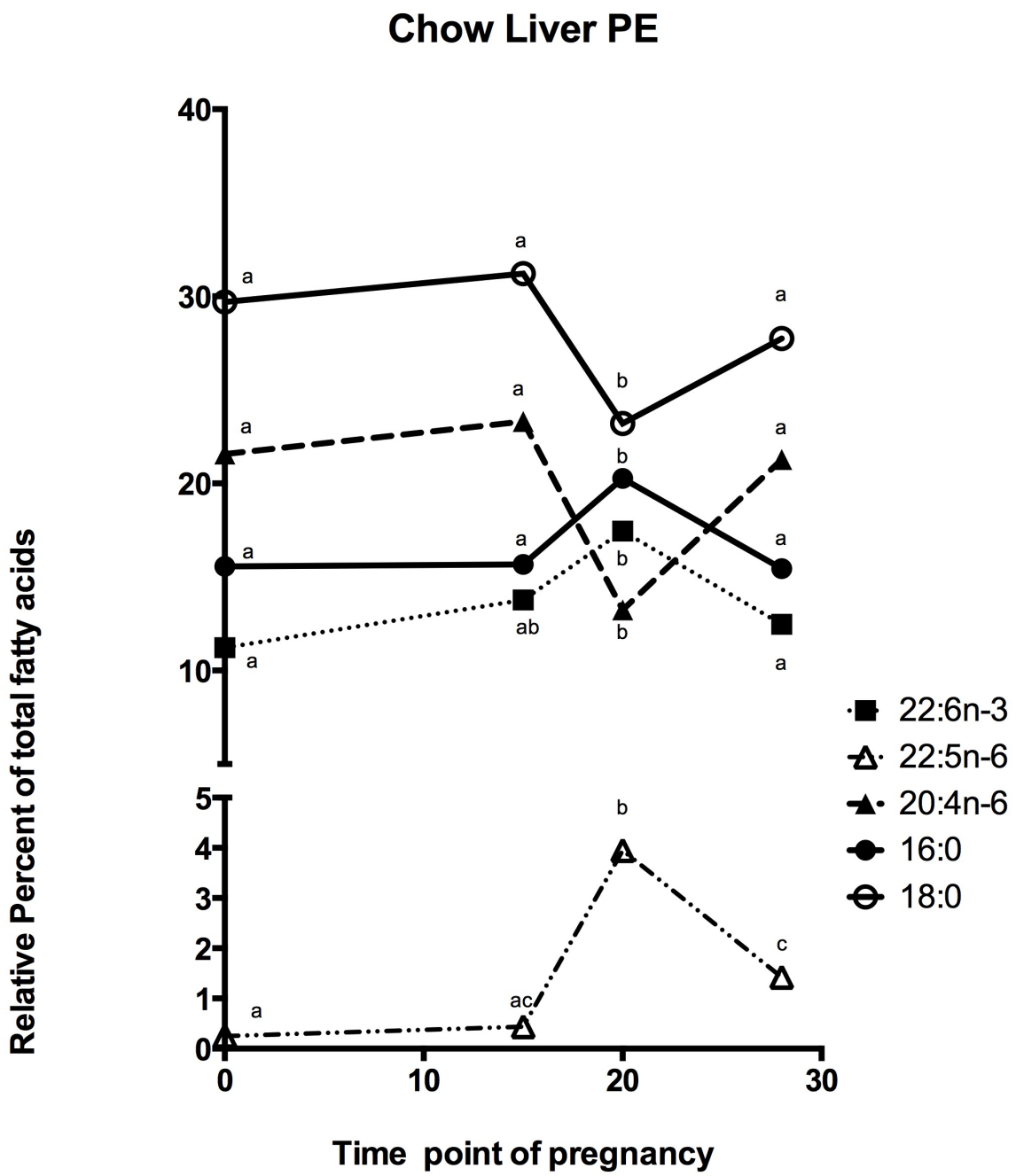

Figure 6.4 Percentage of individual fatty acids in rat liver PE. Each point represents the mean for liver samples (n =18 for baseline and 20 days pregnancy; n=17 for 15 days pregnancy and 7 days post partum) at each time point from non-pregnant female rats and dams placed on a chow diet. Values with different superscripts are significantly different by Tukey's post hoc test ($p<0.05$) following significant F-value by one-way ANOVA.

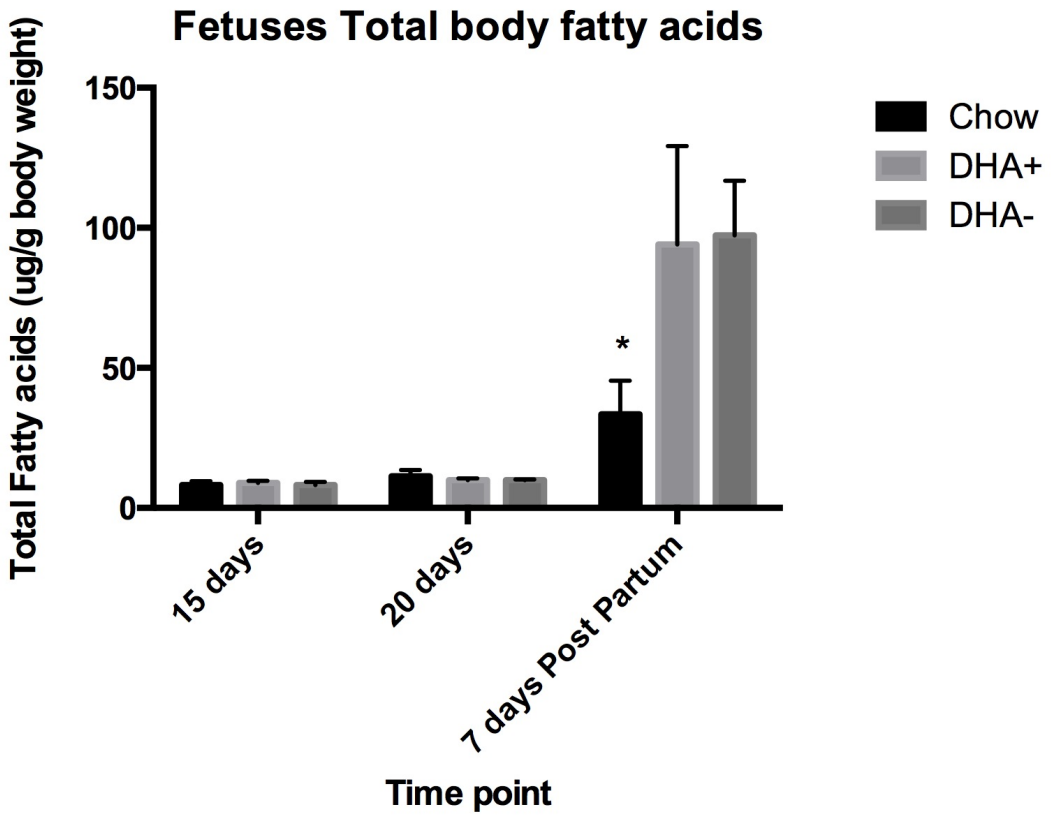

Figure 6.5 Absolute concentrations of fetuses' whole body fatty acids. Each column represents the mean for whole body fetus samples (n =18 for 20 days pregnancy; n=17 for 15 days pregnancy and 7 days post partum) at each time point from dams placed on a high DHA TWD (TWD-DHA+), DHA Deficient TWD (TWD-DHA-) or chow diet. Values with "*" are significantly different by Tukey's post hoc test ($p<0.05$) following significant F-value by one-way ANOVA.

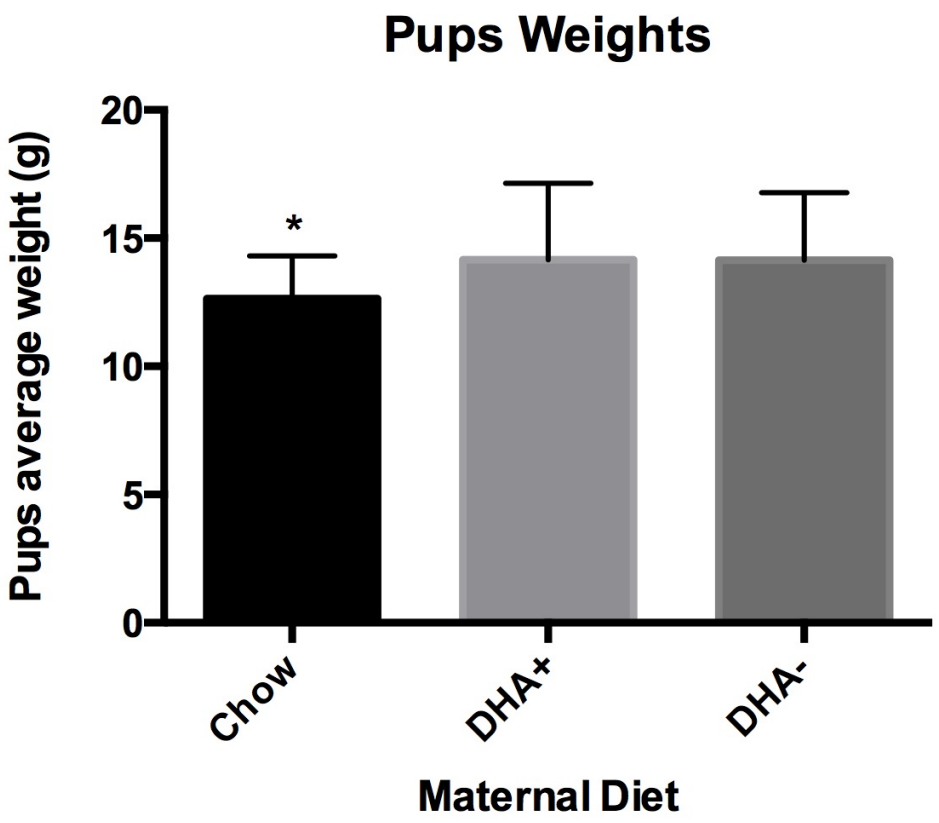

Figure 6.6 Average pups body weight at 7 days post partum. Each column represents the mean for pup body weight from dams placed on a a high DHA TWD (TWD-DHA+), DHA Deficient TWD (TWD-DHA-) or chow diet (n = 60 for TWD-DHA+ ; n=56 for TWD-DHA-, and n=80 for chow). Values with "*" are significantly different by Tukey's post hoc test ($p<0.05$) following significant F-value by one-way ANOVA.

Chapter 7

Discussion

7.1 Discussion

As hypothesized, PEMT mRNA expression, protein levels, and enzyme activity were increased with pregnancy. The expression of PEMT mRNA increased significantly at day 15 of pregnancy in all diet groups, before decreasing dramatically at day 20 and 7 days post partum, while protein PEMT levels tended to be increased at day 20. A dietary effect was observed, with dietary DHA resulting in decreased PEMT mRNA expression at day 15. Despite this decrease in mRNA expression, PEMT protein levels increased at day 20 of pregnancy in both high DHA diet and chow fed animals, and PEMT activity increased significantly at day 20 of pregnancy in high DHA diet fed animals. This trend suggests a time delay between PEMT mRNA expression and actual protein production in maternal liver during pregnancy. The higher protein expression and activity of PEMT in rats fed TWD-DHA+ as compared with rats fed TWD-DHA- at day 20 of pregnancy suggests a positive effect of high DHA intake on PEMT protein expression that was not expected. It is possible that PEMT has a preference for PE high in unsaturated fatty acids (Le Kim et al., 1971); therefore, DHA intake could alter the fatty acid composition of liver PE, which could affect the rate of PE methylation to PC.

Concentrations of DHA in liver and plasma PC increased at 20 days of pregnancy relative to baseline and post partum as hypothesized, but 18:0 levels decreased significantly in relative percentage at that time point. Higher DHA intake of pregnant rats increased the protein expression of PEMT at day 20 of pregnancy and increased DHA incorporation into PC in plasma and liver, which fits our hypothesis. The levels of 16:0

in plasma PC were similar to the increases observed in DHA with pregnancy, but dietary DHA did not appear to have an effect. Dietary DHA increased DHA in PC as expected.

The effect of estrogen on PEMT expression has been reported by several studies, and an estrogen response element (ERE) has been identified ~ 7.5 kb from transcription start site B, TSS (+1) in the PEMT promoter region in both humans and mice (Resseguie et al. 2007; Resseguie et al. 2011). The significant increase in estrogen levels that were observed at day 20 of pregnancy may explain the significant increase in PEMT protein expression and enzymatic activity at that time point. However, the highest level of PEMT mRNA occurred at day 15 in all diet groups, and its activity peaked at day 15 in chow and DHA deficient diet fed animals, while plasma estrogen levels peaked at day 20. Our data suggest that estrogen could be preventing the degradation of PEMT mRNA transcripts, but no evidence of such interaction has been documented in previous studies. Estrogen has been previously shown to affect general protein synthesis and degradation rates in bovine satellite cells (Sollo et al. 2010) and there have been reports of a functional interaction between estradiol stimulated estrogen receptor α (ERα) expression and protein degeneration via proteasomes (Tsai et al. 2004). There were no differences in estrogen levels between rats on different diets (TWD vs. chow); therefore, the effect of TWD (high fat diet) on PEMT protein expression was not caused by a change in serum estrogen levels.

Increased protein expression of PEMT at day 20 of pregnancy in maternal liver was accompanied by a general increase in DHA relative percent in plasma PC. However, there is strong evidence of general lipogenesis occurring at day 20 of pregnancy, an expected adaptation during pregnancy to supply the fetus with sufficient energy (Smith et

al. 1998). Total fatty acid concentrations increased significantly in all plasma pools at day 20 of pregnancy, while liver PC and PE showed no significant changes in total fatty acids. The occurrence of hyperlipidemia in maternal plasma at 20 days of pregnancy meant that the majority of fatty acids that were examined, and not only DHA, showed significantly higher concentrations at that time point compared to baseline, 15 days of pregnancy, and 7 days post partum. However, looking at the relative concentrations of fatty acids reveals differential response of specific fatty acids. DHA percentages increased significantly in plasma PC and plasma PE, in all diet groups at day 20 of pregnancy. Despite the significant increase of DHA percentages in plasma phospholipids in DHA deficient diet fed animals at day 20 of pregnancy, the difference was not significant when looking at the total plasma lipids pool. This suggests either a potential decrease of DHA levels in other plasma lipid fractions such as triacylglycerols (TAG), or a dilution of this increase in DHA content by other fatty acids in the remaining plasma lipid fractions. Other fatty acids, including palmitic acid (16:0) and HUFA with 22 carbons, showed similar trends as DHA. DPA n-6 (22:5n-6) was increased 10-times in plasma PC at 20 days pregnancy as compared with baseline and 15 days of pregnancy in all diet groups, which is in agreement with a recent report (Childs, 2010). The increase of DPA n-6 was particularly large in the DHA deficient diet fed animals suggesting a potential increase in maternal delta 6 desaturase activity (D6D) to meet the fetal demands for DHA (Nakamura and Nara, 2003), but limited available n-3 PUFA substrates.

Contrary to DHA, ARA (20:4n-6) and stearic acid (18:0) relative percent decreased in plasma PC and total lipids at day 20 of pregnancy, but not in plasma PE. PC synthesized *de novo* through the Kennedy pathway, should contain high levels of 16:0

and 20:4n-6, while PC from the PEMT pathway should mimic fatty acid profile of PE, which contains high levels of 18:0 and DHA. The increase in DHA levels in plasma PC observed could not be fully attributed to PEMT activity despite the significant increase in PEMT mRNA at day 15 of pregnancy, and protein expression at day 20 of pregnancy. The concentration of 18:0 in plasma PC was significantly higher at day 20 of pregnancy, but its concentration relative to other fatty acids was decreased at that time point. It appears that *de novo* PC synthesis through the Kennedy pathway is increased at day 20 of pregnancy, based on the higher content of palmitic acid (16:0) relative to other fatty acids in plasma PC. These results are in agreement with previous reports in pregnant rats (Childs et al. 2012, Burdge et al. 1994) and in the developing guinea pig (Burdge et al. 1993). Additionally, the fatty acid composition results suggest that ARA in the sn-2 position of plasma PC molecules is being replaced by DHA. This needs to be confirmed with lipidomic analysis, to characterize the acyl chain composition of plasma PC at the sn-1 and sn-2 positions.

Other possible mechanisms for changing maternal plasma PC fatty acid composition are changes to the phosphatidic acid (PA) substrate pool. Such effects have been previously reported in dams, where increased diacylglycerol (DAG) substrate availability combined with increased CDP enzymes activities were related to increased liver PC and PE synthesis (Burdge et al. 1994). As pregnancy carries a number of metabolic and regulatory changes associated with altered endocrine hormonal levels, the metabolism of fatty acids also changes. Evidence suggests that long chain PUFA synthesis is significantly increased during pregnancy (Burdge and Calder. 2006) likely due to increased desaturases and elongases activities associated with female sex (Kitson

et.al. 2012; Kitson et al. 2013). The increase of plasma and liver DPA n-6 levels suggests that D6D activity is increased in late pregnancy, especially when the dams are fed a diet deficient in DHA that has been proposed to act as a classic feedback inhibitor (Kitson et.al. 2010). With increased PUFA synthesis, incorporation into PA would potentially be increased due to mass action effect, which would subsequently affect the fatty acid composition of the resulting PC and PE synthesized. The increase in PUFA synthesis during pregnancy cannot account for the dramatic increase in plasma DHA levels between day 15 and day 20 of pregnancy. It is possible that specific mechanisms mobilize maternal DHA into the plasma during pregnancy. Adipose tissue has been proposed as a source of 18:3n-3 during pregnancy (Childs et.al.2012). However, DHA is largely associated with protein (Stark and Patterson, 2012) and skeletal muscle appears to be the largest reserve of DHA (Lin and Salem, 2007). While there is little data on the effect of pregnancy on maternal whole body muscle mass, spinal muscular atrophy has been reported during pregnancy (Schonborn et al. 1992). Muscular atrophy during pregnancy could result in the mobilization of fatty acids stores in muscle tissue to the blood stream for placental transport, or for reassembly into plasma phospholipids in the maternal liver.

Specific remodelling of *de novo* synthesized PC and PE could also be responsible for the shift in maternal plasma PC fatty acid composition. The synthesis of PC typically results in 16:0 in the sn-1 position. The 16:0 can be replaced with 18:0 species (Tijburg et al. 1991), but this conversion is reduced at 21 days pregnancy, resulting in increased appearance of 16:0/DHA PC species (Burdge et al. 1994). The increased levels of 16:0 and DHA, and the concurrent reduction of 18:0 and 20:4n-6 levels that we observed in

plasma and liver PC presently, agree with these previous findings. The detailed mechanism of acyl remodeling in phospholipids remains to be fully understood. We hypothesize that pregnancy may also be up-regulating a phospholipase/acyltransferase reaction specific for 16:0 PC and DHA.

The fatty acid composition of fetuses and pups whole body lipids helped to elucidate trends in selective fatty acids transport to the fetus from maternal blood stream via the placenta. Our results suggest a marked increase in DHA delivery to the fetus at day 20 of pregnancy in all diet groups, while other HUFA did not show a similar trend, which has been previously reported (Dutta-Roy, 2000). This trend is hypothesized to be an evolutionary mechanism to supply the fetus with a sufficient amount of DHA necessary to meet the demands of fetal brain development that occurs at this critical stage in pregnancy (Neuringer et.al. 1988). The fetal accretion of DHA was especially high when the mothers were fed a diet high in DHA (TWD-DHA+), as compared with those fed with a DHA deficient diet (TWD-DHA-) or chow. As expected, total fatty acids content increased significantly at 7 days post-partum as the pups grew in size. However, total fatty acids in pups of mothers on the two TWD diets were significantly higher than those whose mothers consumed chow diet despite similar caloric intake at that time point, This trend could be either related to the higher fat content of the TWD diet, or due to a potential effect of the maternal diet on the nutrient density of their breast milk. Breast milk samples were not collected presently, but should be examined in the future.

One limitation to this study is the difference between human and rat brains in terms of fetal development and neurogenesis. Rat pups are born underdeveloped neurologically, particularly the olfactory bulbs, hippocampus, and cerebellum (Altman

and Bayer, 1979) relative to humans. Contrary to rat brains, human brains develop at a higher rate prenatally, and human infants are born with well developed granular neurons (Bayer et.al. 1993). Therefore, since DHA demand is at its highest during brain growth spurt (Innis, 2007; Neuringer et al. 1988), the spike in maternal plasma DHA occurs during the third trimester of pregnancy in women, whereas the brain growth spurt continues postpartum in rats (Bayer et.al. 1993). This could explain the slight decrease in plasma DHA reported in humans (Wijendran, et al. 1999), which does not occur in rats, presently.

The TWD diet used contained significantly higher levels of fat as compared to standard rodent chow that appear to have affected the outcomes of this study, and is considered a limitation in interpreting the effect of dietary DHA. PEMT is regulated by fat intake (Clandinin et al. 1994; Bremer et al. 1961), and increased dietary fat intake is associated with higher blood cholesterol levels that also affect PEMT expression and activity (Keelan et al. 1994). This rodent diet was chosen because it contains types of fatty acids in similar proportions as the diet of Western societies and, therefore, the addition of DHA to the diet would resemble a DHA supplementation regime. Another limitation that was not accounted for with the diets was the effect of insulin on maternal metabolism during pregnancy. Maternal high fat intake not only influences maternal insulin response, but it was also recently associated with insulin resistance in the offspring (Murabayashi et.al. 2013) .Gestational phase insulin resistance is also common during the last phase of pregnancy, and insulin is hypothesized to affect PEMT activity, although this effect is not entirely clear (Hoffman et al.1981; Cabrero et al., 1986; Tashiro et al., 1983; Pangia et al., 1990).

7.2 Conclusion

Pregnancy triggers various maternal adaptations through multiple mechanisms to facilitate the transport and accumulation of fatty acids to and by the fetus. DHA levels in plasma PC rat dams increase during pregnancy compared to baseline and post partum levels. Higher DHA intake of pregnant rats increases the protein expression and activity of PEMT at the end of gestation, and increases DHA incorporation into PC in plasma and liver. While PEMT appears to be involved in influencing the fatty acid composition of plasma PC, the particularly large increase in DHA content in plasma PC during the late stages of pregnancy indicates other mechanisms such as selective acyl remodeling of PC, and PC *de novo* synthesis could also be involved. PEMT seems to have a supporting role in mobilizing maternal DHA into plasma.

Future studies should focus on elucidating the mechanism of DHA incorporation into phospholipids in the Kennedy pathway for phospholipid synthesis, as well as Lands' cycle for phospholipid acyl remodeling. Microarrays could be used to relate the differences in gene expression to different stages in pregnancy, especially to compare day 15 and day 20. Microarrays provide an analytical advantage in that the gene expression of many genes can be examined at once, thereby providing a highly efficient method for studying gene expression. Based on the results obtained from the microarray, the specific genes would be targeted for subsequent analysis with RT-PCR and western blotting. MS/MS based lipidomics analyses could also be pursued to confirm specific phospholipid acyl species, such as 16:0/DHA PC or 18:0/DHA PC. This information could provide definitive answers to confirm whether phospholipid remodeling is indeed a major contributor to the change in maternal phospholipid composition during pregnancy.

The essential nature of omega-3 fatty acids during the late stages in pregnancy has long been demonstrated, and DHA levels have been shown to increase during critical stages of fetal development. Through this research, we elucidated specific mechanisms of DHA mobilization to maternal plasma during pregnancy. PEMT was found to be up-regulated in late pregnancy, but its up-regulation does not appear to be the only pregnancy adaptation contributing to maternal plasma DHA levels. Higher DHA intake during pregnancy was also found to increase PEMT expression, which a novel observation. According to our knowledge this is the first use of total western diet for rodent models in pregnant rats that could allow better insights on DHA intake during pregnancy in the North American population. This research, combined with our dietary intervention, is a step forward in understanding the effects of pregnancy on PUFA metabolism and related fetal outcomes, and could be used in the future to help determine DHA requirements and define intake recommendations.

Appendix A. Plasma Total Lipids

Table A.1. Relative percent of fatty acids in plasma total lipids in chow fed rats at baseline, during pregnancy, and 7 days post partum

Name	baseline	15 days	20 days	7 days post partum
C 14:0	0.32 ± 0.02	0.30 ± 0.11	0.26 ± 0.03	0.29 ± 0.01
C 16:0	14.64 ± 0.64[a]	16.15 ± 0.76[a]	20.38 ± 1.14[b]	15.81 ± 1.53[a]
C 18:0	15.58 ± 1.11[a]	13.68 ± 1.42[a]	8.58 ± 0.96[b]	13.83 ± 1.96[a]
C 20:0	0.11 ± 0.02[a]	0.08 ± 0.01[b]	0.06 ± 0.02[b]	0.08 ± 0.01[b]
C 22:0	0.24 ± 0.02[a]	0.17 ± 0.05[ab]	0.07 ± 0.02[c]	0.15 ± 0.04[b]
C 24:0	0.59 ± 0.02[a]	0.39 ± 0.11[b]	0.18 ± 0.02[c]	0.42 ± 0.10[b]
SFAs	32.20 ± 0.75[a]	31.15 ± 1.11[ab]	29.79 ± 1.17[b]	30.94 ± 1.02[ab]
C 14:1	0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01
C 16:1	0.63 ± 0.14[a]	0.68 ± 0.17	0.72 ± 0.14	0.69 ± 0.15
C 18:1n-7	1.14 ± 0.06[a]	1.27 ± 0.06[ab]	1.37 ± 0.06[b]	1.36 ± 0.08[ab]
C 18:1n-9	5.76 ± 0.43[a]	8.92 ± 1.67[ab]	11.34 ± 0.96[b]	10.77 ± 2.21[ab]
C 20:1n-9	0.06 ± 0.01	0.09 ± 0.02	0.11 ± 0.03	0.12 ± 0.04
C 22:1n-9	0.20 ± 0.03[a]	0.13 ± 0.02[ab]	0.04 ± 0.01[c]	0.06 ± 0.04[bc]
C 24:1n-9	0.36 ± 0.04[a]	0.27 ± 0.08[a]	0.07 ± 0.02[b]	0.26 ± 0.08[a]
MUFAs	8.18 ± 0.42	11.38 ± 1.74	13.67 ± 1.06	13.3 ± 2.29
C 18:2n-6	18.11 ± 1.78[a]	21.00 ± 3.21[ab]	22.68 ± 1.38[b]	21.43 ± 2.30[b]
C 18:3n-6	0.39 ± 0.03[a]	0.69 ± 0.18[a]	0.63 ± 0.05[a]	1.18 ± 0.20[b]
C 20:2n-6	0.12 ± 0.02[a]	0.14 ± 0.02[a]	0.22 ± 0.03[b]	0.19 ± 0.02[b]
C 20:3n-6	0.26 ± 0.02[a]	0.27 ± 0.06[a]	0.25 ± 0.04[a]	0.52 ± 0.07[b]
C 20:4n-6	34.10 ± 1.03[a]	27.03 ± 3.74[b]	19.19 ± 1.63[c]	24.27 ± 3.57[bc]
C 22:2n-6	0.05 ± 0.01[a]	0.04 ± 0.01[ab]	0.02 ± 0.01[c]	0.02 ± 0.01[bc]
C 22:4n-6	0.22 ± 0.02[a]	0.35 ± 0.11[a]	1.3 ± 0.23[b]	0.33 ± 0.05[a]
N-6	53.44 ± 1.04[a]	49.77 ± 0.95[ab]	45.88 ± 1.16[b]	48.55 ± 1.37[ab]
C 18:3n-3	0.48 ± 0.03[a]	0.72 ± 0.33[b]	1.09 ± 0.1[c]	0.48 ± 0.2[a]
C 20:3n-3	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01
C 20:5n-3	0.57 ± 0.08[a]	0.89 ± 0.35[b]	0.61 ± 0.20[ab]	0.83 ± 0.15[ab]
C 22:5n-3	0.43 ± 0.03[a]	0.61 ± 0.10[a]	1.49 ± 0.23[c]	0.86 ± 0.14[b]
C 22:6n-3	3.12 ± 0.13[a]	4.23 ± 0.75[a]	6.25 ± 1.01[b]	3.21 ± 0.49[a]
N-3	4.61 ± 0.12[a]	6.47 ± 0.56[b]	9.45 ± 0.89[c]	5.39 ± 0.58[ab]
HUFA	38.90 ± 1.11[a]	33.66 ± 4.29[ab]	30.71 ± 1.79[b]	30.65 ± 3.90[b]
PUFA	58.05 ± 0.99	56.25 ± 1.05	55.33 ± 0.76	53.94 ± 1.45
Total	208.50 ± 13.58[a]	288.01 ± 80.75[a]	502.49 ± 90.87[b]	319.38 ± 97.11[a]

Values with different superscripts are significantly different by Tukey's post hoc test ($p<0.05$) following significant F-value by one-way ANOVA.

Table A.2. Relative percent of fatty acids in plasma total lipids in high DHA diet (TWD DHA+) fed rats at baseline, during pregnancy, and 7 days post partum

Name	baseline	15 days	20 days	7 days post partum
C 14:0	0.45 ± 0.05^{a}	0.61 ± 0.07^{b}	0.58 ± 0.05^{ab}	0.66 ± 0.10^{b}
C 16:0	15.96 ± 0.69^{a}	18.10 ± 0.96^{ab}	21.42 ± 0.92^{c}	19.80 ± 1.73^{bc}
C 18:0	15.61 ± 0.46^{a}	14.27 ± 0.57^{ab}	10.30 ± 0.93^{c}	12.20 ± 1.07^{bc}
C 20:0	0.10 ± 0.01^{a}	0.09 ± 0.01^{a}	0.06 ± 0.01^{b}	0.08 ± 0.01^{ab}
C 22:0	0.24 ± 0.02^{a}	0.22 ± 0.02^{a}	0.07 ± 0.01^{b}	0.13 ± 0.03^{c}
C 24:0	0.56 ± 0.04^{a}	0.42 ± 0.06^{b}	0.16 ± 0.02^{c}	0.30 ± 0.07^{bc}
SFA	33.52 ± 0.44	34.31 ± 0.92	32.89 ± 0.78	33.64 ± 1.12
C 14:1	0.03 ± 0.01^{a}	0.05 ± 0.01^{b}	0.03 ± 0.01^{a}	0.04 ± 0.01^{ab}
C 16:1	0.90 ± 0.11^{a}	1.16 ± 0.19^{ab}	1.12 ± 0.25^{ab}	1.50 ± 0.28^{b}
C 18:1n-7	1.12 ± 0.06^{a}	1.20 ± 0.07^{ab}	1.41 ± 0.11^{bc}	1.60 ± 0.13^{c}
C 18:1n-9	8.47 ± 0.68^{a}	13.01 ± 1.91^{ab}	18.36 ± 2.66^{bc}	21.09 ± 5.18^{c}
C 20:1n-9	0.06 ± 0.01^{a}	0.08 ± 0.03^{ab}	0.14 ± 0.03^{b}	0.10 ± 0.04^{ab}
C 22:1n-9	0.24 ± 0.02^{a}	0.18 ± 0.04^{a}	0.07 ± 0.04^{b}	0.06 ± 0.04^{b}
C 24:1n-9	0.37 ± 0.07^{ac}	0.43 ± 0.05^{a}	0.11 ± 0.02^{b}	0.26 ± 0.10^{c}
MUFA	11.20 ± 0.73^{a}	15.93 ± 2.32^{ab}	21.24 ± 2.92^{bc}	24.67 ± 5.43^{c}
C 18:2n-6	16.67 ± 0.72	15.84 ± 0.61	14.26 ± 0.48	16.20 ± 1.76
C 18:3n-6	0.28 ± 0.04^{a}	0.43 ± 0.09^{ab}	0.64 ± 0.14^{bc}	0.89 ± 0.25^{c}
C 20:2n-6	0.08 ± 0.01	0.08 ± 0.02	0.11 ± 0.02	0.08 ± 0.01
C 20:3n-6	0.31 ± 0.04^{a}	0.26 ± 0.05^{a}	0.15 ± 0.02^{b}	0.31 ± 0.03^{a}
C 20:4n-6	30.36 ± 1.55^{a}	23.72 ± 2.09^{b}	16.70 ± 1.89^{c}	17.4 ± 5.41^{bc}
C 22:2n-6	0.04 ± 0.01^{a}	0.05 ± 0.01^{b}	0.02 ± 0.01^{c}	0.03 ± 0.01^{ac}
C 22:4n-6	0.17 ± 0.01^{a}	0.18 ± 0.04^{a}	0.46 ± 0.12^{b}	0.11 ± 0.04^{a}
C 22:5n-6	0.13 ± 0.02^{a}	0.13 ± 0.04^{a}	0.74 ± 0.25^{b}	0.09 ± 0.03^{a}
N-6	48.04 ± 0.98^{a}	40.70 ± 1.64^{b}	33.07 ± 1.73^{c}	35.09 ± 4.89^{c}
C 18:3n-3	0.40 ± 0.05	0.47 ± 0.07	0.50 ± 0.08	0.31 ± 0.05
C 20:3n-3	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01
C 20:5n-3	0.62 ± 0.10^{ab}	0.77 ± 0.10^{a}	0.39 ± 0.11^{b}	0.59 ± 0.13^{ab}
C 22:5n-3	0.25 ± 0.02^{a}	0.36 ± 0.10^{a}	0.62 ± 0.11^{b}	0.22 ± 0.05^{a}
C 22:6n-3	4.91 ± 0.41^{a}	6.08 ± 0.69^{b}	9.58 ± 1.12^{c}	3.69 ± 1.28^{a}
N-3	6.19 ± 0.45^{a}	7.7 ± 0.76^{b}	11.1 ± 1.04^{c}	4.82 ± 1.32^{d}
HUFA	36.77 ± 1.27^{a}	31.53 ± 2.72^{ab}	28.66 ± 3.00^{bc}	22.39 ± 6.64^{c}
PUFA	54.23 ± 0.83^{a}	48.40 ± 2.13^{b}	44.18 ± 2.66^{bc}	39.91 ± 5.98^{c}
Total	221.71 ± 13.56^{a}	196.38 ± 24.91^{a}	508.76 ± 165.81^{b}	238.65 ± 23.31^{a}

Values with different superscripts are significantly different by Tukey's post hoc test ($p<0.05$) following significant F-value by one-way ANOVA.

Table A.3. Relative percent of fatty acids in plasma total lipids in DHA deficient diet (TWD DHA-) fed rats at baseline, during pregnancy, and 7 days post partum

Name	baseline	15 days	20 days	7 days post partum
C 14:0	0.39 ± 0.06[a]	0.54 ± 0.04[b]	0.58 ± 0.06[b]	0.67 ± 0.11[b]
C 16:0	14.52 ± 1.19[a]	16.85 ± 0.50[b]	21.27 ± 0.79[c]	18.41 ± 2.16[b]
C 18:0	16.32 ± 0.93[a]	14.86 ± 0.94[ab]	9.58 ± 0.52[c]	12.95 ± 2.16[b]
C 20:0	0.09 ± 0.01[a]	0.09 ± 0.01[a]	0.05 ± 0.01[b]	0.08 ± 0.01[a]
C 22:0	0.23 ± 0.03[a]	0.21 ± 0.03[a]	0.07 ± 0.01[b]	0.11 ± 0.04[b]
C 24:0	0.52 ± 0.06[a]	0.44 ± 0.04[a]	0.17 ± 0.03[b]	0.27 ± 0.13[b]
SFA	32.63 ± 0.81	33.58 ± 1.01	31.96 ± 0.88	33.02 ± 0.22
C 14:1	0.02 ± 0.01[a]	0.04 ± 0.01[b]	0.04 ± 0.01[b]	0.04 ± 0.01[b]
C 16:1	0.70 ± 0.17[a]	1.20 ± 0.14[b]	1.40 ± 0.18[b]	1.52 ± 0.42[b]
C 18:1n-7	1.10 ± 0.06[a]	1.30 ± 0.09[ab]	1.43 ± 0.05[b]	1.73 ± 0.31[c]
C 18:1n-9	7.79 ± 0.92[a]	13.19 ± 0.67[b]	21.20 ± 1.48[c]	22.91 ± 6.11[c]
C 20:1n-9	0.05 ± 0.01[a]	0.11 ± 0.04[ab]	0.12 ± 0.03[b]	0.12 ± 0.04[b]
C 22:1n-9	0.22 ± 0.05[a]	0.19 ± 0.06[a]	0.04 ± 0.01[b]	0.05 ± 0.02[b]
C 24:1n-9	0.36 ± 0.05[ac]	0.41 ± 0.04[a]	0.11 ± 0.02[b]	0.24 ± 0.13[c]
MUFA	10.27 ± 1.12[a]	16.46 ± 0.62[b]	24.32 ± 1.57[c]	26.62 ± 6.63[c]
C 18:2n-6	15.63 ± 0.83	15.02 ± 1.01	14.93 ± 0.89	14.07 ± 1.16
C 18:3n-6	0.39 ± 0.02[a]	0.63 ± 0.09[a]	1.06 ± 0.17[b]	1.34 ± 0.26[b]
C 20:2n-6	0.08 ± 0.01	0.08 ± 0.01	0.10 ± 0.02	0.09 ± 0.01
C 20:3n-6	0.27 ± 0.03[a]	0.18 ± 0.02[ab]	0.11 ± 0.01[b]	0.26 ± 0.06[a]
C 20:4n-6	34.46 ± 2.38[a]	27.24 ± 1.29[b]	17.31 ± 1.78[c]	19.6 ± 7.26[c]
C 22:2n-6	0.03 ± 0.01[ab]	0.04 ± 0.01[b]	0.01 ± 0.01[c]	0.03 ± 0.01[ac]
C 22:4n-6	0.22 ± 0.01[a]	0.23 ± 0.05[a]	0.68 ± 0.11[b]	0.16 ± 0.03[a]
C 22:5n-6	0.29 ± 0.04[a]	0.32 ± 0.12[a]	2.23 ± 0.48[b]	0.35 ± 0.07[a]
N-6	51.38 ± 1.60[a]	43.76 ± 0.72[b]	36.44 ± 1.30[c]	35.89 ± 6.15[c]
C 18:3n-3	0.36 ± 0.03	0.46 ± 0.04	0.51 ± 0.04	0.22 ± 0.07
C 20:3n-3	Not detected	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01
C 20:5n-3	0.40 ± 0.04	0.37 ± 0.07	0.25 ± 0.07	0.23 ± 0.04
C 22:5n-3	0.36 ± 0.03[a]	0.36 ± 0.07[a]	0.61 ± 0.06[b]	0.30 ± 0.04[a]
C 22:6n-3	3.41 ± 0.28[a]	3.71 ± 0.3[a]	3.94 ± 0.35[a]	1.63 ± 0.73[b]
N-3	4.54 ± 0.27[a]	4.90 ± 0.32[a]	5.32 ± 0.33[a]	2.39 ± 0.69[b]
HUFA	39.42 ± 2.58[a]	32.42 ± 1.42[ab]	25.13 ± 2.14[bc]	22.54 ± 8.09[c]
PUFA	55.92 ± 1.81[a]	48.66 ± 0.94[b]	41.76 ± 1.49[c]	38.28 ± 6.83[c]
Total	233.29 ± 26.33[a]	185.55 ± 19.98[a]	588.21 ± 64.92[b]	233.04 ± 50.12[a]

Values with different superscripts are significantly different by Tukey's post hoc test ($p<0.05$) following significant F-value by one-way ANOVA.

Table A.4. Concentration of fatty acids in plasma total lipids in chow fed rats at baseline, during pregnancy, and 7 days post partum (µg/100µl plasma)

Name	baseline	15 days	20 days	7 days post partum
C 14:0	0.67 ± 0.06	0.86 ± 0.32	1.32 ± 0.20	0.95 ± 0.33
C 16:0	30.99 ± 2.10^{a}	47.50 ± 15.45^{a}	103.58 ± 18.59^{b}	52.45 ± 21.04^{a}
C 18:0	33.02 ± 3.36	39.18 ± 8.06	43.15 ± 4.86	43.41 ± 5.82
C 20:0	0.23 ± 0.04	0.22 ± 0.05	0.31 ± 0.09	0.24 ± 0.05
C 22:0	0.50 ± 0.04^{a}	0.46 ± 0.07^{ab}	0.36 ± 0.06^{b}	0.47 ± 0.10^{ab}
C 24:0	1.25 ± 0.11^{a}	1.10 ± 0.22^{ab}	0.91 ± 0.05^{b}	1.31 ± 0.24^{a}
SFA	68.21 ± 4.68^{a}	90.38 ± 23.63^{a}	150.86 ± 21.99^{b}	99.95 ± 27.02^{a}
C 14:1	0.03 ± 0.01	0.04 ± 0.02	0.05 ± 0.02	0.03 ± 0.01
C 16:1	1.36 ± 0.38	2.00 ± 0.79	3.70 ± 1.25	2.34 ± 1.27
C 18:1n-7	2.41 ± 0.21^{a}	3.71 ± 1.01^{a}	7.03 ± 1.58^{b}	4.40 ± 1.30^{a}
C 18:1n-9	12.17 ± 0.56^{a}	26.81 ± 11.41^{ab}	58.29 ± 15.57^{b}	36.76 ± 19.58^{ab}
C 20:1n-9	0.14 ± 0.01^{a}	0.26 ± 0.08^{a}	0.59 ± 0.23^{b}	0.38 ± 0.15^{ab}
C 22:1n-9	0.42 ± 0.07^{a}	0.37 ± 0.07^{ab}	0.19 ± 0.06^{b}	0.20 ± 0.14^{b}
C 24:1n-9	0.76 ± 0.11^{a}	0.75 ± 0.10^{a}	0.36 ± 0.06^{b}	0.79 ± 0.15^{a}
MUFA	17.31 ± 1.01^{a}	33.97 ± 13.27^{ab}	70.22 ± 18.45^{b}	45.01 ± 22.25^{ab}
C 18:2n-6	38.32 ± 4.04^{a}	62.14 ± 22.62^{ab}	114.97 ± 18.44^{c}	71.25 ± 28.85^{b}
C 18:3n-6	0.83 ± 0.09^{a}	2.12 ± 1.13^{ab}	3.22 ± 0.88^{b}	3.98 ± 2.16^{b}
C 20:3n-6	0.55 ± 0.06^{a}	0.75 ± 0.15^{a}	1.29 ± 0.32^{b}	1.65 ± 0.33^{b}
C 20:2n-6	0.26 ± 0.04^{a}	0.39 ± 0.08^{ac}	1.12 ± 0.39^{b}	0.61 ± 0.17^{c}
C 20:4n-6	72.29 ± 6.14	77.51 ± 18.14	97.96 ± 22.43	76.38 ± 12.23
C 22:2n-6	0.11 ± 0.02^{a}	0.10 ± 0.01^{ab}	0.09 ± 0.02^{ab}	0.06 ± 0.01^{b}
C 22:4n-6	0.47 ± 0.05^{a}	0.99 ± 0.33^{a}	6.68 ± 1.94^{b}	1.07 ± 0.42^{a}
C 22:5n-6	0.39 ± 0.06^{a}	0.78 ± 0.33^{a}	8.19 ± 1.98^{b}	2.05 ± 0.84^{a}
N-6	113.22 ± 8.06^{a}	144.78 ± 39.2^{a}	233.53 ± 43.97^{b}	157.05 ± 43.53^{a}
C 18:3n-3	1.01 ± 0.08^{a}	2.20 ± 1.26^{a}	5.53 ± 1.04^{b}	1.69 ± 1.19^{a}
C 20:3n-3	0.02 ± 0.01^{a}	0.03 ± 0.02^{a}	0.07 ± 0.01^{b}	0.02 ± 0.01^{a}
C 20:5n-3	1.22 ± 0.24^{a}	2.67 ± 1.33^{ab}	3.22 ± 1.73^{b}	2.74 ± 1.02^{b}
C 22:5n-3	0.92 ± 0.09^{a}	1.77 ± 0.45^{ab}	7.71 ± 2.54^{c}	2.78 ± 0.89^{b}
C 22:6n-3	6.60 ± 0.45^{a}	12.22 ± 3.38^{a}	31.36 ± 4.37^{b}	10.15 ± 2.01^{a}
N-3	9.77 ± 0.76^{a}	18.89 ± 5.31^{a}	47.88 ± 8.26^{b}	17.37 ± 4.86^{a}
HUFA	82.45 ± 6.91^{a}	96.72 ± 22.92^{a}	156.48 ± 32.27^{b}	96.84 ± 16.67^{a}
PUFA	122.99 ± 8.73^{a}	163.67 ± 44.31^{a}	281.41 ± 50.99^{b}	174.42 ± 48.11^{a}
Total	208.50 ± 13.58^{a}	288.01 ± 80.75^{a}	502.49 ± 90.87^{b}	319.38 ± 97.11^{a}

Values with different superscripts are significantly different by Tukey's post hoc test ($p<0.05$) following significant F-value by one-way ANOVA

Table A.5. Concentration of fatty acids in plasma total lipids in high DHA diet (TWD DHA+) fed rats at baseline, during pregnancy, and 7 days post partum (µg/100µl plasma)

Name	baseline	15 days	20 days	7 days post partum
C 14:0	1.01 ± 0.14[a]	1.20 ± 0.12[a]	3.08 ± 1.26[b]	1.60 ± 0.36[a]
C 16:0	35.74 ± 2.21[a]	35.95 ± 4.25[a]	111.38 ± 39.12[b]	48.41 ± 8.84[a]
C 18:0	35.00 ± 2.70[a]	28.37 ± 3.39[a]	52.21 ± 12.78[b]	29.48 ± 2.01[a]
C 20:0	0.21 ± 0.02[a]	0.18 ± 0.01[a]	0.30 ± 0.09[b]	0.19 ± 0.02[a]
C 22:0	0.53 ± 0.06[a]	0.43 ± 0.05[ab]	0.35 ± 0.07[b]	0.32 ± 0.06[b]
C 24:0	1.26 ± 0.13[a]	0.83 ± 0.09[b]	0.82 ± 0.16[b]	0.72 ± 0.15[b]
SFA	75.09 ± 4.50[a]	68.14 ± 7.08[a]	169.61 ± 53.3[b]	81.87 ± 9.97[a]
C 14:1	0.07 ± 0.02[a]	0.09 ± 0.02[a]	0.17 ± 0.09[b]	0.09 ± 0.02[a]
C 16:1	2.01 ± 0.29[a]	2.37 ± 0.49[a]	6.07 ± 3.13[b]	3.67 ± 0.99[ab]
C 18:1n-7	2.52 ± 0.24[a]	2.38 ± 0.35[a]	7.39 ± 2.78[b]	3.89 ± 0.57[a]
C 18:1n-9	18.92 ± 1.14[a]	26.04 ± 5.86[a]	98.63 ± 46.51[b]	51.93 ± 16.39[a]
C 20:1n-9	0.13 ± 0.01[a]	0.17 ± 0.06[a]	0.73 ± 0.37[b]	0.25 ± 0.09[a]
C 22:1n-9	0.54 ± 0.03[a]	0.35 ± 0.07[b]	0.32 ± 0.17[bc]	0.15 ± 0.09[c]
C 24:1n-9	0.84 ± 0.17[a]	0.86 ± 0.10[a]	0.54 ± 0.10[b]	0.63 ± 0.18[ab]
MUFA	25.05 ± 1.43[a]	31.90 ± 7.16[a]	113.87 ± 52.83[b]	60.64 ± 17.67[a]
C 18:2n-6	37.29 ± 1.46[a]	31.51 ± 3.88[a]	74.06 ± 25.52[b]	39.55 ± 6.88[a]
C 18:3n-6	0.62 ± 0.09[a]	0.87 ± 0.26[a]	3.34 ± 1.32[b]	2.17 ± 0.69[ab]
C 20:2n-6	0.18 ± 0.03[a]	0.17 ± 0.05[a]	0.59 ± 0.31[b]	0.20 ± 0.03[a]
C 20:3n-6	0.68 ± 0.07	0.53 ± 0.14	0.77 ± 0.28	0.75 ± 0.11
C 20:4n-6	68.17 ± 7.24[ac]	47.25 ± 7.40[ab]	84.10 ± 18.26[c]	41.42 ± 10.5[b]
C 22:2n-6	0.09 ± 0.01	0.09 ± 0.02	0.09 ± 0.02	0.06 ± 0.01
C 22:4n-6	0.38 ± 0.02[a]	0.36 ± 0.11[a]	2.46 ± 1.22[b]	0.26 ± 0.09[a]
C 22:5n-6	0.30 ± 0.04[a]	0.25 ± 0.08[a]	3.64 ± 0.98[b]	0.20 ± 0.07[a]
N-6	107.72 ± 8.22[a]	81.02 ± 10.69[a]	169.03 ± 46.44[b]	84.62 ± 9.4[a]
C 18:3n-3	0.88 ± 0.09[a]	0.93 ± 0.20[a]	2.68 ± 1.32[b]	0.76 ± 0.13[a]
C 20:3n-3	0.02 ± 0.01[a]	0.02 ± 0.01[a]	0.05 ± 0.02[b]	0.02 ± 0.01[a]
C 20:5n-3	1.38 ± 0.22	1.53 ± 0.23	2.13 ± 1.02	1.42 ± 0.32
C 22:5n-3	0.57 ± 0.03[a]	0.73 ± 0.25[a]	3.19 ± 1.10[b]	0.53 ± 0.11[a]
C 22:6n-3	11.00 ± 1.03[a]	12.11 ± 2.06[a]	48.19 ± 10.36[b]	8.80 ± 2.47[a]
N-3	13.86 ± 1.11[a]	15.32 ± 2.47[a]	56.25 ± 13.62[b]	11.53 ± 2.37[a]
HUFA	82.50 ± 7.65[a]	62.78 ± 9.80[a]	144.53 ± 32.19[b]	53.35 ± 12.52[a]
PUFA	121.58 ± 8.76[a]	96.34 ± 12.87[a]	225.28 ± 59.96[b]	96.15 ± 10.92[a]
Total	221.71 ± 13.56[a]	196.38 ± 24.91[a]	508.76 ± 165.81[b]	238.65 ± 23.31[a]

Values with different superscripts are significantly different by Tukey's post hoc test ($p<0.05$) following significant F-value by one-way ANOVA

Table A.6. Concentration of fatty acids in plasma total lipids in DHA deficient diet (TWD DHA-) fed rats at baseline, during pregnancy, and 7 days post partum (µg/100µl plasma)

Name	baseline	15 days	20 days	7 days post partum
C 14:0	0.92 ± 0.11[a]	1.02 ± 0.08[a]	3.47 ± 0.50[b]	1.61 ± 0.51[a]
C 16:0	34.18 ± 3.93[a]	31.73 ± 3.99[a]	127.54 ± 14.19[b]	44.57 ± 14.3[a]
C 18:0	38.50 ± 4.82[a]	27.98 ± 3.81[a]	57.24 ± 4.20[b]	30.17 ± 3.77[a]
C 20:0	0.20 ± 0.02[a]	0.18 ± 0.03[a]	0.31 ± 0.03[b]	0.19 ± 0.01[a]
C 22:0	0.53 ± 0.10[a]	0.39 ± 0.06[bc]	0.40 ± 0.03[c]	0.26 ± 0.04[b]
C 24:0	1.24 ± 0.23[a]	0.82 ± 0.12[bc]	1.01 ± 0.11[c]	0.61 ± 0.19[b]
SFA	76.89 ± 7.53[a]	63.21 ± 7.79[a]	191.46 ± 17.99[b]	78.63 ± 17.19[a]
C 14:1	0.06 ± 0.01[a]	0.08 ± 0.01[a]	0.24 ± 0.05[b]	0.10 ± 0.04[a]
C 16:1	1.64 ± 0.36[a]	2.25 ± 0.29[a]	8.39 ± 1.58[b]	3.74 ± 1.62[a]
C 18:1n-7	2.60 ± 0.30[a]	2.44 ± 0.25[a]	8.57 ± 1.01[b]	4.15 ± 1.32[a]
C 18:1n-9	18.25 ± 1.84[a]	24.82 ± 3.18[a]	127.75 ± 21.85[b]	56.22 ± 23.57[b]
C 20:1n-9	0.13 ± 0.02[a]	0.20 ± 0.06[a]	0.69 ± 0.20[b]	0.28 ± 0.12[a]
C 22:1n-9	0.52 ± 0.08[a]	0.35 ± 0.08[ab]	0.21 ± 0.08[bc]	0.13 ± 0.06[c]
C 24:1n-9	0.86 ± 0.17[a]	0.76 ± 0.08[ab]	0.62 ± 0.1[ab]	0.53 ± 0.21[b]
MUFA	24.07 ± 2.34[a]	30.93 ± 3.44[a]	146.50 ± 24.17[b]	65.19 ± 26.43[a]
C 18:2n-6	36.81 ± 3.72[a]	28.33 ± 4.28[a]	89.72 ± 12.82[b]	33.76 ± 9.10[a]
C 18:3n-6	0.93 ± 0.13[a]	1.19 ± 0.21[ab]	6.41 ± 1.46[c]	3.28 ± 1.3[b]
C 20:2n-6	0.19 ± 0.02[a]	0.15 ± 0.02[a]	0.62 ± 0.20[b]	0.20 ± 0.04[a]
C 20:3n-6	0.63 ± 0.04	0.34 ± 0.05	0.63 ± 0.08	0.62 ± 0.19
C 20:4n-6	81.72 ± 13.19[a]	51.09 ± 4.63[b]	103.35 ± 10.7[a]	44.59 ± 10.49[b]
C 22:2n-6	0.08 ± 0.02	0.08 ± 0.02	0.09 ± 0.01	0.07 ± 0.02
C 22:4n-6	0.52 ± 0.06[a]	0.44 ± 0.09[a]	4.09 ± 0.98[b]	0.38 ± 0.10[a]
C 22:5n-6	0.68 ± 0.12[a]	0.59 ± 0.19[a]	13.50 ± 3.53[b]	0.82 ± 0.22[a]
N-6	121.56 ± 16.28[a]	82.21 ± 8.38[a]	218.41 ± 23.13[b]	83.71 ± 11.97[a]
C 18:3n-3	0.84 ± 0.09[a]	0.87 ± 0.14[a]	3.10 ± 0.53[b]	0.53 ± 0.22[a]
C 20:3n-3	0.01 ± 0.01[a]	0.02 ± 0.01[a]	0.04 ± 0.01[b]	0.02 ± 0.01[a]
C 20:5n-3	0.95 ± 0.17	0.69 ± 0.15	1.51 ± 0.49	0.55 ± 0.19
C 22:5n-3	0.85 ± 0.12[a]	0.67 ± 0.14[a]	3.67 ± 0.47[b]	0.74 ± 0.25[a]
C 22:6n-3	8.12 ± 1.48[a]	6.94 ± 0.68[a]	23.52 ± 2.26[b]	3.69 ± 1.10[a]
N-3	10.77 ± 1.76[a]	9.20 ± 0.95[a]	31.84 ± 2.98[b]	5.52 ± 1.08[a]
HUFA	93.48 ± 14.97[a]	60.79 ± 5.20[ab]	150.31 ± 16.2[c]	51.40 ± 11.80[b]
PUFA	132.33 ± 18.01[a]	91.41 ± 9.21[a]	250.25 ± 25.69[b]	89.22 ± 12.89[a]
Total	233.29 ± 26.33[a]	185.55 ± 19.98[a]	588.21 ± 64.92[b]	233.04 ± 50.12[a]

Values with different superscripts are significantly different by Tukey's post hoc test ($p<0.05$) following significant F-value by one-way ANOVA

Appendix B. Plasma PC

Table B.1. Relative percent of fatty acids in plasma PC in chow fed rats at baseline, during pregnancy, and 7 days post partum

Name	baseline	15 days	20 days	7 days post partum
C 14:0	0.41 ± 0.22	0.56 ± 0.13	0.69 ± 0.09	0.74 ± 0.18
C 16:0	16.86 ± 0.99[a]	18.35 ± 1.46[a]	26.76 ± 1.73[b]	18.32 ± 0.66[a]
C 18:0	25.46 ± 2.33[a]	28.88 ± 2.18[ab]	21.55 ± 1.02[c]	29.64 ± 1.65[b]
C 20:0	0.18 ± 0.04	0.15 ± 0.07	0.13 ± 0.01	0.20 ± 0.06
C 22:0	0.25 ± 0.08[a]	0.14 ± 0.05[b]	0.10 ± 0.02[b]	0.10 ± 0.02[b]
C 24:0	0.22 ± 0.04	0.20 ± 0.08	0.24 ± 0.04	0.17 ± 0.05
SFA	43.92 ± 1.26[a]	49.27 ± 2.34[b]	51.06 ± 1.73[b]	50.47 ± 2.18[b]
C 14:1	0.05 ± 0.01[a]	0.04 ± 0.01[a]	Not detected [b]	0.03 ± 0.01[ab]
C 16:1	0.58 ± 0.17	0.74 ± 0.17	0.78 ± 0.12	0.64 ± 0.09
C 18:1n-7	1.49 ± 0.12[a]	1.25 ± 0.11[ab]	1.12 ± 0.1[b]	1.42 ± 0.17[a]
C 18:1n-9	9.54 ± 6.09[a]	4.30 ± 0.92[b]	4.43 ± 0.31[ab]	6.54 ± 1.02[ab]
C 20:1n-9	0.36 ± 0.25	0.13 ± 0.05	0.10 ± 0.02	0.12 ± 0.01
C 22:1n-9	0.20 ± 0.04[ab]	0.27 ± 0.07[a]	0.10 ± 0.03[b]	0.23 ± 0.03[ab]
C 24:1n-9	0.24 ± 0.04	0.26 ± 0.17	0.05 ± 0.03	0.10 ± 0.03
MUFA	12.49 ± 6.38[a]	7.02 ± 0.99[ab]	6.59 ± 0.25[b]	9.09 ± 1.14[ab]
C 18:2n-6	15.78 ± 2.34[a]	12.56 ± 1.53[b]	11.46 ± 1.85[b]	14.42 ± 1.93[ab]
C 18:3n-6	0.74 ± 0.81	0.23 ± 0.07	0.10 ± 0.02	0.15 ± 0.02
C 20:2n-6	0.26 ± 0.08[a]	0.18 ± 0.02[b]	0.16 ± 0.02[b]	0.24 ± 0.03[a]
C 20:3n-6	0.41 ± 0.11[a]	0.37 ± 0.0[ab]	0.22 ± 0.03[b]	0.78 ± 0.08[c]
C 20:4n-6	18.04 ± 3.56[a]	19.54 ± 1.23[a]	11.61 ± 2.05[b]	15.22 ± 1.78[ab]
C 22:2n-6	0.13 ± 0.09	0.12 ± 0.07	0.07 ± 0.05	0.07 ± 0.02
C 22:4n-6	0.32 ± 0.08[a]	0.23 ± 0.06[a]	0.61 ± 0.06[b]	0.33 ± 0.07[a]
C 22:5n-6	0.30 ± 0.04[a]	0.33 ± 0.12[a]	3.36 ± 0.43[b]	0.99 ± 0.32[a]
N-6	35.98 ± 4.91[a]	33.57 ± 1.72[a]	27.60 ± 2.68[b]	32.20 ± 3.06[ab]
C 18:3n-3	0.18 ± 0.10	0.10 ± 0.02	0.12 ± 0.02	0.08 ± 0.01
C 20:3n-3	0.05 ± 0.01	0.08 ± 0.05	0.04 ± 0.01	0.02 ± 0.01
C 20:5n-3	0.21 ± 0.08[a]	0.16 ± 0.07[ab]	0.08 ± 0.01[b]	0.11 ± 0.02[ab]
C 22:5n-3	0.84 ± 0.15[a]	0.80 ± 0.11[a]	1.17 ± 0.12[b]	1.36 ± 0.27[b]
C 22:6n-3	5.69 ± 0.86[a]	6.89 ± 1.45[a]	12.67 ± 1.13[b]	5.69 ± 0.95[a]
N-3	6.98 ± 1.03[a]	8.03 ± 1.45[a]	14.09 ± 1.09[b]	7.26 ± 1.17[a]
HUFA	25.86 ± 4.62	28.41 ± 2.48	29.77 ± 1.5	24.50 ± 2.46
PUFA	42.96 ± 5.84	41.60 ± 2.44	41.69 ± 1.71	39.46 ± 2.98
Total	106.81 ± 34.92[a]	106.13 ± 27.14[a]	184.00 ± 35.52[b]	179.90 ± 20.84[b]

Values with different superscripts are significantly different by Tukey's post hoc test ($p<0.05$) following significant F-value by one-way ANOVA

Table B.2. Relative percent of fatty acids in plasma PC in high DHA diet (TWD DHA+) fed rats at baseline, during pregnancy, and 7 days post partum

Name	baseline	15 days	20 days	7 days post partum
C 16:0	17.40 ± 0.68[a]	18.05 ± 1.46[a]	24.54 ± 2.14[c]	21.09 ± 1.10[b]
C 18:0	27.99 ± 0.59[a]	29.93 ± 1.49[a]	23.77 ± 1.66[b]	28.06 ± 1.68[a]
C 20:0	0.18 ± 0.02[a]	0.19 ± 0.06[ab]	0.12 ± 0.02[a]	0.28 ± 0.07[b]
C 22:0	0.20 ± 0.05[a]	0.15 ± 0.06[ab]	0.09 ± 0.02[b]	0.15 ± 0.06[ab]
C 24:0	0.31 ± 0.08	0.24 ± 0.07	0.22 ± 0.03	0.26 ± 0.14
SFA	47.09 ± 1.14[a]	51.24 ± 1.24[b]	50.73 ± 0.88[ab]	53.23 ± 1.65[b]
C 16:1	0.72 ± 0.14	1.24 ± 0.20	0.88 ± 0.46	1.11 ± 0.32
C 18:1n-7	1.33 ± 0.10[a]	1.03 ± 0.06[b]	1.04 ± 0.11[b]	1.30 ± 0.19[a]
C 18:1n-9	6.69 ± 1.02	6.06 ± 1.59	5.62 ± 1.06	10.07 ± 2.70
C 20:1n-9	0.21 ± 0.09	0.14 ± 0.07	0.10 ± 0.03	0.09 ± 0.03
C 22:1n-9	0.26 ± 0.08[a]	0.43 ± 0.18[b]	0.09 ± 0.02[c]	0.32 ± 0.07[ab]
C 24:1n-9	0.21 ± 0.07	0.22 ± 0.18	0.05 ± 0.03	0.17 ± 0.11
MUFA	9.50 ± 0.97	9.19 ± 2.14	7.78 ± 1.11	13.15 ± 2.51
C 18:2n-6	14.75 ± 0.68[a]	10.79 ± 0.77[bc]	8.34 ± 0.69[c]	12.58 ± 1.87[ab]
C 18:3n-6	0.15 ± 0.02	0.20 ± 0.12	0.08 ± 0.04	0.17 ± 0.07
C 20:2n-6	0.21 ± 0.01[a]	0.13 ± 0.04[b]	0.08 ± 0.01[b]	0.11 ± 0.02[b]
C 20:3n-6	0.51 ± 0.08[a]	0.47 ± 0.08[a]	0.22 ± 0.03[b]	0.52 ± 0.12[a]
C 20:4n-6	17.69 ± 0.6[a]	15.67 ± 2.79[ac]	10.30 ± 1.09[b]	11.87 ± 2.38[bc]
C 22:2n-6	0.09 ± 0.01	0.13 ± 0.12	0.06 ± 0.02	0.08 ± 0.05
C 22:4n-6	0.35 ± 0.04[a]	0.16 ± 0.05[b]	0.34 ± 0.10[a]	0.12 ± 0.03[b]
C 22:5n-6	0.20 ± 0.06[a]	0.16 ± 0.06[a]	1.62 ± 0.43[b]	0.14 ± 0.05[a]
N-6	33.94 ± 0.70[a]	27.71 ± 2.53[b]	21.05 ± 0.28[c]	25.58 ± 2.18[bc]
C 18:3n-3	0.13 ± 0.02	0.09 ± 0.06	0.09 ± 0.04	0.16 ± 0.21
C 20:3n-3	0.07 ± 0.05	0.07 ± 0.08	0.03 ± 0.01	0.04 ± 0.04
C 20:5n-3	0.19 ± 0.03[a]	0.17 ± 0.04[ab]	0.06 ± 0.02[b]	0.19 ± 0.10[a]
C 22:5n-3	0.49 ± 0.02[ab]	0.50 ± 0.13[ab]	0.72 ± 0.10[a]	0.30 ± 0.04[b]
C 22:6n-3	8.25 ± 0.89[ac]	10.39 ± 0.98[a]	18.75 ± 1.31[b]	5.86 ± 1.84[c]
N-3	9.14 ± 0.90[a]	11.23 ± 0.91[a]	19.65 ± 1.31[b]	6.55 ± 1.62[c]
HUFA	27.75 ± 1.08[ab]	27.60 ± 3.2[a]	32.04 ± 1.18[a]	19.03 ± 3.74[b]
PUFA	43.09 ± 0.81[a]	38.94 ± 2.84[a]	40.70 ± 1.13[a]	32.12 ± 3.09[b]
Total	107.63 ± 20.35[a]	93.06 ± 23.54[a]	197.99 ± 25.67[b]	129.25 ± 32.39[a]

Values with different superscripts are significantly different by Tukey's post hoc test ($p<0.05$) following significant F-value by one-way ANOVA

Table B.3. Relative percent of fatty acids in plasma PC in DHA deficient diet (TWD DHA-) fed rats at baseline, during pregnancy, and 7 days post partum

Name	baseline	15 days	20 days	7 days post partum
C 14:0	0.35 ± 0.08[a]	0.69 ± 0.18[ab]	0.87 ± 0.19[b]	1.11 ± 0.20[b]
C 16:0	15.73 ± 0.83[a]	17.06 ± 0.95[a]	23.64 ± 0.85[c]	19.82 ± 0.78[b]
C 18:0	27.36 ± 3.57[a]	31.15 ± 0.88[b]	23.27 ± 1.06[c]	29.26 ± 1.98[ab]
C 20:0	0.16 ± 0.02[ab]	0.13 ± 0.04[a]	0.12 ± 0.04[a]	0.25 ± 0.05[b]
C 22:0	0.19 ± 0.07	0.11 ± 0.05	0.10 ± 0.05	0.12 ± 0.03
C 24:0	0.21 ± 0.05	0.19 ± 0.05	0.21 ± 0.04	0.2 ± 0.06
SFA	44.38 ± 2.71[a]	50.41 ± 0.99[b]	49.56 ± 1.85[b]	52.38 ± 2.30[b]
C 14:1	0.03 ± 0.02[ab]	0.05 ± 0.03[a]	Not detected [b]	0.07 ± 0.02[a]
C 16:1	0.69 ± 0.25	1.07 ± 0.37	0.68 ± 0.20	0.82 ± 0.29
C 18:1n-7	1.30 ± 0.10[ab]	1.11 ± 0.09[a]	1.15 ± 0.09[a]	1.44 ± 0.18[b]
C 18:1n-9	8.09 ± 4.01	5.33 ± 1.09	7.53 ± 1.20	9.95 ± 1.04
C 20:1n-9	0.41 ± 0.31[a]	0.08 ± 0.03[b]	0.08 ± 0.03[b]	0.09 ± 0.01[b]
C 22:1n-9	0.19 ± 0.06[ab]	0.32 ± 0.10[a]	0.10 ± 0.04[b]	0.31 ± 0.04[a]
C 24:1n-9	0.31 ± 0.26	0.16 ± 0.07	0.08 ± 0.07	0.12 ± 0.03
MUFA	11.04 ± 4.21	8.14 ± 1.68	9.64 ± 1.32	12.8 ± 1.27
C 18:2n-6	13.16 ± 1.39[a]	10.59 ± 1.31[ab]	8.69 ± 0.57[b]	12.1 ± 2.14[a]
C 18:3n-6	2.09 ± 3.02[a]	0.16 ± 0.02[b]	0.15 ± 0.08[b]	0.24 ± 0.07[ab]
C 20:2n-6	0.17 ± 0.03[a]	0.09 ± 0.01[b]	0.09 ± 0.02[b]	0.11 ± 0.01[ab]
C 20:3n-6	0.43 ± 0.11[a]	0.33 ± 0.03[ab]	0.19 ± 0.03[b]	0.45 ± 0.06[a]
C 20:4n-6	19.76 ± 3.22[a]	20.41 ± 4.18[a]	13.37 ± 1.31[b]	15.95 ± 1.78[ab]
C 22:2n-6	0.08 ± 0.06	0.08 ± 0.02	0.05 ± 0.04	0.06 ± 0.02
C 22:4n-6	0.35 ± 0.05[a]	0.25 ± 0.08[ac]	0.68 ± 0.11[b]	0.17 ± 0.03[c]
C 22:5n-6	0.45 ± 0.09[a]	0.53 ± 0.25[a]	5.7 ± 1.45[b]	0.64 ± 0.09[a]
N-6	36.48 ± 1.66[a]	32.43 ± 3.36[ab]	28.93 ± 1.13[b]	29.72 ± 2.63[b]
C 18:3n-3	0.22 ± 0.21	0.08 ± 0.03	0.09 ± 0.07	0.07 ± 0.03
C 20:3n-3	0.04 ± 0.01	0.04 ± 0.03	0.03 ± 0.02	0.02 ± 0.01
C 20:5n-3	0.22 ± 0.11[a]	0.10 ± 0.03[b]	0.05 ± 0.01[b]	0.09 ± 0.03[b]
C 22:5n-3	0.62 ± 0.09[a]	0.59 ± 0.14[a]	0.89 ± 0.09[b]	0.46 ± 0.06[a]
C 22:6n-3	6.20 ± 0.82[ac]	7.61 ± 0.93[ab]	9.99 ± 1.82[b]	3.01 ± 0.83[c]
N-3	7.30 ± 0.90[a]	8.41 ± 1.04[a]	11.05 ± 1.83[b]	3.65 ± 0.81[c]
HUFA	28.06 ± 4.32[a]	29.85 ± 3.23[a]	30.91 ± 2.86[a]	20.80 ± 1.96[b]
PUFA	43.78 ± 2.41[a]	40.84 ± 2.57[a]	39.98 ± 2.76[a]	33.37 ± 2.04[b]
Total	124.17 ± 23.53[a]	96.30 ± 29.76[a]	215.91 ± 24.9[b]	135.38 ± 15.97[a]

Values with different superscripts are significantly different by Tukey's post hoc test ($p<0.05$) following significant F-value by one-way ANOVA

Table B.4. Concentration of fatty acids in plasma PC in chow fed rats at baseline, during pregnancy, and 7 days post partum (µg/100µl plasma)

Name	baseline	15 days	20 days	7 days post partum
C 14:0	0.51 ± 0.49	0.62 ± 0.29	1.25 ± 0.13	1.32 ± 0.28
C 16:0	18.35 ± 7.08[a]	19.79 ± 4.88[a]	49.11 ± 6.9[b]	33.17 ± 2.68[c]
C 18:0	26.89 ± 6.71[a]	31.28 ± 7.88[a]	40.02 ± 8.7[ab]	53.93 ± 7.64[b]
C 20:0	0.20 ± 0.12[a]	0.16 ± 0.04[a]	0.24 ± 0.03[ab]	0.36 ± 0.1[b]
C 22:0	0.28 ± 0.18	0.15 ± 0.05	0.18 ± 0.04	0.18 ± 0.03
C 24:0	0.23 ± 0.05[a]	0.20 ± 0.03[a]	0.44 ± 0.07[b]	0.31 ± 0.06[ab]
SFA	47.06 ± 14.70[a]	53.32 ± 13.14[a]	94.09 ± 15.21[b]	91.62 ± 10.4[b]
C 14:1	0.06 ± 0.03[a]	0.05 ± 0.02[ab]	Not Detected	0.06 ± 0.02[a]
C 16:1	0.59 ± 0.15	0.77 ± 0.06	1.42 ± 0.23	1.15 ± 0.09
C 18:1n-7	1.63 ± 0.65[a]	1.35 ± 0.31[a]	2.07 ± 0.46[ab]	2.57 ± 0.32[b]
C 18:1n-9	11.94 ± 12.56	4.83 ± 2.45	8.25 ± 2.03	11.76 ± 1.29
C 20:1n-9	0.45 ± 0.51	0.14 ± 0.03	0.18 ± 0.04	0.21 ± 0.01
C 22:1n-9	0.22 ± 0.08[a]	0.27 ± 0.02[a]	0.17 ± 0.03[a]	0.42 ± 0.05[b]
C 24:1n-9	0.25 ± 0.08	0.25 ± 0.11	0.09 ± 0.04	0.18 ± 0.05
MUFA	15.17 ± 13.93	7.68 ± 2.77	12.22 ± 2.53	16.36 ± 1.27
C 18:2n-6	16.4 ± 2.83[ab]	13.41 ± 2.62[a]	21.55 ± 7.15[bc]	26.15 ± 4.22[c]
C 18:3n-6	0.75 ± 0.8	0.27 ± 0.16	0.18 ± 0.05	0.27 ± 0.05
C 20:2n-6	0.27 ± 0.05[ab]	0.19 ± 0.04[a]	0.31 ± 0.08[b]	0.43 ± 0.06[c]
C 20:3n-6	0.42 ± 0.07[a]	0.40 ± 0.1[a]	0.41 ± 0.08[a]	1.41 ± 0.20[b]
C 20:4n-6	18.74 ± 4.01	21.43 ± 6.61	22.01 ± 7.30	27.82 ± 5.76
C 22:2n-6	0.13 ± 0.08	0.12 ± 0.07	0.12 ± 0.07	0.13 ± 0.03
C 22:4n-6	0.33 ± 0.04[ac]	0.25 ± 0.06[a]	1.13 ± 0.24[b]	0.60 ± 0.18[c]
C 22:5n-6	0.31 ± 0.08[a]	0.35 ± 0.12[a]	6.19 ± 1.26[b]	1.82 ± 0.77[a]
N-6	37.35 ± 6.03[a]	36.42 ± 9.47[a]	51.88 ± 14.65[ab]	58.63 ± 9.77[b]
C 18:3n-3	0.19 ± 0.09	0.11 ± 0.02	0.22 ± 0.09	0.15 ± 0.04
C 20:3n-3	0.05 ± 0.02	0.08 ± 0.05	0.08 ± 0.03	0.04 ± 0.01
C 20:5n-3	0.22 ± 0.05	0.16 ± 0.04	0.16 ± 0.05	0.21 ± 0.04
C 22:5n-3	0.86 ± 0.09[a]	0.85 ± 0.16[a]	2.19 ± 0.51[b]	2.48 ± 0.69[b]
C 22:6n-3	5.91 ± 1.01[a]	7.52 ± 2.39[a]	23.17 ± 2.73[b]	10.43 ± 2.63[a]
N-3	7.23 ± 1.10[a]	8.72 ± 2.54[a]	25.81 ± 3.35[b]	13.3 ± 3.31[a]
HUFA	26.85 ± 5.15[a]	31.05 ± 9.06[a]	55.32 ± 11.69[b]	44.8 ± 9.21[ab]
PUFA	44.58 ± 7.04[a]	45.14 ± 11.65[a]	77.69 ± 17.86[b]	71.92 ± 11.86[b]
Total	106.81 ± 34.92[a]	106.13 ± 27.14[a]	184.00 ± 35.52[b]	179.90 ± 20.84[b]

Values with different superscripts are significantly different by Tukey's post hoc test ($p<0.05$) following significant F-value by one-way ANOVA

Table B.5. Concentration of fatty acids in plasma PC in high DHA diet (TWD DHA+) fed rats at baseline, during pregnancy, and 7 days post partum (µg/100µl plasma)

Name	baseline	15 days	20 days	7 days post partum
C 14:0	0.46 ± 0.09[a]	0.82 ± 0.03[a]	1.42 ± 0.45[ab]	1.96 ± 1.21[b]
C 16:0	18.71 ± 3.06[ac]	16.70 ± 3.49[a]	48.91 ± 6.83[b]	27.78 ± 7.67[c]
C 18:0	30.20 ± 5.55[a]	28.20 ± 7.93[a]	47.60 ± 8.24[b]	36.54 ± 8.16[ab]
C 20:0	0.20 ± 0.04[a]	0.17 ± 0.02[a]	0.24 ± 0.05[ab]	0.37 ± 0.14[b]
C 22:0	0.21 ± 0.03	0.13 ± 0.02	0.18 ± 0.04	0.19 ± 0.05
C 24:0	0.33 ± 0.06[ab]	0.21 ± 0.03[a]	0.43 ± 0.1[b]	0.32 ± 0.14[ab]
SFA	50.69 ± 8.49[a]	47.79 ± 11.15[a]	101.33 ± 14.23[b]	69.61 ± 16.72[a]
C 14:1	0.05 ± 0.01[ab]	0.05 ± 0.01[ab]	0.01 ± 0.01[a]	0.08 ± 0.05[b]
C 16:1	0.76 ± 0.10[a]	1.13 ± 0.17[ab]	1.83 ± 1.24[b]	1.42 ± 0.47[ab]
C 18:1n-7	1.44 ± 0.27[ab]	0.96 ± 0.22[a]	2.07 ± 0.34[b]	1.72 ± 0.53[b]
C 18:1n-9	7.32 ± 2.16	5.41 ± 0.71	11.18 ± 2.17	13.38 ± 5.88
C 20:1n-9	0.24 ± 0.11	0.12 ± 0.03	0.19 ± 0.06	0.11 ± 0.02
C 22:1n-9	0.27 ± 0.06[ab]	0.37 ± 0.09[bc]	0.17 ± 0.03[a]	0.41 ± 0.07[c]
C 24:1n-9	0.23 ± 0.10	0.17 ± 0.11	0.09 ± 0.05	0.20 ± 0.09
MUFA	10.34 ± 2.57[ab]	8.22 ± 0.80[a]	15.56 ± 3.23[b]	17.35 ± 6.30[ab]
C 18:2n-6	15.89 ± 2.81	10.1 ± 2.55	16.60 ± 1.97	16.44 ± 4.55
C 18:3n-6	0.16 ± 0.05	0.16 ± 0.06	0.16 ± 0.08	0.21 ± 0.05
C 20:2n-6	0.23 ± 0.03[a]	0.12 ± 0.03[b]	0.17 ± 0.02[ab]	0.14 ± 0.02[ab]
C 20:3n-6	0.54 ± 0.06[ab]	0.44 ± 0.13[a]	0.43 ± 0.03[ab]	0.66 ± 0.14[b]
C 20:4n-6	19.19 ± 4.29	15.23 ± 6.32	20.70 ± 4.44	15.64 ± 4.59
C 22:2n-6	0.10 ± 0.03	0.11 ± 0.09	0.11 ± 0.04	0.09 ± 0.03
C 22:4n-6	0.37 ± 0.05[ab]	0.15 ± 0.03[a]	0.69 ± 0.22[b]	0.15 ± 0.06[a]
C 22:5n-6	0.21 ± 0.05[a]	0.14 ± 0.04[a]	3.20 ± 0.79[b]	0.17 ± 0.05[a]
N-6	36.69 ± 7.23	26.44 ± 8.78	42.06 ± 5.95	33.49 ± 8.03
C 18:3n-3	0.14 ± 0.03	0.08 ± 0.03	0.17 ± 0.07	0.16 ± 0.15
C 20:3n-3	0.07 ± 0.04	0.06 ± 0.05	0.06 ± 0.01	0.05 ± 0.03
C 20:5n-3	0.21 ± 0.03	0.15 ± 0.03	0.12 ± 0.04	0.23 ± 0.12
C 22:5n-3	0.53 ± 0.10[a]	0.46 ± 0.12[a]	1.43 ± 0.29[b]	0.40 ± 0.13[a]
C 22:6n-3	8.96 ± 2.35[a]	9.85 ± 3.24[a]	37.25 ± 3.55[b]	7.96 ± 3.67[a]
N-3	9.92 ± 2.46[a]	10.60 ± 3.29[a]	39.04 ± 3.75[b]	8.80 ± 3.60[a]
HUFA	30.09 ± 6.69[a]	26.48 ± 9.72[a]	63.88 ± 7.72[b]	25.25 ± 8.09[a]
PUFA	46.61 ± 9.51[a]	37.05 ± 11.98[a]	81.10 ± 9.22[b]	42.29 ± 11.24[a]
Total	107.63 ± 20.35[a]	93.06 ± 23.54[a]	197.99 ± 25.67[b]	129.25 ± 32.39[a]

Values with different superscripts are significantly different by Tukey's post hoc test ($p<0.05$) following significant F-value by one-way ANOVA

Table B.6. Concentration of fatty acids in plasma PC in DHA deficient diet (TWD DHA-) fed rats at baseline, during pregnancy, and 7 days post partum (µg/100µl plasma)

Name	baseline	15 days	20 days	7 days post partum
C 14:0	0.45 ± 0.16[a]	0.63 ± 0.06[ab]	1.89 ± 0.41[c]	1.54 ± 0.37[bc]
C 16:0	19.70 ± 3.83[ac]	16.31 ± 4.21[a]	51.34 ± 4.82[b]	27.17 ± 2.77[c]
C 18:0	33.96 ± 6.72[a]	30.27 ± 9.72[a]	50.51 ± 4.59[b]	40.16 ± 5.03[ab]
C 20:0	0.20 ± 0.05[ab]	0.12 ± 0.01[a]	0.26 ± 0.07[ab]	0.35 ± 0.09[b]
C 22:0	0.25 ± 0.12[a]	0.10 ± 0.01[b]	0.21 ± 0.08[ab]	0.16 ± 0.02[ab]
C 24:0	0.27 ± 0.08[a]	0.17 ± 0.02[a]	0.46 ± 0.07[b]	0.27 ± 0.05[a]
SFA	55.3 ± 9.77[a]	48.61 ± 14.13[a]	107.54 ± 8.59[b]	71.88 ± 8.18[ab]
C 14:1	0.04 ± 0.03[ab]	0.04 ± 0.01[ab]	Not Detected	0.09 ± 0.03[b]
C 16:1	0.89 ± 0.45[a]	0.97 ± 0.15[ab]	1.44 ± 0.31[b]	1.10 ± 0.35[ab]
C 18:1n-7	1.62 ± 0.27[ac]	1.07 ± 0.31[a]	2.51 ± 0.42[b]	1.98 ± 0.27[bc]
C 18:1n-9	10.38 ± 6.06[ab]	4.96 ± 0.97[a]	16.41 ± 3.35[b]	13.73 ± 2.5[ab]
C 20:1n-9	0.54 ± 0.47[a]	0.07 ± 0.01[b]	0.16 ± 0.05[ab]	0.13 ± 0.02[ab]
C 22:1n-9	0.24 ± 0.09[a]	0.29 ± 0.02[a]	0.21 ± 0.08[a]	0.42 ± 0.04[b]
C 24:1n-9	0.41 ± 0.41	0.14 ± 0.04	0.17 ± 0.13	0.16 ± 0.03
MUFA	14.16 ± 6.8	7.56 ± 1.37	20.94 ± 3.53	17.61 ± 2.77
C 18:2n-6	16.29 ± 2.61[ab]	10.23 ± 3.33[a]	18.91 ± 2.4[b]	16.5 ± 2.74[ab]
C 18:3n-6	2.88 ± 4.27[a]	0.15 ± 0.03[b]	0.32 ± 0.14[ab]	0.34 ± 0.12[ab]
C 20:2n-6	0.21 ± 0.04[a]	0.08 ± 0.02[b]	0.20 ± 0.03[a]	0.14 ± 0.01[ab]
C 20:3n-6	0.51 ± 0.07[ab]	0.31 ± 0.10[a]	0.42 ± 0.04[ab]	0.62 ± 0.14[b]
C 20:4n-6	24.58 ± 5.71	20.65 ± 9.54	29.15 ± 4.72	22.02 ± 4.47
C 22:2n-6	0.11 ± 0.09	0.07 ± 0.02	0.11 ± 0.07	0.08 ± 0.03
C 22:4n-6	0.43 ± 0.08[a]	0.22 ± 0.02[a]	1.49 ± 0.35[b]	0.24 ± 0.06[a]
C 22:5n-6	0.55 ± 0.13[a]	0.47 ± 0.14[a]	12.53 ± 3.85[b]	0.89 ± 0.22[a]
N-6	45.56 ± 8.35[ab]	32.20 ± 12.8[a]	63.13 ± 9.03[b]	40.83 ± 6.37[a]
C 18:3n-3	0.30 ± 0.30	0.07 ± 0.02	0.18 ± 0.13	0.09 ± 0.05
C 20:3n-3	0.05 ± 0.02	0.04 ± 0.02	0.07 ± 0.04	0.02 ± 0.01
C 20:5n-3	0.27 ± 0.15[a]	0.09 ± 0.03[b]	0.12 ± 0.03[b]	0.12 ± 0.04[b]
C 22:5n-3	0.77 ± 0.11[a]	0.55 ± 0.14[a]	1.93 ± 0.29[b]	0.64 ± 0.14[a]
C 22:6n-3	7.76 ± 1.89[a]	7.19 ± 1.62[a]	21.99 ± 6.00[b]	4.18 ± 1.38[a]
N-3	9.15 ± 2.22[a]	7.94 ± 1.74[a]	24.29 ± 6.17[b]	5.06 ± 1.44[a]
HUFA	34.93 ± 8.01[a]	29.53 ± 11.20[a]	67.71 ± 13.04[b]	28.74 ± 5.57[a]
PUFA	54.71 ± 10.41[a]	40.13 ± 14.4[a]	87.43 ± 14.91[b]	45.89 ± 6.74[a]
Total	124.17 ± 23.53[a]	96.30 ± 29.76[a]	215.91 ± 24.9[b]	135.38 ± 15.97[a]

Values with different superscripts are significantly different by Tukey's post hoc test ($p<0.05$) following significant F-value by one-way ANOVA

Appendix C. Plasma PE

Table C.1. Relative percent of fatty acids in plasma PE in chow fed rats at baseline, during pregnancy, and 7 days post partum

Name	baseline	15 days	20 days	7 days post partum
C 14:0	2.47 ± 2.48	2.14 ± 0.34	2.29 ± 0.16	2.87 ± 0.53
C 16:0	15.18 ± 5.29	15.11 ± 1.45	17.38 ± 1.50	18.79 ± 1.02
C 18:0	23.86 ± 5.51[a]	33.44 ± 2.74[b]	34.60 ± 2.80[b]	38.38 ± 2.37[ab]
C 20:0	0.42 ± 0.09	0.40 ± 0.11	0.44 ± 0.05	0.53 ± 0.04
C 22:0	0.48 ± 0.15	0.38 ± 0.14	0.50 ± 0.06	0.30 ± 0.03[b]
C 24:0	0.90 ± 0.46	0.66 ± 0.23	0.60 ± 0.08	0.40 ± 0.04
SFA	45.54 ± 7.37[a]	55.48 ± 1.14[b]	60.62 ± 3.26[b]	65.69 ± 1.47
C 14:1	0.09 ± 0.05	0.16 ± 0.06	0.01 ± 0.01	0.10 ± 0.04
C 16:1	2.92 ± 1.67	3.50 ± 0.40	1.94 ± 0.11	2.23 ± 0.47[b]
C 18:1n-7	1.13 ± 1.08	0.57 ± 0.11	0.59 ± 0.22	0.50 ± 0.05[ab]
C 18:1n-9	18.46 ± 8.23[a]	7.86 ± 1.15[ab]	9.86 ± 2.64[ab]	5.91 ± 1.23
C 20:1n-9	0.36 ± 0.31[ab]	0.65 ± 0.18[a]	0.27 ± 0.08[b]	0.25 ± 0.07[ab]
C 22:1n-9	0.59 ± 0.21[ab]	1.33 ± 0.28[a]	0.40 ± 0.14[b]	0.92 ± 0.14[ab]
C 24:1n-9	0.71 ± 0.20	0.59 ± 0.26	0.35 ± 0.07	0.35 ± 0.12[b]
MUFA	24.33 ± 7.13[a]	14.75 ± 1.28[ab]	13.48 ± 2.65[b]	10.27 ± 1.34[b]
C 18:2n-6	4.37 ± 1.36	3.58 ± 0.87	2.71 ± 0.40	2.86 ± 0.57
C 18:3n-6	2.81 ± 4.07	0.48 ± 0.14	0.22 ± 0.02	0.44 ± 0.07[a]
C 20:2n-6	0.28 ± 0.13	0.24 ± 0.14	0.09 ± 0.01	0.11 ± 0.02
C 20:3n-6	0.40 ± 0.22[a]	0.50 ± 0.08[a]	0.23 ± 0.05[a]	0.92 ± 0.08
C 20:4n-6	14.27 ± 4.8	18.26 ± 2.40	15.63 ± 1.47	13.83 ± 1.88[b]
C 22:2n-6	0.34 ± 0.20	0.38 ± 0.15	0.16 ± 0.06	0.20 ± 0.04
C 22:4n-6	0.50 ± 0.23	0.45 ± 0.13	0.36 ± 0.08	0.44 ± 0.12[a]
C 22:5n-6	0.76 ± 0.43	0.56 ± 0.30	0.82 ± 0.20	0.36 ± 0.07
N-6	23.73 ± 5.69	24.45 ± 1.73	20.22 ± 1.81	19.16 ± 1.90[ab]
C 18:3n-3	0.69 ± 0.67	0.35 ± 0.13	0.26 ± 0.06	0.29 ± 0.07[b]
C 20:3n-3	0.28 ± 0.17	0.29 ± 0.17	0.24 ± 0.05	0.11 ± 0.03
C 20:5n-3	0.70 ± 0.34[a]	0.29 ± 0.08[ab]	0.08 ± 0.02[b]	0.14 ± 0.05
C 22:5n-3	0.75 ± 0.35	0.66 ± 0.22	0.53 ± 0.11	0.73 ± 0.16[b]
C 22:6n-3	1.26 ± 0.22[a]	1.84 ± 0.33[ab]	3.16 ± 0.67[b]	1.53 ± 0.15
N-3	3.68 ± 0.84	3.44 ± 0.65	4.27 ± 0.65	2.81 ± 0.25[b]
HUFA	18.91 ± 6.22	22.86 ± 2.38	21.05 ± 1.36	18.07 ± 2.3
PUFA	27.41 ± 6.43	27.89 ± 1.80	24.49 ± 1.73	21.97 ± 2.14
Total	17.68 ± 10.20	12.18 ± 1.87	21.43 ± 1.17	18.97 ± 3.36

Values with different superscripts are significantly different by Tukey's post hoc test ($p<0.05$) following significant F-value by one-way ANOVA

Table C.2. Relative percent of fatty acids in plasma PE in high DHA diet (TWD DHA+) fed rats at baseline, during pregnancy, and 7 days post partum

Name	baseline	15 days	20 days	7 days post partum
C 14:0	1.66 ± 0.63	2.49 ± 0.34	3.06 ± 0.52	2.88 ± 0.40
C 16:0	15.58 ± 2.98^{a}	17.30 ± 0.81^{ab}	17.07 ± 1.56^{a}	24.56 ± 6.55^{b}
C 18:0	20.97 ± 4.78^{a}	32.49 ± 1.75^{b}	30.13 ± 3.49^{b}	34.20 ± 6.78^{b}
C 20:0	0.61 ± 0.22	0.47 ± 0.04	0.44 ± 0.03	0.58 ± 0.11
C 22:0	0.78 ± 0.33	0.46 ± 0.11	0.41 ± 0.14	0.38 ± 0.08
C 24:0	1.26 ± 0.76^{a}	0.72 ± 0.15^{ab}	0.38 ± 0.10^{b}	0.52 ± 0.27^{ab}
SFA	43.22 ± 5.44^{a}	57.61 ± 1.13^{b}	57.63 ± 3.48^{b}	66.85 ± 0.65^{b}
C 14:1	0.26 ± 0.13^{a}	0.22 ± 0.02^{a}	0.01 ± 0.01^{b}	0.12 ± 0.02^{ab}
C 16:1	3.35 ± 1.79	4.50 ± 0.36	2.70 ± 0.82	2.66 ± 0.82
C 18:1n-7	0.93 ± 0.32	0.55 ± 0.03	0.74 ± 0.20	0.65 ± 0.28
C 18:1n-9	21.57 ± 10.07^{a}	9.26 ± 0.91^{b}	13.17 ± 3.77^{ab}	7.93 ± 2.81^{b}
C 20:1n-9	0.46 ± 0.18	0.63 ± 0.15	0.26 ± 0.06	0.37 ± 0.23
C 22:1n-9	0.94 ± 0.51^{ab}	1.50 ± 0.10^{a}	0.43 ± 0.14^{b}	1.09 ± 0.22^{ab}
C 24:1n-9	0.92 ± 0.62^{a}	0.60 ± 0.12^{ab}	0.27 ± 0.12^{b}	0.33 ± 0.14^{ab}
MUFA	28.64 ± 8.71^{a}	17.34 ± 1.13^{b}	17.61 ± 4.25^{b}	13.21 ± 2.08^{b}
C 18:2n-6	4.68 ± 1.46^{a}	2.30 ± 0.19^{b}	2.79 ± 0.68^{ab}	1.69 ± 0.19^{b}
C 18:3n-6	0.47 ± 0.23	0.45 ± 0.09	0.29 ± 0.12	0.35 ± 0.07
C 20:2n-6	0.50 ± 0.25^{a}	0.23 ± 0.09^{ab}	0.07 ± 0.03^{b}	0.09 ± 0.04^{b}
C 20:3n-6	0.48 ± 0.28	0.53 ± 0.09	0.25 ± 0.05	0.49 ± 0.14
C 20:4n-6	9.24 ± 4.43	15.22 ± 1.87	14.36 ± 2.00	9.62 ± 2.59
C 22:2n-6	0.59 ± 0.36	0.37 ± 0.08	0.15 ± 0.12	0.22 ± 0.05
C 22:4n-6	1.14 ± 0.79^{a}	0.38 ± 0.07^{b}	0.20 ± 0.04^{b}	0.27 ± 0.13^{b}
C 22:5n-6	1.13 ± 0.68^{a}	0.47 ± 0.17^{ab}	0.37 ± 0.10^{b}	0.34 ± 0.10^{ab}
N-6	18.23 ± 3.36	19.94 ± 1.68	18.47 ± 1.72	13.07 ± 3.20
C 18:3n-3	0.56 ± 0.27	0.32 ± 0.06	0.30 ± 0.22	0.24 ± 0.10
C 20:3n-3	0.41 ± 0.24	0.29 ± 0.20	0.17 ± 0.10	0.10 ± 0.04
C 20:5n-3	0.95 ± 0.54^{a}	0.26 ± 0.07^{b}	0.13 ± 0.08^{b}	0.13 ± 0.01^{b}
C 22:5n-3	1.27 ± 0.64^{a}	0.46 ± 0.13^{b}	0.36 ± 0.14^{b}	0.50 ± 0.10^{ab}
C 22:6n-3	2.48 ± 1.32^{ab}	2.07 ± 0.39^{a}	3.64 ± 1.07^{b}	2.21 ± 0.56^{ab}
N-3	5.67 ± 2.83	3.40 ± 0.34	4.60 ± 1.42	3.18 ± 0.75
HUFA	17.11 ± 5.3	19.68 ± 1.96	19.47 ± 2.80	13.66 ± 3.54
PUFA	23.90 ± 4.65	23.34 ± 1.84	23.07 ± 2.31	16.25 ± 3.93
Total	13.65 ± 11.06^{ab}	11.06 ± 1.09^{a}	23.40 ± 2.72^{b}	19.03 ± 2.78^{ab}

Values with different superscripts are significantly different by Tukey's post hoc test ($p<0.05$) following significant F-value by one-way ANOVA

Table C.3. Relative percent of fatty acids in plasma PE in DHA deficient diet (TWD DHA-) fed rats at baseline, during pregnancy, and 7 days post partum

Name	baseline	15 days	20 days	7 days post partum
C 14:0	1.84 ± 0.47	2.81 ± 0.33	2.33 ± 0.28	3.40 ± 0.53
C 16:0	16.21 ± 3.34[a]	17.22 ± 1.40[ab]	16.65 ± 1.11[ab]	23.05 ± 3.5[b]
C 18:0	25.69 ± 3.34[a]	30.86 ± 2.48[ab]	34.49 ± 1.32[b]	32.59 ± 3.14[ab]
C 20:0	0.55 ± 0.20	0.54 ± 0.10	0.43 ± 0.07	0.62 ± 0.16
C 22:0	0.79 ± 0.51	0.61 ± 0.09	0.44 ± 0.11	0.43 ± 0.12
C 24:0	1.07 ± 0.66	0.87 ± 0.23	0.49 ± 0.13	0.56 ± 0.16
SFA	48.10 ± 3.99[a]	57.11 ± 1.74[b]	59.70 ± 2.30[b]	64.62 ± 5.64[b]
C 14:1	0.23 ± 0.14[a]	0.24 ± 0.05[a]	0.02 ± 0.03[b]	0.16 ± 0.05[ab]
C 16:1	3.11 ± 0.60[ab]	4.66 ± 0.76[a]	2.03 ± 0.80[b]	2.59 ± 0.53[ab]
C 18:1n-7	1.04 ± 0.50	0.63 ± 0.08	0.61 ± 0.11	0.82 ± 0.36
C 18:1n-9	18.30 ± 7.04	10.26 ± 2.17	10.11 ± 1.70	11.57 ± 4.40
C 20:1n-9	0.37 ± 0.20[ab]	0.70 ± 0.22[a]	0.19 ± 0.03[b]	0.19 ± 0.03[b]
C 22:1n-9	0.84 ± 0.41[a]	1.73 ± 0.79[b]	0.44 ± 0.08[a]	0.92 ± 0.21[a]
C 24:1n-9	0.71 ± 0.43	0.55 ± 0.12	0.29 ± 0.17	0.43 ± 0.15
MUFA	24.73 ± 6.37[a]	18.85 ± 2.59[ab]	13.74 ± 1.79[b]	16.72 ± 4.66[ab]
C 18:2n-6	4.18 ± 1.83	2.32 ± 0.33	2.19 ± 0.18	2.89 ± 1.36
C 18:3n-6	1.40 ± 2.63	0.49 ± 0.08	0.27 ± 0.08	0.35 ± 0.10
C 20:2n-6	0.56 ± 0.36[a]	0.16 ± 0.05[b]	0.08 ± 0.02[b]	0.07 ± 0.05[b]
C 20:3n-6	0.37 ± 0.18	0.50 ± 0.12	0.24 ± 0.07	0.44 ± 0.11
C 20:4n-6	13.17 ± 3.78[ab]	14.64 ± 2.42[ab]	16.20 ± 1.43[a]	8.74 ± 1.68[b]
C 22:2n-6	0.63 ± 0.54[a]	0.32 ± 0.07[ab]	0.14 ± 0.04[b]	0.15 ± 0.07[ab]
C 22:4n-6	0.89 ± 0.49	0.50 ± 0.11	0.44 ± 0.07	0.24 ± 0.04
C 22:5n-6	0.91 ± 0.47[ab]	0.62 ± 0.2[a]	1.43 ± 0.41[b]	0.28 ± 0.12[a]
N-6	22.11 ± 4.26[a]	19.55 ± 2.46[ab]	21.00 ± 1.83[a]	13.15 ± 2.41[b]
C 18:3n-3	0.43 ± 0.15	0.28 ± 0.06	0.22 ± 0.04	0.23 ± 0.05
C 20:3n-3	0.36 ± 0.21	0.29 ± 0.11	0.16 ± 0.05	0.12 ± 0.03
C 20:5n-3	0.72 ± 0.41[a]	0.30 ± 0.11[ab]	0.07 ± 0.05[b]	0.15 ± 0.06[b]
C 22:5n-3	1.17 ± 0.78[a]	0.54 ± 0.16[ab]	0.45 ± 0.10[ab]	0.36 ± 0.10[b]
C 22:6n-3	1.49 ± 0.33[a]	1.31 ± 0.16[a]	2.81 ± 0.56[b]	1.14 ± 0.23[a]
N-3	4.17 ± 1.62	2.72 ± 0.43	3.72 ± 0.56	2.00 ± 0.4
HUFA	19.08 ± 5.16[ab]	18.69 ± 2.29[ab]	21.82 ± 2.14[a]	11.46 ± 2.16[b]
PUFA	26.28 ± 4.97[a]	22.27 ± 2.46[ab]	24.72 ± 2.19[a]	15.15 ± 2.73[b]
Total	13.18 ± 5.37[ab]	11.18 ± 1.19[a]	22.07 ± 1.95[b]	19.71 ± 1.95[ab]

Values with different superscripts are significantly different by Tukey's post hoc test ($p<0.05$) following significant F-value by one-way ANOVA

Table C.4. Concentration of fatty acids in plasma PE in chow fed rats at baseline, during pregnancy, and 7 days post partum (µg/100µl plasma)

Name	baseline	15 days	20 days	7 days post partum
C 14:0	0.5 ± 0.71	0.27 ± 0.06	0.50 ± 0.02	0.55 ± 0.11
C 16:0	2.91 ± 2.22	1.87 ± 0.34	3.78 ± 0.38	3.62 ± 0.56
C 18:0	4.00 ± 1.57[a]	4.16 ± 0.83[a]	7.54 ± 0.92[b]	7.47 ± 1.59[b]
C 20:0	0.07 ± 0.03	0.05 ± 0.01	0.09 ± 0.01	0.10 ± 0.02
C 22:0	0.08 ± 0.02[ab]	0.05 ± 0.01[a]	0.11 ± 0.02[b]	0.06 ± 0.01[ab]
C 24:0	0.13 ± 0.03	0.08 ± 0.02	0.13 ± 0.02	0.08 ± 0.01
SFA	8.03 ± 4.1[a]	6.87 ± 0.99[ab]	13.19 ± 1.23[c]	12.70 ± 2.1[ac]
C 14:1	0.01 ± 0.01[ab]	0.02 ± 0.01[a]	Not Detected	0.02 ± 0.01[a]
C 16:1	0.43 ± 0.15	0.43 ± 0.07	0.42 ± 0.04	0.42 ± 0.07
C 18:1n-7	0.25 ± 0.32	0.07 ± 0.02	0.13 ± 0.04	0.09 ± 0.01
C 18:1n-9	3.86 ± 3.94	0.99 ± 0.26	2.12 ± 0.45	1.13 ± 0.24
C 20:1n-9	0.08 ± 0.09	0.08 ± 0.01	0.06 ± 0.02	0.05 ± 0.02
C 22:1n-9	0.09 ± 0.02[a]	0.16 ± 0.01[ab]	0.09 ± 0.03[a]	0.18 ± 0.05[b]
C 24:1n-9	0.12 ± 0.05	0.07 ± 0.02	0.08 ± 0.02	0.07 ± 0.03
MUFA	4.85 ± 4.13	1.83 ± 0.33	2.91 ± 0.42	1.97 ± 0.30
C 18:2n-6	0.75 ± 0.46	0.45 ± 0.15	0.59 ± 0.09	0.55 ± 0.14
C 18:3n-6	0.79 ± 1.54	0.06 ± 0.01	0.05 ± 0.01	0.09 ± 0.02
C 20:2n-6	0.04 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
C 20:3n-6	0.06 ± 0.01[a]	0.06 ± 0.01[a]	0.05 ± 0.01[a]	0.18 ± 0.04[b]
C 20:4n-6	2.29 ± 0.68	2.28 ± 0.56	3.4 ± 0.43	2.72 ± 0.77
C 22:2n-6	0.05 ± 0.01	0.05 ± 0.02	0.04 ± 0.01	0.04 ± 0.01
C 22:4n-6	0.07 ± 0.01	0.05 ± 0.01	0.08 ± 0.02	0.09 ± 0.04
C 22:5n-6	0.11 ± 0.04[ab]	0.07 ± 0.03[a]	0.18 ± 0.05[b]	0.07 ± 0.02[a]
N-6	4.16 ± 2.55	3.05 ± 0.62	4.40 ± 0.54	3.76 ± 0.96
C 18:3n-3	0.17 ± 0.28	0.04 ± 0.01	0.06 ± 0.01	0.06 ± 0.02
C 20:3n-3	0.04 ± 0.01	0.03 ± 0.02	0.05 ± 0.01	0.02 ± 0.01
C 20:5n-3	0.10 ± 0.03[a]	0.04 ± 0.01[b]	0.02 ± 0.01[b]	0.03 ± 0.01[b]
C 22:5n-3	0.11 ± 0.02	0.08 ± 0.02	0.11 ± 0.03	0.14 ± 0.05
C 22:6n-3	0.22 ± 0.12[a]	0.23 ± 0.06[a]	0.69 ± 0.14[b]	0.30 ± 0.07[a]
N-3	0.64 ± 0.38[ab]	0.42 ± 0.06[a]	0.93 ± 0.15[b]	0.55 ± 0.13[ab]
HUFA	3.00 ± 0.77[a]	2.84 ± 0.59[a]	4.58 ± 0.46[b]	3.55 ± 0.98[ab]
PUFA	4.80 ± 2.93	3.47 ± 0.65	5.33 ± 0.55	4.31 ± 1.09
Total	17.68 ± 10.2	12.18 ± 1.87	21.43 ± 1.17	18.97 ± 3.36

Values with different superscripts are significantly different by Tukey's post hoc test ($p<0.05$) following significant F-value by one-way ANOVA

Table C.5. Concentration of fatty acids in plasma PE in high DHA diet (TWD DHA+) fed rats at baseline, during pregnancy, and 7 days post partum (µg/100µl plasma)

Name	baseline	15 days	20 days	7 days post partum
C 14:0	0.20 ± 0.11[a]	0.28 ± 0.07[ab]	0.73 ± 0.16[b]	0.58 ± 0.15[ab]
C 16:0	2.27 ± 2.14	1.95 ± 0.28	4.07 ± 0.62	4.99 ± 2.10
C 18:0	2.8 ± 1.81[a]	3.64 ± 0.21[a]	7.17 ± 1.22[b]	6.64 ± 0.68[b]
C 20:0	0.07 ± 0.03	0.05 ± 0.01	0.10 ± 0.01	0.11 ± 0.01
C 22:0	0.08 ± 0.02	0.05 ± 0.02	0.10 ± 0.03	0.07 ± 0.01
C 24:0	0.12 ± 0.01	0.08 ± 0.02	0.09 ± 0.03	0.10 ± 0.04
SFA	5.87 ± 4.17[a]	6.48 ± 0.71[a]	13.7 ± 1.64[b]	13.24 ± 2.12[b]
C 14:1	0.03 ± 0.01[a]	0.02 ± 0[a]	Not Detected	0.02 ± 0.01[a]
C 16:1	0.35 ± 0.11[a]	0.51 ± 0.09[ab]	0.63 ± 0.15[b]	0.53 ± 0.18[ab]
C 18:1n-7	0.16 ± 0.20	0.06 ± 0.01	0.18 ± 0.06	0.13 ± 0.08
C 18:1n-9	3.92 ± 5.06	1.04 ± 0.15	3.16 ± 1.05	1.62 ± 0.82
C 20:1n-9	0.06 ± 0.03	0.07 ± 0.02	0.06 ± 0.02	0.07 ± 0.04
C 22:1n-9	0.09 ± 0.02[a]	0.17 ± 0.02[bc]	0.10 ± 0.03[ab]	0.21 ± 0.01[c]
C 24:1n-9	0.08 ± 0.03	0.07 ± 0.02	0.06 ± 0.03	0.06 ± 0.02
MUFA	4.72 ± 5.37	1.96 ± 0.28	4.20 ± 1.17	2.66 ± 0.83
C 18:2n-6	0.77 ± 0.90	0.26 ± 0.04	0.67 ± 0.20	0.33 ± 0.02
C 18:3n-6	0.05 ± 0.03	0.05 ± 0.01	0.07 ± 0.03	0.07 ± 0.01
C 20:2n-6	0.05 ± 0.01[a]	0.03 ± 0.01[b]	0.02 ± 0.01[b]	0.02 ± 0.01[b]
C 20:3n-6	0.05 ± 0.02[a]	0.06 ± 0.01[ab]	0.06 ± 0.01[ab]	0.09 ± 0.020[b]
C 20:4n-6	1.29 ± 0.99[a]	1.70 ± 0.17[a]	3.4 ± 0.52[b]	1.85 ± 0.19[a]
C 22:2n-6	0.05 ± 0.02	0.04 ± 0.01	0.04 ± 0.02	0.04 ± 0.01
C 22:4n-6	0.10 ± 0.03[a]	0.04 ± 0.01[b]	0.05 ± 0.01[b]	0.05 ± 0.02[b]
C 22:5n-6	0.11 ± 0.03	0.05 ± 0.02	0.09 ± 0.03	0.07 ± 0.01
N-6	2.49 ± 1.65	2.24 ± 0.2	4.39 ± 0.56	2.52 ± 0.21
C 18:3n-3	0.06 ± 0.02	0.04 ± 0.01	0.07 ± 0.06	0.05 ± 0.01
C 20:3n-3	0.04 ± 0.01	0.03 ± 0.03	0.04 ± 0.02	0.02 ± 0.01
C 20:5n-3	0.1 ± 0.03[a]	0.03 ± 0.01[b]	0.03 ± 0.02[b]	0.03 ± 0.01[b]
C 22:5n-3	0.13 ± 0.05[a]	0.05 ± 0.02[b]	0.09 ± 0.04[ab]	0.10 ± 0.01[ab]
C 22:6n-3	0.25 ± 0.03[a]	0.23 ± 0.03[a]	0.88 ± 0.33[b]	0.43 ± 0.07[a]
N-3	0.57 ± 0.07[a]	0.38 ± 0.04[a]	1.11 ± 0.43[b]	0.62 ± 0.07[a]
HUFA	2.07 ± 1.00[a]	2.20 ± 0.18[a]	4.63 ± 0.87[b]	2.63 ± 0.27[a]
PUFA	3.06 ± 1.67[ab]	2.62 ± 0.23[a]	5.50 ± 0.89[b]	3.14 ± 0.27[ab]
Total	13.65 ± 11.06[ab]	11.06 ± 1.09[a]	23.40 ± 2.72[b]	19.03 ± 2.78[ab]

Values with different superscripts are significantly different by Tukey's post hoc test ($p<0.05$) following significant F-value by one-way ANOVA

Table C.6. Concentration of fatty acids in plasma PE in DHA deficient diet (TWD DHA-) fed rats at baseline, during pregnancy, and 7 days post partum (µg/100µl plasma)

Name	baseline	15 days	20 days	7 days post partum
C 14:0	0.25 ± 0.14	0.32 ± 0.06	0.52 ± 0.08	0.70 ± 0.18
C 16:0	2.25 ± 1.21^{ab}	1.96 ± 0.21^{a}	3.74 ± 0.39^{ab}	4.76 ± 1.15^{b}
C 18:0	3.41 ± 1.43^{a}	3.50 ± 0.33^{a}	7.75 ± 0.67^{b}	6.63 ± 0.70^{b}
C 20:0	0.07 ± 0.04^{a}	0.06 ± 0.01^{a}	0.10 ± 0.02^{ab}	0.13 ± 0.04^{b}
C 22:0	0.09 ± 0.03	0.07 ± 0.01	0.10 ± 0.03	0.09 ± 0.03
C 24:0	0.12 ± 0.03	0.10 ± 0.02	0.11 ± 0.04	0.11 ± 0.04
SFA	6.43 ± 2.77^{a}	6.48 ± 0.51^{a}	13.42 ± 1.21^{b}	13.24 ± 2.13^{b}
C 14:1	0.02 ± 0.01^{a}	0.03 ± 0.01^{a}	Not Detected	0.03 ± 0.01^{a}
C 16:1	0.40 ± 0.13	0.53 ± 0.11	0.47 ± 0.21	0.53 ± 0.15
C 18:1n-7	0.15 ± 0.12	0.07 ± 0.01	0.14 ± 0.02	0.17 ± 0.09
C 18:1n-9	2.53 ± 1.45	1.19 ± 0.34	2.27 ± 0.39	2.35 ± 0.96
C 20:1n-9	0.04 ± 0.02	0.08 ± 0.02	0.04 ± 0.01	0.04 ± 0.01
C 24:1n-9	0.08 ± 0.02	0.06 ± 0.01	0.07 ± 0.04	0.09 ± 0.03
MUFA	3.34 ± 1.61	2.16 ± 0.44	3.09 ± 0.51	3.41 ± 1.05
C 18:2n-6	0.56 ± 0.29	0.27 ± 0.05	0.49 ± 0.07	0.59 ± 0.30
C 18:3n-6	0.25 ± 0.51	0.06 ± 0.01	0.06 ± 0.02	0.07 ± 0.01
C 20:2n-6	0.06 ± 0.03^{a}	0.02 ± 0.01^{b}	0.02 ± 0.01^{b}	0.01 ± 0.01^{b}
C 20:3n-6	0.04 ± 0.01^{a}	0.06 ± 0.01^{ab}	0.05 ± 0.02^{ab}	0.09 ± 0.02^{b}
C 20:4n-6	1.75 ± 0.92^{a}	1.67 ± 0.39^{a}	3.64 ± 0.44^{b}	1.77 ± 0.22^{a}
C 22:2n-6	0.06 ± 0.03	0.04 ± 0.01	0.03 ± 0.01	0.03 ± 0.01
C 22:4n-6	0.10 ± 0.02^{a}	0.06 ± 0.01^{b}	0.10 ± 0.02^{a}	0.05 ± 0.01^{b}
C 22:5n-6	0.10 ± 0.02^{a}	0.07 ± 0.02^{a}	0.32 ± 0.10^{b}	0.05 ± 0.02^{a}
N-6	2.92 ± 1.38^{ab}	2.23 ± 0.42^{a}	4.73 ± 0.60^{b}	2.66 ± 0.31^{ab}
C 18:3n-3	0.06 ± 0.04	0.03 ± 0.01	0.05 ± 0.01	0.05 ± 0.01
C 20:3n-3	0.04 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.02 ± 0.01
C 20:5n-3	0.08 ± 0.02^{a}	0.03 ± 0.01^{b}	0.02 ± 0.01^{b}	0.03 ± 0.01^{b}
C 22:1n-9	0.09 ± 0.01^{a}	0.19 ± 0.08^{b}	0.10 ± 0.02^{a}	0.18 ± 0.02^{b}
C 22:5n-3	0.13 ± 0.04^{a}	0.06 ± 0.01^{b}	0.10 ± 0.03^{ab}	0.07 ± 0.02^{ab}
C 22:6n-3	0.18 ± 0.05^{a}	0.15 ± 0.02^{a}	0.63 ± 0.13^{b}	0.23 ± 0.02^{a}
N-3	0.49 ± 0.08^{ab}	0.31 ± 0.02^{a}	0.84 ± 0.15^{b}	0.40 ± 0.04^{a}
HUFA	2.42 ± 0.92^{a}	2.13 ± 0.37^{a}	4.91 ± 0.65^{b}	2.31 ± 0.23^{z}
PUFA	3.41 ± 1.42^{a}	2.54 ± 0.41^{b}	5.56 ± 0.72^{a}	3.06 ± 0.33^{ab}
Total	13.18 ± 5.37^{ab}	11.18 ± 1.19^{a}	22.07 ± 1.95^{b}	19.71 ± 1.95^{ab}

Values with different superscripts are significantly different by Tukey's post hoc test ($p<0.05$) following significant F-value by one-way ANOVA

Appendix D. Liver PC

Table D.1. Relative percent of fatty acids in liver PC in chow fed rats at baseline, during pregnancy, and 7 days post partum

Name	baseline	15 days	20 days	7 days post partum
C 14:0	0.45 ± 0.17	0.49 ± 0.26	0.49 ± 0.13	0.4 ± 0.09
C 16:0	17.59 ± 0.58[a]	17.40 ± 0.99[a]	26.3 ± 1.22[b]	17.19 ± 1.00[a]
C 18:0	28.95 ± 1.17[a]	28.71 ± 1.26[a]	19.67 ± 1.42[b]	28.06 ± 0.77[a]
C 20:0	0.08 ± 0.02	0.08 ± 0.02	0.07 ± 0.02	0.06 ± 0.01
C 22:0	0.09 ± 0.05	0.07 ± 0.04	0.08 ± 0.04	0.05 ± 0.02
C 24:0	0.08 ± 0.05	0.09 ± 0.07	0.11 ± 0.02	0.10 ± 0.03
SFAs	48.98 ± 2.39	47.37 ± 1.24	47.47 ± 0.76	46.95 ± 1.36
C 14:1	0.01 ± 0.01	0.02 ± 0.02	0.02 ± 0.01	0.01 ± 0.01
C 16:1	0.27 ± 0.03	0.43 ± 0.13	0.36 ± 0.11	0.32 ± 0.07
C 18:1n-7	1.35 ± 0.08[ab]	1.27 ± 0.12[ab]	1.14 ± 0.09[a]	1.49 ± 0.17[b]
C 18:1n-9	4.86 ± 1.24	5.05 ± 0.87	6.14 ± 1.05	6.72 ± 1.16
C 20:1n-9	0.10 ± 0.03[ab]	0.08 ± 0.03[a]	0.07 ± 0.01[a]	0.14 ± 0.03[b]
C 22:1n-9	0.22 ± 0.10[a]	0.17 ± 0.10[ab]	0.14 ± 0.08[ab]	0.09 ± 0.03[b]
C 24:1n-9	0.13 ± 0.08	0.13 ± 0.11	0.08 ± 0.03	0.06 ± 0.02
MUFA	10.78 ± 0.86[a]	10.37 ± 0.89[ab]	8.39 ± 0.60[b]	11.06 ± 1.18[ab]
C 18:2n-6	0.25 ± 0.03[a]	0.35 ± 0.08[a]	0.31 ± 0.04[a]	0.61 ± 0.12[b]
C 18:3n-6	0.17 ± 0.04	0.17 ± 0.02	0.19 ± 0.02	0.21 ± 0.02
C 20:2n-6	0.33 ± 0.05[a]	0.29 ± 0.05[ab]	0.16 ± 0.01[b]	0.61 ± 0.08[c]
C 20:3n-6	25.34 ± 1.59[a]	24.39 ± 1.42[a]	16.61 ± 1.06[b]	22.24 ± 1.08[a]
C 20:4n-6	0.05 ± 0.02	0.08 ± 0.08	0.05 ± 0.01	0.04 ± 0.01
C 22:2n-6	0.20 ± 0.05[a]	0.20 ± 0.07[a]	0.55 ± 0.04[b]	0.23 ± 0.05[a]
C 22:4n-6	0.17 ± 0.03[a]	0.27 ± 0.15[a]	3.00 ± 0.40[b]	0.89 ± 0.23[a]
C 22:5n-6	37.30 ± 1.97[a]	36.13 ± 1.69[a]	29.26 ± 1.60[b]	35.88 ± 1.53[a]
N-6	0.10 ± 0.01	0.12 ± 0.04	0.14 ± 0.01	0.10 ± 0.02
C 18:3n-3	0.03 ± 0.02	0.02 ± 0.02	0.05 ± 0.02	0.01 ± 0.01
C 20:3n-3	0.17 ± 0.04	0.19 ± 0.04	0.15 ± 0.03	0.23 ± 0.03
C 20:5n-3	0.53 ± 0.11[a]	0.67 ± 0.11[a]	1.06 ± 0.11[b]	1.10 ± 0.11[b]
C 22:5n-3	5.98 ± 10.01[a]	7.51 ± 1.23[a]	13.31 ± 1.4o[b]	6.07 ± 0.23[a]
C 22:6n-3	5.82 ± 2.58[a]	8.52 ± 1.28[b]	14.71 ± 1.33[c]	7.52 ± 0.23[ab]
N-3	31.76 ± 1.69	33.55 ± 2.36	34.89 ± 1.48	30.25 ± 2.28
HUFA	6.99 ± 1.28	7.18 ± 1.15	7.96 ± 1.18	8.87 ± 1.19
PUFA	43.11 ± 1.67	44.65 ± 2.28	43.98 ± 1.54	43.39 ± 1.64
Total	31.82 ± 5.26	35.60 ± 5.20	33.36 ± 14.22	42.05 ± 15.84

Values with different superscripts are significantly different by Tukey's post hoc test ($p<0.05$) following significant F-value by one-way ANOVA

Table D.2. Relative percent of fatty acids in liver PC in high DHA diet (TWD DHA+) fed rats at baseline, during pregnancy, and 7 days post partum

Name	baseline	15 days	20 days	7 days post partum
C 14:0	0.62 ± 0.13	0.55 ± 0.13	0.61 ± 0.15	0.55 ± 0.13
C 16:0	18.19 ± 1.68^{a}	17.61 ± 1.7^{a}	24.14 ± 2.17^{b}	19.96 ± 1.88^{a}
C 18:0	26.09 ± 6.12^{ab}	29.36 ± 1.19^{a}	22.59 ± 1.69^{b}	24.08 ± 2.01^{b}
C 20:0	0.08 ± 0.02	0.08 ± 0.01	0.08 ± 0.03	0.06 ± 0.01
C 22:0	0.10 ± 0.04	0.08 ± 0.05	0.09 ± 0.02	0.05 ± 0.02
C 24:0	0.12 ± 0.04	0.10 ± 0.06	0.08 ± 0.03	0.09 ± 0.02
SFA	48.38 ± 1.14	48.37 ± 1.41	48.21 ± 1.82	46.10 ± 0.69
C 14:1	0.03 ± 0.02	0.03 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
C 16:1	0.27 ± 0.06^{a}	0.51 ± 0.06^{b}	0.41 ± 0.2^{ab}	0.43 ± 0.13^{ab}
C 18:1n-7	1.22 ± 0.08^{a}	1.13 ± 0.1^{a}	1.06 ± 0.11^{a}	1.54 ± 0.24^{b}
C 18:1n-9	5.58 ± 1.39^{a}	6.26 ± 1.25^{a}	6.84 ± 1.4^{a}	9.8 ± 2.05^{b}
C 22:1n-9	0.24 ± 0.05^{a}	0.18 ± 0.06^{ab}	0.13 ± 0.03^{ab}	0.11 ± 0.02^{b}
C 20:1n-9	0.09 ± 0.02	0.07 ± 0.03	0.07 ± 0.01	0.10 ± 0.02
C 24:1n-9	0.18 ± 0.07^{a}	0.14 ± 0.08^{ab}	0.07 ± 0.02^{ab}	0.06 ± 0.01^{b}
MUFA	7.65 ± 1.48^{a}	8.34 ± 1.37^{a}	8.6 ± 1.41^{a}	12.03 ± 2.22^{b}
C 18:2n-6	9.83 ± 0.33^{a}	8.98 ± 0.73^{a}	5.93 ± 0.91^{b}	10.39 ± 2.00^{a}
C 18:3n-6	0.20 ± 0.06^{a}	0.21 ± 0.04^{a}	0.23 ± 0.05^{a}	0.61 ± 0.28^{b}
C 20:2n-6	0.11 ± 0.04	0.09 ± 0.03	0.1 ± 0.01	0.09 ± 0.02
C 20:3n-6	0.36 ± 0.09^{ac}	0.33 ± 0.05^{a}	0.15 ± 0.03^{b}	0.47 ± 0.12^{c}
C 20:4n-6	23.89 ± 1.40^{a}	22.38 ± 1.83^{ab}	16 .00± 1.21^{c}	20.47 ± 2.44^{b}
C 22:2n-6	0.05 ± 0.02	0.08 ± 0.08	0.04 ± 0.01	0.02 ± 0.01
C 22:4n-6	0.18 ± 0.03^{ab}	0.13 ± 0.09^{a}	0.29 ± 0.08^{b}	0.06 ± 0.02^{ac}
C 22:5n-6	0.16 ± 0.10^{a}	0.11 ± 0.05^{a}	1.30 ± 0.36^{b}	0.10 ± 0.03^{a}
N-6	34.78 ± 1.44^{a}	32.32 ± 1.75^{a}	24.03 ± 1.37^{b}	32.22 ± 1.44^{a}
C 18:3n-3	0.09 ± 0.04	0.07 ± 0.01	0.07 ± 0.01	0.06 ± 0.01
C 20:3n-3	0.03 ± 0.01	0.05 ± 0.07	0.04 ± 0.02	0.01 ± 0.01
C 20:5n-3	0.15 ± 0.04^{a}	0.26 ± 0.04^{ab}	0.12 ± 0.02^{a}	0.41 ± 0.24^{b}
C 22:5n-3	0.35 ± 0.12^{a}	0.31 ± 0.06^{a}	0.58 ± 0.09^{b}	0.27 ± 0.05^{a}
C 22:6n-3	7.40 ± 1.19^{a}	9.63 ± 0.55^{b}	17.41 ± 1.29^{c}	7.41 ± 2.31^{a}
N-3	8.03 ± 1.08^{a}	10.33 ± 0.53^{a}	18.22 ± 1.28^{b}	8.16 ± 2.17^{a}
HUFA	32.53 ± 0.99^{ab}	33.21 ± 1.98^{a}	35.89 ± 1.79^{a}	27.78 ± 5.61^{b}
PUFA	42.81 ± 1.11	42.64 ± 1.91	42.25 ± 1.54	40.38 ± 2.37
Total	28.92 ± 6.52	32.61 ± 6.23	38.49 ± 4.06	29.37 ± 6.40

Values with different superscripts are significantly different by Tukey's post hoc test ($p<0.05$) following significant F-value by one-way ANOVA

Table D.3. Relative percent of fatty acids in liver PC in DHA deficient diet (TWD DHA-) fed rats at baseline, during pregnancy, and 7 days post partum

Name	baseline	15 days	20 days	7 days post partum
C 14:0	0.60 ± 0.09	0.46 ± 0.12	0.69 ± 0.09	0.61 ± 0.09
C 16:0	17.42 ± 1.15[a]	16.02 ± 0.87[a]	23.87 ± 0.73[b]	18.04 ± 0.75[a]
C 18:0	28.69 ± 1.58[a]	30.66 ± 0.75[a]	21.44 ± 1.57[b]	25.20 ± 1.15[a]
C 20:0	0.09 ± 0.03	0.06 ± 0.01	0.07 ± 0.02	0.06 ± 0.02
C 22:0	0.09 ± 0.03	0.07 ± 0.01	0.11 ± 0.04	0.07 ± 0.02
C 24:0	0.10 ± 0.02	0.09 ± 0.01	0.13 ± 0.03	0.11 ± 0.02
SFA	47.82 ± 1.60	47.57 ± 1.07	46.89 ± 0.98	45.55 ± 0.56
C 14:1	0.03 ± 0.02	0.02 ± 0.01	0.03 ± 0.01	0.02 ± 0.01
C 16:1	0.27 ± 0.05	0.40 ± 0.12	0.44 ± 0.12	0.48 ± 0.11
C 18:1n-7	1.31 ± 0.04[a]	1.16 ± 0.08[a]	1.21 ± 0.06[a]	1.61 ± 0.18[b]
C 18:1n-9	6.81 ± 1.48[ab]	5.78 ± 1.52[a]	9.23 ± 1.13[bc]	11.02 ± 1.86[c]
C 20:1n-9	0.09 ± 0.01[ab]	0.06 ± 0.01[a]	0.08 ± 0.02[ab]	0.12 ± 0.02[b]
C 22:1n-9	0.21 ± 0.05	0.18 ± 0.05	0.16 ± 0.06	0.13 ± 0.04
C 24:1n-9	0.12 ± 0.02	0.11 ± 0.02	0.08 ± 0.02	0.07 ± 0.02
MUFA	8.87 ± 1.50[a]	7.73 ± 1.56[a]	11.24 ± 1.26[ab]	13.49 ± 2.07[b]
C 18:2n-6	9.17 ± 0.74[a]	8.37 ± 1.35[ab]	7.01 ± 0.53[b]	8.79 ± 0.88[ab]
C 18:3n-6	0.23 ± 0.02[a]	0.29 ± 0.05[a]	0.43 ± 0.08[a]	0.95 ± 0.16[b]
C 20:2n-6	0.10 ± 0.01	0.07 ± 0.01	0.09 ± 0.02	0.09 ± 0.02
C 20:3n-6	0.35 ± 0.06[a]	0.22 ± 0.04[b]	0.13 ± 0.01[b]	0.43 ± 0.08[a]
C 20:4n-6	24.99 ± 1.68[a]	26.26 ± 0.93[a]	18.95 ± 1.99[b]	23.41 ± 2.08[a]
C 22:2n-6	0.05 ± 0.01	0.06 ± 0.03	0.06 ± 0.03	0.03 ± 0.01
C 22:4n-6	0.19 ± 0.04[a]	0.15 ± 0.01[a]	0.54 ± 0.07[b]	0.13 ± 0.03[a]
C 22:5n-6	0.27 ± 0.10[a]	0.42 ± 0.18[a]	4.07 ± 1.08[b]	0.68 ± 0.07[a]
N-6	35.35 ± 1.38[a]	35.84 ± 1.04[a]	31.28 ± 1.27[b]	34.51 ± 1.79[a]
C 18:3n-3	0.09 ± 0.02	0.07 ± 0.03	0.07 ± 0.01	0.05 ± 0.02
C 20:3n-3	0.02 ± 0.01	0.02 ± 0.01	0.06 ± 0.03	0.02 ± 0.01
C 20:5n-3	0.12 ± 0.06	0.10 ± 0.03	0.11 ± 0.01	0.17 ± 0.04
C 22:5n-3	0.39 ± 0.08[a]	0.42 ± 0.06[a]	0.72 ± 0.06[b]	0.48 ± 0.09[a]
C 22:6n-3	5.79 ± 0.49[ac]	7.27 ± 0.43[a]	8.39 ± 0.84[ab]	3.90 ± 1.07[c]
N-3	6.41 ± 0.46[ab]	7.87 ± 0.4[a]	9.34 ± 0.89[ac]	4.62 ± 1.00[b]
HUFA	32.13 ± 1.78[ab]	34.85 ± 1.28[a]	32.95 ± 1.06[ab]	29.21 ± 2.98[b]
PUFA	41.76 ± 1.54[ab]	43.71 ± 0.98[a]	40.61 ± 0.99[ab]	39.12 ± 2.54[b]
Total	32.87 ± 4.00	37.07 ± 5.81	37.50 ± 5.34	32.10 ± 3.61

Values with different superscripts are significantly different by Tukey's post hoc test ($p<0.05$) following significant F-value by one-way ANOVA

Table D.4. Concentration of fatty acids in liver PC in chow fed rats at baseline, during pregnancy, and 7 days post partum (µg/mg)

Name	baseline	15 days	20 days	7 days post partum
C 14:0	0.14 ± 0.04	0.17 ± 0.06	0.15 ± 0.04	0.17 ± 0.06
C 16:0	5.62 ± 0.70	6.24 ± 0.98	8.78 ± 3.73	7.27 ± 2.73
C 18:0	9.33 ± 1.84^{ab}	10.29 ± 1.45^{ab}	6.63 ± 2.83^{a}	11.92 ± 4.57^{b}
C 20:0	0.03 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	0.03 ± 0.01
C 22:0	0.03 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
C 24:0	0.03 ± 0.02	0.03 ± 0.02	0.04 ± 0.01	0.04 ± 0.01
SFA	15.81 ± 3.38	16.95 ± 2.11	15.87 ± 6.61	19.88 ± 7.43
C 14:1	Not Detected	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01
C 16:1	0.09 ± 0.02	0.15 ± 0.04	0.12 ± 0.08	0.14 ± 0.06
C 18:1n-7	0.44 ± 0.09^{ab}	0.46 ± 0.10^{ab}	0.38 ± 0.16^{a}	0.62 ± 0.19^{b}
C 18:1n-9	1.53 ± 0.31^{a}	1.80 ± 0.38^{ab}	2.00 ± 0.70^{ab}	2.84 ± 1.13^{b}
C 20:1n-9	0.03 ± 0.01^{a}	0.03 ± 0.01^{a}	0.02 ± 0.01^{a}	0.06 ± 0.01^{b}
C 22:1n-9	0.07 ± 0.03	0.06 ± 0.02	0.04 ± 0.01	0.04 ± 0.01
C 24:1n-9	0.04 ± 0.02	0.04 ± 0.03	0.02 ± 0.01	0.03 ± 0.01
MUFA	2.21 ± 0.32^{a}	2.56 ± 0.46^{ab}	2.6 ± 0.94^{ab}	3.73 ± 1.37^{b}
C 18:2n-6	3.46 ± 0.62^{a}	3.75 ± 0.78^{a}	2.82 ± 1.23^{b}	4.78 ± 2.27^{a}
C 18:3n-6	0.08 ± 0.01^{a}	0.12 ± 0.02^{a}	0.1 ± 0.05^{a}	0.26 ± 0.12^{b}
C 20:2n-6	0.05 ± 0.01^{a}	0.06 ± 0.01^{ab}	0.06 ± 0.03^{ab}	0.08 ± 0.02^{b}
C 20:3n-6	0.10 ± 0.02^{a}	0.11 ± 0.03^{a}	0.05 ± 0.02^{a}	0.27 ± 0.15^{b}
C 20:4n-6	8.18 ± 1.79	8.79 ± 1.57	5.59 ± 2.47	9.41 ± 3.46
C 22:2n-6	0.02 ± 0.01	0.03 ± 0.02	0.02 ± 0.01	0.02 ± 0.01
C 22:4n-6	0.06 ± 0.02^{a}	0.07 ± 0.02^{a}	0.19 ± 0.09^{b}	0.09 ± 0.03^{a}
C 22:5n-6	0.06 ± 0.02^{a}	0.10 ± 0.05^{a}	1.04 ± 0.55^{b}	0.36 ± 0.08^{a}
N-6	12.02 ± 2.44	13.02 ± 2.31	9.88 ± 4.42	15.27 ± 6.04
C 18:3n-3	0.03 ± 0.01	0.04 ± 0.01	0.05 ± 0.02	0.04 ± 0.02
C 20:3n-3	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01
C 20:5n-3	0.05 ± 0.02	0.07 ± 0.02	0.05 ± 0.03	0.10 ± 0.05
C 22:5n-3	0.17 ± 0.05^{a}	0.24 ± 0.06^{ab}	0.36 ± 0.18^{b}	0.46 ± 0.16^{cb}
C 22:6n-3	1.92 ± 0.45^{a}	2.71 ± 0.65^{ab}	4.54 ± 2.19^{b}	2.55 ± 0.87^{a}
N-3	1.78 ± 0.76^{a}	3.07 ± 0.70^{ab}	5.01 ± 2.40^{b}	3.17 ± 1.10^{ab}
HUFA	10.15 ± 1.35	12.09 ± 2.21	11.84 ± 5.44	12.82 ± 4.95
PUFA	13.80 ± 1.91	16.09 ± 2.88	14.89 ± 6.75	18.44 ± 7.14
Total	31.82 ± 5.26	35.60 ± 5.20	33.36 ± 14.22	42.05 ± 15.84

Values with different superscripts are significantly different by Tukey's post hoc test ($p<0.05$) following significant F-value by one-way ANOVA

Table D.5. Concentration of fatty acids in liver PC in high DHA diet (TWD DHA+) fed rats at baseline, during pregnancy, and 7 days post partum (µg/mg)

Name	baseline	15 days	20 days	7 days post partum
C 14:0	0.19 ± 0.08	0.18 ± 0.02	0.24 ± 0.06	0.16 ± 0.03
C 16:0	5.49 ± 1.30[a]	5.71 ± 0.87[a]	9.40 ± 1.42[b]	5.88 ± 0.90[a]
C 18:0	8.57 ± 1.59	9.69 ± 2.12	8.78 ± 1.21	7.27 ± 2.21
C 20:0	0.02 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.02 ± 0.01
C 22:0	0.03 ± 0.02	0.02 ± 0.01	0.03 ± 0.01	0.02 ± 0.01
C 24:0	0.04 ± 0.02	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01
SFA	14.56 ± 2.83	15.83 ± 2.84	18.76 ± 2.38	13.75 ± 3.04
C 14:1	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	Not Detected
C 16:1	0.08 ± 0.03	0.17 ± 0.04	0.15 ± 0.07	0.13 ± 0.03
C 18:1n-7	0.37 ± 0.06	0.37 ± 0.06	0.41 ± 0.05	0.45 ± 0.07
C 18:1n-9	1.65 ± 0.36	2.02 ± 0.40	2.68 ± 0.72	2.84 ± 0.41
C 20:1n-9	0.03 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.03 ± 0.01
C 22:1n-9	0.07 ± 0.02[a]	0.06 ± 0.02[ab]	0.05 ± 0.02[ab]	0.03 ± 0.01[b]
C 24:1n-9	0.05 ± 0.02[a]	0.04 ± 0.02[ab]	0.03 ± 0.01[ab]	0.02 ± 0.01[b]
MUFA	2.27 ± 0.42	2.69 ± 0.45	3.37 ± 0.76	3.49 ± 0.43
C 18:2n-6	2.95 ± 0.52	2.97 ± 0.69	2.30 ± 0.43	3.01 ± 0.36
C 18:3n-6	0.06 ± 0.01[a]	0.07 ± 0.02[a]	0.09 ± 0.02[ab]	0.18 ± 0.07[b]
C 20:2n-6	0.03 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.02 ± 0.01
C 20:3n-6	0.11 ± 0.02	0.11 ± 0.03	0.06 ± 0.01	0.14 ± 0.03
C 20:4n-6	7.16 ± 1.19	7.43 ± 1.82	6.19 ± 0.57	6.20 ± 2.09
C 22:2n-6	0.02 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.01
C 22:4n-6	0.05 ± 0.01[ab]	0.04 ± 0.01[a]	0.11 ± 0.04[b]	0.02 ± 0.01[a]
C 22:5n-6	0.05 ± 0.03[a]	0.03 ± 0.01[a]	0.50 ± 0.16[b]	0.03 ± 0.01[a]
N-6	10.43 ± 1.7	10.70 ± 2.46	9.31 ± 0.87	9.61 ± 2.13
C 18:3n-3	0.03 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.02 ± 0.01
C 20:3n-3	0.01 ± 0.01[ab]	0.01 ± 0.01[ab]	0.02 ± 0.01[b]	Not Detected
C 20:5n-3	0.05 ± 0.02[a]	0.09 ± 0.02[ab]	0.05 ± 0.01[a]	0.12 ± 0.07[b]
C 22:5n-3	0.11 ± 0.04	0.10 ± 0.02	0.23 ± 0.05	0.08 ± 0.02
C 22:6n-3	2.24 ± 0.62[a]	3.17 ± 0.64[a]	6.74 ± 0.58[b]	2.30 ± 1.27[a]
N-3	2.43 ± 0.64[a]	3.39 ± 0.66[a]	7.05 ± 0.62[b]	2.52 ± 1.28[a]
HUFA	9.77 ± 1.71	10.98 ± 2.46	13.90 ± 1.01	8.54 ± 3.68
PUFA	12.85 ± 2.24	14.09 ± 3.09	16.37 ± 1.21	12.13 ± 3.33
Total	28.92 ± 6.52	32.61 ± 6.23	38.49 ± 4.06	29.37 ± 6.40

Values with different superscripts are significantly different by Tukey's post hoc test ($p<0.05$) following significant F-value by one-way ANOVA

Table D.6. Concentration of fatty acids in liver PC in DHA deficient diet (TWD DHA-) fed rats at baseline, during pregnancy, and 7 days post partum (µg/mg)

Name	baseline	15 days	20 days	7 days post partum
C 14:0	0.20 ± 0.04	0.18 ± 0.07	0.26 ± 0.05	0.20 ± 0.03
C 16:0	5.82 ± 0.83[a]	5.96 ± 0.66[a]	9.08 ± 1.45[b]	5.88 ± 0.45[a]
C 18:0	9.57 ± 1.23	11.49 ± 1.88	8.13 ± 1.31	8.25 ± 1.13
C 20:0	0.03 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.02 ± 0.01
C 22:0	0.03 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.02 ± 0.01
C 24:0	0.03 ± 0.01	0.03 ± 0.01	0.05 ± 0.01	0.04 ± 0.01
SFA	15.96 ± 1.98	17.79 ± 2.6	17.81 ± 2.61	14.89 ± 1.58
C 14:1	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 001
C 16:1	0.09 ± 0.02	0.16 ± 0.07	0.17 ± 0.05	0.16 ± 0.04
C 18:1n-7	0.44 ± 0.05	0.43 ± 0.04	0.46 ± 0.07	0.53 ± 0.09
C 18:1n-9	2.28 ± 0.56[a]	2.20 ± 0.84[a]	3.50 ± 0.61[b]	3.59 ± 0.66[b]
C 20:1n-9	0.03 ± 0.01[a]	0.02 ± 0.01[a]	0.03 ± 0.01[ab]	0.04 ± 0.01[b]
C 22:1n-9	0.07 ± 0.02	0.07 ± 0.02	0.06 ± 0.02	0.04 ± 0.02
C 24:1n-9	0.04 ± 0.01	0.04 ± 0.01	0.03 ± 0.01	0.02 ± 0.01
MUFA	2.96 ± 0.61	2.93 ± 0.95	4.26 ± 0.71	4.4 ± 0.77
C 18:2n-6	3.05 ± 0.33	3.07 ± 0.20	2.67 ± 0.48	2.87 ± 0.41
C 18:3n-6	0.08 ± 0.01[a]	0.11 ± 0.02[a]	0.16 ± 0.04[a]	0.31 ± 0.04[b]
C 20:2n-6	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01
C 20:3n-6	0.12 ± 0.02	0.08 ± 0.01	0.05 ± 0.01	0.14 ± 0.02
C 20:4n-6	8.36 ± 1.31	9.86 ± 1.81	7.22 ± 1.46	7.68 ± 1.27
C 22:2n-6	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.01 ± 0.01
C 22:4n-6	0.06 ± 0.01[a]	0.06 ± 0.01[a]	0.20 ± 0.02[b]	0.04 ± 0.01[a]
C 22:5n-6	0.09 ± 0.03[a]	0.16 ± 0.10[a]	1.52 ± 0.38[b]	0.22 ± 0.03[a]
N-6	11.80 ± 1.54	13.39 ± 1.86	11.89 ± 1.82	11.3 ± 1.52
C 18:3n-3	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.02 ± 0.01
C 20:3n-3	0.01 ± 0.01[a]	0.01 ± 0.01[a]	0.02 ± 0.01[b]	0.01 ± 0.01[a]
C 20:5n-3	0.04 ± 0.02	0.04 ± 0.01	0.04 ± 0.01	0.06 ± 0.01
C 22:5n-3	0.13 ± 0.03	0.15 ± 0.01	0.27 ± 0.05	0.16 ± 0.03
C 22:6n-3	1.94 ± 0.32[ab]	2.73 ± 0.57[ab]	3.18 ± 0.56[a]	1.28 ± 0.41[b]
N-3	2.14 ± 0.33[ab]	2.96 ± 0.58[ab]	3.55 ± 0.61[a]	1.52 ± 0.41[b]
HUFA	10.74 ± 1.61	13.09 ± 2.48	12.52 ± 1.81	9.58 ± 1.65
PUFA	13.95 ± 1.83	16.35 ± 2.43	15.43 ± 2.28	12.82 ± 1.85
Total	32.87 ± 4.00	37.07 ± 5.81	37.50 ± 5.34	32.10 ± 3.61

Values with different superscripts are significantly different by Tukey's post hoc test ($p<0.05$) following significant F-value by one-way ANOVA

Appendix E. Liver PE

Table E.1. Relative percent of fatty acids in liver PE in chow fed rats at baseline, during pregnancy, and 7 days post partum

Name	baseline	15 days	20 days	7 days post partum
C 14:0	0.75 ± 0.32^{ab}	0.63 ± 0.20^{ab}	0.92 ± 0.28^{a}	0.43 ± 0.13^{b}
C 16:0	15.56 ± 1.62^{a}	15.67 ± 2.02^{a}	20.27 ± 0.81^{b}	15.45 ± 0.41^{a}
C 18:0	29.70 ± 2.31^{a}	31.22 ± 3.54^{a}	23.20 ± 1.13^{b}	27.75 ± 0.77^{a}
C 20:0	0.14 ± 0.04^{a}	0.10 ± 0.02^{ab}	0.14 ± 0.03^{a}	0.05 ± 0.01^{b}
C 22:0	0.12 ± 0.03^{ab}	0.13 ± 0.08^{ab}	0.17 ± 0.07^{a}	0.06 ± 0.02^{b}
C 24:0	0.13 ± 0.05	0.13 ± 0.07	0.19 ± 0.06	0.14 ± 0.03
SFA	47.68 ± 0.89^{a}	48.47 ± 1.31^{a}	46.77 ± 1.53^{ab}	45.11 ± 0.45^{b}
C 14:1	0.03 ± 0.01	0.03 ± 0.02	0.06 ± 0.03	0.02 ± 0.01
C 16:1	0.13 ± 0.02^{a}	0.46 ± 0.35^{ab}	0.86 ± 0.49^{b}	0.29 ± 0.09^{a}
C 18:1n-7	1.02 ± 0.11^{ab}	0.84 ± 0.11^{ab}	0.73 ± 0.15^{a}	1.05 ± 0.14^{b}
C 18:1n-9	6.65 ± 2.58	3.94 ± 1.02	9.26 ± 3.64	6.83 ± 1.55
C 20:1n-9	0.12 ± 0.05	0.07 ± 0.03	0.09 ± 0.02	0.12 ± 0.02
C 22:1n-9	0.40 ± 0.10^{a}	0.34 ± 0.12^{a}	0.40 ± 0.11^{a}	0.15 ± 0.05^{b}
C 24:1n-9	0.20 ± 0.09	0.17 ± 0.13	0.16 ± 0.06	0.10 ± 0.02
MUFA	8.53 ± 2.69	5.89 ± 1.57	11.58 ± 3.85	8.58 ± 1.68
C 18:2n-6	5.98 ± 1.07^{a}	4.48 ± 0.24^{ab}	2.86 ± 0.42^{b}	5.64 ± 0.97^{a}
C 18:3n-6	0.16 ± 0.04^{a}	0.14 ± 0.06^{a}	0.09 ± 0.03^{a}	0.28 ± 0.03^{b}
C 20:2n-6	0.12 ± 0.04	0.13 ± 0.03	0.13 ± 0.03	0.16 ± 0.02
C 20:3n-6	0.29 ± 0.03^{a}	0.29 ± 0.06^{a}	0.12 ± 0.03^{b}	0.58 ± 0.10^{c}
C 20:4n-6	21.57 ± 3.21^{a}	23.31 ± 3.07^{a}	13.24 ± 1.20^{b}	21.27 ± 1.58^{a}
C 22:2n-6	0.10 ± 0.05^{ab}	0.13 ± 0.07^{a}	0.09 ± 0.04^{ab}	0.04 ± 0.01^{b}
C 22:4n-6	0.54 ± 0.09^{a}	0.56 ± 0.12^{a}	1.07 ± 0.11^{b}	0.68 ± 0.08^{a}
C 22:5n-6	0.25 ± 0.06^{a}	0.44 ± 0.25^{ac}	3.95 ± 0.71^{b}	1.42 ± 0.39^{c}
N-6	29.01 ± 3.29^{a}	29.48 ± 2.81^{a}	21.54 ± 1.66^{b}	30.09 ± 2.18^{a}
C 18:3n-3	0.21 ± 0.10	0.13 ± 0.03	0.16 ± 0.05	0.09 ± 0.03
C 20:3n-3	0.05 ± 0.02^{ab}	0.07 ± 0.05^{ab}	0.11 ± 0.04^{a}	0.02 ± 0.01^{b}
C 20:5n-3	0.24 ± 0.07^{ab}	0.25 ± 0.05^{ab}	0.11 ± 0.02^{a}	0.37 ± 0.09^{b}
C 22:5n-3	1.09 ± 0.26^{a}	1.27 ± 0.19^{a}	1.27 ± 0.19^{a}	2.20 ± 0.23^{b}
C 22:6n-3	11.22 ± 2.16^{a}	13.77 ± 2.35^{ab}	17.46 ± 1.82^{b}	12.47 ± 1.07^{a}
N-3	12.81 ± 1.92^{a}	15.49 ± 2.44^{ab}	19.11 ± 1.88^{b}	15.14 ± 1.33^{a}
HUFA	35.25 ± 3.82	39.96 ± 1.58	37.32 ± 3.61	38.83 ± 1.81
PUFA	41.82 ± 3.33	44.97 ± 1.31	40.65 ± 3.40	45.22 ± 1.99
Total	10.05 ± 1.49	14.33 ± 2.59	10.69 ± 5.01	16.64 ± 9.68

Values with different superscripts are significantly different by Tukey's post hoc test ($p<0.05$) following significant F-value by one-way ANOVA

Table E.2. Relative percent of fatty acids in liver PE in high DHA diet (TWD DHA+) fed rats at baseline, during pregnancy, and 7 days post partum

Name	baseline	15 days	20 days	7 days post partum
C 14:0	0.92 ± 0.37	0.77 ± 0.19	0.68 ± 0.20	0.68 ± 0.13
C 16:0	15.22 ± 1.03^{ab}	14.96 ± 0.54^{ab}	17.32 ± 1.32^{a}	14.51 ± 0.94^{b}
C 18:0	28.97 ± 2.66	30.16 ± 0.87	26.79 ± 1.74	27.75 ± 1.95
C 20:0	0.12 ± 0.05	0.10 ± 0.02	0.10 ± 0.04	0.07 ± 0.02
C 22:0	0.13 ± 0.05	0.12 ± 0.02	0.13 ± 0.01	0.10 ± 0.03
C 24:0	0.14 ± 0.03	0.13 ± 0.03	0.13 ± 0.03	0.16 ± 0.03
SFA	46.81 ± 2.25	46.99 ± 1.07	47.00 ± 0.31	44.99 ± 1.12
C 14:1	0.05 ± 0.06	0.04 ± 0.01	0.04 ± 0.01	0.03 ± 0
C 16:1	0.12 ± 0.04^{a}	0.66 ± 0.12^{b}	0.45 ± 0.23^{ab}	0.49 ± 0.16^{ab}
C 18:1n-7	0.85 ± 0.26^{ab}	0.60± 0.07^{a}	0.60 ± 0.05^{a}	1.04 ± 0.21^{b}
C 18:1n-9	7.09 ± 3.42	5.99 ± 2.35	7.85 ± 2.81	8.39 ± 2.38
C 20:1n-9	0.11 ± 0.06	0.07 ± 0.01	0.08 ± 0.02	0.12 ± 0.02
C 22:1n-9	0.38 ± 0.11^{a}	0.29 ± 0.06^{ab}	0.39 ± 0.16^{a}	0.18 ± 0.05^{b}
C 24:1n-9	0.23 ± 0.07	0.20 ± 0.07	0.11 ± 0.03	0.42 ± 0.67
MUFA	8.93 ± 3.75	7.89 ± 2.49	9.54 ± 3.09	10.73 ± 2.34
C 18:2n-6	4.75 ± 2.06^{a}	2.87 ± 0.76^{b}	1.84 ± 0.34^{b}	3.57 ± 0.80^{ab}
C 18:3n-6	0.12 ± 0.04^{a}	0.09 ± 0.03^{a}	0.05 ± 0.01^{ab}	0.25 ± 0.04^{b}
C 20:2n-6	0.08 ± 0.03	0.07 ± 0.01	0.09 ± 0.01	0.06 ± 0.02
C 20:3n-6	0.25 ± 0.03^{ac}	0.21 ± 0.02^{a}	0.10 ± 0.02^{b}	0.33 ± 0.08^{c}
C 20:4n-6	19.72 ± 2.72^{a}	18.19 ± 1.50^{a}	13.41 ± 0.73^{b}	18.56 ± 1.60^{a}
C 22:2n-6	0.08 ± 0.03	0.09 ± 0.02	0.05 ± 0.02	0.05 ± 0.01
C 22:4n-6	0.45 ± 0.06^{ac}	0.30 ± 0.05^{ab}	0.59 ± 0.10^{c}	0.26 ± 0.06^{b}
C 22:5n-6	0.27 ± 0.23^{a}	0.19 ± 0.06^{a}	1.83 ± 0.30^{b}	0.21 ± 0.04^{a}
N-6	25.73 ± 2.73^{a}	22.01 ± 1.51^{ab}	17.96 ± 0.50^{b}	23.29 ± 2.29^{a}
C 18:3n-3	0.33 ± 0.44^{a}	0.10 ± 0.03^{ab}	0.11 ± 0.02^{ab}	0.06 ± 0.02^{b}
C 20:3n-3	0.05 ± 0.03	0.09 ± 0.02	0.07 ± 0.01	0.05 ± 0.02
C 20:5n-3	0.19 ± 0.03^{ab}	0.34 ± 0.07^{ac}	0.10 ± 0.01^{b}	0.49 ± 0.27^{c}
C 22:5n-3	0.68 ± 0.23	0.58 ± 0.08	0.69 ± 0.12	0.53 ± 0.05
C 22:6n-3	15.16 ± 3.51^{a}	21.17 ± 1.72^{b}	23.87 ± 2.04^{b}	17.91 ± 1.67^{a}
N-3	16.41 ± 2.98^{a}	22.29 ± 1.72^{bc}	24.84 ± 2.08^{c}	19.03 ± 1.54^{ab}
HUFA	36.78 ± 5.43	41.07 ± 2.54	40.66 ± 2.62	38.34 ± 1.21
PUFA	42.14 ± 3.53	44.29 ± 2.25	42.80 ± 2.51	42.32 ± 1.71
Total	10.89 ± 0.77	17.37 ± 3.78	13.92 ± 2.39	11.71 ± 1.73

Values with different superscripts are significantly different by Tukey's post hoc test ($p<0.05$) following significant F-value by one-way ANOVA

Table E.3. Relative percent of fatty acids in liver PE in DHA deficient diet (TWD DHA-) fed rats at baseline, during pregnancy, and 7 days post partum

Name	baseline	15 days	20 days	7 days post partum
C 14:0	0.93 ± 0.14	0.68 ± 0.17	0.91 ± 0.28	0.52 ± 0.09
C 16:0	15.73 ± 0.65[a]	13.37 ± 0.63[b]	16.26 ± 1.98[a]	13.75 ± 0.26[a]
C 18:0	28.42 ± 0.91[ab]	32.34 ± 2.86[a]	27.39 ± 3.03[b]	28.15 ± 0.66[ab]
C 20:0	0.12 ± 0.03	0.10 ± 0.01	0.11 ± 0.03	0.06 ± 0.01
C 22:0	0.13 ± 0.02	0.13 ± 0.08	0.16 ± 0.07	0.07 ± 0.03
C 24:0	0.16 ± 0.02	0.16 ± 0.05	0.19 ± 0.06	0.19 ± 0.03
SFA	47.04 ± 0.71[a]	47.36 ± 2.19[a]	46.26 ± 0.54[ab]	44.13 ± 0.97[b]
C 14:1	0.04 ± 0.01	0.03 ± 0.02	0.05 ± 0.02	0.02 ± 0.01
C 16:1	0.21 ± 0.12[a]	0.53 ± 0.24[ab]	0.67 ± 0.34[b]	0.38 ± 0.08[ab]
C 18:1n-7	0.93 ± 0.07[ab]	0.67 ± 0.05[a]	0.72 ± 0.04[a]	1.20 ± 0.22[b]
C 18:1n-9	8.28 ± 2.89	6.34 ± 3.34	8.80 ± 1.56	7.98 ± 0.70
C 20:1n-9	0.14 ± 0.01[a]	0.07 ± 0.03[b]	0.09 ± 0.02[ab]	0.12 ± 0.01[ab]
C 22:1n-9	0.43 ± 0.07[a]	0.31 ± 0.08[ab]	0.34 ± 0.07[a]	0.16 ± 0.03[b]
C 24:1n-9	0.35 ± 0.33	0.22 ± 0.13	0.11 ± 0.03	0.14 ± 0.09
MUFA	10.43 ± 2.81	8.24 ± 3.62	10.82 ± 1.81	10.04 ± 0.6
C 18:2n-6	4.44 ± 0.38[a]	3.05 ± 0.5[ab]	2.35 ± 0.49[b]	3.91 ± 0.45[ab]
C 18:3n-6	0.15 ± 0.02[a]	0.12 ± 0.04[a]	0.09 ± 0.04[a]	0.34 ± 0.05[b]
C 20:2n-6	0.08 ± 0.01	0.07 ± 0.03	0.09 ± 0.03	0.07 ± 0.01
C 20:3n-6	0.25 ± 0.03[ac]	0.17 ± 0.01[ab]	0.10 ± 0.02[b]	0.32 ± 0.08[c]
C 20:4n-6	20.31 ± 2.03[a]	22.00 ± 2.63[a]	15.91 ± 2.79[b]	24.14 ± 1.13[a]
C 22:2n-6	0.08 ± 0.01[ab]	0.12 ± 0.03[a]	0.08 ± 0.02[ab]	0.05 ± 0.01[b]
C 22:4n-6	0.52 ± 0.09[a]	0.5 ± 0.06[a]	1.14 ± 0.15[b]	0.56 ± 0.09[a]
C 22:5n-6	0.46 ± 0.17[a]	0.79 ± 0.35[ac]	7.07 ± 1.51[b]	1.72 ± 0.36[c]
N-6	26.29 ± 2.18[a]	26.82 ± 2.12[ab]	26.82 ± 1.86[ab]	31.11 ± 1.78[b]
C 18:3n-3	0.22 ± 0.08	0.10 ± 0.03	0.13 ± 0.05	0.06 ± 0.02
C 20:3n-3	0.07 ± 0.02[ab]	0.09 ± 0.06[a]	0.10 ± 0.05[a]	0.02 ± 0.01[b]
C 20:5n-3	0.16 ± 0.04	0.14 ± 0.02	0.10 ± 0.01	0.27 ± 0.07
C 22:5n-3	0.83 ± 0.23[a]	0.86 ± 0.13[ab]	1.00 ± 0.11[ab]	1.23 ± 0.28[b]
C 22:6n-3	12.70 ± 1.54	15.64 ± 1.47	13.59 ± 0.80	11.79 ± 1.53
N-3	13.96 ± 1.36	16.84 ± 1.51	14.91 ± 0.81	13.37 ± 1.27
HUFA	35.29 ± 2.67	40.20 ± 2.72	36.37 ± 7.37	40.05 ± 1.14
PUFA	40.26 ± 2.41	43.66 ± 2.23	41.73 ± 1.88	44.48 ± 1.21
Total	11.18 ± 2.93	19.23 ± 8.60	14.67 ± 2.58	11.90 ± 1.84

Values with different superscripts are significantly different by Tukey's post hoc test ($p<0.05$) following significant F-value by one-way ANOVA

Table E.4. Concentration of fatty acids in liver PE in chow fed rats at baseline, during pregnancy, and 7 days post partum (µg/mg)

Name	baseline	15 days	20 days	7 days post partum
C 14:0	0.10 ± 0.03	0.09 ± 0.03	0.09 ± 0.03	0.07 ± 0.03
C 16:0	1.68 ± 0.28	2.24 ± 0.37	2.19 ± 1.04	2.59 ± 1.47
C 18:0	2.91 ± 0.55	4.52 ± 1.10	2.49 ± 1.13	4.68 ± 2.8
C 20:0	0.02 ± 0.01^{ab}	0.01 ± 0.01^{ab}	0.01 ± 0.01^{ab}	0.01 ± 0.01^{b}
C 22:0	0.01 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.01 ± 0.01
C 24:0	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
SFA	4.87 ± 0.87	6.99 ± 1.25	5.00 ± 2.23	7.58 ± 4.36
C 14:1	0.01 ± 0.01	Not Detected	0.01 ± 0.01	Not Detected
C 16:1	0.02 ± 0.01^{a}	0.07 ± 0.05^{ab}	0.08 ± 0.03^{b}	0.05 ± 0.02^{ab}
C 18:1n-7	0.11 ± 0.01	0.12 ± 0.02	0.08 ± 0.03	0.17 ± 0.09
C 18:1n-9	0.83 ± 0.23	0.55 ± 0.12	1.01 ± 0.5	1.09 ± 0.50
C 20:1n-9	0.02 ± 0.01^{ab}	0.01 ± 0.01^{a}	0.01 ± 0.01^{a}	0.02 ± 0.01^{b}
C 22:1n-9	0.04 ± 0.01	0.05 ± 0.01	0.04 ± 0.02	0.02 ± 0.01
C 24:1n-9	0.02 ± 0.01	0.02 ± 0.02	0.02 ± 0.01	0.02 ± 0.01
MUFA	1.04 ± 0.24	0.83 ± 0.19	1.24 ± 0.56	1.37 ± 0.62
C 18:2n-6	0.69 ± 0.06	0.65 ± 0.12	0.32 ± 0.16	0.96 ± 0.66
C 18:3n-6	0.02 ± 0.01^{ab}	0.02 ± 0.01^{ab}	0.01 ± 0.01^{a}	0.05 ± 0.03^{b}
C 20:2n-6	0.01 ± 0.01^{a}	0.02 ± 0.01^{ab}	0.01 ± 0.01^{a}	0.03 ± 0.01^{b}
C 20:3n-6	0.03 ± 0.01^{a}	0.04 ± 0.01^{a}	0.01 ± 0.01^{a}	0.10 ± 0.06^{b}
C 20:4n-6	2.05 ± 0.4	3.39 ± 0.93	1.43 ± 0.71	3.61 ± 2.27
C 22:2n-6	0.01 ± 0.01^{a}	0.02 ± 0.01^{b}	0.01 ± 0.01^{ab}	0.01 ± 0.01^{a}
C 22:4n-6	0.06 ± 0.01	0.08 ± 0.02	0.12 ± 0.06	0.11 ± 0.06
C 22:5n-6	0.03 ± 0.01^{a}	0.06 ± 0.04^{a}	0.44 ± 0.27^{b}	0.23 ± 0.12^{ab}
N-6	2.89 ± 0.44	4.28 ± 1.03	2.36 ± 1.20	5.09 ± 3.16
C 18:3n-3	0.04 ± 0.03^{a}	0.02 ± 0.01^{ab}	0.02 ± 0.01^{b}	0.02 ± 0.01^{b}
C 20:3n-3	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	Not Detected
C 20:5n-3	0.02 ± 0.01^{a}	0.04 ± 0.01^{ab}	0.01 ± 0.01^{a}	0.07 ± 0.06^{b}
C 22:5n-3	0.12 ± 0.03^{a}	0.18 ± 0.05^{a}	0.14 ± 0.07^{a}	0.38 ± 0.23^{b}
C 22:6n-3	1.05 ± 0.23	1.99 ± 0.46	1.92 ± 1.04	2.14 ± 1.32
N-3	1.24 ± 0.24	2.24 ± 0.5	2.10 ± 1.12	2.60 ± 1.61
HUFA	3.36 ± 0.68	5.79 ± 1.23	4.09 ± 2.15	6.62 ± 4.09
PUFA	4.13 ± 0.67	6.51 ± 1.34	4.45 ± 2.31	7.69 ± 4.76
Total	10.05 ± 1.49	14.33 ± 2.59	10.69 ± 5.01	16.64 ± 9.68

Values with different superscripts are significantly different by Tukey's post hoc test ($p<0.05$) following significant F-value by one-way ANOVA

Table E.5. Concentration of fatty acids in liver PE in high DHA diet (TWD DHA+) fed rats at baseline, during pregnancy, and 7 days post partum (µg/mg)

Name	baseline	15 days	20 days	7 days post partum
C 14:0	0.10 ± 0.02	0.13 ± 0.04	0.09 ± 0.03	0.08 ± 0.02
C 16:0	1.72 ± 0.05	2.62 ± 0.56	2.41 ± 0.32	1.72 ± 0.33
C 18:0	3.15 ± 0.26	5.29 ± 1.21	3.77 ± 0.80	3.28 ± 0.64
C 20:0	0.01 ± 0[ab]	0.02 ± 0.01[a]	0.01 ± 0.01[ab]	0.01 ± 0.01[b]
C 22:0	0.01 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.01 ± 0.01
C 24:0	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
SFA	5.19 ± 0.31	8.24 ± 1.86	6.59 ± 1.15	5.32 ± 0.99
C 14:1	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 001	Not Detected
C 16:1	0.01 ± 0.01[a]	0.12 ± 0.03[b]	0.06 ± 0.03[ab]	0.06 ± 0.01[a]
C 18:1n-7	0.09 ± 0.02	0.10 ± 0.02	0.08 ± 0.02	0.12 ± 0.03
C 18:1n-9	0.76 ± 0.15	1.04 ± 0.43	1.08 ± 0.35	0.99 ± 0.29
C 20:1n-9	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 001	0.01 ± 0.01
C 22:1n-9	0.04 ± 0.01[ab]	0.05 ± 0.01[a]	0.06 ± 0.03[a]	0.02 ± 0.01[b]
C 24:1n-9	0.03 ± 0.01	0.04 ± 0.02	0.02 ± 0.01	0.05 ± 0.09
MUFA	0.96 ± 0.16	1.38 ± 0.48	1.31 ± 0.40	1.27 ± 0.32
C 18:2n-6	0.43 ± 0.04	0.50 ± 0.15	0.26 ± 0.08	0.41 ± 0.03
C 18:3n-6	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.03 ± 0.01
C 20:2n-6	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01
C 20:3n-6	0.03 ± 0,01	0.04 ± 0.01	0.01 ± 0.01	0.04 ± 0.01
C 20:4n-6	2.07 ± 0.26	3.17 ± 0.66	1.89 ± 0.38	2.17 ± 0.32
C 22:2n-6	0.01 ± 0.01[a]	0.02 ± 0.01[b]	0.01 ± 0.01[a]	0.01 ± 0.01[a]
C 22:4n-6	0.05 ± 0.01	0.05 ± 0.02	0.08 ± 0.02	0.03 ± 0.01
C 22:5n-6	0.02 ± 0.01[a]	0.03 ± 0.02[ab]	0.25 ± 0.03[b]	0.03 ± 0.01[a]
N-6	2.63 ± 0.31	3.83 ± 0.75	2.52 ± 0.48	2.72 ± 0.33
C 18:3n-3	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.01 ± 0.01
C 20:3n-3	0.01 ± 0.01[a]	0.02 ± 0.01[b]	0.01 ± 0.01[ab]	0.01 ± 0.01[a]
C 20:5n-3	0.03 ± 0.01[a]	0.06 ± 0.01[a]	0.01 ± 0.01[b]	0.05 ± 0.03[ab]
C 22:5n-3	0.06 ± 0.01	0.10 ± 0.02	0.10 ± 0.03	0.06 ± 0.01
C 22:6n-3	1.78 ± 0.11[a]	3.73 ± 1.03[b]	3.36 ± 0.72[bc]	2.13 ± 0.51[ac]
N-3	1.89 ± 0.12[a]	3.92 ± 1.06[b]	3.49 ± 0.75[ab]	2.26 ± 0.51[a]
HUFA	4.03 ± 0.37	7.20 ± 1.72	5.71 ± 1.16	4.52 ± 0.78
PUFA	4.52 ± 0.4	7.75 ± 1.77	6.02 ± 1.21	4.98 ± 0.78
Total	10.89 ± 0.77	17.37 ± 3.78	13.92 ± 2.39	11.71 ± 1.73

Values with different superscripts are significantly different by Tukey's post hoc test ($p<0.05$) following significant F-value by one-way ANOVA

Table E.6. Concentration of fatty acids in liver PE in DHA deficient diet (TWD DHA-) fed rats at baseline, during pregnancy, and 7 days post partum (µg/mg)

Name	baseline	15 days	20 days	7 days post partum
C 14:0	0.10 ± 0.04[ab]	0.13 ± 0.03[a]	0.14 ± 0.04[a]	0.06 ± 0.02[b]
C 16:0	1.79 ± 0.51	2.85 ± 1.49	2.46 ± 0.41	1.66 ± 0.27
C 18:0	3.26 ± 0.75[a]	6.21 ± 2.57[b]	3.98 ± 0.87[ab]	3.40 ± 0.53[a]
C 20:0	0.01 ± 0.01[ab]	0.02 ± 0.01[a]	0.02 ± 0.01[a]	0.01 ± 0.01[b]
C 22:0	0.01 ± 0.01[ab]	0.02 ± 0.01[a]	0.02 ± 0.01[a]	0.01 ± 0.01[b]
C 24:0	0.02 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.02 ± 0.01
SFA	5.37 ± 1.38	9.37 ± 4.06	6.87 ± 1.26	5.33 ± 0.86
C 14:1	Not Detected	0.01 ± 0.01[ab]	0.01 ± 0.01[b]	Not Detected
C 16:1	0.03 ± 0.02[a]	0.11 ± 0.03[b]	0.12 ± 0.04[b]	0.05 ± 0.01[a]
C 18:1n-7	0.11 ± 0.03	0.17 ± 0.13	0.11 ± 0.02	0.14 ± 0.04
C 18:1n-9	0.94 ± 0.66	1.29 ± 0.52	1.32 ± 0.31	0.96 ± 0.16
C 20:1n-9	0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01
C 22:1n-9	0.05 ± 0.02[a]	0.05 ± 0.02[a]	0.05 ± 0.01[a]	0.02 ± 0.01[b]
C 24:1n-9	0.04 ± 0.03	0.04 ± 0.02	0.02 ± 0.01	0.02 ± 0.01
MUFA	1.18 ± 0.73	1.70 ± 0.64	1.63 ± 0.35	1.21 ± 0.20
C 18:2n-6	0.52 ± 0.17	0.96 ± 1.11	0.35 ± 0.09	0.47 ± 0.05
C 18:3n-6	0.02 ± 0.01	0.04 ± 0.04	0.01 ± 0.01	0.04 ± 0.01
C 20:2n-6	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.01
C 20:3n-6	0.03 ± 0.01	0.04 ± 0.02	0.01 ± 0.01	0.04 ± 0.01
C 20:4n-6	2.33 ± 0.37[a]	4.57 ± 2.61[b]	2.23 ± 0.38[a]	2.90 ± 0.39[ab]
C 22:2n-6	0.01 ± 0.02[a]	0.02 ± 0.01[b]	0.01 ± 0.01[a]	0.01 ± 0.01[a]
C 22:4n-6	0.06 ± 0.01[a]	0.08 ± 0.02[a]	0.17 ± 0.04[b]	0.07 ± 0.02[a]
C 22:5n-6	0.06 ± 0.01[a]	0.14 ± 0.06[a]	1.06 ± 0.23[b]	0.20 ± 0.03[a]
N-6	3.04 ± 0.57	5.86 ± 3.76	3.86 ± 0.60	3.73 ± 0.45
C 18:3n-3	0.03 ± 0.02	0.02 ± 0.01	0.02 ± 0.01	0.01 ± 0.01
C 20:3n-3	0.01 ± 0.02[ac]	0.02 ± 0.01[b]	0.02 ± 0.01[ab]	Not Detected
C 20:5n-3	0.02 ± 0.01	0.03 ± 0.01	0.01 ± 0.01	0.03 ± 0.01
C 22:5n-3	0.10 ± 0.02	0.15 ± 0.01	0.15 ± 0.03	0.14 ± 0.02
C 22:6n-3	1.42 ± 0.25	2.64 ± 0.27	2.10 ± 0.48	1.44 ± 0.39
N-3	1.58 ± 0.29	2.85 ± 0.27	2.30 ± 0.52	1.63 ± 0.39
HUFA	4.04 ± 0.65[a]	7.65 ± 2.64[b]	5.45 ± 1.62[ab]	4.83 ± 0.78[ab]
PUFA	4.62 ± 0.84	8.72 ± 3.79	6.16 ± 1.08	5.36 ± 0.81
Total	11.18 ± 2.93	19.23 ± 8.60	14.67 ± 2.58	11.90 ± 1.84

Values with different superscripts are significantly different by Tukey's post hoc test ($p<0.05$) following significant F-value by one-way ANOVA

Appendix F. Pups whole body Total Lipids

Table F.1. Relative percent of fatty acids in whole body pups of chow fed rats during pregnancy, and 7 days post partum

Name	15 days	20 days	7 days post partum
C 14:0	2.04 ± 0.24[a]	2.26 ± 0.18[a]	4.59 ± 1.19[b]
C 16:0	26.74 ± 0.73[a]	26.27 ± 0.73[a]	22.08 ± 0.82[b]
C 18:0	14.53 ± 0.65[a]	13.05 ± 0.16[a]	9.14 ± 1.89[b]
C 20:0	0.28 ± 0.08	0.26 ± 0.01	0.19 ± 0.05
C 22:0	0.16 ± 0.03[a]	0.38 ± 0.04[b]	0.23 ± 0.07[ab]
C 24:0	0.20 ± 0.06[a]	1.05 ± 0.05[b]	0.65 ± 0.22[b]
SFA	45.47 ± 0.74[a]	43.99 ± 0.70[ab]	42.03 ± 1.30[b]
C 14:1	0.03 ± 0.02	Not detected	0.03 ± 0.01
C 16:1	2.61 ± 0.14[a]	2.72 ± 0.26[a]	0.99 ± 0.11[b]
C 18:1n-7	3.54 ± 0.16[a]	3.42 ± 0.15[a]	2.03 ± 0.21[b]
C 18:1n-9	15.90 ± 0.88	15.14 ± 0.42	15.81 ± 1.26
C 20:1n-9	0.32 ± 0.07	0.22 ± 0.01	0.28 ± 0.03
C 22:1n-9	0.40 ± 0.24	0.23 ± 0.05	0.09 ± 0.03
C 24:1n-9	0.26 ± 0.08	0.33 ± 0.03	0.17 ± 0.07
MUFA	23.44 ± 0.87[a]	22.19 ± 0.79[ab]	19.66 ± 1.13[b]
C 18:2n-6	3.49 ± 0.29[a]	7.46 ± 0.70[b]	18.06 ± 2.25[c]
C 18:3n-6	0.25 ± 0.05[a]	0.18 ± 0.02[a]	0.38 ± 0.08[b]
C 20:2n-6	0.36 ± 0.13[a]	0.34 ± 0.09[a]	0.76 ± 0.06[b]
C 20:3n-6	0.52 ± 0.09[a]	0.71 ± 0.09[b]	1.08 ± 0.07[c]
C 20:4n-6	13.88 ± 0.78[a]	11.49 ± 0.10[a]	8.61 ± 2.00[b]
C 22:2n-6	0.10 ± 0.03	0.14 ± 0.09	0.05 ± 0.02
C 22:4n-6	2.96 ± 0.30[a]	1.93 ± 0.08[b]	1.44 ± 0.29[b]
C 22:5n-6	1.57 ± 0.23[a]	1.14 ± 0.12[a]	0.44 ± 0.15[b]
N-6	23.13 ± 0.81[a]	23.39 ± 0.81[a]	30.84 ± 0.89[b]
C 18:3n-3	0.09 ± 0.06	0.21 ± 0.09	0.76 ± 0.24
C 20:3n-3	0.06 ± 0.02	0.03 ± 0.02	0.06 ± 0.01
C 20:5n-3	0.12 ± 0.08[a]	0.16 ± 0.04[ab]	0.25 ± 0.04[b]
C 22:5n-3	0.26 ± 0.12[a]	0.46 ± 0.07[a]	1.03 ± 0.11[b]
C 22:6n-3	2.61 ± 0.23[a]	4.34 ± 0.56[b]	2.57 ± 0.68[a]
N-3	3.15 ± 0.29[a]	5.21 ± 0.72[b]	4.67 ± 0.53[b]
HUFA	21.98 ± 1.10[a]	20.26 ± 0.71[a]	15.48 ± 3.23[b]
PUFA	26.28 ± 0.82[a]	28.59 ± 1.42[a]	35.50 ± 1.22[b]
Total	8.24 ± 1.29	11.30 ± 2.27	33.44 ± 11.97

Values with different superscripts are significantly different by Tukey's post hoc test ($p<0.05$) following significant F-value by one-way ANOVA

Table F.2. Relative percent of fatty acids in whole body pups of high DHA diet (TWD DHA+) fed rats during pregnancy, and 7 days post partum

Name	15 days	20 days	7 days post partum
C 14:0	2.23 ± 0.26[a]	2.48 ± 0.09[a]	4.56 ± 0.72[b]
C 16:0	26.55 ± 0.3[a]	25.06 ± 0.51[a]	20.96 ± 0.66[b]
C 18:0	13.94 ± 0.44[a]	13.22 ± 0.37[a]	6.89 ± 0.88[b]
C 20:0	0.25 ± 0.09[a]	0.30 ± 0.08[a]	0.10 ± 0.04[b]
C 22:0	0.14 ± 0.07[a]	0.35 ± 0.10[b]	0.09 ± 0.04[a]
C 24:0	0.15 ± 0.05[a]	0.79 ± 0.11[b]	0.20 ± 0.13[a]
SFA	45.44 ± 0.66[a]	43.60 ± 0.77[a]	38.23 ± 0.64[b]
C 14:1	0.03 ± 0.01[a]	0.03 ± 0.01[a]	0.12 ± 0.02[b]
C 16:1	2.65 ± 0.15[a]	2.60 ± 0.12[a]	1.54 ± 0.19[b]
C 18:1n-7	3.59 ± 0.10[a]	3.38 ± 0.15[a]	2.29 ± 0.14[b]
C 18:1n-9	17.12 ± 1.03[a]	16.81 ± 1.14[a]	30.33 ± 1.83[b]
C 20:1n-9	0.38 ± 0.05[a]	0.25 ± 0.04[a]	0.51 ± 0.04[c]
C 22:1n-9	0.24 ± 0.13	0.33 ± 0.13	0.07 ± 0.02
C 24:1n-9	0.23 ± 0.09[a]	0.44 ± 0.14[b]	0.09 ± 0.04[a]
MUFA	24.81 ± 1.09[a]	24.12 ± 1.34[a]	35.06 ± 1.79[b]
C 18:2n-6	3.19 ± 0.27[a]	6.35 ± 0.40[b]	12.69 ± 0.35[c]
C 18:3n-6	0.19 ± 0.03[a]	0.18 ± 0.03[a]	0.32 ± 0.05[b]
C 20:2n-6	0.31 ± 0.08[a]	0.31 ± 0.04[a]	0.65 ± 0.05[b]
C 20:3n-6	0.52 ± 0.08[a]	0.72 ± 0.06[b]	0.85 ± 0.05[b]
C 20:4n-6	12.62 ± 0.51[a]	11.00 ± 0.38[a]	4.16 ± 1.24[b]
C 22:2n-6	0.13 ± 0.08[a]	0.06 ± 0.03[ab]	0.03 ± 0.01[b]
C 22:4n-6	2.56 ± 0.16[a]	1.67 ± 0.12[b]	0.83 ± 0.21[b]
C 22:5n-6	1.21 ± 0.13[a]	0.69 ± 0.15[b]	0.11 ± 0.05[c]
N-6	20.73 ± 0.81	20.99 ± 0.71	19.63 ± 1.34
C 18:3n-3	0.07 ± 0.03	0.15 ± 0.07	0.59 ± 0.07
C 20:3n-3	0.07 ± 0.03	0.05 ± 0.02	0.04 ± 0.01
C 20:5n-3	0.10 ± 0.05[a]	0.21 ± 0.05[b]	0.19 ± 0.02[b]
C 22:5n-3	0.27 ± 0.07[a]	0.29 ± 0.09[ab]	0.48 ± 0.13[b]
C 22:6n-3	3.60 ± 0.35[a]	6.00 ± 0.54[b]	2.65 ± 0.81[a]
N-3	4.11 ± 0.31[a]	6.70 ± 0.61[b]	3.95 ± 0.91[a]
HUFA	20.96 ± 0.96[a]	20.64 ± 0.51[a]	9.32 ± 2.46[b]
PUFA	24.85 ± 1.08[ab]	27.70 ± 0.89[a]	23.59 ± 2.23[b]
Total	8.90 ± 0.84[a]	9.96 ± 0.57[a]	94.03 ± 35.11[b]

Values with different superscripts are significantly different by Tukey's post hoc test ($p<0.05$) following significant F-value by one-way ANOVA

Table F.3. Relative percent of fatty acids in whole body pups of DHA deficient diet (TWD DHA-) fed rats during pregnancy, and 7 days post partum

Name	15 days	20 days	7 days post partum
C 14:0	2.40 ± 0.37[a]	2.33 ± 0.32[a]	4.86 ± 1.00[b]
C 16:0	26.50 ± 0.97[a]	25.77 ± 0.69[a]	20.94 ± 1.52[b]
C 18:0	13.87 ± 0.89[a]	12.93 ± 0.40[a]	6.46 ± 0.82[b]
C 20:0	0.25 ± 0.07[a]	0.27 ± 0.05[a]	0.10 ± 0.02[b]
C 22:0	0.20 ± 0.17[a]	0.44 ± 0.07[b]	0.07 ± 0.02[a]
C 24:0	0.26 ± 0.44[a]	0.89 ± 0.06[b]	0.18 ± 0.06[a]
SFA	44.98 ± 1.03[a]	43.31 ± 1.11[a]	38.13 ± 2.43[b]
C 14:1	0.04 ± 0.01[a]	0.02 ± 0.01[a]	0.12 ± 0.02[b]
C 16:1	2.68 ± 0.09[a]	2.69 ± 0.16[a]	1.55 ± 0.29[b]
C 18:1n-7	3.54 ± 0.17[a]	3.44 ± 0.18[a]	2.25 ± 0.16[b]
C 18:1n-9	17.06 ± 0.66[a]	17.56 ± 1.46[a]	31.48 ± 1.25[b]
C 20:1n-9	0.35 ± 0.04[a]	0.26 ± 0.05[a]	0.53 ± 0.10[b]
C 22:1n-9	0.34 ± 0.20	0.31 ± 0.08	0.06 ± 0.01
C 24:1n-9	0.16 ± 0.13[ab]	0.32 ± 0.07[a]	0.09 ± 0.03[b]
MUFA	24.52 ± 0.67[a]	24.77 ± 1.67[a]	36.21 ± 1.30[b]
C 18:2n-6	3.75 ± 1.32[a]	6.19 ± 0.53[b]	12.76 ± 0.4[c]
C 18:3n-6	0.22 ± 0.04	0.25 ± 0.09	0.31 ± 0.05
C 20:2n-6	0.29 ± 0.05[a]	0.30 ± 0.08[a]	0.66 ± 0.10[b]
C 20:3n-6	0.49 ± 0.10[a]	0.63 ± 0.05[a]	0.81 ± 0.09[b]
C 20:4n-6	12.96 ± 1.06[a]	11.69 ± 0.93[a]	4.36 ± 1.02[b]
C 22:2n-6	0.11 ± 0.06	0.08 ± 0.06	0.03 ± 0.01
C 22:4n-6	2.65 ± 0.43[a]	2.05 ± 0.19[b]	0.99 ± 0.39[b]
C 22:5n-6	1.63 ± 0.30[a]	1.85 ± 0.30[a]	0.30 ± 0.03[b]
N-6	22.10 ± 0.92[ab]	23.04 ± 1.24[a]	20.24 ± 1.78[b]
C 18:3n-3	0.14 ± 0.07	0.15 ± 0.01	0.58 ± 0.05
C 20:3n-3	0.09 ± 0.03[a]	0.03 ± 0.01[b]	0.04 ± 0.01[b]
C 20:5n-3	0.09 ± 0.02	0.09 ± 0.05	0.16 ± 0.01
C 22:5n-3	0.20 ± 0.10[a]	0.21 ± 0.12[a]	0.49 ± 0.10[b]
C 22:6n-3	2.71 ± 0.59[a]	3.43 ± 0.41[a]	0.90 ± 0.21[b]
N-3	3.22 ± 0.68	3.90 ± 0.35	2.17 ± 0.26
HUFA	20.82 ± 1.27[a]	19.98 ± 1.27[a]	8.06 ± 1.79[b]
PUFA	25.33 ± 1.53[ab]	26.95 ± 1.35[a]	22.41 ± 2.04[b]
Total	8.15 ± 1.20[a]	9.96 ± 0.22[a]	97.29 ± 19.46[b]

Values with different superscripts are significantly different by Tukey's post hoc test ($p<0.05$) following significant F-value by one-way ANOVA

Table F.4. Concentration of fatty acids in whole body pups of chow fed rats during pregnancy, and 7 days post partum (µg/mg)

Name	15 days	20 days	7 days post partum
C 14:0	0.17 ± 0.01	0.27 ± 0.03	1.68 ± 0.88
C 16:0	2.32 ± 0.38	3.13 ± 0.64	7.66 ± 2.93
C 18:0	1.26 ± 0.22[a]	1.55 ± 0.29[ab]	2.96 ± 0.54[b]
C 20:0	0.02 ± 0.01[a]	0.03 ± 0.01[a]	0.06 ± 0.01[b]
C 22:0	0.01 ± 0.01[a]	0.04 ± 0.01[b]	0.07 ± 0.01[c]
C 24:0	0.02 ± 0.01[a]	0.12 ± 0.02[b]	0.20 ± 0.03[c]
SFA	3.94 ± 0.64	5.24 ± 1.00	14.47 ± 5.21
C 14:1	Not Detected	Not Detected	0.01 ± 0.01
C 16:1	0.23 ± 0.04	0.32 ± 0.07	0.35 ± 0.15
C 18:1n-7	0.31 ± 0.05	0.41 ± 0.08	0.68 ± 0.19
C 18:1n-9	1.37 ± 0.15[a]	1.80 ± 0.36[a]	5.55 ± 2.35[b]
C 20:1n-9	0.03 ± 0.01	0.03 ± 0.01	0.10 ± 0.04
C 22:1n-9	0.03 ± 0.02	0.03 ± 0.01	0.03 ± 0.01
C 24:1n-9	0.02 ± 0.01[a]	0.04 ± 0.01[ab]	0.05 ± 0.01[b]
MUFA	2.02 ± 0.25	2.64 ± 0.52	6.84 ± 2.73
C 18:2n-6	0.30 ± 0.03[a]	0.89 ± 0.20[a]	6.42 ± 2.87[b]
C 18:3n-6	0.02 ± 0.01	0.02 ± 0.01	0.14 ± 0.07
C 20:2n-6	0.03 ± 0.01[a]	0.04 ± 0.01[a]	0.26 ± 0.10[b]
C 20:3n-6	0.04 ± 0.01[a]	0.08 ± 0.01[a]	0.37 ± 0.12[b]
C 20:4n-6	1.21 ± 0.23[a]	1.37 ± 0.28[a]	2.76 ± 0.44[b]
C 22:2n-6	0.01 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
C 22:4n-6	0.26 ± 0.06[a]	0.23 ± 0.04[a]	0.47 ± 0.10[b]
C 22:5n-6	0.14 ± 0.04	0.14 ± 0.04	0.14 ± 0.02
N-6	2.01 ± 0.36[a]	2.79 ± 0.57[a]	10.58 ± 3.68[b]
C 18:3n-3	0.01 ± 0.01[a]	0.03 ± 0.01[a]	0.28 ± 0.17[b]
C 20:3n-3	0.01 ± 0.01[a]	Not Detected	0.02 ± 0.01[b]
C 20:5n-3	0.01 ± 0.01[a]	0.02 ± 0.01[a]	0.09 ± 0.04[b]
C 22:5n-3	0.02 ± 0.01[a]	0.05 ± 0.01[a]	0.34 ± 0.10[b]
C 22:6n-3	0.23 ± 0.04[a]	0.52 ± 0.17[a]	0.82 ± 0.11[b]
N-3	0.27 ± 0.04[a]	0.63 ± 0.20[a]	1.55 ± 0.41[b]
HUFA	1.91 ± 0.36[a]	2.42 ± 0.56[a]	5.01 ± 0.91[b]
PUFA	2.28 ± 0.40[a]	3.42 ± 0.76[a]	12.13 ± 4.09[b]
Total	8.24 ± 1.29	11.30 ± 2.27	33.44 ± 11.97

Values with different superscripts are significantly different by Tukey's post hoc test ($p<0.05$) following significant F-value by one-way ANOVA

Table F.5. Concentration of fatty acids in whole body pups of high DHA diet (TWD DHA+) fed rats during pregnancy, and 7 days post partum (µg/mg)

Name	15 days	20 days	7 days post partum
C 14:0	0.21 ± 0.02[a]	0.26 ± 0.02[a]	4.57 ± 1.99[b]
C 16:0	2.49 ± 0.24[a]	2.62 ± 0.18[a]	20.47 ± 8.00[b]
C 18:0	1.30 ± 0.12[a]	1.38 ± 0.09[a]	6.46 ± 1.87[b]
C 20:0	0.02 ± 0.01[a]	0.03 ± 0.01[a]	0.09 ± 0.01[b]
C 22:0	0.01 ± 0.01[a]	0.04 ± 0.01[b]	0.08 ± 0.01[c]
C 24:0	0.01 ± 0.01[a]	0.08 ± 0.01[b]	0.16 ± 0.03[c]
SFA	4.25 ± 0.43[a]	4.55 ± 0.29[a]	37.19 ± 13.98[b]
C 14:1	Not Detected	Not Detected	0.11 ± 0.05[b]
C 16:1	0.25 ± 0.03[a]	0.27 ± 0.01[a]	1.53 ± 0.69[b]
C 18:1n-7	0.34 ± 0.03[a]	0.35 ± 0.01[a]	2.19 ± 0.76[b]
C 18:1n-9	1.60 ± 0.15[a]	1.75 ± 0.08[a]	29.96 ± 12.6[b]
C 20:1n-9	0.04 ± 0.01[a]	0.03 ± 0.01[a]	0.49 ± 0.19[b]
C 22:1n-9	0.02 ± 0.01[a]	0.03 ± 0.01[ab]	0.06 ± 0.01[b]
C 24:1n-9	0.02 ± 0.01[a]	0.05 ± 0.02[b]	0.08 ± 0.01[c]
MUFA	2.32 ± 0.20[a]	2.51 ± 0.09[a]	34.54 ± 14.28[b]
C 18:2n-6	0.30 ± 0.04[a]	0.66 ± 0.07[a]	12.34 ± 4.64[b]
C 18:3n-6	0.02 ± 0.01[a]	0.02 ± 0.01[a]	0.32 ± 0.16[b]
C 20:2n-6	0.03 ± 0.01[a]	0.03 ± 0.01[a]	0.63 ± 0.21[b]
C 20:3n-6	0.05 ± 0.01[a]	0.08 ± 0.01[a]	0.82 ± 0.29[b]
C 20:4n-6	1.18 ± 0.14[a]	1.15 ± 0.08[a]	3.73 ± 0.72[b]
C 22:2n-6	0.01 ± 0.01[a]	0.01 ± 0.01[a]	0.02 ± 0.01[b]
C 22:4n-6	0.24 ± 0.01[a]	0.17 ± 0.02[a]	0.77 ± 0.21[b]
C 22:5n-6	0.11 ± 0.02[a]	0.07 ± 0.02[a]	0.10 ± 0.02[b]
N-6	1.94 ± 0.20[a]	2.19 ± 0.18[a]	18.72 ± 6.16[b]
C 18:3n-3	0.01 ± 0.01[a]	0.02 ± 0.01[a]	0.58 ± 0.22[b]
C 20:3n-3	0.01 ± 0.01[a]	0.01 ± 0.01[a]	0.04 ± 0.01[b]
C 20:5n-3	0.01 ± 0.01[a]	0.02 ± 0.01[a]	0.18 ± 0.06[b]
C 22:5n-3	0.03 ± 0.01[a]	0.03 ± 0.01[a]	0.43 ± 0.08[b]
C 22:6n-3	0.34 ± 0.05[a]	0.63 ± 0.08[a]	2.36 ± 0.37[b]
N-3	0.39 ± 0.05[a]	0.70 ± 0.10[a]	3.59 ± 0.72[b]
HUFA	1.96 ± 0.21[a]	2.16 ± 0.17[a]	8.42 ± 1.71[b]
PUFA	2.33 ± 0.25[a]	2.90 ± 0.25[a]	22.31 ± 6.87[b]
Total	8.9 ± 0.84[a]	9.96 ± 0.57[a]	94.03 ± 35.11[b]

Values with different superscripts are significantly different by Tukey's post hoc test ($p<0.05$) following significant F-value by one-way ANOVA

Table F.6. Concentration of fatty acids in whole body pups of DHA deficient diet (TWD DHA-) fed rats during pregnancy, and 7 days post partum (µg/mg)

Name	15 days	20 days	7 days post partum
C 14:0	0.21 ± 0.05[a]	0.24 ± 0.03[a]	5.02 ± 1.98[b]
C 16:0	2.27 ± 0.26[a]	2.70 ± 0.12[a]	21.26 ± 5.73[b]
C 18:0	1.18 ± 0.11[a]	1.36 ± 0.06[a]	6.40 ± 0.75[b]
C 20:0	0.02 ± 0.01[a]	0.03 ± 0.01[a]	0.10 ± 0.01[b]
C 22:0	0.02 ± 0.02[a]	0.05 ± 0.01[b]	0.07 ± 0.01[c]
C 24:0	0.03 ± 0.05[a]	0.09 ± 0.01[b]	0.17 ± 0.03[c]
SFA	3.86 ± 0.49[a]	4.54 ± 0.18[a]	38.65 ± 10.07[b]
C 14:1	Not Detected	Not Detected	0.12 ± 0.05[b]
C 16:1	0.23 ± 0.03[a]	0.28 ± 0.02[a]	1.59 ± 0.55[b]
C 18:1n-7	0.30 ± 0.04[a]	0.36 ± 0.02[a]	2.24 ± 0.28[b]
C 18:1n-9	1.47 ± 0.25[a]	1.84 ± 0.17[a]	31.62 ± 6.00[b]
C 20:1n-9	0.03 ± 0.01[a]	0.03 ± 0.01[a]	0.52 ± 0.06[b]
C 22:1n-9	0.03 ± 0.02[a]	0.03 ± 0.01[ab]	0.06 ± 0.01[b]
C 24:1n-9	0.01 ± 0.01[a]	0.03 ± 0.01[a]	0.09 ± 0.01[b]
MUFA	2.11 ± 0.33[a]	2.60 ± 0.2[a]	36.35 ± 6.83[b]
C 18:2n-6	0.33 ± 0.18[a]	0.65 ± 0.05[a]	12.8 ± 2.35[b]
C 18:3n-6	0.02 ± 0.01[a]	0.03 ± 0.01[a]	0.32 ± 0.11[b]
C 20:2n-6	0.03 ± 0.01[a]	0.03 ± 0.01[a]	0.65 ± 0.07[b]
C 20:3n-6	0.04 ± 0.01[a]	0.07 ± 0.01[a]	0.81 ± 0.10[b]
C 20:4n-6	1.11 ± 0.11[a]	1.22 ± 0.08[a]	4.27 ± 0.51[b]
C 22:2n-6	0.01 ± 0.01[a]	0.01 ± 0.01[a]	0.03 ± 0.01[b]
C 22:4n-6	0.22 ± 0.03[a]	0.22 ± 0.02[a]	0.96 ± 0.24[b]
C 22:5n-6	0.14 ± 0.04[a]	0.19 ± 0.03[a]	0.30 ± 0.04[b]
N-6	1.90 ± 0.31[a]	2.41 ± 0.10[a]	20.14 ± 2.7[b]
C 18:3n-3	0.01 ± 0.01[a]	0.02 ± 0.01[a]	0.59 ± 0.16[b]
C 20:3n-3	0.01 ± 0.01[a]	Not Detected	0.04 ± 0.01[b]
C 20:5n-3	0.01 ± 0.01[a]	0.01 ± 0.01[a]	0.16 ± 0.04[b]
C 22:5n-3	0.02 ± 0.01[a]	0.02 ± 0.01[a]	0.48 ± 0.05[b]
C 22:6n-3	0.24 ± 0.08[a]	0.36 ± 0.04[a]	0.88 ± 0.11[b]
N-3	0.28 ± 0.09[a]	0.41 ± 0.04[a]	2.15 ± 0.27[b]
HUFA	1.78 ± 0.23[a]	2.09 ± 0.12[a]	7.89 ± 0.92[b]
PUFA	2.18 ± 0.40[a]	2.82 ± 0.11[a]	22.29 ± 2.96[b]
Total	8.15 ± 1.20[a]	9.96 ± 0.22[a]	97.29 ± 19.46[b]

Values with different superscripts are significantly different by Tukey's post hoc test ($p<0.05$) following significant F-value by one-way ANOVA

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