The Effects of Dietary DHA During Development on Maternal Tissue and Spatial Memory in Female and Male Pups During Adolescence

by

Daniel Marc Enlai Lamontagne-Kam

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Author's Declaration

This thesis consists of material all of which I authored or co-authored: see Statement of Contributions included in the thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

I understand that my thesis may be made electronically available to the public.

Statement of contributions:

The first draft of the study in chapter 5 was written with my supervisor, Dr. Ken D Stark. The study has been published.

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I am the sole author of all other chapters in my thesis.

Abstract:

Sex differences in plasma docosahexaenoic acid (DHA) status during adulthood have been documented previously, and recent studies suggest the effects of DHA supplementation on human cognition are different in females compared with males during infancy, adolescence, and early adulthood. Differences in estradiol concentration are believed to be the predominant factor in sexual dimorphisms in DHA status when DHA is not supplemented. This suggests that sexual dimorphisms begin during adolescence when fed diets not supplemented with DHA, but this has not been examined comprehensively. Therefore, this thesis examined: 1) DHA status in rat maternal, fetal and pup tissues with and without dietary DHA supplementation during pregnancy; 2) the onset of sex differences in the fatty acid composition of plasma, heart, brain and liver from birth to adulthood in rats fed chow diets; and 3) the impact of DHA supplementation during the perinatal period on spatial memory in female and male adolescent pups, and possible relationships to brain lipidomic profiles. Results from these studies show that maternal DHA status decreased significantly during the postpartum period when DHA was not provided in the diet and suggest that DHA could be mobilized from maternal adipose and possibly maternal heart and liver for milk production. Furthermore, results show that sex differences in tissue polyunsaturated fatty acids (PUFA) began at 6 weeks of age. In the spatial memory experiment, DHA supplementation significantly increased latency times during the final learning session of Morris Water Maze (MWM) testing and reduced arachidonic acid (ARA) and n-6 docosapentaenoic acid (DPA) containing phospholipid species in the hippocampus of both males and females. Understanding the effects of DHA supplementation during the perinatal and adolescent periods on the PUFA composition of maternal and pup tissues, and how these effects interact with sexual dimorphisms is critical to understanding dietary requirements of DHA throughout pregnancy and childhood. Additionally, MWM testing and lipidomic analyses

indicate that DHA supplementation may interact with long chain n-6 PUFA metabolism that may be important for spatial memory.

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List of Abbreviations:

AFWD – Adequate Fat Western Diet
ALA – Alpha-linoleic acid
ARA – Arachidonic acid
BHT – Butylated hydroxytoluene
CE – Cholesterol ester
D6D – Delta 6 desaturase
DDA – Data dependent analysis
DHA – Docosahexaenoic acid
DNA – Deoxyribonucleic acid
EDTA - Ethylenediaminetetraacetic acid
ER – Estrogen receptor
EPA – Eicosapentaenoic acid
FID – Flame ionization detector
GC – Gas chromatography
GPR30 – G protein-coupled receptor 30
HPLC – High performance liquid chromatography
LA – Linoleic acid
LCPUFA – Long chain polyunsaturated fatty acid
LDL – Low density lipoprotein
LPL – Lipoprotein lipase
mRNA – Mitochondrial ribonucleic acid
MS – Mass spectrometry
MUFA – Monounsaturated fatty acid
MWM – Morris water maze
NEFA – Non esterified fatty acid
PC – Phosphatidyl choline
PE – Phosphatidyl ethanolamine
PEMT – phosphatidylethanolamine N-methyltransferase
PG – Phosphatidyl glycerol
PI – Phosphatidyl inositol
PS – Phosphatidyl serine
PL- Phospholipid
PLA2 – phospholipase A2
PPAR - peroxisome proliferator-activated receptor alpha
PUFA – Polyunsaturated fatty acid
qPCR – Qualitative polymerase chain reaction
QTOF – Quadrupole time of flight
RBC – Red blood cell
RNA – Ribonucleic acid
RT-qPCR – Reverse transcriptase qualitative polymerase chain reaction
SFA – Saturated fatty acid
TAG – Triacylglycerol
TLC – Thin layer chromatography
TWD – Total Western Diet (High fat)
UHPLC – Ultra high performance liquid chromatography
VLDL – Very low density lipoprotein

1 General Introduction

Docosahexaenoic acid (DHA) is essential to the development and maintenance of the central nervous system, and supports learning and spatial memory performance throughout life [1,2]. The ability of DHA to support learning and spatial memory performance has been linked to neurogenesis and synaptogenesis [3], particularly in the hippocampus, a brain region associated with spatial memory [4]. Recent human trials have reported a sex x diet interaction in young adults supplemented with DHA, where supplementation improved episodic memory in females, and working memory reaction time in males [5], but the mechanism behind this interaction has not been fully explained. Animal models could provide further insight into the mechanisms behind this. Animal models show rats with adequate dietary DHA intakes have shorter latency times during spatial memory testing as compared with n-3 PUFA deficient rats, but this research has been largely limited to male rats [6–10]. Additionally, MUFAs, which are higher in the typical Western diet than regular chow diets used in most studies have been shown to reduce PUFA metabolism [11]. Examining an animal model that incorporates females and Western diets could provide further insight into the mechanism behind a sex x diet interaction in young adults supplemented with DHA.

Sex differences in the interaction between DHA and brain function could be occurring directly through the effect of estradiol on the brain [12,13] or indirectly via the ability of estradiol to increase circulating levels of DHA [14,15], which could then affect brain concentrations [16]. DHA is incorporated at higher levels in the hippocampi of female control mice as compared with ovariectomized mice [13]. In addition, 17β-estradiol increases mRNA and protein expression of hepatic delta 6 desaturase (D6D), providing females with the ability to

synthesize DHA from shorter chain precursors more rapidly than males [14,17], particularly when DHA status is low [14,15,18,19].

The general consensus is that estrogen largely mediates sexual dimorphisms in DHA status. However, when sexual dimorphisms in DHA status begin, and how they relate to differences reported in memory and cognition at infancy [20], adolescence [21], and adulthood [5,22] is not fully explained. Although overall estrogen levels are low during prepubescence, levels in females are higher than males [23]. Female rats also begin adolescence earlier than males [24]. Understanding the influence of estrogen on LCPUFA synthesis and when sexual dimorphisms begin is important. Most DHA is accumulated in the brain between the third trimester and two years of age in humans, and prenatal day 7 to postnatal day 21 in rats [3]. Knowing how much the brain needs, at what stages of life, and how sexual dimorphisms affect DHA supply and demand is important for making dietary recommendations for all stages of life.

Current recommendations for DHA intakes during pregnancy and lactation vary between authoritative bodies. During pregnancy, the International Society for the Study of Fatty Acids and Lipids (ISSFAL) recommends 200mg/d [25], and Health Canada recommends that women continue eating the generally recommended 150g of fish per week [26]. The Institute of Medicine (IOM) has general omega-3 recommendations, but no DHA recommendations specific to pregnancy [27]. Most Canadian women do not meet the recommended 200mg/d of DHA [28]. Quantifying the effect of insufficient DHA consumption during pregnancy and lactation could help in determining the impact insufficient consumption has on public health, and identify better dietary recommendations for pregnant women, and for males and females in general.

This thesis aims to better understand sex differences in DHA metabolism from infancy to adulthood. This will provide insights on the impact of diet on maternal-fetal levels of DHA,

changes in DHA status, and fatty acid composition in males and females during development and adulthood, and the potential impact of sex differences and dietary DHA on spatial memory and lipidomic profiles. Determining what effect these sex differences may have, and how DHA status changes during development in males and females is critical to understanding the dietary requirement of DHA throughout pregnancy and childhood development, and their impact on memory during adolescence.

2 Biochemical Foundations

2.1 DHA status during pregnancy and perinatal uptake

The mechanisms responsible for increasing DHA during pregnancy have not been fully identified, but dietary intakes and *de novo* synthesis could both contribute [1,29–32]. Maternal intakes during pregnancy can be low in North America [28], and are unlikely to match the amount transferred to the fetus [33] for a considerable proportion of the population. Low DHA intakes and status in mothers may also affect the amount of DHA supplied to the newborn during lactation [34,35]. The rate of DHA conversion from alpha linolenic acid (ALA) adults is generally less than 1% [36], and although *de novo* synthesis of DHA is upregulated during pregnancy [30], it may not be adequate to meet fetal and infant demand. Therefore, in situations where *de novo* synthesis and dietary intakes are insufficient, mobilization of maternal stores may be a major provider of DHA for fetuses and newborns, but mothers not consuming DHA can deplete them within two months of delivery [37].

Most research examining maternal DHA mobilization is focused on the liver and adipose depots [31,32,38,39]. However, there are other tissues with considerable concentrations of DHA that could provide DHA during pregnancy and lactation. Skeletal muscle in the rat has both higher relative, and absolute amounts of DHA as compared with white adipose [40], and fatty acid levels in muscle decrease near parturition in bovine models [41]. In addition, gene expression for fatty acid uptake and degradation enzymes are decreased in the muscle during lactation in the rat [42]. The ability for skeletal muscle to mobilize fatty acids during pregnancy could allow mothers to provide fetuses and newborns with a minimal supply of DHA. Similarly, cardiac muscle in rats also has a higher concentration and percentage of DHA relative to white adipose [40]. Cardiac muscle also has a high fatty acid turnover rate [43] with potential

specificity for long chain polyunsaturated fatty acid (LCPUFA) lipolysis [44,45], but whether these lipids are exported for perinatal supply has not been examined. The expression of proteins involved in lipid uptake have been shown to decrease during lactation in skeletal muscle[42], and a similar mechanism could be occurring in cardiac muscle.

Pregnancy-induced lipidemia is well documented [46]. Circulating levels of triacylglycerols (TAG), phospholipids (PL), and nonesterified fatty acids (NEFA) increase during gestation in part due to stimulation from placentally derived leptin [37]. In plasma, DHA increases predominantly in 16:0/DHA phosphatidylcholine (PC) [29]. This increase is due to repackaging of phosphatidylethanolamine (PE) to PC through the Kennedy pathway by increased phosphatidylethanolamine methyl transferase (PEMT) activity [29]. Generally, PE has a higher content of DHA than PC, but PE predominantly resides on the inner cell membrane of phospholipid bilayers [47]. Hepatic PEMT-derived PC appears to be preferentially partitioned to the fetus [48]. Converting DHA-rich hepatic PE to PC allows the DHA to be readily incorporated into the lipoprotein monolayers that are predominantly PC. Once in the circulation, the DHA-rich PC is then accessible to lipases found on the microvillous membrane of the placenta, allowing DHA to be delivered as NEFA to the fetus by passive diffusion [33] or through protein mediated transport [49].

During lactation, circulating NEFA levels are increased by reducing fatty acid oxidation and increasing lipolysis in maternal adipose [50]. Adipose derived NEFA can then be sent to the liver for repackaging as triacylglycerol (TAG) dominant lipoproteins such as very low density lipoproteins (VLDL) before being sent to the mammary gland to support milk synthesis [51], though uptake of albumin-bound NEFA could be occurring as well [52]. Similar to lipolytic signalling from the placenta during pregnancy, lipoprotein lipase (LPL) activity at the mammary

gland is increased via increased prolactin levels [51]. The increase in LPL activity is an adaption to promote the incorporation of DHA into milk, as DHA from the diet is preferentially incorporated into plasma PL and TAG [53]. Lipids derived from intestinal absorption circulate primarily as chylomicrons, while those derived from hepatic synthesis or adipose tissue circulate primarily as VLDL[54].

In all, the combined provision of maternal DHA to the fetus and infant via placental transfer and lactation are important in ensuring adequate perinatal DHA status. Tracer studies have confirmed that increases in maternal dietary DHA consumption increase DHA concentrations in the placenta [55] and breast milk [56], but no tracer studies have yet confirmed to what degree each mechanism of transfer for maternal DHA to the infant is responsible for perinatal DHA status. De novo synthesis of DHA during the perinatal period has also been reported in term and preterm infants [57], but these synthesis pathways do not appear to have as large an impact on infant DHA status compared to adequate maternal DHA [58]. Increases in maternal DHA intakes are linked with increases in perinatal DHA status [58]. However, further research is needed to fully characterize the role of placenta, breast milk, and perinatal de novo synthesis on infant DHA status.

2.2 Sex differences in DHA metabolism

DHA levels in females are generally higher than those of males when dietary intake is controlled [15,59]. Females have higher biosynthesis of DHA from ALA [60]. These differences are believed to be primarily the result of 17β-estradiol, which increases gene expression of FADS2, the gene responsible for Δ6-desaturase (D6D), the rate limiting enzyme in DHA and ARA synthesis [17].

How estrogen increases D6D activity and circulating levels of DHA has not been fully uncovered. The three main forms of estrogens in humans are estriol, estrone, and 17β-estradiol. 17β-estradiol is the most bioactive form in mammals, and functions through genomic and nongenomic mechanisms. For genomic mechanisms, 17β-estradiol binds to estrogen receptors (ERα and/or ERβ) to up- or downregulate gene expression, while for nongenomic mechanisms, it binds primarily to G protein-coupled receptor 30 (GPR30) to mediate secondary messengers through protein phosphorylation and calcium signalling [61]. To date, no mechanisms directly relating estrogen to D6D have been identified. However, genomic mechanisms explaining the role of 17β-estradiol in DHA synthesis have been proposed, involving the upregulation of desaturase, elongase, and peroxisomal enzymes through ERα-activated peroxisome proliferator-activated receptor alpha (PPARα) upregulation [14].

Determining the exact lipid pools in which DHA differs between males and females could lead to a better understanding of the metabolic control of DHA partitioning. A recent review highlighted that sex differences in circulating DHA can be specified to total plasma, plasma PL, and red blood cell (RBC) PC fractions in humans [18,62] and rats [63]. This is in agreement with tracer studies showing DHA elongated from ALA leaves its synthesis pathway as acyl-CoA and incorporates primarily into PC, followed by CE, TAG and NEFA pools in women of reproductive age [59] The higher content in PC could be a result of higher circulating levels of 17β-estradiol, which can increase PEMT gene expression, thereby increasing their conversion of PE 16:0/DHA to PC 16:0/DHA [64].

Sex differences in DHA status may be dependent on low background intakes of omega-3 PUFA [14,65], as these sex differences have been shown to disappear with fish oil supplementation, and are not observed in countries with a high omega-3 intakes and blood levels

[18,66,67]. Most observations of higher blood DHA levels in females have been observed between the ages of 13-50 years old [18]. Although it is assumed that sex differences are associated with the higher concentration of circulating sex hormones that occur during the onset of puberty, this has not been clearly demonstrated.

2.3 Established DHA biomarkers

For fatty acid biomarkers, various tissues can be used, but blood has significant advantages as it is routinely collected and stored in clinical settings. Within blood, there are various fractions and lipid pools that can be examined, including whole blood (that can be collected as dried blood spots), plasma (or serum), erythrocytes, and the buffy coat. Within blood, DHA is found primarily in the PL of the various blood fractions with the fatty acid compositions of plasma and RBC being reported the most often [67,68]. Tissue n-3 LCPUFA status correlates more strongly with RBC than plasma, given that RBC has a lipid bilayer and is composed of a more comprehensive phospholipid profile, unlike plasma, which is dominated by PC lipoproteins [69]. It has also been demonstrated that DHA levels in RBC are better indicators of dietary adherence than other n-3 LCPUFA measures in plasma, as turnover of DHA in this pool is slower [70]. However, the recent identification of plasma PC 16:0/DHA as the specific lipid responsible for most of the increase in plasma DHA during pregnancy suggests that lipidomic analyses has the potential to identify highly informative biomarkers not possible with methods that rely on the chemical isolation and derivatization of fatty acids [29].

While blood fatty acids can be used as biomarkers of DHA intake, the brain strongly retains DHA, even when blood DHA status is low [71]. Although correlations between brain and blood n-3 LCPUFA can be strong when n-3 PUFA deficient rats ($r \geq 0.95$) are included, the correlations become considerably weaker when all animals are adequate in n-3 PUFA, even

when it is primarily obtained as ALA and not preformed DHA [69]. When examining intake ranges above deficiency, correlations between RBC and brain (r = 0.62) are stronger than brain and plasma (r=0.46) [69]. A lipidomic approach examining different brain regions may identify acyl specific lipid species that are highly correlated in brain and blood, and potentially serve as informative biomarkers in the future.

Lipidomic data also have the potential to provide additional insights about metabolism when examining sex differences. Previously, the identification of increased 16:0/DHA PC in late pregnancy led to additional evidence that increased expression of PEMT supports maternal-fetal DHA transfer [29] and not just the synthesis of PC. Changes in acyl specific lipid species allows for systems biology approaches to identify molecular pathways with sexual dimorphism in response to physiological changes and diets. However, with known sex differences in lipoprotein metabolism [72], lipoprotein isolation followed by lipidomic analysis, though currently technically challenging, needs to be considered to improve characterization of the plasma lipidome and our understanding of sex differences in hepatic fatty acid and lipid metabolism.

2.4 Cognitive benefits of DHA

DHA comprises between 15 and 20% of total lipids in the adult rodent brain, and is critical for optimal brain function [3,73]. The majority of DHA in the brain is incorporated within PL [16], but the effect of dietary intervention, and the PL fraction most affected by it is debatable. The PE and PC fractions are the most abundant phospholipids in brain [74], but these phospholipids and their DHA content are relatively resistant to changes in DHA intake other than outright n-3 PUFA deficiency [75]. However, DHA supplementation can increase DHA composition (%wt) of other lipid species, and possibly increase the absolute amounts of DHA

containing phosphatidylserine (PS) [74]. Plasmalogens, which make up an important subclass, and constitute approximately 20% of white matter phospholipid mass [76] may also increase in DHA content during dietary DHA supplementation [77,78]. DHA supplementation may also reduce arachidonic acid (ARA) in PC, PE, PS, and phosphatidylinositol (PI) fractions, even though relative amounts of DHA do not change [75]. Aging may also have a role in sex differences in brain, as brain DHA in lysoPC is higher in aging female as compared with male mice [79].

In multi-generational studies of DHA depletion, male rodents show impaired spatial memory performance in Morris water maze (MWM) studies [6–10]. *In vitro* studies show neurons from the hippocampus, an area critical for spatial memory, generate more synapses in cell cultures with DHA included as compared with cultures without DHA [4]. This observation of increased neural connections could explain the shorter latency times in MWM performance of rats with adequate levels of DHA [6–10], as DHA may be supporting memory formation and increased recall speed.

Cognitive interactions between sex and DHA intake have been reported. In 18-45 year old adults, DHA supplementation improved episodic memory reaction times and working memory accuracy in both sexes, episodic memory accuracy in females only, and working memory reaction times in males only [5]. DHA blood levels also correlate with cognitive test scores twice as strongly in girls as compared with boys, and DHA supplementation shows an inverse relationship with blood levels of n-6 PUFA in females only [21]. DHA supplementation is also linked with reducing depression in women, but not men [22]. Interestingly, in 3 year old Danish girls, an inverse relationship was shown between DHA in RBC and communication as determined by the ASQ-3 [20]. Studies examining the effects of DHA on cognition do not

always reach significance, and its effects remain controversial [80]. This may be partially explained by studies not accounting for sex differences, only measuring effects in males, or not accounting for age, as it is possible the effects of DHA supplementation interact with sex differently at different ages. Understanding the mechanisms behind why DHA supplementation affects females differently than males is important in understanding the cognitive effects of DHA.

3 Rationale, Objectives, and Hypotheses

3.1 Rationale:

Sexual dimorphisms have been overlooked in science [81]. Within the last decade, policies have been put into place to prevent basic and preclinical research from being focused on males only [82,83]. A significant amount of basic research on the benefits of DHA has been conducted using only male specimens [6–10,84–86], despite female and male humans showing different circulating levels of DHA [15,59,60], different memory improvements from DHA supplementation [5], and sexually dimorphic cognitive differences in infants following maternal DHA supplementation [87]. Pregnancy models can provide insights on the effects of sex hormones on fatty acid and lipid metabolism and need to be examined to understand female biology. Plasma DHA increases approaching parturition in humans and rats [29,88], and sex differences in DHA are associated with higher levels of estrogen in females [17,29]. As Canadian women do not meet ISSFAL recommendations for DHA consumption during pregnancy [28], examining the effects of a high fat diet that mimics a Western fatty acid profile with and without DHA on the mother, fetuses, and 7d old pups could provide more insight on maternal adaptations to DHA supplementation and sexual dimorphisms in pup development.

Additionally, circulating DHA levels are often reported to be greater in females relative to males [14,15,18]. This increased capacity is associated with circulating estrogen [14,15] and sex differences in the expression of genes related to PUFA synthesis [14,89]. While the onset of these differences has not been examined comprehensively, human studies suggest differences in circulating DHA begin with puberty [18], but sex-DHA status interactions on communication skills have been observed at 9 months of age [20]. This could be due to differences in DHA status occurring earlier than previously thought. Animal studies examining tissue DHA status

have been limited to single time points in mature rats [14,63], and when examined in growing rats, it has been examined under n-3 PUFA deprivation/repletion models [89]. Puberty in the rat begins between 40-60 days of age [90]. Examining changes in DHA status from 1 to 9 weeks of age could provide insight on how sexual dimorphisms in DHA occur in rats during development.

Finally, DHA supplementation causes sexually dimorphic memory improvements in young adults [5], and multigenerational studies in rats show severe DHA depletion negatively affects spatial memory in MWM [6,9,10]. However, this effect on spatial memory has only been examined in males. Spatial memory and MWM performance are highly associated with hippocampal function [91]. Dietary ALA supplementation increases DHA brain composition in females more than males [92], and DHA supplementation has been shown to improve memory differently in females as compared with males [5]. In the hippocampus, DHA accumulation occurs primarily in PL [93], and DHA uptake and PE levels in the brain are affected by estrogen, as estrogen treatment appears to maintain brain lipid homeostasis in aging and ovariectomized rodents [12]. A lipidomic analysis of the hippocampus compared with blood lipidomics may provide further insight on sexually dimorphic responses to DHA supplementation and identify novel blood biomarkers that are more informative than fatty acid based blood-brain biomarkers.

3.2 Objectives:

Using a rat model, the main objectives of this thesis are to: 1) assess the effect of dietary DHA supplementation and high fat Western diets during pregnancy on tissue DHA status of mothers, fetuses and pups, 2) determine the onset of sex differences in DHA status from birth to adulthood, and 3) determine the impact of DHA supplementation during pregnancy and lactation on the spatial memory of adolescent offspring.

Assessing the effect of dietary DHA on maternal tissue stores involved a pregnancy model with total Western diets with (TWD+) and without DHA (TWD-), and a standard chow diet. Maternal and 7-day old pup tissues were analyzed during pregnancy and lactation. Plasma, RBC, heart, liver, brain, white perirenal adipose and placenta from the mothers were collected to determine how fetal demand for LCPUFA during pregnancy and lactation affected maternal tissue stores of LCPUFA. Brain, heart, and liver from 7-day old pups were also collected and examined for potential dietary effects and sexual dimorphisms in LCPUFA composition.

Upon examination of sexual dimorphisms in brain DHA concentrations of 7-day old pups fed TWD+, an objective of determining when sexual dimorphisms in the LCPUFA status of chow fed Sprague-Dawley rats was established. Plasma, heart, liver, and brain fatty acid compositions were examined.

The potential interaction between dietary DHA supplementation throughout development and sexual dimorphisms at 6 weeks of age was also examined. Spatial memory was then assessed using the Morris water maze, and lipidomic and fatty acid analyses were performed on the offspring to determine the lipid species of the hippocampus and erythrocytes. Correlations were performed to determine whether erythrocytes could be an effective biomarker for assessing the hippocampal lipidome.

3.3 Hypotheses

Study 1: The Effect of Dietary DHA on the Fatty Acid Composition of Maternal Tissues and 7 Day Old Pups

1. At days 20 of pregnancy or 7 days postpartum, mothers fed TWD- and chow will have lower levels of DHA in their adipose, heart, and liver, as compared with mothers fed

TWD+. Maternal brain levels throughout pregnancy and postpartum will not change, regardless of diet.

2. TWD+ pups will have greater levels of DHA in whole body, brain, heart, and liver than TWD- and chow.
3. The tissue compositions of female and male fetuses and pups will be similar.

Study 2: Sex Differences in Fatty Acid Compositions of Plasma, Liver, Brain, and Heart from Birth to Adulthood

1. Plasma, heart, and liver DHA composition will be higher in females than males beginning at 40 days of age.
2. Brain DHA composition (%wt) and concentration will be the same between sexes throughout the experiment (weeks 1 – 9).

Study 3: Examining the Effects of Dietary DHA Supplementation on Spatial Memory and the Hippocampus in Female and Male Rats

1. At week 6, DHA+ females will have shorter latency times than DHA+ males. For both sexes, the DHA supplemented group will have shorter latency times than controls
2. Females will have greater hippocampal DHA levels than males in the supplemented, but not the control group at 7 weeks of age with the increased DHA occurring in PC and PE 16:0/DHA and 18:0/DHA, and PS 18:0/DHA at the expense of n-6 LCPUFA.
3. DHA levels in the RBC PE 16:0/DHA and PE 18:0/DHA will correlate with PE 16:0/DHA and PE 18:0/DHA levels in hippocampal PE at 7 weeks of age.

4 Common Methods

4.1 Animals and Tissue Collection

All animal experiments will be performed in agreement with the policies of the Canadian Council on Animal Care and submitted for approval by the University of Waterloo Animal Care Committee. In all experiments, exsanguinated blood will be collected in the presence of ethylenediaminetetraacetic acid (EDTA) and plasma will be isolated by centrifugation at 4°C and 1,500 *g* for 10 mins. Tissues will be washed in saline (0.9% v/v), weighed, and flash-frozen in liquid nitrogen. After collection, samples will be stored at -80°C until analysis.

4.2 Lipid Extraction

Tissue samples will be pulverized, weighed, placed in an ice bath, and homogenized using a Kinematica PT 1200E Polytron (Kinematica Inc., Bohemia, NY) in 3mL of 2:1 chloroform:methanol containing 50µg/mL 2,6-di-tert-butyl-4-methylphenol (butylated hydroxytoluene, BHT; Sigma-Aldrich, St. Louis, MO, USA) and 10µg of docosatrienoic acid (22:3n-3, Nu Chek Prep Inc., Elysian MN) for gas chromatography analysis, or 500pmol of 17:0/17:0 PC for mass spectrometry analysis as internal standard. Homogenized samples will be left at room temperature overnight in 3mL of 2:1 chloroform:methanol for lipid extraction following a method modified from Folch et al. [94]. Aqueous and organic phases will be separated by aqueous buffer of 500µL of 0.2M sodium phosphate, and the organic phase will be collected.

4.3 Gas Chromatography

The collected organic phase containing the extracted lipids will be dried under nitrogen and methylated in 1mL BF_3-MeOH and 300µL of hexane for one hour at 100°C. The hexane

layer with fatty acid methyl esters will be collected, dried under nitrogen and reconstituted in 65µL of hexane for analysis with a Varian 3900 gas chromatograph equipped with a DB-FFAP 15 m x 0.1 mm injected dose x 0.1 µm film thickness polyethylene glycol capillary column (J and W Scientific from Agilent Technologies, Mississauga, ON) at a 100:1 split ratio. The inlet will be heated to 250ºC with hydrogen used as a carrier gas. A temperature program will be used with an initial column temperature of 150ºC with a 0.25 minute hold, followed by a 35ºC/min ramp to 200ºC and a 8ºC/min ramp until 245ºC will be reached and held for 15 minutes [95]. Fatty acid methyl esters will be measured using a flame ionization detector set at 300°C and a sampling frequency of 50Hz [96].

5 The Effect of Dietary DHA on the Fatty Acid Composition of Maternal Tissues and 7 Day Old Pups

5.1 Introduction

Levels of DHA in plasma increase approaching parturition in both humans [88,97,98] and rats [29,99], likely as an adaptation to meet fetal demand during fetal brain growth. Mechanisms responsible for this increase have not been fully identified, but dietary intake, upregulated *de novo* synthesis and mobilization from maternal tissues are potential mechanisms [1,29–32]. Evidence suggests DHA is mobilized from maternal liver and adipose depots during pregnancy and lactation [31,32,38,39], and it is possible that DHA could also be mobilized from other maternal tissues, particularly those demonstrated to be rich in DHA [40]. The exact roles of these mechanisms, and how they interact with high fat diets typically found in Western societies could provide a better understanding as to how mothers meet fetal and infant DHA demand.

The goal of this study was to investigate if DHA can be mobilized from various maternal tissues in addition to adipose during pregnancy and postpartum, and if this mobilization can be influenced by DHA supplementation and high fat diets. This was examined with a background rodent diet designed to emulate a typical human Western diet, including percentages of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and PUFA [100]. A common rodent chow diet was included as an additional control. Maternal liver, brain, adipose, heart, erythrocytes, and plasma, as well as placenta, and fetal and pup tissues were examined throughout pregnancy and into the postpartum period across all diets. To my knowledge, this is the first study to comprehensively examine changes in the fatty acid compositions of multiple maternal tissue stores, and that of placenta, fetuses, and pups during Western and chow diet interventions.

5.2 Methods

5.2.1 Animals and Pregnancy Model

Seventy-two female Sprague-Dawley rats were purchased from Envigo (Mississauga, Ontario, Canada) and arrived at the University of Waterloo Central Animal Facility at 7 weeks of age. Baseline rats were not bred and fed either a fixed-formula 8640 Teklad 22/5 Rodent Diet (Chow), a TD.110424 total western diet with DHA (TWD+) or a total western diet without DHA (TWD-) (Table 1) for 7 days prior to being sacrificed (n=6 per diet). Rats assigned to the pregnancy groups were housed with male breeders and fed chow diets until confirmation of pregnancy by vaginal plug. Once pregnant, rats were immediately assigned to chow, TWD+, or TWD- diets (Figure 1). Daily food intakes and body weight were measured at baseline, day 15 and 20 of pregnancy, and postpartum. Following an overnight fast, rats were sacrificed at day 15 of pregnancy, day 20 of pregnancy, or 7 days postpartum by exsanguination after anesthesia using isoflurane (n=6 per group). Plasma, RBC, adipose, brain, heart, liver, fetuses, 7 day old pups, and pup brain, hearts, and livers were collected and analysed by gas chromatography as described in the general methods chapter.

5.2.2 Statistical Analyses

All statistical analyses were performed using SPSS release 20.0 (IBM, Chicago, IL, USA). The effect of diet and time was examined across maternal parameters using a two-way ANOVA with interactions. Differences in pup tissues across diets were examined by one-way ANOVA. Individual means were compared by Tukey post hoc testing after significant F-value by ANOVA. All data are presented as means ± SD with significance accepted when $p<0.05$.

5.3 Results

5.3.1 Maternal food intake and body weight

Energy intakes did not differ across the diets despite the higher energy density of the TWD+ and TWD- as compared with chow. Maternal energy intakes were significantly increased in all diets at 7 days postpartum as compared with baseline and gestation across all diets (Figure 2). At 7 days postpartum, chow dams consumed a significantly greater mass of food than TWD+ and TWD- dams (47.5 ± 4.37g vs. 34.0 ± 10.0g and 32.33 ± 12.45g, respectively). Maternal body weight increased from baseline throughout pregnancy with a decrease at postpartum that remained higher than baseline. This was in agreement with previous reports using this pregnant rat model [29]. Maternal and fetal body weights did not differ between diets (data not shown). However, the weights of 7-day old pups from TWD+ and TWD- mothers were significantly higher than those from chow mothers (14.16 ± 2.99g and 14.14 ± 2.63g vs 12.63 ± 1.68g respectively. $p = 0.0001$).

5.3.2 Fatty acid composition of maternal tissues

The TWD+ diet supported increased concentrations of DHA in most maternal tissues during pregnancy while the TWD- and chow diets did not, although the responses of each tissue were not similar (Figure 3). In plasma, there was a significant increase in DHA at day 20 of pregnancy in all three diets, and the DHA concentration with TWD+ feeding was 53% and 78% higher than the DHA concentrations with chow and TWD- feeding, respectively (Figure 3A). These concentrations returned to baseline levels by 7 days postpartum in all the diets. These increases reflected, in part, a general increase in total fatty acids in plasma at 20 days of pregnancy. However, the relative percentage of DHA was significantly higher at 20 days of pregnancy for the TWD+ and chow groups, but not the TWD- diet (Appendix Table 5.1, Appendix

Table 5.2**Error! Reference source not found.**). Concentrations of ARA also changed, increasing above baseline at 20 days of pregnancy in TWD+ and chow rats, and decreasing below baseline at 7 days postpartum in TWD+ rats. The TWD+ and TWD- diets also resulted in higher percentages of saturated and monounsaturated and lower percentages of n-6 PUFA as compared with plasma from the chow diet. In erythrocytes, the increase in DHA concentrations at day 20 was also observed in all the diets, but the increase was more gradual, very subtle in the chow and TWD-, and extended into postpartum for the TWD+ group (Figure 3 B). In contrast to plasma, the total fatty acid concentrations of erythrocytes did not increase at day 20 of pregnancy.

Liver DHA concentrations increased above baseline at day 15 and 20 in the TWD+ group and decreased below baseline at 7 days postpartum in the TWD- group (Figure 3C). The relative percentage of DHA was significantly higher at day 20 in both the chow (6.42±0.78 vs 10.96±2.98) and TWD+ groups (7.42±0.90 vs 11.35±2.45, Appendix Table 5.3). Liver ARA concentrations did not change from baseline in any of the dams. In the TWD+ and TWD- groups, total fatty acid concentrations significantly increased at 7 days postpartum as compared with day 20 of pregnancy (1.7 – 1.9 fold) and were significantly higher than chow at the 7 days postpartum (2.5 – 2.7 fold, Appendix Table 5.3). These increases in liver total fatty acids were driven by very large increases in 16:0 and 18:1n-9, as percentages of n-3 and n-6 PUFA decreased in the TWD+ and TWD- diets.

Adipose DHA concentrations were decreased throughout pregnancy in the TWD- group (Figure 3D). The TWD+ and chow groups had similarly higher DHA concentrations in adipose throughout pregnancy and postpartum with an increase at day 20 of pregnancy that was above baseline levels. There was a tendency for total fatty acid concentrations of adipose to increase

during pregnancy and then decrease postpartum across all diets with the decrease at postpartum in the chow diet being the largest. In the heart, DHA concentrations increased in the TWD+ at day 20 of pregnancy and then returned to baseline levels (Figure 3E). In the TWD- and chow groups, heart DHA concentrations did not increase at day 20 and significantly decreased at 7 days postpartum (Figure 3E) and there was evidence of increased percentages of 22:4n-6 and 22:5n-6 in these groups as compared with the TWD+ group (Appendix Table 5.5). Maternal brain DHA concentrations in the TWD- group were significantly lower than those in the TWD+ group, at day 20 of pregnancy, but none of the values were different from baseline (Figure 3F) and the percentage of brain DHA did not differ in any of the groups (Appendix Table 5.6).

5.3.4 Fatty acid composition of the placenta and pup whole bodies.

Placental DHA concentrations increased in all diets from day 15 to day 20 of pregnancy with the increase in the TWD+ placentas being significantly greater than the other diets (Figure 4A). While there was a tendency for total fatty acid concentrations to also increase from day 15 to day 20 (approximately 30-40%), the percentages of DHA at day 20 remained significantly higher than day 15 for all the diets with TWD+ having the greatest increase (Appendix Table 5.7). In the fetus, whole body DHA concentrations did not differ (Figure 4B) although the percentage of DHA increased in the TWD+ and chow fetuses from day 15 to day 20 (Appendix Table 5.8). After birth, DHA concentrations increased in the pups relative to the fetuses in all the diets with a remarkable increase in the TWD+ diet (7 fold from baseline versus 3.7 fold in TWD- and 3.6 fold in chow, Figure 4B). After birth, total fatty acid concentrations increased significantly in all the pups, however, the concentrations in the TWD+ and TWD- 7-day old pup whole bodies were approximately 3 times the concentration of the chow-fed pups (Appendix Table 5.8).

5.3.5 Fatty acid composition of pup brain, liver and heart.

The pups from the TWD+ group had the highest concentrations of DHA all tissues, although chow had a statistically similar level of DHA in the heart (Figure 5). DHA concentrations in the brain and heart of TWD- group were also significantly lower than the chow group. Differences in total fatty acid concentrations appear to have contributed to this observation as total fatty acid concentrations in the TWD- group were lower in the brain and heart but higher in the liver relative to the chow group (Appendix Table 5.9). In the brain, the percentage of DHA was only different (1.2 times higher) in the TWD+ group, while the percentages of 22:5n-6 and 22:5n-3 were higher in the TWD- and chow pups as compared with the TWD+ pups (Appendix Table 5.9). In both the heart and liver, there were increases in the percentages of 18:1n-9 with the TWD+ and TWD- feeding relative to chow. In the heart, this was offset by lower percentages of 18:2n-6 and 18:3n-6 relative to chow, while in the liver, the TWD+ and TWD- had lower percentages of 18:0 and 20:4n-6 (Appendix Table 5.9).

5.4 Discussion

Results of this study indicate that including preformed DHA can, in general, increase DHA status in various maternal, fetal and pup tissues even when fed a background Western style diet. High fat feeding during pregnancy has been demonstrated to potentially reduce the hepatic DHA status of neonatal pups [101], but surprisingly, manipulating the DHA content of a Western style diet fed rodents during pregnancy has not been examined previously to our knowledge. This is despite the fact that the use of DHA supplements during pregnancy is encouraged for women in Western countries [102].

An increase in DHA concentrations in maternal plasma has been observed previously [29,31,38,39,88,103]. This study also confirms a recent report that the DHA increase occurs

largely in late and not early pregnancy, where it was demonstrated that DHA was specifically increased into 16:0/DHA PC for incorporation into lipoproteins during pregnancy induced hyperlipidemia [29]. The present study demonstrates that the DHA increase in plasma during late pregnancy is even higher when preformed DHA is included in the diet, and that this metabolic adaptation to mobilize DHA to the plasma for potential fetal uptake was also evident in the TWD- diet, a high fat diet without preformed DHA. The increase and decrease in DHA concentrations and percentages in plasma during pregnancy and postpartum have been observed previously in rodents [29] and in humans [88,97]. This rise and fall is associated with changes in lipoprotein levels, but also DHA availability for incorporation into PC [29,104]. Erythrocyte DHA concentrations were also increased above baseline with this increase being higher and persisting into 7 days postpartum in the TWD+ group.

In the other maternal tissues examined, an increase in DHA concentrations at day 20 of pregnancy was observed only in tissues of the TWD+ group. Specifically, liver, adipose, heart and erythrocyte, but not brain DHA concentrations were all increased above baseline at day 20 and they all returned to baseline levels at 7 days postpartum. In the heart and liver, the chow and TWD- groups were generally similar with the DHA concentrations remaining at baseline levels, but lower than the TWD+ group throughout pregnancy. However, at 7 days postpartum, DHA concentrations fell below baseline levels in both the chow and TWD- groups in the heart, and in the TWD- group in the liver. The decrease in heart tissue DHA in both the chow and TWD- was not expected, but losses of DHA in the heart could have been anticipated. Heart tissue has a high concentration of DHA [40] and high fatty acid turnover rate [43], with a potential specificity for lipolysis of LCPUFA such as DHA by phospholipase A2 enzymes [44,45] . It is not clear if the

decrease in DHA was caused by metabolic use of DHA by the heart or by release of DHA from the heart to the circulation. Further examination is required.

In contrast to the response in maternal liver and heart, perirenal adipose tissue DHA concentrations in the chow and TWD+ groups were similar, while the TWD- group was lower throughout pregnancy and postpartum. The DHA content of adipose was actually increased at day 20 in the TWD+ and chow diets suggesting that adipose may have been taking up DHA from plasma, rather than providing it. Previously it has been reported that DHA concentrations in periuterine adipose decrease but that perirenal adipose do not change at 18 days of pregnancy in rats fed low n-3 or ALA adequate diets [38]. In this study, an increase in DHA in perirenal adipose in the chow and TWD+ diets at day 20 but not day 15 of pregnancy was observed.

The lower levels of DHA in the adipose of the TWD- group throughout pregnancy and postpartum suggest that the fetal/pup demand was not being met by the intake of dietary DHA and that adaptations to increase DHA biosynthesis during pregnancy [29,59,105,106] could not meet the demand as well. The TWD- diet contained only 17 µg DHA/g diet (0.01% of total fat) while the chow diet had 226 µg DHA/g diet (0.41% of total fat). In regard to DHA biosynthesis, the total ALA content in the TWD- diet (3.17 mg ALA/g diet) was slightly higher than chow (2.63 mg ALA/g diet), but the % of ALA in total fat in the TWD- (1.8% of total fatty acids) was less than half of the chow diet (4.8% of total fatty acids). Based on a previous study that considered the ratios of linoleic acid (LA) to ALA and the energy % of over 50 diets [107], it is unlikely that the DHA biosynthesis potential of the chow and TWD- diets would differ. However, the previous work was completed in male rats and not pregnant females, and this study included different levels of preformed DHA in the diets. In addition, high MUFA intakes can reduce ALA metabolism [11] and absolute MUFA content in the TWD- diet was 66.9 mg

MUFA/g diet as compared with 13.4 mg MUFA/g diet in the chow diet. Additionally, the different micronutrient composition of the chow and TWD diets could have had an effect on the metabolic outcomes of the pups[108]. Further research on their impact is necessary.

Placental and whole body pup DHA concentrations from this model are consistent with previous reports of maternal DHA status affecting fetal and infant DHA status through both placental transfer [33] and lactation [109]. The TWD+ pups had both higher whole body and tissue specific DHA concentrations when compared to their chow or TWD- counterparts. However, it was interesting that the differences in whole body DHA concentrations were only observed in the 7-day old pups and not in the fetuses. This suggests that maternal adaptations during pregnancy were meeting fetal demand, but that this did not extend into postpartum lactation. Fetal synthesis of DHA could also be occurring, but from the current literature, it is difficult to quantitate DHA synthesis occurring during early infancy from that occurring in utero [33]. Future research is needed to quantify how much perinatal DHA is synthesized from the mother relative to the infant. Tissue fatty acid analyses of the pups indicate that while some of the increased DHA in the TWD+ group is probably excess and accumulating in depots such as liver and possibly adipose (not measured), diets low in DHA can result in slightly, but significantly lower levels in brain and heart. Interestingly, the pup heart was the most sensitive to losses of DHA with the TWD- diet. As mentioned previously, there is the potential for high DHA turnover in the heart [110], but it remains to be seen if this turnover is to meet the metabolic need for DHA by the heart itself or if some of the DHA is released to the circulation for uptake by other tissues. More research understanding DHA metabolism and turnover in the heart is needed. The postpartum observations in this study may be unique to rodent models and not extrapolate to humans as rat pup brain development does not reach the same level as a term

human infant until 12 days postpartum [90]. In this rat model, however, it appears that while adaptations during pregnancy maintain maternal DHA status before birth, these adaptations change postpartum and may not support DHA demand by the pup via lactation.

This study has several limitations. While several maternal and fetal/pup tissues across various time points were screened to identify decreases in tissue levels as evidence of maternal mobilization for fetal/pup transfer, only static measures of tissue fatty acid levels were completed. Also, while several maternal tissues were examined, results of decreases in DHA in heart indicate that maternal skeletal muscle should also be examined, and a recent study suggests that other adipose sites should be examined as well [38]. While the use of a background diet that resembled human fat intakes was novel for DHA supplementation during pregnancy, there were some unanticipated results that made it difficult to compare to the existing literature in the field. The TWD+ and TWD- resulted in significant increases in hepatic total fatty acids at postpartum, suggesting the development of fatty liver. The TWD+ and TWD- resulted in significant increases in hepatic total fatty acids at postpartum only (73.1 ± 25.4 and 77.2 ± 33.3 mg/g) that suggested progression towards fatty liver. Total hepatic fatty acid concentrations of 122 mg/g and percentage shifts towards higher 18:1n-9 and lower 18:0 has been observed previously in rats with steatosis [111].This was not present during pregnancy, and was not reflected in maternal adipose, but it was reflected in the pup whole body total fatty acids. As only one postpartum time point was measured, the duration of this response and the consequences of these diets on maternal and pup health need to be examined in more detail.

5.5 Conclusion

In conclusion, this study provides further evidence that DHA is increased in plasma during late pregnancy across diets with different intakes of DHA and that tissues other than

adipose and liver may be a source of DHA during pregnancy and lactation in order to meet fetal and pup accretion and physiological requirements. These observations also suggest that in this model, maternal adaptations to meet lipid and fatty acid requirements change in the transition from pregnancy to lactation with the pup requirement for DHA during lactation being particularly detrimental to maternal tissue levels when dietary DHA levels are low. Additional work examining the rates of mobilization of DHA from maternal tissues, and the dietary intake required to prevent tissue decreases, could help define dietary requirements during pregnancy.

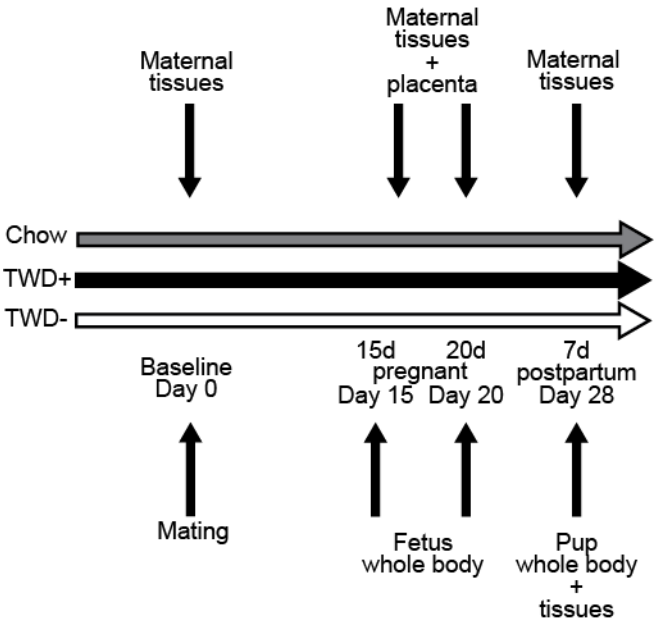

Figure 1: Study design flowchart for pregnancy study. Female Sprague Dawley rats were fed either a fixed formula 8640 Teklad 22/5 Rodent Diet (Chow), a TD.110424 total western diet with DHA (TWD+) or a total western diet without DHA (TWD-). Baseline rats were sacrificed at 9 weeks of age after one week on the diets. Rats assigned to the pregnancy groups were fed chow diets until confirmation of pregnancy and then immediately assigned to one of the 3 diets, and sacrificed at 15d of pregnancy, 20d of pregnancy or 7d postpartum (n=6 for each group).

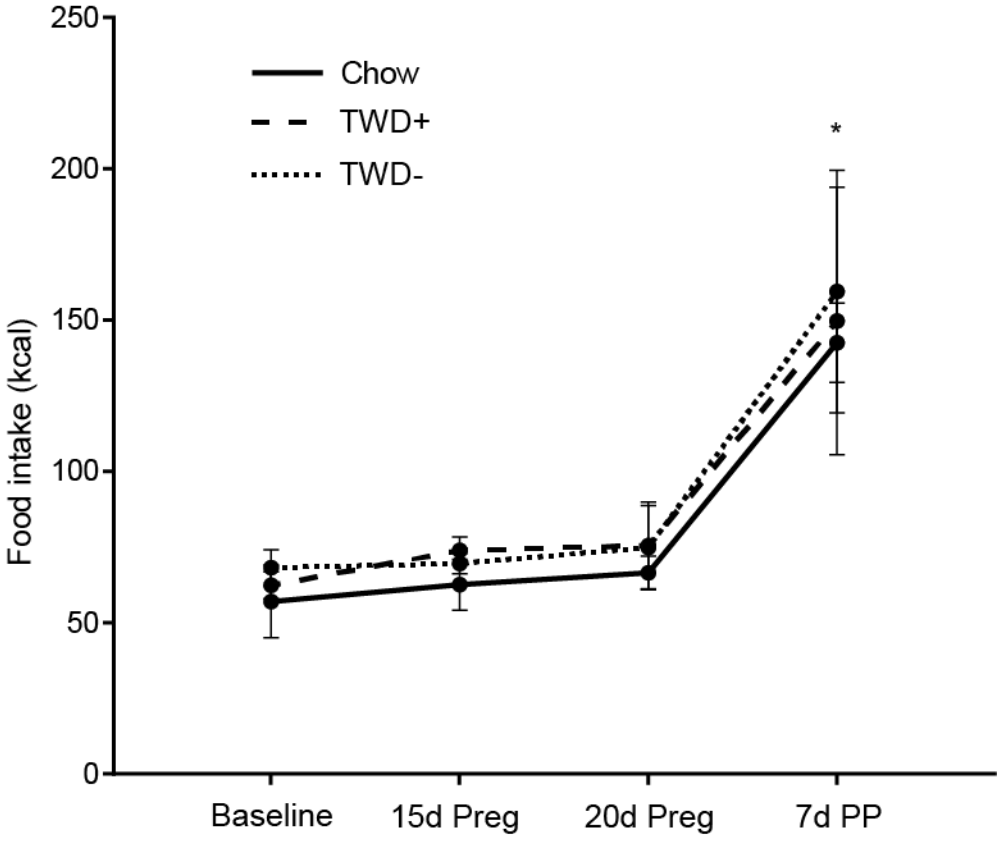

Figure 2: Food intake across pregnancy and the postpartum period. *Significantly higher than intakes at other time points for all diets (main effect of time by two-way ANOVA followed by Tukey post hoc (p<0.05). Mean ± SD, n = 6 for each point.

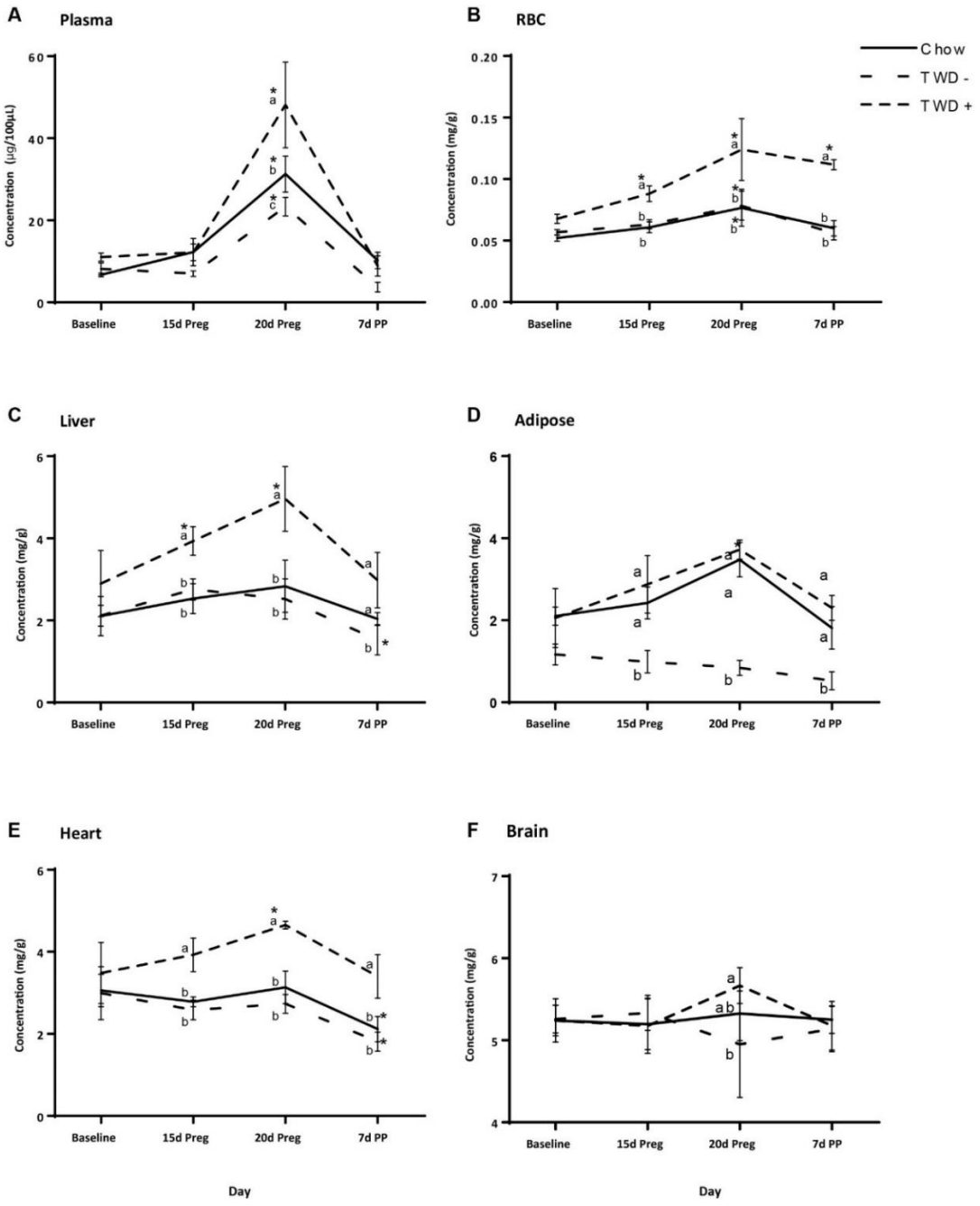

Figure 3: Effects of pregnancy and diet on DHA concentrations of maternal tissues. Different letters indicate diet differences within a timepoint, and * indicate timepoint differences from baseline within a diet. All differences are as determined by Tukey's post hoc following significant ($p<0.05$) F-value by two-way ANOVA. Mean ± SD, n = 6 for each point. TWD+: DHA-supplemented Total Western Diet, TWD-: Total Western Diet.

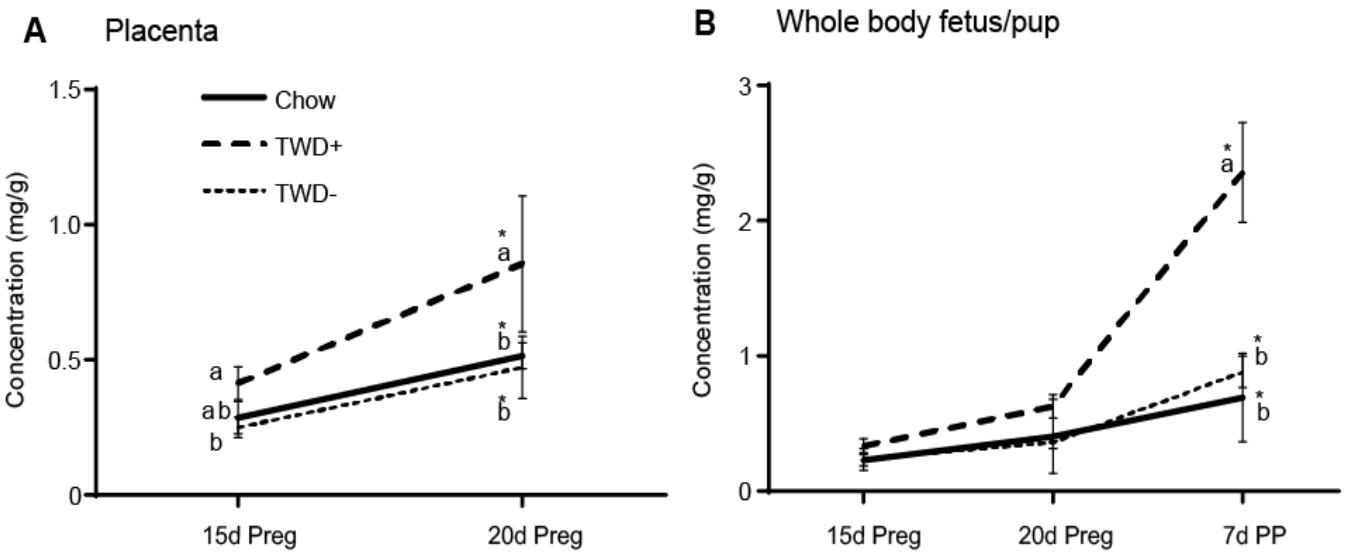

Figure 4: Effects of pregnancy and diet on DHA concentrations of placenta and whole body fetuses and pups. Different letters indicate diet differences within a timepoint, and * indicate timepoint differences from baseline within a diet. All differences are as determined by Tukey's post hoc following significant ($p<0.05$) F-value by two-way ANOVA. Mean ± SD, n = 6 for each point. TWD+: DHA-supplemented Total Western Diet, TWD-: Total Western Diet.

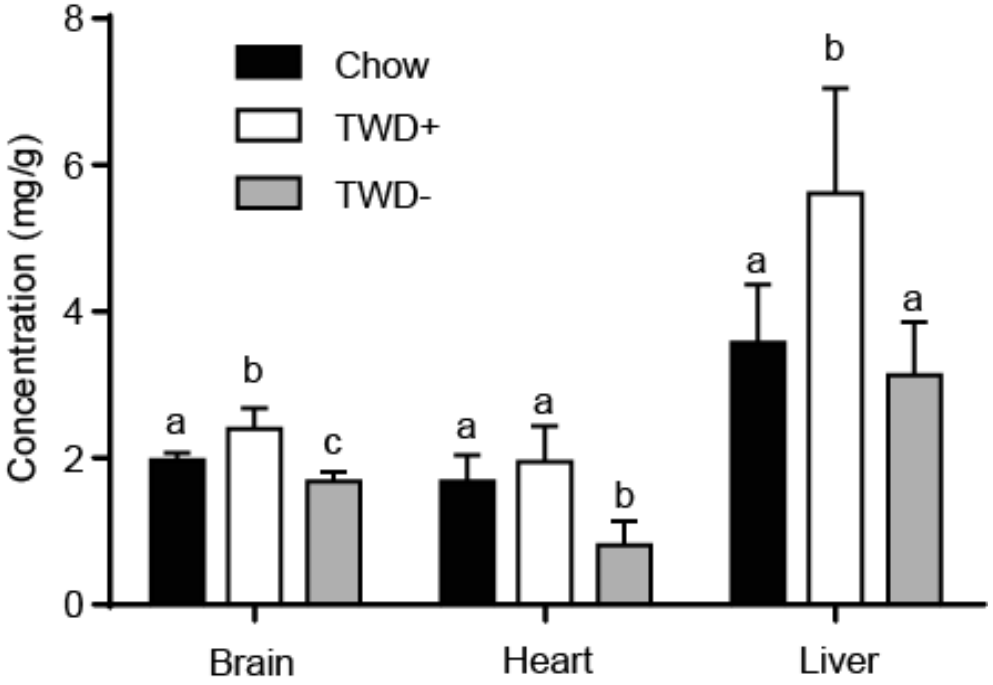

Figure 5: Effects of pregnancy and diet on DHA concentrations of pup tissues. Different letters indicate diet differences. All differences are as determined by Tukey's post hoc following significant ($p<0.05$) F-value by two-way ANOVA. Mean ± SD. TWD+: DHA-supplemented Total Western Diet, TWD-: Total Western Diet.

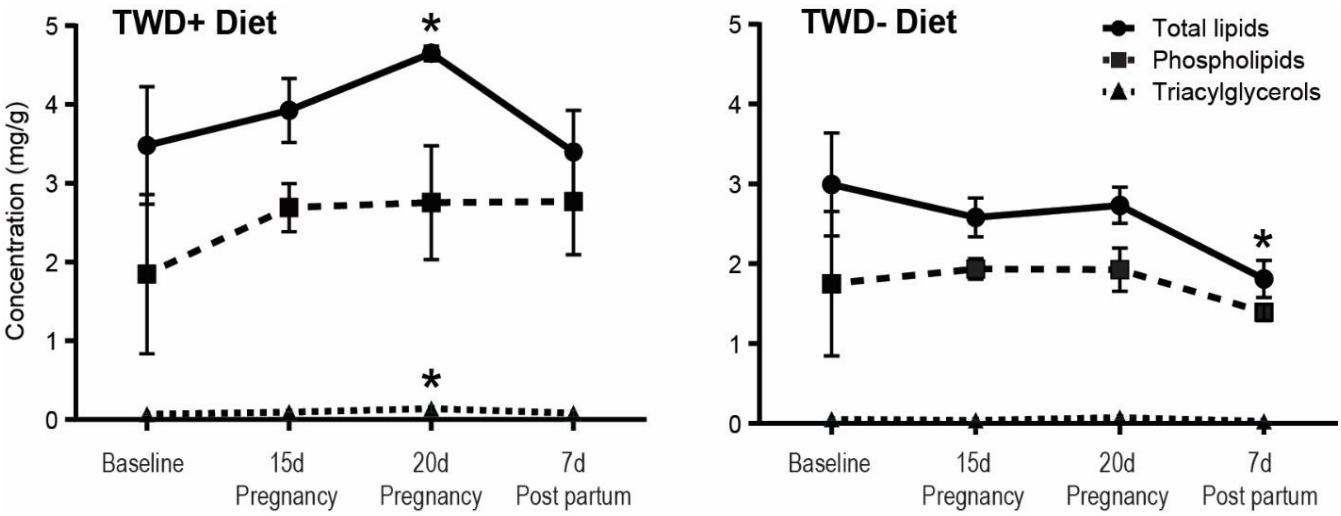

Figure 6: Effects of pregnancy and diet on DHA total lipids, phospholipids, and triacylglycerols in maternal heart. *****Significantly different from baseline ($p<0.05$) as determined by two-tailed student's t-test. Mean ± SEM, n = 6 for each point. TWD+: DHA-supplemented Total Western Diet, TWD-: Total Western Diet.

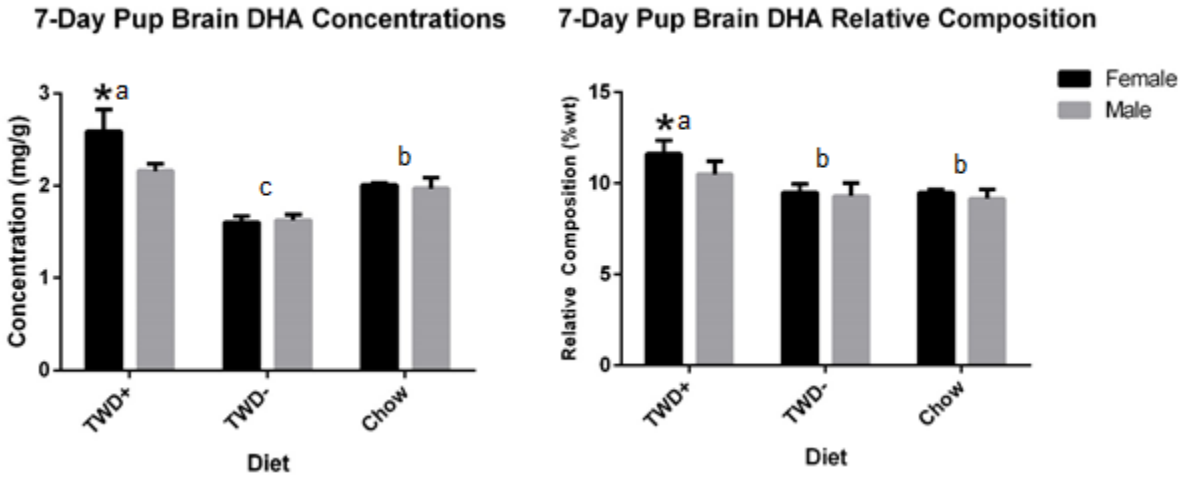

Figure 7: Effects of maternal diet on brain DHA concentrations of male and female pups *Significant differences between male and female pups from mothers of the same diet ($p<0.05$), and letters indicate significant differences between diet as determined by two-tailed student's t-test. Mean ± SD. n=4 for TWD+ and chow. n=3 for TWD-. TWD+: DHA-supplemented Total Western Diet, TWD-: Total Western Diet.

Table 1: Macronutrient and fatty acid composition of chow, and DHA supplemented and unsupplemented Total Western Diets (TWD).

Diet component	Chow	TWD-DHA	TWD+DHA
Macronutrient		*mg/g of diet*	
Protein	22.0	16.8	16.8
Carbohydrate	40.6	54.6	54.6
Fat	5.5	16.7	16.7
Fatty Acid		*% composition*	
C 16:0	13.24 ± 0.11	18.73 ± 0.08	18.81 ± 0.13
C 18:0	3.68 ± 0.02	8.87 ± 0.01	9.16 ± 0.01
Total SFA	18.56 ± 0.13	32.78 ± 0.05	32.74 ± 0.16
C 16:1	0.77 ± 0.01	1.18 ± 0.01	1.14 ± 0.01
C 18:1n-7	1.31 ± 0.01	1.49 ± 0.02	1.52 ± 0.06
C 18:1n-9	21.82 ± 0.26	36.64 ± 0.10	36.79 ± 0.08
Total MUFA	24.34 ± 0.23	39.90 ± 0.10	40.03 ± 0.07
C 18:2n-6	48.51 ± 0.05	20.21 ± 0.04	20.86 ± 0.03
C 20:4n-6	0.14 ± 0.02	0.08 ± 0.01	0.09 ± 0.01
Total n-6 PUFA	48.82 ± 0.03	20.55 ± 0.04	21.23 ± 0.05
C 18:3n-3	4.78 ± 0.01	1.83 ± 0.01	1.90 ± 0.01
C 20:3n-3	0.01 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
C 20:5n-3	0.42 ± 0.01	0.01 ± 0.01	0.01 ± 0.01
C 22:5n-3	0.08 ± 0.01	0.05 ± 0.01	0.03 ± 0.01
C 22:6n-3	0.41 ± 0.02	1.21 ± 0.02	0.01 ± 0.01
Total n-3 PUFA	5.71 ± 0.03	3.12 ± 0.01	1.98 ± 0.01

Data is mean ± SD from triplicate analysis in our laboratory. SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids.

Table 2 : Macronutrient and fatty acid composition of chow vs DHA supplemented (TWD+) and unsupplemented (TWD-) Total Western Diets.

Diet component	Chow	TWD+	TWD-
Energy Density (kcal/g)	3.0	4.4	4.4
Macronutrients (g/100g)			
Protein	22.0	16.8	16.8
Carbohydrate	40.6	54.6	54.6
Fat	5.5	16.7	16.7
Fatty Acid (% wt)			
C 16:0	13.24 ± 0.11	18.73 ± 0.08	18.81 ± 0.13
C 18:0	3.68 ± 0.02	8.87 ± 0.01	9.16 ± 0.01
Total SFA	18.56 ± 0.13	32.78 ± 0.05	32.74 ± 0.16
C 16:1	0.77 ± 0.01	1.18 ± 0.01	1.14 ± 0.01
C 18:1n-7	1.31 ± 0.01	1.49 ± 0.02	1.52 ± 0.06
C 18:1n-9	21.82 ± 0.26	36.64 ± 0.10	36.79 ± 0.08
Total MUFA	24.34 ± 0.23	39.90 ± 0.10	40.03 ± 0.07
C 18:2n-6	48.51 ± 0.05	20.21 ± 0.04	20.86 ± 0.03
C 20:4n-6	0.14 ± 0.02	0.08 ± 0.01	0.09 ± 0.01
Total n-6 PUFA	48.82 ± 0.03	20.55 ± 0.04	21.23 ± 0.05
C 18:3n-3	4.78 ± 0.01	1.83 ± 0.01	1.90 ± 0.01
C 20:3n-3	0.01 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
C 20:5n-3	0.42 ± 0.01	0.01 ± 0.01	0.01 ± 0.01
C 22:5n-3	0.08 ± 0.01	0.05 ± 0.01	0.03 ± 0.01
C 22:6n-3	0.41 ± 0.02	1.21 ± 0.02	0.01 ± 0.01
Total n-3 PUFA	5.71 ± 0.03	3.12 ± 0.01	1.98 ± 0.01
Micronutrients			
Minerals (mg/kg)			
Calcium	11000	2011	2011
Phosphorus	9000	2757	2757
Sodium	4000	7078	7078
Potassium	10000	5333	5333
Magnesium	2000	589	589
Zinc	77.0	25.0	25.0
Copper	24.0	2.6	2.6
Iron	280	31.0	31.0
Vitamins (U/kg)			
Vitamin A (IU)	15800	4300	4300
Vitamin D3 (IU)	3000	391	391
Vitamin E (IU)	150	24.6	24.6
Vitamin B1 (mg)	32.0	3.5	3.5
Vitamin B2 (mg)	9.0	4.4	4.4
Niacin (mg)	66.0	50.6	50.6
Vitamin B6 (mg)	14.0	3.9	3.9
Vitamin B12 (mg)	0.06	11	11
Folate (mg)	3.0	1.3	1.3
Choline (mg)	2380	648	648
Isoflavones (mg/kg)	350-650	n.d.	n.d.

Data is mean ± SD from triplicate analysis in our laboratory. SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids.

Appendix Table 5.1: Fatty acid composition (weight %) of maternal plasma during pregnancy and postpartum

Chow	Baseline	15d Preg	20d Preg	7d PP
16:0	14.54 ± 0.63^{a}	15.99 ± 0.75^{a}	20.31 ± 1.14^{b}	15.73 ± 1.53^{a,1}
18:0	15.50 ± 1.11^{a}	13.56 ± 1.42^{a}	8.54 ± 0.95^{b}	13.76 ± 1.96^{a}
Total SFA	33.46 ± 0.81^{a}	31.60 ± 1.15^{b,1}	30.01 ± 1.16^{b,1}	31.37 ± 1.10^{b,1}
16:1	0.58 ± 0.14	0.67 ± 0.17^{1}	0.74 ± 0.14^{1}	0.70 ± 0.15^{1}
18:1n-7	1.13 ± 0.06^{a}	1.24 ± 0.03^{b}	1.37 ± 0.06^{c}	1.34 ± 0.09^{bc,1}
18:1n-9	5.73 ± 0.43^{a}	8.81 ± 1.64^{b}	11.28 ± 0.96^{b,1}	10.72 ± 2.20^{b,1}
Total MUFA	8.10 ± 0.41^{a}	11.31 ± 1.72^{b}	13.73 ± 1.01^{b,1}	13.27 ± 2.30^{b,1}
18:2n-6	17.96 ± 1.75^{a}	20.78 ± 3.16^{ab,1}	22.60 ± 1.37^{b,1}	21.31 ± 2.29^{ab,1}
20:4n-6	33.82 ± 1.09^{a}	26.79 ± 3.72^{b}	19.05 ± 1.65^{c}	24.16 ± 3.56^{b}
22:4n-6	0.22 ± 0.03^{a}	0.36 ± 0.12^{a}	1.31 ± 0.23^{b,1}	0.35 ± 0.05^{a}
22:5n-6	0.14 ± 0.04^{a}	0.26 ± 0.12^{a}	1.63 ± 0.17^{b,1}	0.65 ± 0.14^{c,1}
Total n-6 PUFA	52.97 ± 1.03^{a}	49.34 ± 0.99^{b,1}	45.72 ± 1.12^{c,1}	48.38 ± 1.40^{b,1}
18:3n-3	0.48 ± 0.03^{a}	0.73 ± 0.32^{a,1}	1.09 ± 0.10^{b,1}	0.48 ± 0.20^{a}
20:5n-3	0.58 ± 0.08	0.89 ± 0.34^{1}	0.61 ± 0.19^{1}	0.83 ± 0.15^{1}
22:5n-3	0.42 ± 0.03^{a}	0.63 ± 0.10^{a}	1.50 ± 0.23^{b,1}	0.88 ± 0.15^{c,1}
22:6n-3	3.16 ± 0.12^{a}	4.25 ± 0.76^{a,1,2}	6.28 ± 1.00^{b,1,2}	3.24 ± 0.49^{a,1,2}
Total n-3 PUFA	4.65 ± 0.10^{a}	6.53 ± 0.58^{b,1,2}	9.49 ± 0.89^{c,1}	5.43 ± 0.59^{a,1}
Total FA (µg/100µL)	235 ± 27^{a}	187 ± 20^{a}	584 ± 64^{b}	233 ± 50^{a}
TWD+	Baseline	15d Preg	20d Preg	7d PP
16:0	15.91 ± 0.67^{a}	18.00 ± 0.98^{b}	21.40 ± 0.93c	19.69 ± 1.72^{bc,2}
18:0	15.56 ± 0.47^{a}	14.19 ± 0.59^{b}	10.30 ± 0.93c	12.13 ± 1.06d
Total SFA	34.26 ± 0.42^{ab}	35.01 ± 0.94^{a,2}	33.24 ± 0.75^{b,2}	34.05 ± 1.04^{ab,2}
16:1	0.84 ± 0.12^{a}	1.13 ± 0.19^{a,2}	1.12 ± 0.25^{a,1,2}	1.55 ± 0.27^{b,2}
18:1n-7	1.09 ± 0.06^{a}	1.17 ± 0.08^{a}	1.41 ± 0.11^{b}	1.57 ± 0.14^{b,1,2}
18:1n-9	8.42 ± 0.69^{a}	12.92 ± 1.88^{a}	18.34 ± 2.67^{b,2}	20.93 ± 5.16^{b,2}
Total MUFA	11.11 ± 0.74^{a}	16.03 ± 2.11^{ab}	21.26 ± 2.92^{bc,2}	24.59 ± 5.43^{c,2}
18:2n-6	16.58 ± 0.69^{a}	15.72 ± 0.62^{ab,2}	14.25 ± 0.49^{b,2}	16.11 ± 1.78^{a,2}
20:4n-6	30.28 ± 1.56^{a}	23.59 ± 2.09^{b}	16.64 ± 1.86^{c}	17.30 ± 5.36^{c}
22:4n-6	0.17 ± 0.02^{a}	0.18 ± 0.05^{a}	0.48 ± 0.13^{b,2}	0.11 ± 0.05^{a}
22:5n-6	0.11 ± 0.03^{a}	0.10 ± 0.04^{a}	0.76 ± 0.25^{b,2}	0.07 ± 0.03^{a,2}
Total n-6 PUFA	47.85 ± 0.99^{a}	40.43 ± 1.66^{b,2}	33.04 ± 1.69^{c,2}	34.92 ± 4.88^{c,2}
18:3n-3	0.40 ± 0.05^{ab}	0.46 ± 0.08^{a,2}	0.50 ± 0.08^{a,2}	0.32 ± 0.05^{b}
20:5n-3	0.63 ± 0.10^{ab}	0.78 ± 0.10^{a,1}	0.40 ± 0.10^{c,1,2}	0.60 ± 0.13^{b,1}
22:5n-3	0.25 ± 0.02^{a}c	0.36 ± 0.11^{a}	0.64 ± 0.11^{b,2}	0.21 ± 0.05^{c,2}
22:6n-3	4.94 ± 0.38^{a}c	6.14 ± 0.68^{a,1}	9.64 ± 1.11^{b,1}	3.73 ± 1.27^{c,1}
Total n-3 PUFA	6.23 ± 0.42^{a}c	7.78 ± 0.77^{a,1}	11.19 ± 1.03^{b,1}	4.86 ± 1.31^{c,1}
Total FA (µg/100µL)	222 ± 13^{a}	197 ± 25^{a}	508 ± 166^{b}	239 ± 23^{a}
TWD-	Baseline	15d Preg	20d Preg	7d PP
16:0	14.44 ± 1.17^{a}	16.74 ± 0.46^{b}	21.26 ± 0.81^{c}	18.33 ± 2.21^{b,2}
18:0	16.23 ± 0.94^{a}	14.78 ± 0.92^{ab}	9.58 ± 0.51^{c}	12.89 ± 2.11^{b}
Total SFA	33.46 ± 0.82^{ab}	34.23 ± 0.87^{a,2}	32.28 ± 0.90^{b,2}	33.47 ± 0.15^{ab,2}
16:1	0.63 ± 0.17^{a}	1.19 ± 0.13^{b,2}	1.41 ± 0.18^{b,2}	1.54 ± 0.43^{b,2}
18:1n-7	1.06 ± 0.05^{a}	1.29 ± 0.09^{ab}	1.43 ± 0.05^{b}	1.72 ± 0.31^{c,2}
18:1n-9	7.73 ± 0.91^{a}	13.09 ± 0.63^{b}	21.17 ± 1.49^{c,3}	22.82 ± 6.15^{c,2}
Total MUFA	10.14 ± 1.10^{a}	16.39 ± 0.59^{b}	24.37 ± 1.57^{c,2}	26.58 ± 6.66^{c,2}
18:2n-6	15.51 ± 0.82	14.89 ± 1.01^{2}	14.93 ± 0.90^{2}	14.01 ± 1.18^{2}
20:4n-6	34.22 ± 2.41^{a}	27.09 ± 1.30^{b}	17.27 ± 1.77^{c}	19.46 ± 7.12^{c}
22:4n-6	0.22 ± 0.02^{a}	0.24 ± 0.05^{a}	0.68 ± 0.12^{b,2}	0.16 ± 0.04^{a}
22:5n-6	0.28 ± 0.04^{a}	0.32 ± 0.13^{a}	2.24 ± 0.48^{b,3}	0.34 ± 0.08^{a,1,2}
Total n-6 PUFA	50.99 ± 1.61^{a}	43.50 ± 0.81^{b,1,2}	36.43 ± 1.26^{c,2}	35.70 ± 5.99^{c,2}
18:3n-3	0.36 ± 0.03^{a}	0.47 ± 0.03^{b,2}	0.51 ± 0.04^{b,2}	0.23 ± 0.07^{c}
20:5n-3	0.41 ± 0.04^{a}	0.39 ± 0.06^{a,2}	0.26 ± 0.06^{b,2}	0.24 ± 0.04^{b,2}
22:5n-3	0.36 ± 0.03^{a}	0.36 ± 0.06^{a}	0.63 ± 0.06^{b,2}	0.30 ± 0.06^{a,2}
22:6n-3	3.43 ± 0.27^{a}	3.74 ± 0.30^{a,2}	3.96 ± 0.36^{a,2}	1.65 ± 0.74^{b,2}
Total n-3 PUFA	4.57 ± 0.25^{a}	4.98 ± 0.31^{ab,2}	5.37 ± 0.33^{b,2}	2.42 ± 0.71^{c,2}
Total FA (µg/100µL)	235 ± 27^{a}	187 ± 20^{a}	584 ± 64^{b}	233 ± 50^{a}

Mean ± SD, n=6 for each group. Values with different alphabetical superscripts across time and different numerical superscripts across diet are significantly different by Tukey's HSD following a significant *F*-value ($p< 0.05$) by two-way ANOVA. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; FA, fatty acids, TWD total western diet with (+) or without (-) docosahexaenoic acid.

Appendix Table 5.2: Fatty acid composition (weight %) of maternal erythrocytes during pregnancy and postpartum

Chow	Baseline	15d Preg	20d Preg	7d PP
16:0	23.25 ± 0.57^{a}	$22.14 \pm 0.18^{b,1}$	$25.10 \pm 0.65^{c,1}$	$24.67 \pm 0.59^{c,1}$
18:0	18.03 ± 0.38	17.96 ± 0.70	16.53 ± 1.65	16.66 ± 0.65
24:0	1.32 ± 0.09	1.31 ± 0.10^{1}	1.30 ± 0.08^{1}	1.24 ± 0.08^{1}
Total SFA	47.55 ± 0.65^{ab}	48.21 ± 0.49^{a}	$47.06 \pm 1.48^{ab,1}$	$46.02 \pm 1.13^{b,1}$
18:1n-7	1.91 ± 0.11^{a}	2.22 ± 0.09^{b}	2.22 ± 0.10^{b}	2.41 ± 0.22^{b}
18:1n-9	5.35 ± 0.24^{a}	6.21 ± 0.42^{ab}	$5.97 \pm 0.75^{ab,1}$	$7.32 \pm 1.43^{b,1}$
24:1n-9	0.66 ± 0.05	0.64 ± 0.04	0.66 ± 0.07^{1}	0.65 ± 0.04^{1}
Total MUFA	8.95 ± 0.26^{a}	10.58 ± 0.82^{ab}	9.55 ± 0.83^{ab}	11.48 ± 2.15^{b}
18:2n-6	9.02 ± 0.46^{ab}	8.28 ± 0.26^{a}	$8.82 \pm 0.53^{a,1}$	$9.75 \pm 0.48^{b,1}$
20:4n-6	22.70 ± 0.58^{a}	22.47 ± 0.51^{ab}	$21.18 \pm 1.10^{bc,1,2}$	$20.36 \pm 0.75^{c,1}$
22:4n-6	$1.81 \pm 0.05^{a,1}$	$1.67 \pm 0.06^{b,1}$	$1.64 \pm 0.04^{bc,1}$	$1.56 \pm 0.08^{c,1}$
22:5n-6	0.71 ± 0.03^{ab}	0.61 ± 0.07^{a}	$0.86 \pm 0.17^{bc,1}$	$1.00 \pm 0.17^{c,1}$
Total n-6 PUFA	35.12 ± 0.62^{a}	33.81 ± 0.55^{ab}	$33.26 \pm 1.31^{b,1}$	$33.55 \pm 0.98^{b,1}$
20:5n-3	0.22 ± 0.02^{a}	$0.27 \pm 0.04^{b,1}$	$0.22 \pm 0.03^{a,1}$	$0.28 \pm 0.03^{b,1}$
22:5n-3	$1.69 \pm 0.04^{a,1}$	$1.59 \pm 0.07^{ab,1}$	$1.54 \pm 0.11^{ab,1}$	$1.52 \pm 0.11^{b,1}$
22:6n-3	2.42 ± 0.08^{a}	$2.75 \pm 0.14^{a,1}$	$3.80 \pm 0.75^{b,1}$	$2.70 \pm 0.20^{a,1}$
Total n-3 PUFA	4.44 ± 0.14^{a}	$4.73 \pm 0.09^{a,1,2}$	$5.70 \pm 0.78^{b,1}$	$4.63 \pm 0.22^{a,1}$
Total FA (mg/g)	2.07 ± 0.07^{ab}	2.16 ± 0.06^{a}	$1.96 \pm 0.12^{b,1}$	$2.11 \pm 0.13^{ab,1}$
TWD+	Baseline	15d Preg	20d Preg	7d PP
16:0	22.81 ± 0.63^{a}	$21.44 \pm 0.55^{b,1,2}$	$23.27 \pm 0.88^{a,2}$	$23.38 \pm 1.04^{a,2}$
18:0	18.18 ± 0.26^{a}	18.84 ± 0.63^{a}	16.91 ± 1.31^{b}	15.94 ± 0.32^{b}
24:0	1.26 ± 0.11^{a}	$1.09 \pm 0.07^{b,2}$	$1.01 \pm 0.06^{b,2}$	$0.96 \pm 0.10^{b,2}$
Total SFA	47.08 ± 0.46^{a}	47.77 ± 0.28^{a}	$45.16 \pm 0.81^{b,2}$	$44.21 \pm 1.00^{b,2}$
18:1n-7	1.83 ± 0.10^{a}	1.96 ± 0.11^{ab}	2.10 ± 0.18^{ab}	2.23 ± 0.26^{b}
18:1n-9	6.41 ± 0.25^{a}	7.40 ± 0.58^{ab}	$7.84 \pm 0.90^{b,2}$	$9.40 \pm 1.29^{c,2}$
24:1n-9	0.66 ± 0.09	0.69 ± 0.03	$0.70 \pm 0.08^{1,2}$	$0.74 \pm 0.06^{1,2}$
Total MUFA	9.94 ± 0.35^{a}	12.00 ± 0.78^{ab}	11.60 ± 1.48^{ab}	13.63 ± 2.08^{b}
18:2n-6	8.71 ± 0.45	7.82 ± 0.40	$7.96 \pm 0.58^{1,2}$	8.62 ± 0.93^{2}
20:4n-6	22.19 ± 0.49^{a}	21.98 ± 0.49^{a}	$20.03 \pm 0.27^{b,1}$	$20.10 \pm 0.79^{b,1}$
22:4n-6	$1.66 \pm 0.05^{a,2}$	$1.37 \pm 0.03^{b,2}$	$1.26 \pm 0.04^{c,2}$	$1.10 \pm 0.08^{d,2}$
22:5n-6	0.64 ± 0.03^{a}	0.51 ± 0.03^{bc}	$0.54 \pm 0.08^{b,2}$	$0.43 \pm 0.07^{c,2}$
Total n-6 PUFA	34.05 ± 0.30^{a}	32.50 ± 0.51^{b}	$30.51 \pm 0.79^{c,2}$	$31.03 \pm 1.49^{bc,2}$
20:5n-3	0.22 ± 0.03^{ab}	$0.29 \pm 0.02^{c,2}$	$0.21 \pm 0.03^{a,1}$	$0.28 \pm 0.05^{bc,1}$
22:5n-3	$1.45 \pm 0.05^{a,2}$	$1.24 \pm 0.04^{b,2}$	$1.13 \pm 0.04^{c,2}$	$1.02 \pm 0.07^{d,2}$
22:6n-3	3.10 ± 0.19^{a}	$3.83 \pm 0.19^{ab,2}$	$5.36 \pm 0.97^{c,2}$	$4.57 \pm 0.27^{bc,2}$
Total n-3 PUFA	4.87 ± 0.20^{a}	$5.45 \pm 0.17^{ab,1}$	$6.78 \pm 0.94^{c,2}$	$5.96 \pm 0.31^{b,2}$
Total FA (mg/g)	2.09 ± 0.06^{a}	2.25 ± 0.08^{bc}	$2.19 \pm 0.07^{ab,2}$	$2.34 \pm 0.06^{c,2}$
TWD-	Baseline	15d Preg	20d Preg	7d PP
16:0	22.46 ± 0.48^{a}	$20.78 \pm 0.54^{b,2}$	$23.36 \pm 0.49^{c,2}$	$23.46 \pm 0.56^{c,1,2}$
18:0	18.33 ± 0.35^{a}	18.76 ± 0.40^{a}	16.27 ± 0.46^{b}	16.35 ± 1.03^{b}
24:0	1.26 ± 0.11^{a}	$1.14 \pm 0.08^{ab,1,2}$	$1.14 \pm 0.07^{ab,1,2}$	$1.04 \pm 0.07^{b,2}$
Total SFA	46.75 ± 0.32^{a}	47.28 ± 0.70^{a}	$45.52 \pm 0.80^{b,1,2}$	$44.99 \pm 0.90^{b,1,2}$
18:1n-7	1.88 ± 0.04^{a}	2.11 ± 0.11^{bc}	2.06 ± 0.10^{ab}	2.26 ± 0.19^{b}
18:1n-9	6.45 ± 0.33^{a}	7.68 ± 0.58^{b}	$7.52 \pm 0.37^{b,2}$	$9.07 \pm 0.85^{c,2}$
24:1n-9	0.69 ± 0.05^{a}	0.74 ± 0.06^{ab}	$0.82 \pm 0.07^{b,2}$	$0.82 \pm 0.07^{b,2}$
Total MUFA	10.07 ± 0.39^{a}	12.52 ± 0.75^{b}	11.18 ± 0.55^{a}	13.09 ± 1.11^{b}
18:2n-6	8.74 ± 0.27^{a}	7.30 ± 0.59^{b}	$7.35 \pm 0.40^{b,2}$	$8.32 \pm 0.90^{a,2}$
20:4n-6	22.60 ± 0.37^{ab}	23.15 ± 0.85^{a}	$21.76 \pm 0.45^{b,2}$	$22.14 \pm 0.73^{ab,2}$
22:4n-6	$1.72 \pm 0.02^{1,2}$	1.59 ± 0.09^{1}	1.70 ± 0.10^{1}	$1.61 \pm 0.11^{1,2}$
22:5n-6	0.71 ± 0.05^{a}	0.61 ± 0.07^{a}	$1.19 \pm 0.20^{b,3}$	$1.03 \pm 0.13^{b,1}$
Total n-6 PUFA	34.63 ± 0.20^{a}	33.39 ± 0.73^{bc}	$32.68 \pm 0.25^{b,1}$	$33.91 \pm 0.31^{c,1}$
20:5n-3	0.18 ± 0.02^{a}	$0.20 \pm 0.02^{a,3}$	$0.15 \pm 0.01^{b,2}$	$0.18 \pm 0.03^{a,2}$
22:5n-3	$1.53 \pm 0.08^{a,2}$	$1.29 \pm 0.06^{b,2}$	$1.21 \pm 0.08^{bc,2}$	$1.16 \pm 0.03^{c,2}$
22:6n-3	2.58 ± 0.08^{a}	$2.77 \pm 0.10^{a,1}$	$3.55 \pm 0.44^{b,1}$	$2.40 \pm 0.18^{a,1}$
Total n-3 PUFA	4.40 ± 0.08^{a}	$4.35 \pm 0.07^{a,2}$	$4.99 \pm 0.45^{b,1}$	$3.83 \pm 0.14^{c,1}$
Total FA (mg/g)	2.11 ± 0.06^{a}	2.22 ± 0.12^{ab}	$2.11 \pm 0.09^{a,1}$	$2.30 \pm 0.07^{b,2}$

Mean ± SD, n=6 for each group. Values with different alphabetical superscripts across time and different numerical superscripts across diet are significantly different by Tukey's HSD following a significant *F*-value ($p < 0.05$) by two-way ANOVA. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; FA, fatty acids, TWD total western diet with (+) or without (-) docosahexaenoic acid.

Appendix Table 5.3: Fatty acid composition (weight %) of maternal liver during pregnancy and postpartum

Chow	Baseline	15d Preg	20d Preg	7d PP
16:0	17.40 ± 0.78^{a}	16.75 ± 0.90^{a}	20.77 ± 2.87^{b}	16.22 ± 0.43^{a,1}
18:0	20.36 ± 1.54^{a}	23.25 ± 0.99^{b,1}	19.60 ± 1.69^{a,1}	23.14 ± 0.89^{b,1}
Total SFA	39.95 ± 1.20^{a}	42.26 ± 1.20^{b,1}	42.50 ± 1.37^{b,1}	41.57 ± 0.80^{ab,1}
16:1	0.61 ± 0.12	0.50 ± 0.24	0.42 ± 0.10	0.57 ± 0.10^{1}
18:1n-7	1.70 ± 0.09^{a}	1.55 ± 0.08^{ab}	1.47 ± 0.18^{b}	1.77 ± 0.16^{a}
18:1n-9	7.81 ± 2.72^{1}	6.42 ± 1.44^{1}	5.49 ± 1.16^{1}	7.54 ± 1.20^{1}
Total MUFA	10.46 ± 2.84^{1}	8.85 ± 1.62^{1}	7.72 ± 1.39^{1}	10.25 ± 1.33^{1}
18:2n-6	19.11 ± 1.19^{a}	15.99 ± 2.17^{b}	14.00 ± 2.57^{b}	15.47 ± 0.92^{b,1}
20:4n-6	19.80 ± 1.93	20.97 ± 1.34^{1}	18.46 ± 2.11^{1}	19.72 ± 0.57^{1}
Total n-6 PUFA	40.45 ± 2.69^{a}	38.68 ± 1.37^{ab,1}	36.53 ± 3.13^{b,1}	38.27 ± 0.46^{ab,1}
18:3n-3	0.66 ± 0.11^{a}	0.45 ± 0.18^{b}	0.43 ± 0.10^{b}	0.27 ± 0.04^{b}
20:5n-3	0.38 ± 0.07	0.38 ± 0.14^{1}	0.26 ± 0.08	0.36 ± 0.07^{1}
22:5n-3	0.72 ± 0.19^{a}	0.82 ± 0.13^{a,1}	1.14 ± 0.25^{b,1}	1.40 ± 0.15^{b,1}
22:6n-3	6.42 ± 0.78^{a}	8.02 ± 1.43^{a,1,2}	10.96 ± 2.98^{b,1}	7.16 ± 0.54^{a,1}
Total n-3 PUFA	8.22 ± 0.55^{a,1,2}	9.71 ± 1.31^{a,1,2}	12.81 ± 3.05^{b,1}	9.22 ± 0.56^{a,1}
Total (mg/g)	32.3 ± 4.6	31.7 ± 3.5	26.7 ± 5.0	28.4 ± 2.4^{1}
TWD+	Baseline	15d Preg	20d Preg	7d PP
16:0	18.76 ± 1.78^{a}	19.10 ± 1.16^{a}	21.16 ± 1.42^{ab}	24.42 ± 3.43^{b,2}
18:0	17.50 ± 3.43^{a}	18.48 ± 1.98^{a,2}	15.24 ± 1.79^{a,2}	9.64 ± 2.84^{b,2}
Total SFA	38.61 ± 2.03^{ab}	39.52 ± 0.94^{a,1,2}	38.06 ± 1.97^{ab,2}	35.90 ± 1.89^{b,2}
16:1	0.78 ± 0.23^{a}	0.86 ± 0.12^{a}	0.94 ± 0.11^{a}	2.83 ± 1.09^{b,2}
18:1n-7	1.66 ± 0.08	1.55 ± 0.07	1.65 ± 0.10	1.72 ± 0.31
18:1n-9	12.73 ± 4.09^{a,2}	15.47 ± 1.66^{a,2}	17.27 ± 2.50^{a,2}	31.46 ± 6.80^{b,2}
Total MUFA	15.47 ± 4.26^{a,1,2}	18.24 ± 1.70^{a,2}	20.18 ± 2.51^{a,2}	36.30 ± 7.54^{b,2}
18:2n-6	18.73 ± 1.97^{a}	15.26 ± 1.65^{ab}	13.72 ± 3.10^{b}	12.48 ± 2.97^{b,2}
20:4n-6	15.84 ± 3.52^{a}	14.43 ± 1.72^{a,2}	12.38 ± 1.33^{a,2}	7.30 ± 2.98^{b,2}
Total n-6 PUFA	35.98 ± 3.13^{a}	30.79 ± 0.75^{ab,2}	28.04 ± 2.20^{b,2}	20.89 ± 5.50^{c,2}
18:3n-3	0.70 ± 0.13^{a}	0.47 ± 0.10^{b}	0.48 ± 0.15^{b}	0.31 ± 0.15^{b}
20:5n-3	0.37 ± 0.09^{ab}	0.45 ± 0.06^{a,1}	0.26 ± 0.11^{b}	0.26 ± 0.13^{b,1,2}
22:5n-3	0.51 ± 0.14^{a}	0.38 ± 0.06^{a,2}	0.54 ± 0.14^{a,2}	0.18 ± 0.09^{b,2}
22:6n-3	7.42 ± 0.91^{b}	9.01 ± 0.96^{ab,1}	11.34 ± 2.45^{a,1}	4.48 ± 2.15^{c,1,2}
Total n-3 PUFA	9.04 ± 0.81^{b,1}	10.35 ± 0.91^{ab,1}	12.66 ± 2.30^{a,1}	5.25 ± 2.44^{c,2}
Total (mg/g)	38.3 ± 8.9^{a}	43.6 ± 6.3^{a}	44.4 ± 8.1^{a}	77.2 ± 33.3^{b,2}
TWD-	Baseline	15d Preg	20d Preg	7d PP
16:0	18.53 ± 1.19^{a}	18.71 ± 0.90^{a}	20.90 ± 0.66^{a}	25.22 ± 3.07^{b,2}
18:0	17.51 ± 1.33^{a}	17.20 ± 3.19^{a,2}	16.60 ± 1.11^{a,1,2}	9.97 ± 2.53^{b,2}
Total SFA	38.09 ± 0.54	38.42 ± 3.54^{2}	39.46 ± 1.21^{2}	37.13 ± 1.40^{2}
16:1	0.83 ± 0.14^{a}	1.04 ± 0.22^{a}	1.07 ± 0.23^{a}	3.24 ± 0.80^{b,2}
18:1n-7	1.82 ± 0.03	1.67 ± 0.05	1.64 ± 0.04	1.70 ± 0.29
18:1n-9	14.70 ± 1.41^{a,2}	17.44 ± 4.08^{a,2}	16.00 ± 2.13^{a,2}	33.58 ± 4.98^{b,2}
Total MUFA	17.72 ± 1.52^{a,2}	20.47 ± 4.25^{a,2}	19.03 ± 2.33^{a,2}	38.80 ± 5.18^{b,2}
18:2n-6	17.72 ± 0.69^{a}	15.90 ± 2.93^{a}	11.95 ± 1.16^{b}	9.39 ± 0.92^{b,2}
20:4n-6	16.74 ± 1.45^{a}	15.42 ± 2.83^{a,2}	15.02 ± 1.13^{a,1,2}	8.27 ± 3.18^{b,2}
Total n-6 PUFA	35.95 ± 1.25^{a}	32.87 ± 0.68^{ab,2}	32.36 ± 1.24^{b,2}	19.28 ± 4.20^{c,2}
18:3n-3	0.62 ± 0.06^{a}	0.52 ± 0.19^{ab}	0.35 ± 0.07^{b}	0.16 ± 0.03^{c}
20:5n-3	0.26 ± 0.09^{a}	0.23 ± 0.06^{ab,2}	0.15 ± 0.04^{bc}	0.10 ± 0.03^{c,2}
22:5n-3	0.47 ± 0.07^{a}	0.42 ± 0.06^{a,2}	0.68 ± 0.06^{b,2}	0.27 ± 0.09^{c,2}
22:6n-3	5.58 ± 0.85^{a}	5.76 ± 1.24^{a,2}	6.73 ± 0.70^{a,2}	2.30 ± 1.12^{b,2}
Total n-3 PUFA	6.95 ± 0.81^{a,2}	6.97 ± 1.04^{a,2}	7.94 ± 0.66^{a,2}	2.83 ± 1.22^{b,2}
Total (mg/g)	37.6 ± 2.5^{a}	49.3 ± 12.4^{a}	37.5 ± 8.7^{a}	73.1 ± 25.4^{b,2}

Mean ± SD, n=6 for each group. Values with different alphabetical superscripts across time and different numerical superscripts across diet are significantly different by Tukey's HSD following a significant *F*-value ($p< 0.05$) by two-way ANOVA. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; FA, fatty acids, TWD total western diet with (+) or without (-) docosahexaenoic acid.

Appendix Table 5.4: Fatty acid composition (weight %) of maternal adipose during pregnancy and postpartum

Chow	Baseline	15d Preg	20d Preg	7d PP
16:0	23.17 ± 0.70	20.47 ± 2.12	21.33 ± 1.28	20.94 ± 1.24
18:0	4.26 ± 0.31	4.88 ± 0.85	3.98 ± 0.32	4.77 ± 0.48
Total SFA	29.53 ± 0.83	26.94 ± 2.49	26.88 ± 1.35	27.48 ± 1.30
16:1	3.29 ± 0.46	2.42 ± 1.14	2.59 ± 0.63	2.13 ± 0.63
18:1n-7	2.24 ± 0.11	2.22 ± 0.15	2.05 ± 0.13	2.12 ± 0.12
18:1n-9	28.63 ± 3.56	27.29 ± 1.51	25.84 ± 0.70	27.24 ± 0.87
Total MUFA	34.52 ± 4.02	32.28 ± 2.47	30.77 ± 1.16	31.89 ± 1.31
18:2n-6	31.08 ± 4.15	35.38 ± 4.42	36.30 ± 1.89	35.55 ± 2.26
20:4n-6	0.81 ± 0.22	0.79 ± 0.03	0.86 ± 0.11	0.79 ± 0.06
22:4n-6	0.21 ± 0.06^{a}	0.24 ± 0.03^{a}	0.25 ± 0.02^{a}	0.33 ± 0.04^{b}
22:5n-6	0.08 ± 0.02^{a}	0.09 ± 0.02^{a}	0.12 ± 0.02^{a}	0.18 ± 0.04^{b}
Total n-6 PUFA	32.68 ± 4.48	37.01 ± 4.47	38.05 ± 2.05	37.41 ± 2.29
18:3n-3	1.97 ± 0.26^{ab}	2.13 ± 0.25^{ab}	2.27 ± 0.17^{a}	1.71 ± 0.16^{b}
20:5n-3	0.05 ± 0.02	0.08 ± 0.03	0.08 ± 0.02	0.04 ± 0.01
22:5n-3	0.14 ± 0.05	0.17 ± 0.03	0.17 ± 0.03	0.19 ± 0.02
22:6n-3	0.29 ± 0.02	0.36 ± 0.03	0.37 ± 0.06	0.37 ± 0.05
Total n-3 PUFA	2.47 ± 0.34^{ab}	2.77 ± 0.33^{ab}	2.91 ± 0.27^{a}	2.33 ± 0.20^{b}
Total FA (mg/g)	655 ± 116^{a}	602 ± 46^{ab}	867 ± 44^{c}	450 ± 115^{b,1}
TWD+	Baseline	15d Preg	20d Preg	7d PP
16:0	23.06 ± 1.42	22.51 ± 0.61	22.44 ± 0.85	22.04 ± 1.34
18:0	4.44 ± 0.11	4.47 ± 0.23	4.57 ± 0.45	4.52 ± 0.55
Total SFA	29.87 ± 1.68	29.76 ± 0.69	29.82 ± 0.78	29.50 ± 0.94
16:1	3.37 ± 0.71	3.64 ± 0.27	3.51 ± 0.46	3.25 ± 0.77
18:1n-7	2.09 ± 0.21^{a}	2.18 ± 0.20^{ab}	2.37 ± 0.13^{bc}	2.50 ± 0.06^{c}
18:1n-9	32.43 ± 2.92^{a}	39.33 ± 0.54^{b}	39.58 ± 0.71^{b}	40.63 ± 1.08^{b}
Total MUFA	38.26 ± 3.53^{a}	45.58 ± 0.35^{b}	45.90 ± 0.84^{b}	46.87 ± 1.09^{b}
18:2n-6	27.53 ± 4.44^{a}	20.89 ± 0.58^{b}	20.65 ± 1.18^{b}	20.66 ± 1.42^{b}
20:4n-6	0.62 ± 0.18^{a}	0.34 ± 0.04^{b}	0.35 ± 0.03^{b}	0.30 ± 0.04^{b}
22:4n-6	0.15 ± 0.06^{a}	0.07 ± 0.01^{b}	0.08 ± 0.01^{b}	0.09 ± 0.02^{b}
22:5n-6	0.05 ± 0.02^{a}	0.03 ± 0.01^{b}	0.04 ± 0.01^{ab}	0.04 ± 0.01^{ab}
Total n-6 PUFA	28.78 ± 4.76^{a}	21.65 ± 0.60^{b}	21.43 ± 1.19^{b}	21.42 ± 1.51^{b}
18:3n-3	1.74 ± 0.26^{a}	1.22 ± 0.07^{b}	1.19 ± 0.08^{b}	1.02 ± 0.10^{b}
20:5n-3	0.04 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
22:5n-3	0.10 ± 0.04	0.07 ± 0.01	0.06 ± 0.01	0.06 ± 0.02
22:6n-3	0.28 ± 0.08^{a}	0.39 ± 0.07^{b}	0.40 ± 0.03^{b}	0.31 ± 0.05^{ab}
Total n-3 PUFA	2.17 ± 0.33^{a}	1.72 ± 0.13^{b}	1.69 ± 0.11^{b}	1.44 ± 0.13^{b}
Total FA (mg/g)	667 ± 108^{a}	666 ± 72^{a}	834 ± 22^{b}	673 ± 44^{a,2}
TWD-	Baseline	15d Preg	20d Preg	7d PP
16:0	22.03 ± 0.99^{ab}	22.13 ± 0.97^{ab}	23.10 ± 0.61^{a}	20.80 ± 1.23^{b}
18:0	4.44 ± 0.20^{ab}	4.13 ± 0.16^{a}	4.28 ± 0.16^{a}	4.95 ± 0.51^{b}
Total SFA	28.92 ± 0.96	28.68 ± 1.08	30.09 ± 0.70	28.60 ± 0.98
16:1	2.04 ± 1.83^{a}	4.00 ± 0.33^{b}	4.01 ± 0.32^{b}	2.65 ± 0.79^{ab}
18:1n-7	2.39 ± 0.06^{ac}	1.95 ± 0.11^{b}	2.27 ± 0.14^{a}	2.57 ± 0.12^{c}
18:1n-9	34.18 ± 0.55^{a}	39.60 ± 0.83^{b}	39.94 ± 0.69^{b}	41.27 ± 0.17^{c}
Total MUFA	39.01 ± 1.83^{a}	45.99 ± 1.03^{b}	46.68 ± 0.65^{b}	47.01 ± 0.71^{b}
18:2n-6	26.60 ± 1.45^{a}	21.13 ± 1.59^{b}	19.98 ± 0.79^{b}	21.37 ± 1.45^{b}
20:4n-6	0.63 ± 0.07^{a}	0.43 ± 0.10^{b}	0.37 ± 0.07^{b}	0.31 ± 0.04^{b}
22:4n-6	0.18 ± 0.02^{a}	0.11 ± 0.03^{b}	0.10 ± 0.02^{b}	0.11 ± 0.03^{b}
22:5n-6	0.07 ± 0.02	0.05 ± 0.02	0.06 ± 0.02	0.07 ± 0.01
Total n-6 PUFA	27.92 ± 1.60^{a}	22.08 ± 1.76^{b}	20.81 ± 0.92^{b}	22.19 ± 1.49^{b}
18:3n-3	1.64 ± 0.05^{a}	1.25 ± 0.12^{b}	1.14 ± 0.07^{b}	0.91 ± 0.13^{c}
20:5n-3	0.03 ± 0.01^{a}	0.03 ± 0.01^{ab}	0.02 ± 0.01^{b}	0.02 ± 0.01^{b}
22:5n-3	0.10 ± 0.01^{a}	0.06 ± 0.02^{b}	0.05 ± 0.01^{b}	0.06 ± 0.01^{b}
22:6n-3	0.15 ± 0.02^{a}	0.12 ± 0.03^{ab}	0.09 ± 0.02^{b}	0.08 ± 0.01^{b}
Total n-3 PUFA	1.93 ± 0.08^{a}	1.47 ± 0.17^{b}	1.31 ± 0.10^{bc}	1.08 ± 0.14^{c}
Total FA (mg/g)	700 ± 47^{ab}	735 ± 33^{ab}	861 ± 83^{a}	589 ± 195^{b,1,2}

Mean ± SD. Values with different alphabetical superscripts across time and different numerical superscripts across diet are significantly different by Tukey's HSD following a significant *F*-value ($p < 0.05$) by two-way ANOVA. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; FA, fatty acids, TWD total western diet with (+) or without (-) docosahexaenoic acid. Tissue collected was white perirenal adipose.

Appendix Table 5.5: Fatty acid composition (weight %) of maternal heart during pregnancy and postpartum

Chow	Baseline	15d Preg	20d Preg	7d PP
16:0	15.72 ± 1.53^{a}	13.03 ± 0.33^{b}	13.27 ± 0.77^{b}	12.59 ± 0.59^{b}
18:0	17.82 ± 3.53	20.96 ± 0.72	19.45 ± 1.01	19.85 ± 0.88
Total SFA	38.20 ± 2.87^{a}	37.92 ± 0.70^{ab}	36.19 ± 0.65^{ab}	35.52 ± 0.66^{b}
16:1	0.94 ± 0.59^{a}	0.28 ± 0.07^{b}	0.34 ± 0.13^{b}	0.33 ± 0.05^{b}
18:1n-7	2.82 ± 0.12^{a}	2.93 ± 0.08^{ab}	2.77 ± 0.11^{a}	3.01 ± 0.11^{b}
18:1n-9	9.68 ± 4.66	5.62 ± 0.90	6.68 ± 1.37	6.52 ± 1.32^{1}
Total MUFA	14.21 ± 5.08	9.63 ± 1.04	10.36 ± 1.53	10.57 ± 1.46^{1}
18:2n-6	19.90 ± 3.00	19.52 ± 1.94^{1}	19.34 ± 0.79^{1}	22.36 ± 1.28^{1}
20:4n-6	13.62 ± 2.97^{a}	16.61 ± 0.88^{b,1,2}	17.67 ± 0.87^{b,1}	15.97 ± 0.83^{ab}
22:4n-6	0.71 ± 0.10^{a}	0.88 ± 0.10^{a,1}	1.23 ± 0.16^{b,1}	1.07 ± 0.07^{b,1}
22:5n-6	0.89 ± 0.21^{ab}	0.83 ± 0.17^{a,1,2}	1.31 ± 0.26^{bc,1}	1.75 ± 0.36^{c,1}
Total n-6 PUFA	35.62 ± 2.22^{a}	38.39 ± 1.17^{b,1}	40.16 ± 0.59^{bc,1}	41.89 ± 0.75^{c,1}
18:3n-3	0.56 ± 0.35	0.32 ± 0.05	0.38 ± 0.06	0.27 ± 0.06
22:5n-3	1.32 ± 0.31^{a}	1.48 ± 0.03^{ab,1}	1.63 ± 0.10^{b,1}	1.53 ± 0.05^{ab,1}
22:6n-3	8.71 ± 2.69	10.45 ± 0.78^{1,2}	10.08 ± 1.00^{1}	8.51 ± 1.47^{1}
Total n-3 PUFA	10.70 ± 2.48	12.36 ± 0.69	12.19 ± 1.03^{1}	10.42 ± 1.38^{1}
Total FA (mg/g)	36.8 ± 8.5^{a}	26.3 ± 1.1^{b}	31.1 ± 5.6^{ab}	27.2 ± 1.2^{b}
TWD+	Baseline	15d Preg	20d Preg	7d PP
16:0	15.22 ± 1.10^{a}	14.90 ± 1.53^{ab}	13.20 ± 0.51^{b}	13.24 ± 1.16^{b}
18:0	20.02 ± 2.37	19.48 ± 1.98	19.89 ± 0.20	19.06 ± 1.55
Total SFA	38.91 ± 2.03^{a}	37.81 ± 0.86^{ab}	36.59 ± 0.39^{bc}	35.62 ± 0.61^{c}
16:1	0.69 ± 0.42	0.77 ± 0.56	0.38 ± 0.07	0.56 ± 0.29
18:1n-7	2.87 ± 0.06^{a}	2.78 ± 0.09^{a}	2.84 ± 0.13^{a}	3.06 ± 0.10^{b}
18:1n-9	8.61 ± 3.67	11.03 ± 3.51	9.23 ± 0.66	11.07 ± 3.68^{1,2}
Total MUFA	12.85 ± 3.84	15.28 ± 4.02	13.09 ± 0.80	15.47 ± 4.14^{1,2}
18:2n-6	17.48 ± 2.90	15.70 ± 1.02^{2}	14.35 ± 0.71^{2}	17.15 ± 2.26^{2}
20:4n-6	15.19 ± 1.65^{ab}	13.71 ± 1.95^{a,1}	16.49 ± 0.89^{b,1}	14.21 ± 1.67^{ab}
22:4n-6	0.67 ± 0.09^{a}	0.45 ± 0.07^{b,2}	0.63 ± 0.11^{a,2}	0.61 ± 0.14^{ab,2}
22:5n-6	0.80 ± 0.11^{a}	0.33 ± 0.07^{b,1}	0.50 ± 0.07^{c,2}	0.39 ± 0.05^{bc,2}
Total n-6 PUFA	34.64 ± 1.76^{a}	30.68 ± 2.42^{b,2}	32.51 ± 0.88^{ab,2}	32.97 ± 2.63^{ab,2}
18:3n-3	0.39 ± 0.22	0.26 ± 0.09	0.20 ± 0.05	0.19 ± 0.05
22:5n-3	1.16 ± 0.19^{a}	0.74 ± 0.10^{b,2}	0.84 ± 0.05^{b,2}	0.73 ± 0.10^{b,2}
22:6n-3	11.22 ± 3.49^{a}	13.37 ± 1.95^{ab,2}	15.19 ± 0.83^{b,2}	13.46 ± 1.64^{ab,2}
Total n-3 PUFA	12.87 ± 3.21	14.48 ± 1.97	16.31 ± 0.90^{2}	14.48 ± 1.69^{2}
Total FA (mg/g)	31.9 ± 4.2	29.1 ± 3.1	30.3 ± 2.1	27.7 ± 3.8
TWD-	Baseline	15d Preg	20d Preg	7d PP
16:0	14.56 ± 1.63	13.35 ± 0.51	12.91 ± 0.46	12.79 ± 1.29
18:0	20.03 ± 1.28^{ab}	21.22 ± 0.55^{a}	19.68 ± 0.42^{ab}	18.90 ± 1.44^{b}
Total SFA	38.46 ± 1.79^{a}	38.35 ± 0.78^{a}	35.96 ± 0.61^{b}	35.22 ± 0.73^{b}
16:1	0.68 ± 0.28	0.41 ± 0.07	0.45 ± 0.08	0.61 ± 0.29
18:1n-7	2.94 ± 0.15	2.93 ± 0.13	2.86 ± 0.08	3.03 ± 0.18
18:1n-9	8.53 ± 2.31^{ab}	8.30 ± 0.62^{a}	10.81 ± 0.85^{ab}	12.30 ± 4.05^{b,2}
Total MUFA	12.93 ± 2.36^{ab}	12.48 ± 0.63^{a}	14.65 ± 1.03^{ab}	16.82 ± 4.13^{b,2}
18:2n-6	16.92 ± 1.31^{ab}	15.78 ± 0.82^{ab,2}	15.18 ± 1.01^{a,2}	18.09 ± 2.19^{b,2}
20:4n-6	16.37 ± 1.91^{a}	17.95 ± 0.53^{ab,2}	18.88 ± 0.53^{b,2}	16.36 ± 1.79^{a}
22:4n-6	0.83 ± 0.19^{a}	0.84 ± 0.11^{a,1}	1.10 ± 0.09^{b,1}	1.02 ± 0.14^{ab,1}
22:5n-6	1.20 ± 0.46^{a}	1.06 ± 0.35^{a,2}	2.06 ± 0.43^{b,3}	2.06 ± 0.54^{b,1}
Total n-6 PUFA	35.86 ± 1.80	36.12 ± 0.54^{1}	37.71 ± 0.97^{1}	38.15 ± 3.10^{1}
18:3n-3	0.36 ± 0.11^{a}	0.22 ± 0.03^{b}	0.20 ± 0.02^{b}	0.21 ± 0.05^{b}
22:5n-3	1.44 ± 0.22^{a}	1.28 ± 0.09^{ab,1}	1.15 ± 0.10^{b,3}	1.20 ± 0.23^{ab,3}
22:6n-3	9.85 ± 2.71^{a}	9.48 ± 0.36^{a,1}	8.78 ± 0.66^{ab,1}	6.69 ± 0.77^{b,1}
Total n-3 PUFA	11.75 ± 2.54^{a}	11.08 ± 0.31^{a}	10.19 ± 0.63^{ab,1}	8.18 ± 0.95^{b,1}
Total FA (mg/g)	30.7 ± 4.2	26.7 ± 1.7	30.7 ± 2.5	29.7 ± 4.0

Mean ± SD, n=6 for each group. Values with different alphabetical superscripts across time and different numerical superscripts across diet are significantly different by Tukey's HSD following a significant *F*-value ($p < 0.05$) by two-way ANOVA. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; FA, fatty acids, TWD total western diet with (+) or without (-) docosahexaenoic acid.

Appendix Table 5.6: Fatty acid composition (weight %) of maternal brain during pregnancy and postpartum

Chow	Baseline	15d Preg	20d Preg	7d PP
16:0	18.17 ± 0.71^{a}	19.91 ± 1.18^{b}	18.70 ± 0.89^{ab}	19.96 ± 0.39^{b}
18:0	18.81 ± 0.34	19.22 ± 0.68	19.02 ± 0.33	18.60 ± 0.58
24:0	1.19 ± 0.17^{a}	0.89 ± 0.14^{b}	0.93 ± 0.13^{b}	0.85 ± 0.07^{b}
Total SFA	44.93 ± 0.69^{a}	46.03 ± 1.28^{ab}	46.60 ± 0.82^{b}	46.40 ± 0.67^{b}
18:1n-7	3.52 ± 0.10	3.52 ± 0.19	3.67 ± 0.13	3.59 ± 0.06
18:1n-9	16.14 ± 0.32	16.06 ± 0.41	16.83 ± 0.75	16.72 ± 0.47
24:1n-9	1.68 ± 0.25	1.34 ± 0.27	1.48 ± 0.26	1.30 ± 0.15
Total MUFA	24.80 ± 0.97	23.73 ± 1.15	25.72 ± 1.70	24.78 ± 0.88
18:2n-6	0.92 ± 0.09	0.82 ± 0.07	0.83 ± 0.07	1.35 ± 1.01
20:4n-6	8.95 ± 0.30	9.10 ± 0.62	8.87 ± 0.36	8.88 ± 0.40
22:4n-6	2.96 ± 0.03^{a}	2.73 ± 0.12^{b}	2.65 ± 0.05^{b}	2.74 ± 0.10^{b}
22:5n-6	0.61 ± 0.05^{ac}	0.50 ± 0.05^{b}	0.54 ± 0.04^{ab,1}	0.63 ± 0.05^{c,1}
Total n-6 PUFA	14.09 ± 0.36	13.69 ± 0.68	13.42 ± 0.44	14.19 ± 0.70^{1}
20:5n-3	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
22:5n-3	0.16 ± 0.01^{a}	0.13 ± 0.01^{b,1}	0.14 ± 0.01^{b}	0.16 ± 0.01^{a,1}
22:6n-3	12.12 ± 0.79	12.08 ± 0.88	11.82 ± 0.81	12.20 ± 0.56
Total n-3 PUFA	12.32 ± 0.79	12.25 ± 0.88	12.00 ± 0.82	12.42 ± 0.53
Total FA (mg/g)	41.8 ± 1.9	41.3 ± 1.8	44.1 ± 1.5	42.2 ± 1.7
TWD+	Baseline	15d Preg	20d Preg	7d PP
16:0	18.00 ± 0.18^{a}	19.01 ± 0.64^{ab}	18.91 ± 1.01^{ab}	19.36 ± 0.52^{b}
18:0	18.88 ± 0.56	19.00 ± 0.27	18.82 ± 0.62	18.44 ± 0.36
24:0	1.16 ± 0.12^{a}	0.85 ± 0.17^{b}	0.90 ± 0.21^{ab}	0.94 ± 0.13^{ab}
Total SFA	45.41 ± 0.50	46.18 ± 0.95	46.49 ± 1.42	46.51 ± 0.42
18:1n-7	3.49 ± 0.08	3.50 ± 0.28	2.95 ± 1.42	3.63 ± 0.18
18:1n-9	16.13 ± 0.13	16.42 ± 0.96	17.06 ± 1.15	17.04 ± 0.64
24:1n-9	1.66 ± 0.16	1.28 ± 0.29	1.40 ± 0.35	1.48 ± 0.19
Total MUFA	24.79 ± 0.39	24.46 ± 2.09	25.18 ± 1.78	25.76 ± 1.42
18:2n-6	0.86 ± 0.08	0.76 ± 0.06	1.08 ± 0.79	0.93 ± 0.18
20:4n-6	8.86 ± 0.26	8.96 ± 0.42	8.58 ± 0.50	8.44 ± 0.39
22:4n-6	2.91 ± 0.14^{a}	2.70 ± 0.03^{b}	2.55 ± 0.10^{b}	2.58 ± 0.07^{b}
22:5n-6	0.57 ± 0.06^{a}	0.44 ± 0.03^{b}	0.40 ± 0.03^{bc,2}	0.35 ± 0.03^{c,2}
Total n-6 PUFA	13.83 ± 0.40^{a}	13.40 ± 0.45^{ab}	13.16 ± 0.71^{ab}	12.90 ± 0.45^{b,2}
20:5n-3	0.01 ± 0.01^{a}	0.01 ± 0.01^{a}	0.02 ± 0.01^{b}	0.02 ± 0.01^{b}
22:5n-3	0.15 ± 0.01^{a}	0.13 ± 0.01^{b,1,2}	0.14 ± 0.01^{b}	0.14 ± 0.01^{ab,1,2}
22:6n-3	12.19 ± 0.22	12.02 ± 0.65	12.34 ± 1.05	12.47 ± 0.48
Total n-3 PUFA	12.37 ± 0.22	12.17 ± 0.65	12.54 ± 1.04	12.66 ± 0.47
Total FA (mg/g)	41.5 ± 1.8^{a}	41.5 ± 1.0^{a}	44.9 ± 2.1^{b}	40.6 ± 1.4^{a}
TWD-	Baseline	15d Preg	20d Preg	7d PP
16:0	17.69 ± 0.63^{a}	19.45 ± 0.86^{b}	19.08 ± 0.33^{ab}	19.10 ± 1.38^{ab}
18:0	18.54 ± 0.30^{ab}	18.92 ± 0.43^{ab}	19.22 ± 0.49^{a}	18.36 ± 0.64^{b}
24:0	1.28 ± 0.10^{a}	0.92 ± 0.13^{b}	0.90 ± 0.18^{b}	0.95 ± 0.23^{b}
Total SFA	44.96 ± 0.38^{a}	45.58 ± 1.19^{ab}	46.69 ± 0.74^{b}	46.15 ± 1.29^{ab}
18:1n-7	3.55 ± 0.04	3.51 ± 0.14	3.59 ± 0.19	3.81 ± 0.31
18:1n-9	16.24 ± 0.34^{a}	16.45 ± 0.28^{ab}	16.72 ± 0.71^{ab}	17.40 ± 1.12^{b}
24:1n-9	1.86 ± 0.16	1.42 ± 0.28	1.38 ± 0.32	1.62 ± 0.50
Total MUFA	25.45 ± 0.80	24.48 ± 0.85	25.19 ± 1.56	26.81 ± 2.82
18:2n-6	0.82 ± 0.02^{ab}	0.70 ± 0.05^{a}	0.86 ± 0.13^{b}	0.85 ± 0.13^{b}
20:4n-6	8.75 ± 0.25	8.79 ± 0.40	9.20 ± 0.54	8.32 ± 0.89
22:4n-6	2.90 ± 0.06^{a}	2.64 ± 0.14^{b}	2.75 ± 0.11^{ab}	2.68 ± 0.22^{ab}
22:5n-6	0.58 ± 0.04^{ab}	0.47 ± 0.07^{a}	0.63 ± 0.09^{ab,1}	0.64 ± 0.16^{b,1}
Total n-6 PUFA	13.71 ± 0.26	13.12 ± 0.55	13.96 ± 0.63	13.09 ± 1.08^{2}
20:5n-3	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.04 ± 0.06
22:5n-3	0.15 ± 0.01^{a}	0.11 ± 0.01^{b,2}	0.13 ± 0.01^{c}	0.12 ± 0.01^{bc,2}
22:6n-3	11.84 ± 0.58	11.70 ± 0.67	11.84 ± 0.68	11.52 ± 0.98
Total n-3 PUFA	12.02 ± 0.58	11.84 ± 0.68	12.01 ± 0.68	11.72 ± 0.98
Total FA (mg/g)	42.8 ± 1.0	43.4 ± 1.5	41.0 ± 5.3	43.7 ± 1.9

Mean ± SD. Values with different alphabetical superscripts across time and different numerical superscripts across diet are significantly different by Tukey's HSD following a significant *F*-value ($p < 0.05$) by two-way ANOVA. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; FA, fatty acids, TWD total western diet with (+) or without (-) docosahexaenoic acid.

Appendix Table 5.7: Fatty acid composition (weight %) of the placenta during pregnancy

	Chow		TWD+		TWD-	
	15d Preg	20d Preg	15d Preg	20d Preg	15d Preg	20d Preg
16:0	18.44 ± 0.74^{a}	20.51 ± 0.90^{b}	18.76 ± 0.92^{a}	20.36 ± 0.47^{b}	18.28 ± 0.77^{a}	20.33 ± 0.63^{b}
18:0	20.32 ± 1.52^{a}	15.42 ± 2.50^{c}	20.21 ± 1.19^{a}	17.44 ± 0.54^{b}	20.64 ± 0.99^{a}	17.20 ± 0.77^{b}
24:0	0.99 ± 0.16^{a}	1.41 ± 0.44^{b}	0.89 ± 0.10^{a}	1.54 ± 0.23^{b}	0.90 ± 0.13^{a}	1.50 ± 0.17^{b}
Total SFA	44.43 ± 2.05^{a}	40.86 ± 2.53^{b}	44.68 ± 1.18^{a}	43.88 ± 1.19^{a}	44.44 ± 0.73^{a}	43.21 ± 1.46^{a}
16:1	0.96 ± 0.27	1.03 ± 0.56	1.06 ± 0.15	0.83 ± 0.07	1.12 ± 0.23	0.88 ± 0.09
18:1n-7	2.41 ± 0.17^{a}	2.11 ± 0.18^{b}	2.32 ± 0.24^{ab}	2.16 ± 0.09^{b}	2.41 ± 0.22^{a}	2.15 ± 0.11^{b}
18:1n-9	10.12 ± 2.12^{a}	12.37 ± 5.51^{ab}	12.64 ± 1.76^{ab}	13.88 ± 0.85^{b}	12.48 ± 1.24^{ab}	14.48 ± 1.19^{b}
Total MUFA	15.24 ± 2.33^{a}	16.70 ± 5.91^{ab}	17.97 ± 1.93^{ab}	18.73 ± 1.03^{ab}	17.96 ± 1.54^{ab}	19.02 ± 1.34^{b}
18:2n-6	13.00 ± 2.46^{b}	15.54 ± 3.40^{a}	10.75 ± 1.15^{bc}	10.61 ± 0.39^{c}	10.18 ± 0.80^{c}	10.36 ± 0.75^{c}
20:4n-6	16.23 ± 1.60^{bc}	12.83 ± 2.88^{a}	15.01 ± 1.00^{bc}	14.35 ± 0.68^{ab}	16.29 ± 0.89^{c}	14.79 ± 0.63^{bc}
22:4n-6	3.21 ± 0.37^{a}	2.28 ± 0.89^{bc}	2.65 ± 0.23^{b}	1.77 ± 0.25^{c}	3.35 ± 0.36^{a}	2.11 ± 0.23^{bc}
22:5n-6	0.48 ± 0.09^{a}	1.40 ± 0.36^{b}	0.32 ± 0.09^{a}	1.15 ± 0.60^{b}	0.57 ± 0.17^{a}	2.53 ± 0.77^{c}
Total n-6 PUFA	34.94 ± 1.65^{a}	33.58 ± 2.46^{ab}	30.56 ± 1.36^{cd}	29.37 ± 1.44^{d}	32.23 ± 1.10^{bc}	31.18 ± 1.11^{cd}
20:5n-3	0.25 ± 0.07^{ab}	0.21 ± 0.06^{ab}	0.28 ± 0.09^{a}	0.21 ± 0.05^{ab}	0.17 ± 0.05^{b}	0.17 ± 0.08^{b}
22:5n-3	0.61 ± 0.08^{b}	0.77 ± 0.22^{a}	0.54 ± 0.08^{bc}	0.55 ± 0.12^{bc}	0.50 ± 0.11^{bc}	0.43 ± 0.08^{c}
22:6n-3	2.33 ± 0.44^{a}	3.13 ± 0.84^{bc}	3.26 ± 0.40^{c}	5.15 ± 0.87^{d}	2.11 ± 0.25^{a}	3.10 ± 0.85^{bc}
Total n-3 PUFA	3.65 ± 0.36^{ab}	4.73 ± 0.85^{c}	4.44 ± 0.46^{bc}	6.22 ± 0.96^{d}	3.13 ± 0.29^{a}	3.98 ± 0.87^{abc}
Total FA (mg/g)	11.98 ± 1.31^{a}	17.00 ± 5.35^{c}	12.42 ± 0.84^{ab}	16.40 ± 4.59^{bc}	11.73 ± 1.19^{a}	14.97 ± 0.83^{abc}

Mean ± SD, n=6 for each group. Values with a different superscript are significantly different by Tukey's HSD following a significant *F*-value ($p < 0.05$) by two-way ANOVA. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; FA, fatty acids, TWD total western diet with (+) or without (-) docosahexaenoic acid.

Appendix Table 5.8: Fatty acid composition (weight %) of whole body fetuses and pups

Chow	15d Preg	20d Preg	7d PP
16:0	26.55 ± 0.72[a]	26.46 ± 0.79[a]	21.99 ± 0.83[b]
18:0	14.43 ± 0.64[a]	13.14 ± 0.17[a]	9.10 ± 1.88[b,1]
Total SFA	45.55 ± 0.61[a]	44.58 ± 0.75[a]	42.16 ± 1.28[b,1]
16:1	2.59 ± 0.14[a]	2.74 ± 0.26[a]	0.99 ± 0.12[b,1]
18:1n-7	3.51 ± 0.16[a]	3.44 ± 0.16[a]	2.02 ± 0.21[b]
18:1n-9	15.79 ± 0.88	15.25 ± 0.45	15.75 ± 1.26[1]
Total MUFA	23.28 ± 0.87[a]	22.35 ± 0.84[a]	19.58 ± 1.13[b,1]
18:2n-6	3.46 ± 0.29[a]	7.51 ± 0.70[b]	18.00 ± 2.25[c,1]
20:4n-6	13.79 ± 0.77[a]	11.57 ± 0.13[a]	8.57 ± 1.99[b,1]
22:4n-6	2.94 ± 0.30[a]	1.94 ± 0.08[b]	1.44 ± 0.29[b,1]
22:5n-6	1.56 ± 0.23[a]	1.15 ± 0.13[b,1]	0.44 ± 0.14[c]
Total n-6 PUFA	22.97 ± 0.80[a,1]	23.55 ± 0.77[a,1]	30.72 ± 0.89[b,1]
20:5n-3	0.12 ± 0.08[a]	0.16 ± 0.04[ab,1,2]	0.25 ± 0.04[b]
22:5n-3	0.26 ± 0.12[a]	0.46 ± 0.07[a,1]	1.02 ± 0.11[b,1]
22:6n-3	2.59 ± 0.23[a]	4.37 ± 0.56[b,1]	2.56 ± 0.67[a,1]
Total n-3 PUFA	3.13 ± 0.29[a]	5.24 ± 0.72[b,1]	4.65 ± 0.52[b,1]
Total FA (mg/g)	8.24 ± 1.29[a]	11.37 ± 2.28[a]	33.57 ± 11.99[b,1]
TWD+	15d Preg	20d Preg	7d PP
16:0	26.50 ± 0.35[a]	24.96 ± 0.55[b]	20.89 ± 0.68[c]
18:0	13.91 ± 0.41[a]	13.17 ± 0.37[a]	6.87 ± 0.87[b,2]
Total SFA	45.61 ± 0.65[a]	43.76 ± 0.83[b]	38.35 ± 0.62[c,2]
16:1	2.65 ± 0.16[a]	2.59 ± 0.12[a]	1.53 ± 0.19[b,2]
18:1n-7	3.58 ± 0.11[a]	3.36 ± 0.15[b]	2.28 ± 0.14[c]
18:1n-9	17.09 ± 1.00[a]	16.74 ± 1.11[a]	30.22 ± 1.85[b,2]
Total MUFA	24.77 ± 1.07[a]	24.02 ± 1.28[a]	34.94 ± 1.82[b,2]
18:2n-6	3.18 ± 0.26[a]	6.33 ± 0.41[b]	12.64 ± 0.35[c,2]
20:4n-6	12.60 ± 0.51[a]	10.96 ± 0.40[b]	4.14 ± 1.23[c,2]
22:4n-6	2.56 ± 0.16[a]	1.66 ± 0.13[b]	0.83 ± 0.21[c,2]
22:5n-6	1.21 ± 0.13[a]	0.69 ± 0.14[b,2]	0.11 ± 0.05[c]
Total n-6 PUFA	20.69 ± 0.79[2]	20.91 ± 0.76[2]	19.56 ± 1.32[2]
20:5n-3	0.10 ± 0.05[a]	0.21 ± 0.04[b,1]	0.19 ± 0.02[b]
22:5n-3	0.27 ± 0.07[a]	0.29 ± 0.09[a,1,2]	0.48 ± 0.13[b,2]
22:6n-3	3.59 ± 0.35[a]	5.97 ± 0.54[b,2]	2.64 ± 0.81[c,1]
Total n-3 PUFA	4.10 ± 0.30[a]	6.68 ± 0.61[b,2]	3.94 ± 0.90[a,1]
Total FA (mg/g)	8.90 ± 0.84[a]	10.03 ± 0.57[a]	94.35 ± 35.17[b,2]
TWD-	15d Preg	20d Preg	7d PP
16:0	26.41 ± 0.86[a]	25.87 ± 0.75[a]	20.86 ± 1.52[b]
18:0	13.82 ± 0.84[a]	12.98 ± 0.37[a]	6.43 ± 0.81[b,2]
Total SFA	45.16 ± 0.94[a]	43.78 ± 1.12[a]	38.26 ± 2.41[b,2]
16:1	2.68 ± 0.09[a]	2.70 ± 0.16[a]	1.55 ± 0.29[b,2]
18:1n-7	3.53 ± 0.17[a]	3.45 ± 0.18[a]	2.24 ± 0.16[b]
18:1n-9	17.01 ± 0.67[a]	17.62 ± 1.49[a]	31.36 ± 1.24[b,2]
Total MUFA	24.44 ± 0.71[a]	24.87 ± 1.70[a]	36.06 ± 1.30[b,2]
18:2n-6	3.74 ± 1.33[a]	6.21 ± 0.52[b]	12.71 ± 0.39[c,2]
20:4n-6	12.92 ± 1.05[a]	11.73 ± 0.90[a]	4.34 ± 1.01[b,2]
22:4n-6	2.64 ± 0.43[a]	2.06 ± 0.19[a]	0.99 ± 0.39[b,1,2]
22:5n-6	1.63 ± 0.30[a]	1.85 ± 0.30[a,3]	0.30 ± 0.03[b]
Total n-6 PUFA	22.04 ± 0.97[ab,1,2]	23.13 ± 1.19[a,1,2]	20.16 ± 1.77[b,2]
20:5n-3	0.09 ± 0.02[a]	0.09 ± 0.05[a,2]	0.16 ± 0.02[b]
22:5n-3	0.20 ± 0.10[a]	0.21 ± 0.12[a,2]	0.48 ± 0.10[b,2]
22:6n-3	2.70 ± 0.60[a]	3.44 ± 0.41[a,1]	0.90 ± 0.22[b,2]
Total n-3 PUFA	3.22 ± 0.68[a]	3.92 ± 0.35[a,3]	2.16 ± 0.26[b,2]
Total FA (mg/g)	8.15 ± 1.20[a]	10.03 ± 0.22[a]	97.70 ± 19.51[b,2]

Mean ± SD, n=6 for each group. Values with different alphabetical superscripts across time and different numerical superscripts across diet are significantly different by Tukey's HSD following a significant *F*-value ($p < 0.05$) by two-way ANOVA. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; FA, fatty acids, TWD total western diet with (+) or without (-) docosahexaenoic acid.

Appendix Table 5.9: Fatty acid composition (weight %) of 7-day old pup tissues

	Chow	TWD+	TWD-
Brain			
14:0	1.70 ± 0.22[b]	2.11 ± 0.37[a]	2.04 ± 0.30[a]
16:0	31.52 ± 0.65[a]	31.45 ± 0.87[a]	30.44 ± 0.97[b]
18:0	16.30 ± 0.46	16.24 ± 0.30	16.50 ± 0.35
Total SFA	53.97 ± 1.21[b]	52.23 ± 1.17[a]	50.79 ± 1.44[a]
16:1	1.47 ± 0.11[a]	1.45 ± 0.19[a]	1.29 ± 0.10[b]
18:1n-7	2.20 ± 0.06	2.21 ± 0.09	2.21 ± 0.07
18:1n-9	10.49 ± 0.76	10.59 ± 0.31	10.52 ± 0.39
Total MUFA	14.77 ± 0.88	15.00 ± 0.96	14.51 ± 0.45
18:2n-6	1.37 ± 0.88	0.91 ± 0.04	0.90 ± 0.08
20:4n-6	12.21 ± 0.47[a]	11.93 ± 0.28[a]	13.40 ± 0.61[b]
22:4n-6	2.40 ± 0.11[a]	2.44 ± 0.22[a]	2.89 ± 0.23[b]
22:5n-6	1.61 ± 0.11[c]	1.15 ± 0.12[a]	2.65 ± 0.15[b]
Total n-6 PUFA	18.24 ± 0.60[c]	17.09 ± 0.56[a]	20.46 ± 0.99[b]
20:5n-3	0.03 ± 0.01[ab]	0.05 ± 0.02[a]	0.03 ± 0.01[b]
22:5n-3	0.26 ± 0.02[c]	0.18 ± 0.02[a]	0.30 ± 0.05[b]
22:6n-3	9.33 ± 0.39[b]	11.27 ± 0.68[a]	9.37 ± 0.56[b]
Total n-3 PUFA	9.64 ± 0.37[b]	11.52 ± 0.70[a]	9.72 ± 0.57[b]
Total FA (mg/g)	20.47 ± 1.37[a]	20.41 ± 2.00[a]	17.08 ± 1.39[b]
Heart			
14:0	0.82 ± 0.48	0.95 ± 0.39	1.09 ± 0.23
16:0	16.19 ± 0.77	16.69 ± 0.77	16.58 ± 0.66
18:0	18.93 ± 1.32	19.27 ± 1.54	19.22 ± 1.25
Total SFA	39.89 ± 1.04	40.54 ± 1.12	39.89 ± 2.19
18:1n-7	3.18 ± 0.38	3.40 ± 0.14	3.37 ± 0.23
18:1n-9	5.60 ± 1.58[b]	9.19 ± 3.29[a]	9.19 ± 2.17[a]
Total MUFA	9.91 ± 1.42[b]	14.08 ± 3.32[a]	14.92 ± 4.36[a]
18:2n-6	7.18 ± 1.09[b]	5.78 ± 0.76[a]	5.37 ± 0.42[a]
18:3n-6	2.34 ± 0.88[b]	0.06 ± 0.02[a]	0.03 ± 0.01[a]
20:3n-6	1.07 ± 0.10	1.17 ± 0.10	1.04 ± 0.17
20:4n-6	23.18 ± 2.03	21.83 ± 2.38	22.89 ± 1.85
22:4n-6	0.39 ± 0.96[c]	2.24 ± 0.24[a]	3.70 ± 0.45[b]
22:5n-6	0.18 ± 0.47[a]	0.29 ± 0.09[a]	1.33 ± 0.22[b]
Total n-6 PUFA	35.35 ± 1.28[b]	32.13 ± 1.91[a]	35.24 ± 1.99[b]
20:5n-3	0.15 ± 0.28	0.05 ± 0.02	0.04 ± 0.01
22:5n-3	0.23 ± 0.08[b]	0.15 ± 0.02[a]	0.10 ± 0.02[a]
22:6n-3	6.68 ± 1.04[c]	10.09 ± 1.63[a]	4.67 ± 0.92[b]
Total n-3 PUFA	10.30 ± 0.92[a]	11.83 ± 1.74[a]	7.44 ± 1.37[b]
Total FA (mg/g)	23.98 ± 3.52[b]	19.17 ± 3.99[ab]	16.38 ± 4.06[a]
Liver			
16:0	20.77 ± 1.05[b]	19.24 ± 1.46[ab]	19.06 ± 0.95[a]
18:0	15.58 ± 0.87[b]	13.29 ± 2.26[a]	12.10 ± 1.78[a]
Total SFA	39.05 ± 0.93[b]	35.20 ± 1.97[a]	34.21 ± 1.21[a]
18:1n-7	1.38 ± 0.06[b]	1.61 ± 0.13[a]	1.58 ± 0.10[a]
18:1n-9	3.71 ± 0.94[b]	10.46 ± 2.47[a]	12.09 ± 2.25[a]
Total MUFA	5.83 ± 0.98[b]	13.11 ± 2.46[a]	14.76 ± 2.33[a]
18:2n-6	9.73 ± 1.16	9.40 ± 1.05	9.87 ± 1.03
20:4n-6	19.79 ± 0.75[c]	15.71 ± 2.01[a]	17.54 ± 0.92[b]
22:4n-6	0.92 ± 0.11[a]	1.24 ± 0.34[a]	2.08 ± 0.75[b]
22:5n-6	0.43 ± 0.07[c]	0.23 ± 0.11[a]	1.21 ± 0.23[b]
Total n-6 PUFA	32.40 ± 1.30[b]	28.20 ± 1.44[a]	32.40 ± 0.94[b]
20:5n-3	0.39 ± 0.11[b]	0.50 ± 0.08[a]	0.33 ± 0.06[b]
22:5n-3	2.47 ± 0.29[b]	1.52 ± 0.27[a]	2.52 ± 0.66[b]
22:6n-3	13.39 ± 1.50[c]	15.79 ± 1.13[a]	9.04 ± 1.57[b]
Total n-3 PUFA	16.44 ± 1.39[a]	18.03 ± 1.17[a]	12.17 ± 1.66[b]
Total FA (mg/g)	25.12 ± 5.06[b]	33.80 ± 9.05[ab]	35.39 ± 5.81[a]

Mean ± SD, n=6 for each group. Values with a different superscript are significantly different by Tukey's HSD following a significant *F*-value ($p < 0.05$) by one-way ANOVA. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; FA, fatty acids, TWD total western diet with (+) or without (-) docosahexaenoic acid

6 Sex Differences in Fatty Acid Compositions of Plasma, Liver, Brain, and Heart from Birth to Adulthood

6.1 Introduction

The effects of estrogen on LCPUFA and long-chain MUFA and SFA synthesis from shorter chain precursors has been reported [15,105,112]. Sex differences have been reported to occur predominantly when diets are controlled for low DHA intakes [18]. However, recent studies reporting sexual dimorphisms in communication skills at 3 years of age following DHA supplementation during infancy [20], and the results from chapter 5 showing a sexual dimorphism in the brain DHA concentrations of 7-day pups warrant further investigation into when sexual dimorphisms in fatty status begin. To my knowledge, the fatty acid compositions of plasma, brain, heart, and liver of female and male pups from weeks 1 to 9 have not yet been examined. This study is the first, and provides insight into how tissue fatty acid compositions change during development in rats fed a regular chow diet.

6.2 Methods

6.2.1: Study Design

Five male and five female pups fed Teklad 22/5 Rodent Diet chow were sacrificed each week from weeks 1 to 9. Pups were sacrificed after an overnight fast, except for pups 1-3 weeks old who were left with their mothers until sacrifice. Plasma, RBC, brain, hearts, and livers were collected and analyzed by gas chromatography as described above.

6.2.2 Diets

Rats were fed the chow diets outlined in Table 1 from weeks 4 to 9, and mothers at weeks 1 to three were fed chow as well. Since 1 to 3-week-old rats were not fasted, stomach contents were

collected, and their compositions at each week are outlined in Appendix Table 6.1 and Appendix Table 6.2.

6.2.3 Statistical Analyses

All statistical analyses were performed using SPSS release 20.0 (IBM, Chicago, IL, USA). The effect of sex and time was examined across offspring tissue fatty acid and body weight data using a two-way ANOVA with interactions. Differences in tissues across time were examined by one-way ANOVA. Individual means were compared by Tukey post hoc testing after significant F-value by ANOVA. All data are presented as means ± SD with significance accepted when $p<0.05$.

6.3 Results

6.3.1 Body Weights over Time

Males were significantly heavier than females from week 6 to week 9 (Figure 8). In both sexes, body weights increased each week except between weeks 2 and 3, and weeks 7 and 8, where body weights remained equal.

6.3.2 Changes in the Fatty Acid Composition of Plasma

Relative levels of DHA and n-3 docosapentaenoic acid (DPAn-3) were significantly affected by a sex x time interaction. Both DHA and DPAn-3 decreased below baseline at week 3 and returned to baseline levels at week 4. DPAn-3 then decreased below baseline again at week 5 in females and week 6 in males (Appendix Table 6.3 and 6.4). At week 9, females had higher plasma relative levels of DHA than males (Figure 9). Relative levels of ALA were higher in males than females overall, but no sex x time interaction occurred in ALA relative levels or concentrations. No effect of sex was detected in DHA concentrations, which were highest in both sexes at weeks 2, 4, and 5.

Relative levels of ARA increased above baseline at week 5 in females, and week 7 in males and were higher in females at week 9 (Appendix Table 6.3 and Appendix Table 6.4 13). Conversely, while LA levels increased above baseline at week 3 and returned to baseline levels at week 4 in both sexes, LA levels in males increased above baseline again at week 9, and were higher than females at weeks 7, 8, and 9 (Figure 9). Concentrations of ARA were not different between sexes. Concentrations of LA were higher in males at weeks 7, 8, and 9.

In both sexes, total MUFA concentrations increased above baseline at week 5, before returning to baseline at week 7. Additionally, the relative composition of saturates were sexually dimorphic. Females had higher relative levels of 18:0 acid at weeks 7, 8, and 9, while males had higher levels of 16:0 acid at weeks 8 and 9 (Appendix Table 6.3 and Appendix Table 6.4).

Total fatty acid concentrations in plasma were not significant for sex x time interactions or sexual dimorphisms but were affected by time. Fatty acid concentrations increased above baseline at 2, 5, and 6 weeks of age (Appendix Table 6.3 and Appendix Table 6.4).

6.3.3 Changes in the Fatty Acid Composition of Liver

Female livers had higher relative amounts of DHA than males at week 9, but males had higher concentrations at weeks 6 and 9 (Figure 9). In both sexes, relative compositions of DHA and total n-3 PUFA decreased from weeks 2 to 9, while relative percent compositions of ALA increased from weeks 1 to 9. In females, relative levels and concentrations of ALA increased and remained above baseline levels starting a week 3, whereas in males relative levels and concentrations of ALA increased above baseline levels at week 4 (Appendix Table 6.5 and Appendix Table 6.6). Fatty acid composition (weight %) of male liver).

Males had a higher relative composition of total hepatic n-6 PUFA than females at weeks 6, 8, and 9, but differences within individual fatty acids were subtle. There was a significant sex x time interaction for relative LA compositions, which increased above baseline at week 2 in females and at week 3 in males (Appendix Table 6.5 and Appendix Table 6.6). Relative ARA levels were higher in males than females at week 6, but concentrations of LA and ARA were not significant for sex differences or sex x time interactions.

Relative compositions of total MUFA increased above baseline at week 3 in both sexes, suggesting a weaning effect. A sex x time interaction was also shown in 18:1n-7 and 18:1n-9. In females, 18:1n-7 started at the same relative percentage as males, and remained at baseline levels at week 9, whereas males increased significantly. Relative percentages of 18:1n-9 for females were equal to males at baseline, but increased significantly for females while males remained at baseline levels at week 9. hepatic total SFAs had a significant time effect, and 16:0 and 18:0 were significant for sex x time interactions. Palmitic acid dropped from week 1 to 9, and at week 8, was significantly lower in females. Conversely, 18:0 increased from week 1 to 9, and was significantly higher in females at weeks 8 and 9. Total concentrations were significant for sex differences, and were higher in females (Appendix Table 6.5 and Appendix Table 6.6).

6.3.4 Changes in the Fatty Acid Composition of Heart and Brain

DHA concentrations in the brain were significantly higher in females, but there was no sex x time interaction, and relative compositions of DHA and ARA, and ARA concentrations showed no sexual dimorphisms (Figure 9). Interestingly, brain relative percentages of 18:2n-6 began at the same levels in males and females, but were higher in males at week 9 (Appendix Table 6.7 and Appendix Table 6.8).

Relative fatty acid compositions in brain and heart were affected primarily by time. In the brain, relative compositions of DHA in both sexes peaked at weeks 3, 4 and 5, while ARA composition was highest at week 1 and decreased until week 9. In the heart, DHA relative composition or concentrations were not significant for sex x time interactions (Figure 9) but were significantly higher in females (Appendix Table 6.9 and Appendix Table 6.10). Total fatty acid concentrations in the brain were affected by time, whereas those in the heart were more stable. Total fatty acid concentrations in the brain increased above baseline immediately from week 1 to 2, and peaked at weeks 6, 7, and 9, in both sexes.

6.4 Discussion

To my knowledge, this study is the first to demonstrate that in the plasma and liver, 18 carbon PUFA increase above baseline levels in males before females, whereas LCPUFA increase above baseline in females first. This also appears to be the first study to show that total fatty acid concentrations in plasma, liver, brain, and heart peak during adolescence before decreasing entering adulthood.

Higher levels of ARA in the plasma and liver of adult females, and LA in in that of adult males, have been reported previously [105,113] and are confirmed in this study. Sexual dimorphisms in circulating levels of DHA and ARA have been linked to differences in estrogen [14,15,17]. In the rat, puberty begins in females and males at postnatal days 32-34 and 45-48 respectively [24,114,115]. This study goes to further show that these differences also interact with development. The relative percentage of plasma ARA increasing above baseline in females prior to males and remaining higher in females at adulthood supports the theory of elongation and desaturation being related to estrogen. However, further research directly linking estrogen to levels of circulating ARA increasing in females before males is required.

Elongation and desaturation enzymes critical to fatty acid remodelling and synthesis are found in the liver [116]. Greater relative percentages of DHA in female livers at week 9 despite similar relative percentages of ALA found in female and male livers at that time support previous reports of sexual dimorphisms in the expression and activity of elongase and desaturase enzymes [17,63], hepatic export and uptake of fatty acids [117–119], and fatty acid metabolism [120]. In particular, higher relative percentages of DHA in female livers despite similar ALA amounts supports previous data of females having greater expression and activity of delta 5 and delta 6 desaturase [105]. This increase in the relative percent of hepatic DHA in females as compared with the higher hepatic relative percentage of ARA in males, despite similar levels of LA, suggests females may also have a greater selectivity for elongating and desaturating DHA precursors.

Concentrations and relative percentages of the DHA precursor ALA could be increasing above baseline earlier in female as compared with male livers due to a variety of mechanisms. Despite being low compared to adolescence and adulthood, estrogen levels are higher in prepubescent females than males [23], in rats, females begin adolescence earlier than males [24], and females have a higher expression of fatty acid transport proteins [117,118,121]. This, combined with the chow diet being composed of more ALA than DHA could explain the greater amounts of LCPUFA precursors in female livers, but further research is required to confirm which combination or individual mechanism leads to the earlier increase of hepatic ALA in females.

Sex differences in liver and plasma fatty acids were not limited to PUFA. Sexual dimorphisms in relative percent [122] and concentrations of 16:0 and 18:0 of plasma is in agreement with previous animal models. Differences in plasma 16:0 concentrations alone has been shown in some animal models [112,123], and differences in 18:0 concentrations alone in others [122], but this study shows differences in both occurring simultaneously. Females show lower relative percentages of 16:0 starting at week 7,

and higher percentages of 18:0 at week 8. Relative percentages of 16:0 decrease from week 7 to week 9 in both sexes. However, 18:0 increases from week 7 to week 9 in females only. No sex differences in MUFA were shown, although higher levels of 16:1 [112] and 18:1n-7 have been reported at later timepoints than those observed in this study [122]. This suggests that at a young age, females may have a greater ability to convert 16:0 to other fatty acids and are converting it to 18:0 at a greater rate than males.

Hepatic sexual dimorphisms in 18:0, 18:1n-9 and 18:1n-7 concentrations [112,123] were confirmed, but differences in 16:0 [112] were not, possibly as a result of not separating PL from TAG and other lipid fractions. Higher relative percentages of hepatic 18:1n-7 in males, and nearly significantly higher hepatic levels of 18:1n9 in females at week 6 ($p = 0.059$), occurred alongside higher relative percentages of 18:0 in females and higher amounts of 16:0 in males. This is in agreement with previous studies indicating that estradiol and progesterone increase the protein levels of stearoyl-CoA desaturase 1 (SCD1) [110], a delta-9 desaturase that desaturates 18:0 to 18:1n-9 [124]. However, these findings are not in agreement with previous findings of 18:1n-7 in the PL fraction being increased in 12 week old ovariectomized rats given progesterone and estradiol [123]. This could be due to differences occurring in older age, an interaction with additional sex hormones affected by ovariectomy models, or differences in PL that were not detected in the total lipid extracts of this study.

In contrast to the plasma and hepatic fatty acid changes, brain and heart tissue were relatively stable. There were no sex x time interactions for relative percentages or concentrations of DHA or ARA, but there was a sex difference for DHA concentrations being generally higher in female brain. The lack of sex differences in the relative percentage of DHA is in agreement with previous data [92,105]. In contrast to DHA, brain PUFA and MUFA did show sex x time interactions. The 18:1n-9 spike in females at week 6, and the nearly significant ($p=0.055$) sex differences in total brain fatty acid

concentrations were unexpected, but could have implications for sex differences shown in high fat diet rodent models [125].

The heart had fewer significant concentration sex x time interactions than the brain, but relative percentage measures were similar, and in both the brain and the heart, LA had a significant sex x time interaction. The greater amounts of LA in the male brain is in agreement with previous data [126], but the greater amounts of LA in the male heart is not [105]. These sex differences in tissue LA relative percentages could be the result of higher levels of plasma LA in males, which are significantly higher beginning at week 7, being deposited at a greater rate in male tissues.

In both sexes, the peaking of total fatty acids in plasma at weeks 2 and 5 suggest high amounts of fatty acid turnover during weaning and puberty, and higher circulating fatty acid concentrations at week 5 appeared to increase the fatty acid concentrations of surrounding tissues. Total fatty acid concentrations in liver appeared to be reflected in plasma total fatty acid concentrations, with amounts peaking at week 5 before decreasing. This supports studies showing the liver playing a role in fatty acid remodelling [116], and the sexual dimorphisms in remodelling could play a role in sexual dimorphisms shown in other tissues [105]. Total fatty acid concentrations in the brain peaked at week 6, and were maintained at those concentrations for weeks 7 and 9, in agreement with findings that most fatty acid accumulation in the brain occurs during early development [3]. However, relative levels of fatty acids continued to change in the brain despite the steady total fatty acid concentration. As the brain developed, the relative percentage of ARA decreased in both sexes while DHA remained steady, supporting previous literature showing a specific need for DHA in the brain [73].

The design of this pilot study limited its findings. The fatty acid data is limited to total lipid fatty acid analyses, and differences between PL, TAG, and other lipid classes were not determined. Pups were chosen out of convenience and from mothers excluded by a previous study as a result of a failure to

observe the vaginal plug after breeding. As a result, the block design of the litters were limited to one litter per time point when multiple litters should have been used [127]. Pups being weaned abruptly at 3 weeks of age could be affecting plasma and tissue concentrations at weeks 4 and 5, as free living rats are breastfed for longer, and weaned more gradually [128]. Additionally, sex hormones also factor into adipose tissue lipolysis [129], and sexual dimorphisms have been shown in intestinal fatty acid uptake [130–132]. Collecting adipose tissue and intestines could have provided more insights into how sexual dimorphisms in tissues were affecting sexual dimorphisms in the plasma, and vice versa. Finally, these findings are limited to rats fed a standard chow diet high in LA, and providing the rats with different diets during development could have interacted with changes in tissue fatty acid composition [126]. Further research including adipose tissue analysis, and the interaction of different diets and maturity on sexual dimorphisms should be conducted.

6.5 Conclusion

This study confirms previous reports of sexual dimorphisms in n-3 and n-6 PUFA, SFA, and MUFA, and provides evidence that these dimorphisms interact with development. In chow fed rats, females have higher levels of circulating ARA beginning at 8 weeks of age, while males have higher levels of plasma LA beginning at weeks 7, which appear to influence brain and heart LA levels. Relative percentages of hepatic ALA and LA appear to increase above baseline earlier in females than males, potentially playing a role in DHA and ARA levels, which could impact development. Finally, the peak in total fatty acid concentrations occurring around puberty in plasma and brain suggest further research investigating the influence of diets, developmental stages, and sexual dimorphisms on tissue fatty acid composition should be conducted.

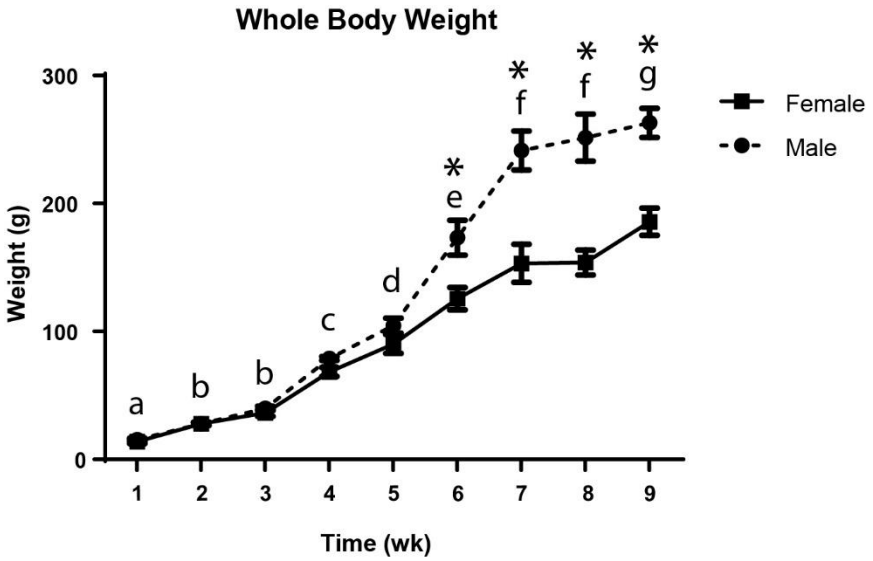

Figure 8: Whole Body Weights *****Significant sex differences, and letters represent significant time differences determined by Tukey's post hoc following significant two-way ANOVA ($p<0.05$). Mean ± SD, n = 5 at each point.

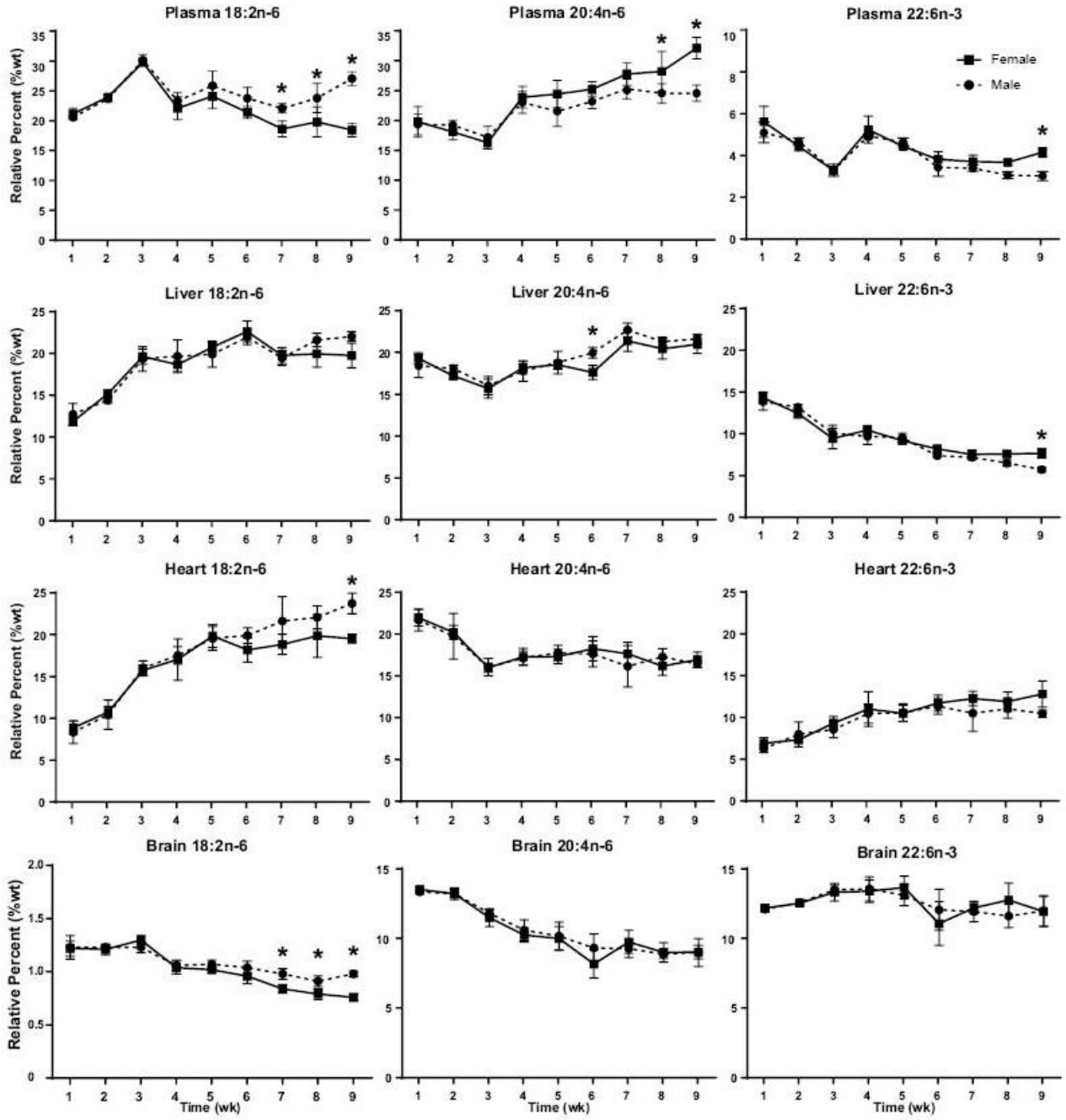

Figure 9: Relative percent (%wt) levels of LA, ARA and DHA in male and female tissues *****Significant sex difference determined by Tukey's post hoc following significant two-way ANOVA ($p<0.05$). Mean ± SD, n = 5 at each point.

Appendix Table 6.1: Fatty acid composition (weight %) of female stomach contents

	Week 1	Week 2	Week 3	P Sex x time	P Sex Effect
14:0	9.53 ± 0.75^{a}	10.67 ± 0.55^{b}	7.80 ± 0.54^{c}	0.84	0.14
16:0	22.90 ± 0.99^{a}	23.79 ± 0.68^{a}	17.18 ± 0.21^{b}	0.91	0.09
18:0	3.20 ± 0.18^{a}	3.26 ± 0.11^{a}	2.98 ± 0.03^{b}	0.41	0.02
22:0	0.04 ± 0.01^{a}	0.04 ± 0.01^{a}	0.08 ± 0.02^{b}	0.63	0.66
24:0	0.06 ± 0.01^{a}	0.06 ± 0.01^{a}	0.10 ± 0.02^{b}	0.72	0.75
Total SFA	53.91 ± 1.94^{a}	57.91 ± 1.43^{b}	50.95 ± 2.90^{a}	0.68	0.67
16:1	1.24 ± 0.11^{a}	0.86 ± 0.06^{b}	1.00 ± 0.05^{c}	0.20	<0.01
18:1n-7	1.34 ± 0.15^{a}	0.96 ± 0.06^{b}	1.07 ± 0.05^{b}	0.91	0.04
18:1n-9	15.26 ± 0.90^{a}	13.77 ± 0.57^{b}	14.35, ± 0.50^{ab}	0.97	0.11
24:1n-9	0.03 ± 0.01^{a}	0.02, ± 0.01^{ab}	0.02 ± 0.01^{b}	0.71	0.56
Total MUFA	18.20 ± 1.21^{a}	15.89 ± 0.69^{b}	16.70 ± 0.53^{b}	0.94	0.07
18:2n-6	20.75 ± 0.41^{a}	21.02 ± 0.57^{a}	27.43 ± 2.29^{b}	0.62	0.97
18:3n-6	0.52 ± 0.05^{a}	0.32 ± 0.02^{b}	0.13 ± 0.01^{c}	0.14	0.14
20:2n-6	0.59 ± 0.06^{a}	0.43 ± 0.03^{b}	0.24 ± 0.02^{c}	0.53	0.03
20:3n-6	0.64 ± 0.04^{a}	0.32 ± 0.02^{b}	0.12 ± 0.01^{c}	0.84	0.37
20:4n-6	1.40 ± 0.17^{a}	0.80 ± 0.03^{b}	0.50 ± 0.04^{c}	0.59	0.04
22:4n-6	0.29 ± 0.06^{a}	0.15 ± 0.01^{b}	0.09 ± 0.02^{b}	0.53	0.02
22:5n-6	0.05 ± 0.01^{a}	0.03 ± 0.01^{b}	0.03 ± 0.01^{b}	0.24	0.03
Total n-6 PUFA	24.28 ± 0.76^{a}	23.10 ± 0.66^{a}	28.55 ± 2.19^{b}	0.62	0.94
18:3n-3	1.79 ± 0.06^{a}	2.01 ± 0.03^{a}	2.88 ± 0.26^{b}	0.57	0.58
20:5n-3	0.45 ± 0.02^{a}	0.35 ± 0.01^{b}	0.28 ± 0.02^{c}	0.36	0.91
22:5n-3	0.36 ± 0.05^{a}	0.30 ± 0.02^{b}	0.22 ± 0.02^{c}	0.76	0.04
22:6n-3	0.43 ± 0.04^{a}	0.33 ± 0.01^{b}	0.35 ± 0.01^{b}	0.74	0.58
Total n-3 PUFA	3.10 ± 0.06^{a}	3.06 ± 0.07^{a}	3.77 ± 0.26^{b}	0.56	0.79
20:3n-9	0.06 ± 0.01^{a}	0.04 ± 0.01^{b}	0.04 ± 0.01^{b}	0.36	0.03
Total FA (mg/g)	262 ± 17^{a}	264 ± 37^{a}	115 ± 37^{b}	0.56	0.94

Mean ± SD, n=5. Different alphabetical superscripts represent differences across time and different numerical superscripts represent sex x time interactions significantly different by Tukey's HSD following a significant *F*-value ($p< 0.05$) by two-way ANOVA. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; FA, fatty acids.

Appendix Table 6.2: Fatty acid composition (weight %) of male stomach contents

	Week 1	Week 2	Week 3	P Sex x time	P Sex Effect
14:0	9.71 ± 0.76^{ab}	11.07 ± 0.15^{a}	8.33 ± 1.13^{b}	0.84	0.14
16:0	22.88 ± 0.66^{a}	24.18 ± 0.33^{b}	17.52 ± 0.49^{c}	0.91	0.09
18:0	3.20 ± 0.14^{a}	3.19 ± 0.05^{a}	2.85 ± 0.12^{b}	0.41	0.02
22:0	0.04 ± 0.01^{a}	0.04 ± 0.01^{a}	0.09 ± 0.03^{b}	0.63	0.66
24:0	0.06 ± 0.01^{a}	0.06 ± 0.01^{a}	0.09 ± 0.02^{b}	0.72	0.75
Total SFA	54.65 ± 2.16^{ab}	58.92 ± 0.51^{a}	50.97 ± 4.50^{b}	0.68	0.67
16:1	1.18 ± 0.14^{a}	0.82 ± 0.03^{b}	0.90 ± 0.06^{b}	0.20	<0.01
18:1n-7	1.30 ± 0.14^{a}	0.92 ± 0.03^{b}	1.02 ± 0.06^{b}	0.91	0.04
18:1n-9	15.07 ± 1.01^{a}	13.35 ± 0.24^{b}	13.94 ± 0.82^{ab}	0.97	0.11
24:1n-9	0.03 ± 0.01^{a}	0.02 ± 0.01^{a}	0.02 ± 0.01^{a}	0.71	0.56
Total MUFA	17.91 ± 1.30^{a}	15.37 ± 0.30^{b}	16.13 ± 0.89^{b}	0.94	0.07
18:2n-6	20.54 ± 0.60^{a}	20.62 ± 0.17^{a}	27.91 ± 3.35^{b}	0.62	0.97
18:3n-6	0.48 ± 0.08^{a}	0.30 ± 0.01^{b}	0.13 ± 0.01^{c}	0.14	0.14
20:2n-6	0.64 ± 0.07^{a}	0.41 ± 0.02^{b}	0.20 ± 0.02^{c}	0.53	0.03
20:3n-6	0.64 ± 0.04^{a}	0.31 ± 0.02^{b}	0.11 ± 0.01^{c}	0.84	0.37
20:4n-6	1.35 ± 0.13^{a}	0.77 ± 0.02^{b}	0.46 ± 0.04^{c}	0.59	0.04
22:4n-6	0.30 ± 0.04^{a}	0.14 ± 0.01^{b}	0.08 ± 0.01^{c}	0.53	0.02
22:5n-6	0.05 ± 0.01^{a}	0.03 ± 0.01^{b}	0.02 ± 0.01^{b}	0.24	0.03
Total n-6 PUFA	24.03 ± 0.86^{a}	22.61 ± 0.22^{a}	28.92 ± 3.26^{b}	0.62	0.94
18:3n-3	1.78 ± 0.04^{a}	2.01 ± 0.02^{a}	2.99 ± 0.39^{b}	0.57	0.58
20:5n-3	0.46 ± 0.02^{a}	0.34 ± 0.01^{b}	0.29 ± 0.04^{b}	0.36	0.91
22:5n-3	0.39 ± 0.04^{a}	0.28 ± 0.01^{b}	0.20 ± 0.03^{c}	0.76	0.04
22:6n-3	0.43 ± 0.03^{a}	0.33 ± 0.01^{b}	0.35 ± 0.04^{b}	0.74	0.58
Total n-3 PUFA	3.13 ± 0.09^{a}	3.02 ± 0.03^{a}	3.87 ± 0.45^{b}	0.56	0.79
20:3n-9	0.06 ± 0.01^{a}	0.04 ± 0.01^{b}	0.03 ± 0.01^{b}	0.36	0.03
Total FA (mg/g)	271 ± 15^{a}	272 ± 23^{a}	105 ± 31^{b}	0.56	0.94

Mean ± SD, n=5. Different alphabetical superscripts represent differences across time and different numerical superscripts represent sex x time interactions significantly different by Tukey's HSD following a significant *F*-value ($p < 0.05$) by two-way ANOVA. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; FA, fatty acids.

Appendix Table 6.3: Fatty acid composition (weight %) of female plasma

	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9
14:0	2.44 ± 0.75^{ab}	3.14 ± 0.55^{a}	2.17 ± 0.38^{b}	0.68 ± 0.08^{c}	0.40 ± 0.02^{c}	0.43 ± 0.04^{c}	0.36 ± 0.05^{c}	0.31 ± 0.03^{c}	0.25 ± 0.03^{c}
16:0	21.44 ± 1.16^{6,7,8}	22.99 ± 0.47^{8}	19.90 ± 0.27^{4,5,6}	18.37 ± 0.33^{2,3,4}	17.37 ± 0.48^{2,3,4}	19.30 ± 0.97^{3,4,5}	19.18 ± 1.46^{3,4,5}	16.76 ± 1.32^{1,2}	15.13 ± 0.61^{1}
18:0	9.17 ± 0.44^{1}	9.57 ± 0.52^{1}	10.16 ± 0.62^{1,2}	10.30 ± 0.79^{1,2,3}	11.80 ± 1.20^{2,3,4}	11.89 ± 0.67^{3,4}	13.49 ± 0.96^{4,5}	15.15 ± 1.25^{5,6}	16.28 ± 0.55^{6}
22:0	0.14 ± 0.01^{a}	0.20 ± 0.02^{bcdf}	0.18 ± 0.02^{abc}	0.15 ± 0.03^{ab}	0.22 ± 0.02^{cdef}	0.20 ± 0.02^{bcdf}	0.24 ± 0.02^{de}	0.26 ± 0.06^{e}	0.17 ± 0.02^{af}
24:0	0.38 ± 0.07^{ade}	0.39 ± 0.03^{ab}	0.42 ± 0.03^{ac}	0.28 ± 0.04^{d}	0.38 ± 0.03^{ade}	0.45 ± 0.03^{bcefh}	0.56 ± 0.04^{fg}	0.59 ± 0.10^{g}	0.39 ± 0.04^{adh}
Σ SFA	36.29 ± 1.35^{4,5,6,7}	38.70 ± 0.94^{7}	35.07 ± 0.47^{3,4}	31.26 ± 1.32^{1,2}	30.99 ± 0.95^{1,2}	34.80 ± 1.91^{3,4}	37.07 ± 1.42^{4,5,6,7}	35.53 ± 1.28^{3,4,5,6}	33.50 ± 0.71^{2,3}
16:1	0.57 ± 0.09^{ad}	0.43 ± 0.03^{a}	0.47 ± 0.05^{ad}	0.74 ± 0.11^{bce}	0.75 ± 0.05^{be}	0.89 ± 0.07^{b}	0.76 ± 0.08^{be}	0.57 ± 0.07^{acf}	0.64 ± 0.11^{def}
18:1n-7	1.11 ± 0.05^{a}	0.86 ± 0.02^{b}	1.14 ± 0.04^{a}	1.53 ± 0.15^{c}	1.50 ± 0.09^{c}	1.40 ± 0.08^{cd}	1.23 ± 0.08^{ade}	1.37 ± 0.11^{cef}	1.19 ± 0.12^{af}
18:1n-9	7.63 ± 1.34^{ab}	7.23 ± 0.46^{ab}	7.84 ± 0.69^{ab}	8.17 ± 0.87^{a}	8.33 ± 0.83^{a}	7.61 ± 0.33^{ab}	6.48 ± 0.67^{bd}	6.47 ± 0.72^{bcd}	5.57 ± 0.35^{d}
24:1n-9	0.24 ± 0.04^{ad}	0.17 ± 0.04^{bcg}	0.22 ± 0.03^{abd}	0.14 ± 0.02^{c}	0.19 ± 0.02^{abc}	0.28 ± 0.02^{dfh}	0.36 ± 0.02^{e}	0.35 ± 0.05^{ef}	0.23 ± 0.03^{agh}
Σ MUFA	9.96 ± 1.43^{2,3,4,5}	9.20 ± 0.49^{1,2,3,4}	9.94 ± 0.72^{2,3,4,5}	10.78 ± 1.00^{3,4,5,6}	11.09 ± 0.87^{5,6}	10.37 ± 0.35^{2,3,4,5,6}	9.04 ± 0.76^{1,2,3}	9.02 ± 0.77^{1,2}	7.78 ± 0.39^{1}
18:2n-6	21.15 ± 0.95^{1,2,3,4}	23.96 ± 0.26^{4,5,6}	29.77 ± 0.49^{7}	22.10 ± 1.91^{2,3,4}	24.12 ± 2.01^{4,5,6}	21.40 ± 0.99^{1,2,3,4}	18.63 ± 1.31^{1}	19.78 ± 2.51^{1,2}	18.44 ± 1.15^{1}
18:3n-6	0.45 ± 0.04^{2,3,4,5}	0.33 ± 0.01^{1,2}	0.33 ± 0.06^{1,2}	0.59 ± 0.11^{5,6}	0.59 ± 0.06^{4,5,6}	0.56 ± 0.03^{3,4,5,6}	0.38 ± 0.07^{2}	0.37 ± 0.09^{2}	0.44 ± 0.04^{2,3,4}
20:2n-6	0.38 ± 0.05^{8,9}	0.34 ± 0.02^{7,8,9}	0.26 ± 0.03^{3,4,5}	0.27 ± 0.02^{4,5,6,7}	0.26 ± 0.01^{3,4,5}	0.19 ± 0.01^{1,2,3}	0.17 ± 0.02^{1}	0.20 ± 0.06^{1,2,3,4}	0.17 ± 0.03^{1,2}
20:3n-6	0.95 ± 0.03^{8}	0.67 ± 0.02^{7}	0.41 ± 0.02^{4,5,6}	0.28 ± 0.02^{1,2}	0.29 ± 0.02^{1,2}	0.25 ± 0.02^{1}	0.29 ± 0.03^{1,2}	0.35 ± 0.08^{2,3,4,5}	0.31 ± 0.04^{1,2,3}
20:4n-6	19.78 ± 2.59^{1,2,3,4}	18.09 ± 1.32^{1,2}	16.30 ± 1.04^{1}	23.90 ± 1.81^{4,5,6}	24.43 ± 2.29^{5,6,7}	25.25 ± 1.26^{5,6,7}	27.78 ± 1.91^{6,7}	28.21 ± 3.33^{7,8}	32.14 ± 1.80^{8}
22:4n-6	0.40 ± 0.04^{a}	0.30 ± 0.02^{b}	0.30 ± 0.06^{b}	0.39 ± 0.04^{a}	0.27 ± 0.02^{bc}	0.22 ± 0.04^{c}	0.23 ± 0.03^{bc}	0.24 ± 0.04^{bc}	0.26 ± 0.04^{bc}
22:5n-6	0.20 ± 0.03^{2,3,4}	0.12 ± 0.02^{1,2,3,4}	0.23 ± 0.06^{4,5}	0.33 ± 0.06^{5,6}	0.20 ± 0.01^{2,3,4}	0.22 ± 0.03^{4}	0.23 ± 0.14^{4,5}	0.15 ± 0.02^{1,2,3,4}	0.13 ± 0.03^{1,2,3,4}
Σ n-6 PUFA	43.40 ± 1.74^{a}	43.86 ± 1.51^{a}	47.64 ± 1.02^{b}	47.94 ± 0.85^{b}	50.18 ± 0.68^{bc}	48.20 ± 1.54^{b}	47.85 ± 1.84^{b}	49.42 ± 1.29^{bc}	51.94 ± 1.03^{c}
18:3n-3*****	0.66 ± 0.17^{ab}	0.73 ± 0.10^{ab}	0.97 ± 0.13^{a}	0.92 ± 0.22^{ab}	0.92 ± 0.15^{ab}	0.87 ± 0.06^{ab}	0.70 ± 0.17^{ab}	0.65 ± 0.18^{ab}	0.64 ± 0.10^{b}
20:5n-3*****	1.14 ± 0.13^{acde}	1.10 ± 0.07^{acdef}	0.75 ± 0.04^{b}	1.36 ± 0.23^{a}	1.31 ± 0.13^{a}	0.96 ± 0.11^{bc}	0.87 ± 0.16^{bd}	0.88 ± 0.10^{be}	0.84 ± 0.09^{bf}
22:5n-3	1.45 ± 0.20^{5,6}	1.53 ± 0.09^{6}	0.99 ± 0.08^{4}	1.27 ± 0.09^{5}	0.82 ± 0.06^{3,4}	0.54 ± 0.06^{1,2}	0.39 ± 0.15^{1}	0.51 ± 0.04^{1,2}	0.54 ± 0.05^{1,2}
22:6n-3	5.61 ± 0.74^{8}	4.44 ± 0.22^{4,5,6}	3.29 ± 0.17^{1,2}	5.24 ± 0.64^{7,8}	4.43 ± 0.11^{4,5,6}	3.81 ± 0.37^{2,3,4}	3.71 ± 0.30^{1,2,3,4}	3.67 ± 0.13^{1,2,3}	4.15 ± 0.23^{3,4,5}
Σ n-3 PUFA	8.92 ± 0.85^{a}	7.85 ± 0.27^{bd}	6.05 ± 0.17^{c}	8.84 ± 0.82^{ab}	7.52 ± 0.40^{d}	6.21 ± 0.51^{c}	5.70 ± 0.52^{c}	5.74 ± 0.24^{c}	6.19 ± 0.19^{c}
20:3n-9	0.13 ± 0.03^{ab}	0.14 ± 0.02^{a}	0.15 ± 0.04^{a}	0.15 ± 0.02^{a}	0.09 ± 0.01^{bc}	0.06 ± 0.01^{cd}	0.04 ± 0.01^{d}	0.04 ± 0.01^{de}	0.05 ± 0.01^{cdf}
Σ FA (mg/g)	69 ± 13^{a}	144 ± 22^{b}	92 ± 18^{ac}	94 ± 36^{ac}	132 ± 22^{bcde}	112 ± 11^{ab}	97 ± 7^{ad}	98 ± 22^{ae}	82 ± 15^{a}

Mean ± SD, n=5. Different numerical superscripts represent sex x time interactions and different alphabetical superscripts represent differences across time that are significantly different by Tukey's HSD following a significant *F*-value ($p< 0.05$) by two-way ANOVA. *Indicates a significant main effect of sex by two-way ANOVA ($p< 0.05$). SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; FA, fatty acids.

Appendix Table 6.4: Fatty acid composition (weight %) of male plasma

	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9
14:0	2.68 ± 0.48^{a}	2.94 ± 0.23^{a}	1.92 ± 0.59^{b}	0.73 ± 0.04^{c}	0.49 ± 0.07^{c}	0.49 ± 0.07^{c}	0.38 ± 0.14^{c}	0.31 ± 0.01^{c}	0.28 ± 0.05^{c}
16:0	22.06 ± 1.50^{7,8}	22.96 ± 0.58^{8}	19.82 ± 0.66^{4,5,6}	18.90 ± 0.44^{3,4,5}	18.42 ± 0.62^{2,3,4}	20.58 ± 1.04^{5,6,7}	20.76 ± 0.68^{5,6,7}	19.83 ± 0.79^{4,5,6}	17.79 ± 0.79^{2,3}
18:0	9.33 ± 0.56^{1}	9.89 ± 0.36^{1}	10.34 ± 0.58^{1,2,3}	9.45 ± 0.60^{1}	10.58 ± 1.33^{1,2,3}	10.17 ± 0.43^{1,2}	10.65 ± 0.41^{1,2,3}	10.74 ± 0.54^{1,2,3}	10.76 ± 0.46^{1,2,3}
22:0	0.17 ± 0.07^{ab}	0.19 ± 0.02^{abc}	0.19 ± 0.02^{abcd}	0.13 ± 0.01^{a}	0.20 ± 0.02^{bcdf}	0.24 ± 0.03^{cd}	0.25 ± 0.01^{d}	0.25 ± 0.02^{cde}	0.15 ± 0.02^{af}
24:0	0.43 ± 0.05^{ae}	0.39 ± 0.03^{ace}	0.43 ± 0.03^{ae}	0.28 ± 0.04^{b}	0.34 ± 0.04^{bc}	0.45 ± 0.04^{a}	0.55 ± 0.04^{d}	0.56 ± 0.07^{d}	0.36 ± 0.01^{be}
Σ SFA	37.89 ± 1.56^{5,6,7}	38.09 ± 0.75^{6,7}	34.62 ± 0.86^{3,4}	30.78 ± 1.03^{1}	30.82 ± 0.80^{1}	34.43 ± 1.86^{3,4}	35.42 ± 0.54^{3,4,5}	34.96 ± 1.37^{3,4}	30.57 ± 0.44^{1}
16:1	0.56 ± 0.03^{a}	0.43 ± 0.01^{a}	0.44 ± 0.02^{a}	0.78 ± 0.11^{b}	0.88 ± 0.09^{bc}	0.96 ± 0.07^{c}	0.76 ± 0.15^{b}	0.55 ± 0.07^{a}	0.55 ± 0.04^{a}
18:1n-7	1.06 ± 0.04^{a}	0.88 ± 0.03^{b}	1.12 ± 0.02^{a}	1.54 ± 0.12^{cd}	1.64 ± 0.06^{ce}	1.57 ± 0.08^{cd}	1.43 ± 0.05^{d}	1.75 ± 0.08^{e}	1.75 ± 0.08^{ef}
18:1n-9	7.46 ± 0.67^{adef}	7.10 ± 0.29^{abf}	7.34 ± 0.59^{acf}	8.30 ± 0.76^{adeg}	8.93 ± 0.65^{d}	7.66 ± 0.90^{adef}	7.00 ± 1.00^{bcef}	6.61 ± 0.43^{f}	6.93 ± 0.47^{bcfg}
24:1n-9	0.25 ± 0.02^{ad}	0.18 ± 0.03^{bc}	0.22 ± 0.02^{ab}	0.14 ± 0.03^{c}	0.17 ± 0.03^{bc}	0.31 ± 0.02^{d}	0.38 ± 0.02^{e}	0.38 ± 0.05^{e}	0.17 ± 0.03^{bc}
Σ MUFA	9.74 ± 0.68^{2,3,4,5}	9.05 ± 0.29^{1,2,3}	9.38 ± 0.63^{1,2,3,4,5}	10.92 ± 0.91^{4,5,6}	12.00 ± 0.68^{6}	10.72 ± 0.92^{2,3,4,5,6}	9.80 ± 1.18^{2,3,4,5}	9.56 ± 0.36^{2,3,4,5}	9.61 ± 0.46^{2,3,4,5}
18:2n-6	20.57 ± 0.24^{1,2,3}	23.69 ± 0.58^{3,4,5}	30.14 ± 0.96^{7}	23.32 ± 1.34^{3,4,5}	25.85 ± 2.45^{5,6}	23.74 ± 1.85^{3,4,5,6}	22.08 ± 0.77^{2,3,4}	23.78 ± 2.45^{3,4,5,6}	27.04 ± 0.74^{6,7}
18:3n-6	0.42 ± 0.03^{2,3}	0.34 ± 0.01^{1,2}	0.38 ± 0.07^{2}	0.64 ± 0.15^{6}	0.54 ± 0.10^{3,4,5,6}	0.33 ± 0.03^{1,2}	0.21 ± 0.03^{1}	0.21 ± 0.02^{1}	0.20 ± 0.05^{1}
20:2n-6	0.41 ± 0.06^{9}	0.34 ± 0.02^{6,7,8,9}	0.25 ± 0.04^{2,3,4}	0.27 ± 0.04^{4,5,6,7}	0.25 ± 0.01^{3,4}	0.25 ± 0.04^{3,4}	0.23 ± 0.01^{1,2,3,4}	0.27 ± 0.02^{3,4,5,6}	0.33 ± 0.04^{5,6,7,8}
20:3n-6	0.96 ± 0.08^{8}	0.67 ± 0.01^{7}	0.44 ± 0.04^{6}	0.26 ± 0.02^{1,2}	0.29 ± 0.03^{1,2}	0.34 ± 0.03^{1,2,3,4,5}	0.32 ± 0.03^{1,2,3,4}	0.40 ± 0.06^{3,4,5,6}	0.43 ± 0.06^{5,6}
20:4n-6	19.36 ± 1.72^{1,2,3}	19.24 ± 0.79^{1,2,3}	17.19 ± 1.83^{1}	23.04 ± 1.85^{3,4,5}	21.53 ± 2.52^{2,3,4,5}	23.20 ± 1.21^{3,4,5}	25.29 ± 1.67^{5,6,7}	24.57 ± 1.62^{5,6,7}	24.56 ± 1.35^{5,6,7}
22:4n-6	0.36 ± 0.02^{ac}	0.27 ± 0.02^{bd}	0.29 ± 0.04^{ab}	0.37 ± 0.05^{c}	0.27 ± 0.03^{bd}	0.22 ± 0.02^{d}	0.24 ± 0.02^{bd}	0.23 ± 0.04^{bd}	0.29 ± 0.05^{bd}
22:5n-6	0.13 ± 0.02^{1,2,3,4}	0.10 ± 0.01^{1,2}	0.23 ± 0.07^{4,5}	0.37 ± 0.05^{6}	0.21 ± 0.04^{3,4}	0.12 ± 0.02^{1,2,3,4}	0.11 ± 0.01^{1,2,3}	0.10 ± 0.02^{1,2}	0.07 ± 0.01^{1}
Σ n-6 PUFA	42.28 ± 1.68^{a}	44.69 ± 0.84^{a}	48.99 ± 1.07^{b}	48.32 ± 0.75^{b}	49.00 ± 0.40^{b}	48.32 ± 1.22^{b}	48.61 ± 1.55^{b}	49.68 ± 1.33^{b}	52.97 ± 0.74^{c}
18:3n-3*****	0.67 ± 0.07^{a}	0.73 ± 0.06^{ab}	0.87 ± 0.18^{ab}	0.99 ± 0.18^{ab}	1.03 ± 0.19^{b}	0.90 ± 0.22^{ab}	0.73 ± 0.12^{ab}	0.76 ± 0.12^{ab}	0.98 ± 0.15^{ab}
20:5n-3*****	1.09 ± 0.07^{acf}	1.07 ± 0.07^{acf}	0.80 ± 0.05^{a}	1.39 ± 0.20^{bg}	1.39 ± 0.19^{b}	1.13 ± 0.17^{bcdeg}	0.97 ± 0.10^{adh}	0.96 ± 0.08^{aeh}	1.10 ± 0.17^{fgh}
22:5n-3	1.30 ± 0.13^{5}	1.45 ± 0.11^{5,6}	1.02 ± 0.13^{4}	1.32 ± 0.10^{5,6}	0.84 ± 0.05^{3,4}	0.60 ± 0.06^{1,2}	0.58 ± 0.05^{1,2}	0.59 ± 0.04^{1,2}	0.69 ± 0.13^{2,3}
22:6n-3	5.10 ± 0.49^{6,7,8}	4.67 ± 0.17^{5,6,7}	3.29 ± 0.29^{1,2}	4.94 ± 0.08^{6,7,8}	4.63 ± 0.22^{5,6,7}	3.43 ± 0.43^{1,2,3}	3.39 ± 0.14^{1,2}	3.06 ± 0.16^{1,2}	3.02 ± 0.22^{1}
Σ n-3 PUFA	8.22 ± 0.42^{a}	7.95 ± 0.30^{a}	6.01 ± 0.31^{b}	8.67 ± 0.38^{a}	7.94 ± 0.37^{a}	6.10 ± 0.71^{b}	5.70 ± 0.16^{b}	5.41 ± 0.14^{b}	5.83 ± 0.49^{b}
20:3n-9	0.11 ± 0.01^{ad}	0.13 ± 0.01^{ab}	0.16 ± 0.03^{b}	0.15 ± 0.02^{bc}	0.09 ± 0.01^{de}	0.05 ± 0.01^{ef}	0.04 ± 0.01^{f}	0.04 ± 0.01^{fg}	0.04 ± 0.01^{fh}
Σ FA (mg/g)	88 ± 17^{a}	136 ± 13^{b}	94 ± 25^{a}	114 ± 19^{ab}	131 ± 24^{b}	109 ± 24^{ab}	109 ± 4^{ab}	105 ± 6^{ab}	83 ± 7^{a}

Mean ± SD, n=5. Different numerical superscripts represent sex x time interactions and different alphabetical superscripts represent differences across time that are significantly different by Tukey's HSD following a significant *F*-value ($p< 0.05$) by two-way ANOVA. *Indicates a significant main effect of sex by two-way ANOVA ($p< 0.05$). SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; FA, fatty acids.

Appendix Table 6.5: Fatty acid composition (weight %) of female liver

	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9
14:0	0.91 ± 0.17^{ac}	1.26 ± 0.15^{ab}	1.43 ± 0.51^{b}	0.59 ± 0.07^{cd}	0.34 ± 0.02^{dg}	0.28 ± 0.03^{deg}	0.19 ± 0.02^{dfg}	0.16 ± 0.02^{g}	0.18 ± 0.02^{dgh}
16:0	23.26 ± 0.46^{5}	23.17 ± 0.25^{5}	20.16 ± 0.50^{4}	18.79 ± 0.38^{3}	17.49 ± 0.33^{2}	16.90 ± 0.31^{2}	16.37 ± 0.41^{2}	15.02 ± 0.80^{1}	16.47 ± 0.37^{2}
18:0	$15.94 \pm 0.41^{1,2,3}$	15.09 ± 0.53^{1}	$15.77 \pm 0.95^{1,2}$	$16.48 \pm 0.57^{1,2,3,4}$	$17.37 \pm 0.64^{2,3,4,5}$	$16.89 \pm 1.30^{1,2,3,4}$	$20.03 \pm 0.48^{6,7}$	20.84 ± 1.63^{7}	$20.19 \pm 0.88^{6,7}$
22:0	$0.17 \pm 0.02^{2,3,4}$	0.12 ± 0.01^{1}	$0.14 \pm 0.01^{1,2}$	$0.17 \pm 0.02^{2,3,4}$	$0.19 \pm 0.01^{3,4,5}$	$0.15 \pm 0.02^{1,2,3}$	$0.19 \pm 0.03^{3,4,5}$	$0.21 \pm 0.02^{4,5,6}$	$0.14 \pm 0.01^{1,2}$
24:0	$0.53 \pm 0.09^{1,2,3,4,5}$	$0.54 \pm 0.04^{2,3,4,5,6}$	$0.49 \pm 0.06^{1,2,3}$	$0.48 \pm 0.05^{1,2}$	$0.52 \pm 0.02^{1,2,3,4,5}$	$0.46 \pm 0.06^{1,2}$	$0.60 \pm 0.02^{3,4,5,6,7}$	0.74 ± 0.06^{8}	$0.44 \pm 0.05^{1,2}$
Σ SFA	42.29 ± 0.51^{a}	41.41 ± 0.39^{a}	39.51 ± 0.28^{b}	37.59 ± 0.86^{c}	37.01 ± 0.47^{cd}	35.76 ± 1.46^{d}	38.55 ± 0.57^{bc}	38.21 ± 1.14^{bc}	38.50 ± 0.93^{bc}
16:1*****	0.23 ± 0.04^{a}	0.18 ± 0.02^{a}	0.34 ± 0.14^{ab}	0.48 ± 0.07^{b}	0.53 ± 0.09^{bcd}	0.70 ± 0.09^{d}	0.52 ± 0.08^{bde}	0.46 ± 0.13^{bf}	0.48 ± 0.08^{bg}
18:1n-7	$1.53 \pm 0.03^{3,4}$	$1.31 \pm 0.03^{1,2}$	$1.65 \pm 0.04^{4,5,6}$	$1.74 \pm 0.03^{5,6,7}$	$1.88 \pm 0.07^{7,8}$	$1.89 \pm 0.10^{7,8,9}$	$1.79 \pm 0.16^{5,6,7}$	$1.89 \pm 0.15^{7,8,9}$	$1.67 \pm 0.09^{4,5,6}$
18:1n-9	3.77 ± 0.40^{1}	$4.96 \pm 0.35^{1,2,3,4}$	$7.14 \pm 1.37^{6,7}$	$6.53 \pm 0.58^{4,5,6,7}$	$6.73 \pm 0.50^{5,6,7}$	7.70 ± 0.79^{7}	$5.87 \pm 0.66^{2,3,4,5,6}$	$6.26 \pm 0.95^{4,5,6,7}$	$5.91 \pm 0.61^{2,3,4,5,6}$
24:1n-9*****	0.25 ± 0.03^{a}	0.21 ± 0.02^{acd}	0.15 ± 0.03^{bef}	0.17 ± 0.01^{bcef}	0.20 ± 0.01^{bdg}	0.15 ± 0.02^{e}	0.20 ± 0.02^{dfg}	0.22 ± 0.03^{ag}	0.13 ± 0.02^{eh}
Σ MUFA	6.08 ± 0.38^{1}	$6.88 \pm 0.34^{1,2,3}$	$9.49 \pm 1.55^{4,5,6}$	$9.09 \pm 0.65^{4,5,6}$	$9.51 \pm 0.62^{5,6}$	10.61 ± 0.90^{6}	$8.53 \pm 0.57^{3,4,5}$	$9.00 \pm 0.87^{4,5,6}$	$8.34 \pm 0.61^{3,4,5}$
18:2n-6	11.87 ± 0.49^{1}	15.17 ± 0.47^{2}	$19.63 \pm 0.93^{3,4,5,6}$	18.68 ± 0.84^{3}	$20.77 \pm 0.58^{3,4,5,6,7}$	22.63 ± 1.23^{7}	$19.75 \pm 0.96^{3,4,5,6}$	$19.97 \pm 1.59^{3,4,5,6}$	$19.77 \pm 1.47^{3,4,5,6}$
18:3n-6	$0.12 \pm 0.01^{1,2}$	0.04 ± 0.01^{1}	0.06 ± 0.01^{1}	$0.45 \pm 0.04^{5,6}$	$0.43 \pm 0.08^{4,5,6}$	0.50 ± 0.06^{6}	$0.30 \pm 0.10^{3,4,5}$	$0.35 \pm 0.15^{4,5,6}$	$0.38 \pm 0.06^{4,5,6}$
20:2n-6	$0.48 \pm 0.03^{4,5,6}$	$0.56 \pm 0.02^{5,6,7}$	$0.56 \pm 0.03^{5,6,7}$	$0.47 \pm 0.02^{3,4,5}$	$0.46 \pm 0.03^{3,4,5}$	$0.37 \pm 0.02^{1,2,3}$	0.34 ± 0.08^{1}	$0.35 \pm 0.09^{1,2}$	0.34 ± 0.04^{1}
20:3n-6	$0.82 \pm 0.02^{7,8}$	0.75 ± 0.04^{7}	$0.70 \pm 0.02^{6,7}$	0.33 ± 0.02^{1}	$0.37 \pm 0.02^{1,2,3}$	0.32 ± 0.01^{1}	$0.38 \pm 0.04^{1,2,3}$	$0.47 \pm 0.13^{2,3,4,5}$	$0.45 \pm 0.06^{1,2,3,4,5}$
20:4n-6	$19.29 \pm 0.68^{3,4,5,6}$	$17.19 \pm 0.32^{1,2,3}$	15.69 ± 1.11^{1}	$18.17 \pm 0.72^{2,3,4}$	$18.54 \pm 0.53^{3,4,5}$	$17.62 \pm 0.83^{1,2,3}$	$21.39 \pm 1.25^{6,7,8}$	$20.45 \pm 1.23^{5,6,7}$	$20.97 \pm 1.09^{6,7,8}$
22:4n-6	$0.76 \pm 0.03^{6,7}$	$0.67 \pm 0.04^{5,6}$	0.70 ± 0.13^{6}	$0.55 \pm 0.02^{4,5}$	$0.43 \pm 0.02^{1,2,3}$	$0.40 \pm 0.03^{1,2,3}$	$0.36 \pm 0.04^{1,2,3}$	$0.41 \pm 0.03^{1,2,3}$	$0.47 \pm 0.04^{3,4}$
22:5n-6	$0.24 \pm 0.01^{2,3,4}$	$0.20 \pm 0.01^{1,2,3,4}$	0.50 ± 0.12^{5}	0.49 ± 0.08^{5}	$0.26 \pm 0.05^{3,4}$	$0.27 \pm 0.05^{3,4}$	$0.16 \pm 0.04^{1,2,3,4}$	$0.18 \pm 0.07^{1,2,3,4}$	$0.20 \pm 0.02^{1,2,3,4}$
Σ n-6 PUFA	33.63 ± 0.42^{1}	34.59 ± 0.22^{1}	37.86 ± 0.48^{2}	$39.14 \pm 0.30^{3,4}$	$41.27 \pm 0.18^{6,7}$	$42.14 \pm 0.63^{7,8}$	$42.70 \pm 1.09^{8,9}$	$42.21 \pm 0.45^{7,8}$	$42.60 \pm 0.60^{8,9}$
18:3n-3	0.21 ± 0.03^{1}	$0.42 \pm 0.04^{1,2,3,4}$	$0.59 \pm 0.12^{3,4,5,6}$	$0.69 \pm 0.10^{5,6}$	$0.75 \pm 0.09^{5,6,7}$	0.99 ± 0.10^{7}	$0.71 \pm 0.15^{5,6}$	$0.77 \pm 0.18^{6,7}$	$0.77 \pm 0.15^{5,6,7}$
20:5n-3	0.43 ± 0.03^{acgi}	0.52 ± 0.02^{abd}	0.35 ± 0.03^{c}	0.62 ± 0.14^{d}	0.64 ± 0.05^{de}	0.63 ± 0.06^{df}	0.58 ± 0.10^{bdg}	0.61 ± 0.09^{dh}	0.60 ± 0.09^{bdi}
22:5n-3	$2.37 \pm 0.18^{5,6}$	3.39 ± 0.12^{7}	2.50 ± 0.30^{6}	2.02 ± 0.11^{4}	1.44 ± 0.10^{3}	1.02 ± 0.05^{1}	0.86 ± 0.13^{1}	0.98 ± 0.05^{1}	0.98 ± 0.07^{1}
22:6n-3	14.33 ± 0.69^{7}	12.44 ± 0.56^{6}	$9.44 \pm 1.22^{4,5}$	10.44 ± 0.53^{5}	$9.18 \pm 0.37^{4,5}$	$8.19 \pm 0.43^{3,4}$	$7.53 \pm 0.52^{2,3}$	$7.60 \pm 0.34^{2,3}$	$7.69 \pm 0.55^{2,3}$
Σ n-3 PUFA	17.41 ± 0.50^{7}	16.85 ± 0.53^{7}	$12.95 \pm 1.37^{5,6}$	13.82 ± 0.64^{6}	$12.07 \pm 0.33^{4,5}$	$10.87 \pm 0.43^{3,4}$	$9.73 \pm 0.52^{2,3}$	$10.00 \pm 0.15^{2,3}$	$10.07 \pm 0.37^{2,3}$
20:3n-9	0.08 ± 0.01^{ad}	0.12 ± 0.01^{bc}	0.12 ± 0.02^{b}	0.09 ± 0.01^{ac}	0.06 ± 0.01^{dgh}	0.05 ± 0.01^{def}	0.03 ± 0.01^{f}	0.03 ± 0.01^{fg}	0.03 ± 0.01^{fh}
Σ FA (mg/g) *****	26 ± 4^{a}	35 ± 2^{b}	35 ± 6^{ab}	44 ± 4^{cde}	43 ± 2^{bc}	48 ± 5^{c}	41 ± 3^{bc}	36 ± 5^{bd}	38 ± 5^{be}

Mean ± SD, n=5. Different numerical superscripts represent sex x time interactions and different alphabetical superscripts represent differences across time that are significantly different by Tukey's HSD following a significant *F*-value ($p < 0.05$) by two-way ANOVA. *Indicates a significant main effect of sex by two-way ANOVA ($p < 0.05$). SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; FA, fatty acids.

Appendix Table 6.6: Fatty acid composition (weight %) of male liver

	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9
14:0	1.23 ± 0.44[a]	1.12 ± 0.04[a]	1.23 ± 0.22[a]	0.57 ± 0.14[b]	0.32 ± 0.03[bc]	0.26 ± 0.04[bc]	0.16 ± 0.01[c]	0.14 ± 0.01[bd]	0.19 ± 0.04[bc]
16:0	23.20 ± 0.96[5]	23.02 ± 0.36[5]	20.39 ± 0.45[4]	18.71 ± 0.58[3]	17.52 ± 0.49[2]	17.43 ± 0.61[2]	17.38 ± 0.46[2]	16.68 ± 0.37[2]	17.48 ± 0.64[2]
18:0	15.07 ± 1.48[1]	15.12 ± 0.24[1]	15.88 ± 1.18[1,2]	15.75 ± 1.13[1,2]	17.65 ± 0.84[2,3,4,5]	17.28 ± 0.63[2,3,4,5]	19.05 ± 0.44[5,6,7]	18.40 ± 0.33[4,5,6]	17.95 ± 0.67[3,4,5]
22:0	0.17 ± 0.03[2,3]	0.14 ± 0.01[1,2]	0.15 ± 0.02[1,2]	0.19 ± 0.02[3,4,5]	0.19 ± 0.03[3,4,5]	0.19 ± 0.01[3,4,5]	0.24 ± 0.02[6]	0.22 ± 0.01[5,6]	0.14 ± 0.01[1,2]
24:0	0.62 ± 0.09[4,5,6,7]	0.64 ± 0.02[5,6,7,8]	0.51 ± 0.06[1,2,3,4]	0.48 ± 0.07[1,2]	0.50 ± 0.04[1,2,3,4,5]	0.48 ± 0.03[1,2]	0.65 ± 0.05[6,7,8]	0.68 ± 0.03[7,8]	0.41 ± 0.02[1]
Σ SFA	41.88 ± 0.27[a]	41.27 ± 0.35[ab]	39.56 ± 0.73[bd]	36.84 ± 1.50[c]	37.28 ± 1.34[ce]	36.72 ± 0.90[c]	38.77 ± 0.46[defg]	37.43 ± 0.23[bf]	37.39 ± 0.57[cg]
16:1*	0.23 ± 0.02[ae]	0.17 ± 0.01[a]	0.31 ± 0.11[aceg]	0.55 ± 0.09[bd]	0.51 ± 0.06[bd]	0.60 ± 0.09[b]	0.44 ± 0.06[cdf]	0.34 ± 0.03[efg]	0.40 ± 0.11[dg]
18:1n-7	1.45 ± 0.08[2,3]	1.22 ± 0.01[1]	1.61 ± 0.04[3,4,5]	1.81 ± 0.03[6,7]	1.89 ± 0.06[7,8,9]	2.06 ± 0.08[9]	1.99 ± 0.05[8,9]	2.30 ± 0.12[10]	2.26 ± 0.12[10]
18:1n-9	4.34 ± 0.68[1,2]	4.51 ± 0.21[1,2,3]	6.37 ± 0.74[4,5,6,7]	7.03 ± 1.13[6,7]	6.68 ± 1.21[5,6,7]	6.07 ± 0.49[3,4,5,6,7]	4.92 ± 0.38[1,2,3,4]	5.11 ± 0.15[1,2,3,4,5]	5.35 ± 0.65[1,2,3,4,5]
24:1n-9*	0.25 ± 0.03[a]	0.24 ± 0.01[ac]	0.18 ± 0.02[bf]	0.18 ± 0.02[bde]	0.20 ± 0.03[bcde]	0.17 ± 0.02[bf]	0.23 ± 0.02[ad]	0.23 ± 0.01[ae]	0.14 ± 0.01[f]
Σ MUFA	6.57 ± 0.59[1,2]	6.34 ± 0.20[1]	8.66 ± 0.85[4,5]	9.75 ± 1.21[5,6]	9.45 ± 1.22[4,5,6]	9.08 ± 0.50[4,5,6]	7.74 ± 0.39[1,2,3,4]	8.19 ± 0.15[2,3,4,5]	8.36 ± 0.76[3,4,5]
18:2n-6	12.72 ± 1.32[1,2]	14.42 ± 0.38[2]	19.36 ± 1.46[3,4]	19.67 ± 1.97[3,4,5,6]	19.91 ± 1.53[3,4,5,6]	21.95 ± 0.88[5,6,7]	19.47 ± 0.89[3,4,5]	21.62 ± 0.80[4,5,6,7]	22.03 ± 0.58[6,7]
18:3n-6	0.14 ± 0.04[1,2,3]	0.06 ± 0.04[1]	0.05 ± 0.01[1]	0.50 ± 0.14[6]	0.41 ± 0.17[4,5,6]	0.27 ± 0.03[2,3,4]	0.14 ± 0.02[1,2,3]	0.16 ± 0.03[1,2,3]	0.16 ± 0.02[1,2,3]
20:2n-6	0.52 ± 0.03[4,5,6,7]	0.52 ± 0.01[4,5,6,7]	0.59 ± 0.05[7]	0.47 ± 0.02[4,5]	0.45 ± 0.03[2,3,4]	0.50 ± 0.06[4,5,6,7]	0.46 ± 0.03[3,4,5]	0.51 ± 0.06[4,5,6,7]	0.58 ± 0.06[6,7]
20:3n-6	0.89 ± 0.10[8]	0.73 ± 0.01[7]	0.74 ± 0.07[7]	0.34 ± 0.02[1,2]	0.37 ± 0.04[1,2,3]	0.47 ± 0.05[3,4,5]	0.45 ± 0.06[1,2,3,4]	0.58 ± 0.11[5,6]	0.58 ± 0.06[4,5,6]
20:4n-6	18.40 ± 1.40[3,4,5]	18.10 ± 0.41[2,3,4]	16.05 ± 1.09[1,2]	17.79 ± 1.23[1,2,3]	18.80 ± 1.35[3,4,5]	19.97 ± 0.65[4,5,6,7]	22.69 ± 0.83[8]	21.30 ± 0.54[6,7,8]	21.64 ± 0.53[7,8]
22:4n-6	0.82 ± 0.05[7]	0.68 ± 0.02[6]	0.64 ± 0.10[5,6]	0.55 ± 0.04[4,5]	0.44 ± 0.06[2,3,4]	0.31 ± 0.08[1]	0.33 ± 0.03[1,2]	0.33 ± 0.03[1,2]	0.33 ± 0.02[1,2]
22:5n-6	0.25 ± 0.03[3,4]	0.19 ± 0.01[1,2,3,4]	0.48 ± 0.13[5]	0.50 ± 0.09[5]	0.29 ± 0.10[4]	0.13 ± 0.03[1,2,3]	0.09 ± 0.01[1]	0.10 ± 0.01[1,2]	0.09 ± 0.02[1]
Σ n-6 PUFA	33.77 ± 0.38[1]	34.72 ± 0.28[1]	37.93 ± 0.40[2,3]	39.84 ± 1.07[4,5]	40.69 ± 0.55[5,6]	43.62 ± 0.38[9,10]	43.66 ± 0.49[9,10]	44.63 ± 0.45[10,11]	45.44 ± 0.76[11]
18:3n-3	0.30 ± 0.10[1,2]	0.39 ± 0.02[1,2,3]	0.51 ± 0.12[2,3,4,5]	0.78 ± 0.17[6,7]	0.67 ± 0.16[4,5,6]	0.77 ± 0.13[5,6,7]	0.53 ± 0.06[2,3,4,5,6]	0.69 ± 0.07[5,6]	0.70 ± 0.08[5,6]
20:5n-3	0.51 ± 0.07[acefhi]	0.56 ± 0.03[abd]	0.36 ± 0.05[c]	0.69 ± 0.12[d]	0.58 ± 0.09[bdeg]	0.62 ± 0.09[bdfg]	0.51 ± 0.03[acghi]	0.60 ± 0.03[bdh]	0.61 ± 0.09[bdi]
22:5n-3	2.67 ± 0.32[6]	3.25 ± 0.13[7]	2.69 ± 0.16[6]	2.02 ± 0.24[4,5]	1.40 ± 0.10[2,3]	1.17 ± 0.06[1,2,3]	1.14 ± 0.12[1,2,3]	1.19 ± 0.10[1,2,3]	1.08 ± 0.10[1,2]
22:6n-3	13.86 ± 1.01[6,7]	13.17 ± 0.35[6,7]	10.05 ± 0.98[5]	9.69 ± 0.98[5]	9.53 ± 0.54[4,5]	7.39 ± 0.16[2,3]	7.20 ± 0.29[2,3]	6.51 ± 0.40[1,2]	5.71 ± 0.29[1]
Σ n-3 PUFA	17.40 ± 0.53[7]	17.44 ± 0.45[7]	13.69 ± 0.74[6]	13.24 ± 0.89[5,6]	12.24 ± 0.41[5]	10.00 ± 0.29[2,3]	9.43 ± 0.28[1,2]	9.04 ± 0.37[1,2]	8.16 ± 0.20[1]
20:3n-9	0.07 ± 0.01[a]	0.11 ± 0.01[b]	0.12 ± 0.03[b]	0.10 ± 0.02[b]	0.06 ± 0.01[a]	0.04 ± 0.01[c]	0.03 ± 0.01[c]	0.03 ± 0.01[b]	0.03 ± 0.01[c]
Σ FA (mg/g) *	30 ± 2[a]	34 ± 1[a]	32 ± 2[a]	46 ± 6[b]	41 ± 2[bcd]	42 ± 3[bcd]	36 ± 3[ac]	35 ± 2[a]	36 ± 2[ad]

Mean ± SD, n=5. Different numerical superscripts represent sex x time interactions and different alphabetical superscripts represent differences across time that are significantly different by Tukey's HSD following a significant *F*-value ($p < 0.05$) by two-way ANOVA. *Indicates a significant main effect of sex by two-way ANOVA ($p < 0.05$). SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; FA, fatty acids.

Appendix Table 6.7: Fatty acid composition (weight %) of female brain

	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9
14:0	1.67 ± 0.09^{7}	0.95 ± 0.05^{6}	$0.46 \pm 0.06^{2,3,4,5}$	$0.33 \pm 0.05^{1,2,3}$	$0.32 \pm 0.09^{1,2,3}$	$0.27 \pm 0.04^{1,2}$	$0.33 \pm 0.10^{1,2,3}$	$0.39 \pm 0.03^{1,2,3,4}$	$0.55 \pm 0.05^{4,5}$
16:0	29.14 ± 0.51^{a}	26.23 ± 0.44^{b}	21.34 ± 1.01^{c}	19.22 ± 1.16^{cd}	18.63 ± 1.58^{df}	17.61 ± 1.97^{de}	20.24 ± 0.65^{cf}	19.59 ± 1.29^{cd}	19.26 ± 0.93^{cd}
18:0	15.78 ± 0.05^{a}	17.34 ± 0.18^{b}	18.61 ± 0.23^{c}	19.17 ± 0.32^{cd}	19.25 ± 0.22^{cde}	19.56 ± 0.68^{de}	20.09 ± 0.78^{e}	19.38 ± 0.32^{cde}	19.71 ± 0.34^{def}
22:0	0.06 ± 0.02^{a}	0.18 ± 0.04^{ab}	0.41 ± 0.10^{bc}	0.53 ± 0.13^{c}	0.57 ± 0.15^{cd}	0.64 ± 0.22^{ce}	0.44 ± 0.02^{bcf}	0.48 ± 0.14^{cg}	0.61 ± 0.11^{ch}
24:0	0.09 ± 0.03^{a}	0.28 ± 0.07^{a}	0.74 ± 0.19^{b}	1.01 ± 0.22^{b}	1.12 ± 0.29^{b}	1.17 ± 0.34^{b}	0.88 ± 0.03^{b}	0.98 ± 0.25^{b}	1.14 ± 0.20^{b}
Σ SFA	51.02 ± 0.47^{a}	50.39 ± 0.43^{a}	48.07 ± 0.59^{bc}	47.06 ± 0.74^{bc}	46.32 ± 0.87^{b}	47.24 ± 1.47^{bc}	48.22 ± 0.91^{c}	47.34 ± 0.84^{bc}	47.50 ± 0.62^{bc}
16:1	1.35 ± 0.03^{5}	0.82 ± 0.03^{4}	$0.47 \pm 0.03^{2,3}$	$0.36 \pm 0.03^{1,2}$	0.31 ± 0.03^{1}	$0.37 \pm 0.06^{1,2,3}$	$0.38 \pm 0.02^{1,2,3}$	$0.38 \pm 0.03^{1,2,3}$	$0.37 \pm 0.05^{1,2,3}$
18:1n-7	2.32 ± 0.09^{a}	2.70 ± 0.08^{b}	3.00 ± 0.14^{bc}	3.16 ± 0.08^{c}	3.37 ± 0.23^{cdfgh}	3.88 ± 0.36^{e}	3.65 ± 0.05^{ef}	3.58 ± 0.16^{eg}	3.74 ± 0.19^{eh}
18:1n-9	10.36 ± 0.31^{a}	11.19 ± 0.55^{ab}	13.10 ± 0.83^{bc}	14.51 ± 0.72^{cd}	15.57 ± 1.15^{dfh}	18.24 ± 1.78^{e}	16.88 ± 0.29^{ef}	16.24 ± 1.30^{deg}	17.43 ± 1.03^{eh}
24:1n-9	0.07 ± 0.01^{a}	0.18 ± 0.04^{a}	0.51 ± 0.16^{ab}	0.96 ± 0.25^{bc}	1.15 ± 0.36^{cg}	1.36 ± 0.50^{cdg}	0.92 ± 0.03^{bce}	1.12 ± 0.34^{cfg}	1.56 ± 0.30^{g}
Σ MUFA	14.62 ± 0.41^{a}	15.75 ± 0.75^{a}	18.73 ± 1.42^{ab}	21.34 ± 1.52^{bc}	23.14 ± 2.42^{cg}	28.09 ± 3.97^{d}	24.69 ± 0.45^{cde}	24.15 ± 2.37^{cdf}	26.56 ± 2.06^{dg}
18:2n-6	1.22 ± 0.07^{5}	1.20 ± 0.05^{5}	1.30 ± 0.05^{5}	$1.04 \pm 0.06^{3,4}$	$1.02 \pm 0.04^{3,4}$	$0.96 \pm 0.07^{2,3,4}$	$0.84 \pm 0.04^{1,2}$	0.79 ± 0.06^{1}	0.76 ± 0.03^{1}
18:3n-6	0.07 ± 0.01^{7}	$0.02 \pm 0.01^{4,5,6}$	$0.01 \pm 0.01^{1,2,3}$	$0.01 \pm 0.01^{1,2,3}$	0.03 ± 0.01^{6}	$0.01 \pm 0.01^{1,2,3,4}$	$0.01 \pm 0.01^{1,2,3}$	$0.01 \pm 0.01^{1,2,3}$	$0.01 \pm 0.01^{1,2}$
20:2n-6	0.16 ± 0.01^{1}	$0.19 \pm 0.01^{1,2,3,4}$	$0.24 \pm 0.02^{2,3,4}$	$0.23 \pm 0.02^{1,2,3,4}$	$0.24 \pm 0.04^{2,3,4}$	0.25 ± 0.06^{4}	$0.18 \pm 0.02^{1,2,3}$	$0.17 \pm 0.02^{1,2}$	$0.18 \pm 0.02^{1,2,3,4}$
20:3n-6*****	0.48 ± 0.02^{ac}	0.54 ± 0.02^{a}	0.62 ± 0.05^{b}	0.56 ± 0.03^{ab}	0.54 ± 0.06^{ab}	0.50 ± 0.07^{ac}	0.43 ± 0.01^{cd}	0.38 ± 0.03^{d}	0.39 ± 0.02^{de}
20:4n-6	13.52 ± 0.18^{a}	13.20 ± 0.30^{a}	11.50 ± 0.65^{b}	10.25 ± 0.51^{bc}	9.99 ± 0.86^{c}	8.17 ± 0.99^{d}	9.75 ± 0.83^{ce}	9.00 ± 0.69^{cdf}	9.02 ± 0.48^{cdg}
22:4n-6	$3.01 \pm 0.07^{3,4,5,6,7}$	$3.07 \pm 0.07^{5,6,7}$	3.14 ± 0.06^{7}	$3.01 \pm 0.10^{3,4,5,6,7}$	$3.13 \pm 0.09^{6,7}$	$2.75 \pm 0.04^{1,2}$	$2.83 \pm 0.17^{1,2,3,4}$	2.70 ± 0.17^{1}	$2.80 \pm 0.08^{1,2,3}$
22:5n-6	1.42 ± 0.07^{a}	1.21 ± 0.06^{b}	0.89 ± 0.07^{c}	0.77 ± 0.10^{cd}	0.70 ± 0.10^{d}	0.38 ± 0.08^{e}	0.51 ± 0.04^{e}	0.47 ± 0.09^{e}	0.41 ± 0.07^{e}
Σ n-6 PUFA	19.89 ± 0.18^{a}	19.45 ± 0.25^{a}	17.73 ± 0.66^{b}	15.91 ± 0.69^{c}	15.69 ± 0.83^{c}	13.06 ± 0.88^{d}	14.57 ± 0.97^{cd}	13.54 ± 0.83^{d}	13.58 ± 0.57^{d}
18:3n-3	0.01 ± 0.01^{a}	0.01 ± 0.01^{a}	0.03 ± 0.01^{abc}	0.03 ± 0.01^{bc}	0.02 ± 0.01^{ab}	0.04 ± 0.01^{c}	0.03 ± 0.01^{cd}	0.03 ± 0.01^{bce}	0.04 ± 0.01^{cf}
20:5n-3	0.04 ± 0.01^{a}	0.03 ± 0.01^{ab}	0.03 ± 0.01^{abc}	0.03 ± 0.01^{a}	0.03 ± 0.01^{abc}	0.02 ± 0.01^{bce}	0.01 ± 0.01^{ce}	0.02 ± 0.01^{bcde}	0.01 ± 0.01^{e}
22:5n-3*****	0.33 ± 0.02^{ab}	0.34 ± 0.01^{a}	0.29 ± 0.02^{bc}	0.26 ± 0.02^{cd}	0.24 ± 0.01^{d}	0.15 ± 0.02^{e}	0.15 ± 0.01^{e}	0.15 ± 0.02^{e}	0.14 ± 0.01^{e}
22:6n-3	12.19 ± 0.26^{ab}	12.53 ± 0.18^{ab}	13.31 ± 0.63^{a}	13.40 ± 0.82^{a}	13.66 ± 0.82^{a}	11.08 ± 1.56^{b}	12.19 ± 0.41^{ab}	12.75 ± 1.20^{ab}	11.93 ± 1.07^{ab}
Σ n-3 PUFA	12.58 ± 0.27^{ab}	12.92 ± 0.18^{ab}	13.67 ± 0.63^{a}	13.75 ± 0.84^{a}	13.95 ± 0.83^{a}	11.30 ± 1.56^{b}	12.40 ± 0.42^{ab}	12.96 ± 1.20^{ab}	12.14 ± 1.06^{ab}
20:3n-9	0.13 ± 0.01^{ab}	0.14 ± 0.01^{a}	0.11 ± 0.02^{bc}	0.09 ± 0.01^{ceg}	0.10 ± 0.02^{cde}	0.08 ± 0.02^{eg}	0.07 ± 0.01^{efg}	0.06 ± 0.01^{g}	0.07 ± 0.01^{gh}
Σ FA (mg/g)	22 ± 1^{a}	30 ± 1^{b}	34 ± 6^{b}	36 ± 4^{b}	36 ± 2^{b}	56 ± 6^{c}	48 ± 5^{cd}	45 ± 4^{d}	48 ± 3^{cd}

Mean ± SD, n=5. Different numerical superscripts represent sex x time interactions and different alphabetical superscripts represent differences across time that are significantly different by Tukey's HSD following a significant *F*-value ($p < 0.05$) by two-way ANOVA. *Indicates a significant main effect of sex by two-way ANOVA ($p < 0.05$). SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; FA, fatty acids.

Appendix Table 6.8: Fatty acid composition (weight %) of male brain

	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9
14:0	1.82 ± 0.25^{7}	1.05 ± 0.04^{6}	0.44 ± 0.04^{1,2,3,4,5}	0.25 ± 0.11^{1}	0.60 ± 0.11^{5}	0.35 ± 0.04^{1,2,3}	0.42 ± 0.03^{1,2,3,4,5}	0.52 ± 0.06^{3,4,5}	0.62 ± 0.10^{5}
16:0	29.82 ± 0.56^{a}	26.50 ± 0.36^{b}	21.86 ± 0.78^{c}	18.45 ± 0.60^{d}	19.19 ± 1.48^{d}	19.73 ± 1.76^{cd}	20.07 ± 0.81^{cd}	18.85 ± 0.63^{d}	18.61 ± 1.34^{d}
18:0	15.75 ± 0.25^{a}	17.06 ± 0.20^{b}	19.06 ± 0.57^{c}	19.07 ± 0.32^{c}	19.33 ± 0.68^{c}	19.52 ± 0.60^{c}	19.60 ± 0.24^{c}	19.70 ± 0.43^{c}	19.40 ± 0.59^{c}
22:0	0.05 ± 0.01^{a}	0.19 ± 0.05^{ab}	0.40 ± 0.02^{bc}	0.59 ± 0.09^{c}	0.62 ± 0.16^{cd}	0.48 ± 0.17^{ce}	0.44 ± 0.08^{cf}	0.62 ± 0.04^{cg}	0.64 ± 0.18^{ch}
24:0	0.09 ± 0.01^{a}	0.29 ± 0.08^{ab}	0.70 ± 0.02^{bde}	1.14 ± 0.17^{c}	1.23 ± 0.32^{c}	0.89 ± 0.29^{cd}	0.89 ± 0.15^{ce}	1.28 ± 0.09^{c}	1.16 ± 0.30^{c}
Σ SFA	51.52 ± 0.39^{a}	50.28 ± 0.26^{a}	47.80 ± 1.19^{beg}	46.23 ± 0.27^{bc}	46.40 ± 1.06^{bd}	48.34 ± 1.23^{e}	48.40 ± 0.53^{ef}	47.61 ± 0.32^{beg}	46.57 ± 1.02^{cdg}
16:1	1.49 ± 0.18^{6}	0.86 ± 0.03^{4}	0.49 ± 0.03^{3}	0.34 ± 0.01^{1}	0.33 ± 0.02^{1}	0.40 ± 0.05^{1,2,3}	0.36 ± 0.03^{1,2}	0.35 ± 0.02^{1}	0.32 ± 0.05^{1}
18:1n-7	2.28 ± 0.05^{a}	2.65 ± 0.11^{ab}	2.99 ± 0.09^{bc}	3.20 ± 0.12^{cfg}	3.33 ± 0.28^{cde}	3.65 ± 0.33^{eh}	3.63 ± 0.15^{efh}	3.55 ± 0.16^{egh}	3.83 ± 0.29^{h}
18:1n-9	10.22 ± 0.36^{a}	11.17 ± 0.52^{ab}	13.14 ± 0.36^{bc}	14.70 ± 0.76^{cd}	15.83 ± 1.21^{dg}	16.44 ± 1.78^{deg}	16.66 ± 0.90^{dfg}	17.29 ± 0.42^{g}	17.77 ± 1.35^{gh}
24:1n-9	0.12 ± 0.10^{a}	0.18 ± 0.05^{a}	0.50 ± 0.06^{ab}	1.07 ± 0.17^{bcg}	1.22 ± 0.38^{cg}	0.94 ± 0.38^{bcd}	0.98 ± 0.12^{bce}	1.53 ± 0.13^{cfg}	1.59 ± 0.54^{g}
Σ MUFA	14.61 ± 0.40^{a}	15.70 ± 0.72^{a}	18.54 ± 0.34^{ab}	21.86 ± 1.49^{bc}	23.32 ± 2.53^{cg}	24.71 ± 3.63^{cdg}	24.85 ± 1.50^{ceg}	25.90 ± 0.84^{cfg}	27.21 ± 3.03^{g}
18:2n-6	1.23 ± 0.11^{5}	1.23 ± 0.03^{5}	1.23 ± 0.04^{5}	1.06 ± 0.05^{4}	1.07 ± 0.05^{4}	1.04 ± 0.06^{3,4}	0.98 ± 0.05^{3,4}	0.91 ± 0.05^{2,3}	0.98 ± 0.03^{3,4}
18:3n-6	0.07 ± 0.01^{7}	0.03 ± 0.01^{5,6}	0.02 ± 0.01^{3,4,5}	0.02 ± 0.01^{2,3,4,5}	0.03 ± 0.01^{5,6}	0.01 ± 0.01^{1,2,3}	0.01 ± 0.01^{1,2,3}	0.01 ± 0.01^{1,2,3}	0.01 ± 0.01^{1}
20:2n-6	0.16 ± 0.01^{1}	0.19 ± 0.01^{1,2,3,4}	0.22 ± 0.02^{1,2,3,4}	0.25 ± 0.04^{4}	0.24 ± 0.05^{3,4}	0.24 ± 0.05^{2,3,4}	0.24 ± 0.02^{2,3,4}	0.23 ± 0.02^{1,2,3,4}	0.24 ± 0.05^{3,4}
20:3n-6*****	0.46 ± 0.02^{a}	0.55 ± 0.03^{b}	0.62 ± 0.01^{b}	0.59 ± 0.05^{b}	0.57 ± 0.06^{b}	0.46 ± 0.06^{a}	0.46 ± 0.03^{a}	0.44 ± 0.02^{a}	0.44 ± 0.04^{a}
20:4n-6	13.34 ± 0.16^{a}	13.21 ± 0.42^{ab}	11.81 ± 0.26^{bc}	10.59 ± 0.76^{cd}	10.17 ± 0.99^{dg}	9.32 ± 1.01^{deg}	9.27 ± 0.63^{dfg}	8.88 ± 0.33^{g}	8.99 ± 0.99^{gh}
22:4n-6	2.97 ± 0.06^{2,3,4,5,6,7}	3.02 ± 0.02^{3,4,5,6,7}	3.14 ± 0.13^{7}	3.16 ± 0.11^{7}	3.06 ± 0.06^{4,5,6,7}	2.71 ± 0.12^{1}	2.80 ± 0.13^{1,2,3}	2.89 ± 0.14^{1,2,3,4,5}	2.90 ± 0.07^{1,2,3,4,5,6}
22:5n-6	1.49 ± 0.12^{a}	1.19 ± 0.04^{b}	0.89 ± 0.04^{c}	0.81 ± 0.10^{cd}	0.71 ± 0.12^{d}	0.49 ± 0.11^{e}	0.42 ± 0.06^{e}	0.41 ± 0.03^{e}	0.35 ± 0.05^{e}
Σ n-6 PUFA	19.76 ± 0.23^{a}	19.43 ± 0.42^{ab}	17.96 ± 0.43^{bc}	16.55 ± 0.84^{cd}	15.90 ± 1.02^{de}	14.31 ± 1.07^{ef}	14.22 ± 0.69^{f}	13.78 ± 0.40^{fg}	13.94 ± 0.97^{fh}
18:3n-3	0.02 ± 0.01^{ab}	0.01 ± 0.01^{a}	0.03 ± 0.01^{bcf}	0.02 ± 0.01^{abc}	0.01 ± 0.01^{ab}	0.04 ± 0.01^{cf}	0.04 ± 0.01^{cdf}	0.04 ± 0.01^{cef}	0.04 ± 0.01^{f}
20:5n-3	0.04 ± 0.01^{a}	0.03 ± 0.01^{abc}	0.03 ± 0.01^{abc}	0.04 ± 0.02^{ab}	0.02 ± 0.01^{bcd}	0.02 ± 0.01^{cd}	0.01 ± 0.01^{d}	0.01 ± 0.01^{de}	0.02 ± 0.01^{cdf}
22:5n-3*****	0.34 ± 0.02^{ab}	0.34 ± 0.03^{a}	0.29 ± 0.03^{b}	0.30 ± 0.02^{ab}	0.25 ± 0.01^{c}	0.19 ± 0.02^{d}	0.18 ± 0.02^{d}	0.17 ± 0.01^{d}	0.17 ± 0.01^{d}
22:6n-3	12.10 ± 0.17^{ab}	12.57 ± 0.22^{ab}	13.50 ± 0.34^{a}	13.56 ± 0.86^{a}	13.11 ± 0.75^{ab}	12.06 ± 1.48^{ab}	11.93 ± 0.74^{ab}	11.61 ± 0.83^{b}	11.98 ± 1.09^{ab}
Σ n-3 PUFA	12.51 ± 0.17^{ab}	12.98 ± 0.21^{ab}	13.86 ± 0.35^{a}	13.95 ± 0.86^{a}	13.40 ± 0.75^{ab}	12.32 ± 1.48^{ab}	12.18 ± 0.74^{ab}	11.85 ± 0.83^{b}	12.21 ± 1.09^{ab}
20:3n-9	0.13 ± 0.02^{a}	0.14 ± 0.01^{a}	0.10 ± 0.01^{bc}	0.11 ± 0.02^{ab}	0.10 ± 0.01^{b}	0.07 ± 0.02^{cf}	0.07 ± 0.01^{cdf}	0.07 ± 0.01^{cef}	0.06 ± 0.01^{f}
Σ FA (mg/g)	22 ± 1^{a}	30 ± 1^{ab}	34 ± 2^{b}	32 ± 3^{bc}	37 ± 3^{bdf}	47 ± 5^{e}	47 ± 2^{e}	46 ± 4^{ef}	47 ± 9^{e}

Mean ± SD, n=5. Different numerical superscripts represent sex x time interactions and different alphabetical superscripts represent differences across time that are significantly different by Tukey's HSD following a significant *F*-value ($p< 0.05$) by two-way ANOVA. *Indicates a significant main effect of sex by two-way ANOVA ($p< 0.05$). SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; FA, fatty acids.

Appendix Table 6.9: Fatty acid composition (weight %) of female heart

	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9
14:0	1.23 ± 0.18^{a}	1.39 ± 0.25^{a}	1.61 ± 0.43^{a}	0.65 ± 0.27^{b}	0.39 ± 0.23^{bc}	0.15 ± 0.10^{c}	0.22 ± 0.11^{bc}	0.33 ± 0.09^{bc}	0.24 ± 0.13^{bc}
16:0	18.62 ± 0.49^{a}	18.53 ± 0.31^{a}	17.69 ± 0.49^{a}	14.41 ± 0.63^{b}	13.74 ± 0.46^{bc}	13.11 ± 0.85^{bc}	12.72 ± 0.93^{c}	13.15 ± 0.49^{bc}	13.17 ± 1.31^{bc}
18:0	19.01 ± 0.45^{ab}	18.73 ± 0.53^{ab}	17.80 ± 0.71^{a}	19.15 ± 0.89^{ab}	19.72 ± 0.79^{ab}	20.20 ± 1.05^{ab}	20.49 ± 1.23^{b}	20.08 ± 2.25^{ab}	20.16 ± 1.35^{ab}
22:0	0.17 ± 0.02^{a}	0.19 ± 0.01^{ad}	0.25 ± 0.01^{be}	0.31 ± 0.02^{c}	0.25 ± 0.03^{be}	0.25 ± 0.02^{b}	0.21 ± 0.02^{def}	0.24 ± 0.02^{bf}	0.10 ± 0.01^{g}
24:0	0.23 ± 0.02^{acd}	0.25 ± 0.01^{a}	0.27 ± 0.01^{a}	0.25 ± 0.02^{a}	0.17 ± 0.02^{b}	0.19 ± 0.02^{bc}	0.17 ± 0.03^{b}	0.20 ± 0.02^{bd}	0.09 ± 0.02^{e}
Σ SFA	$42.92 \pm 0.27^{3,4,5}$	$43.09 \pm 0.20^{3,4,5}$	41.13 ± 0.44^{3}	38.24 ± 0.45^{2}	$37.22 \pm 0.37^{1,2}$	$37.12 \pm 0.45^{1,2}$	$37.64 \pm 0.50^{1,2}$	$37.63 \pm 2.00^{1,2}$	$37.15 \pm 0.47^{1,2}$
16:1	0.47 ± 0.14	0.22 ± 0.02	0.20 ± 0.05	0.21 ± 0.05	0.35 ± 0.09	0.39 ± 0.18	0.31 ± 0.18	0.38 ± 0.15	0.39 ± 0.19
18:1n-7	3.42 ± 0.07^{ac}	3.46 ± 0.10^{ab}	3.71 ± 0.17^{b}	3.49 ± 0.13^{ab}	3.40 ± 0.12^{ac}	3.48 ± 0.20^{ab}	3.22 ± 0.15^{acd}	3.14 ± 0.04^{cd}	3.01 ± 0.12^{d}
18:1n-9	6.48 ± 1.08^{a}	4.43 ± 0.52^{ab}	3.82 ± 0.65^{b}	4.23 ± 1.12^{ab}	5.09 ± 0.92^{ab}	4.95 ± 1.52^{ab}	4.34 ± 1.38^{ab}	5.14 ± 1.22^{ab}	4.83 ± 1.87^{ab}
24:1n-9	0.16 ± 0.01^{a}	0.12 ± 0.01^{b}	0.11 ± 0.02^{b}	0.10 ± 0.01^{b}	0.06 ± 0.01^{c}	0.06 ± 0.01^{c}	0.07 ± 0.01^{c}	0.07 ± 0.01^{c}	0.05 ± 0.01^{c}
Σ MUFA	11.27 ± 1.15^{a}	8.95 ± 0.47^{ab}	8.38 ± 0.54^{b}	8.61 ± 1.04^{ab}	9.44 ± 0.89^{ab}	9.47 ± 1.54^{ab}	8.71 ± 1.41^{ab}	9.46 ± 1.34^{ab}	9.02 ± 1.95^{ab}
18:2n-6	8.88 ± 0.79^{1}	10.73 ± 0.67^{1}	15.74 ± 0.65^{2}	$17.05 \pm 2.47^{2,3}$	$19.85 \pm 1.37^{3,4,5}$	$18.17 \pm 1.46^{2,3,4}$	$18.85 \pm 1.20^{2,3,4,5}$	$19.87 \pm 2.57^{3,4,5}$	$19.53 \pm 0.53^{3,4,5}$
18:3n-6	0.04 ± 0.01	0.04 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.03 ± 0.01
20:2n-6*****	0.74 ± 0.04^{a}	0.80 ± 0.01^{b}	0.60 ± 0.01^{c}	0.48 ± 0.03^{d}	0.35 ± 0.03^{e}	0.33 ± 0.02^{ef}	0.28 ± 0.01^{fg}	0.27 ± 0.02^{g}	0.26 ± 0.03^{gh}
20:3n-6	1.03 ± 0.06^{a}	1.10 ± 0.02^{a}	0.82 ± 0.03^{b}	0.51 ± 0.03^{c}	0.39 ± 0.01^{d}	0.36 ± 0.04^{d}	0.34 ± 0.02^{d}	0.33 ± 0.03^{d}	0.32 ± 0.04^{d}
20:4n-6	21.97 ± 1.02^{a}	20.22 ± 0.84^{ac}	16.00 ± 0.55^{b}	17.28 ± 1.03^{b}	17.32 ± 0.87^{b}	18.26 ± 1.43^{bc}	17.65 ± 1.37^{b}	16.17 ± 1.09^{b}	16.94 ± 0.93^{b}
22:4n-6	2.09 ± 0.19^{7}	1.81 ± 0.02^{6}	1.43 ± 0.05^{5}	$1.26 \pm 0.03^{4,5}$	$1.05 \pm 0.03^{3,4}$	$1.06 \pm 0.17^{3,4}$	$0.92 \pm 0.07^{2,3}$	$0.84 \pm 0.05^{1,2,3}$	$0.84 \pm 0.16^{1,2,3}$
22:5n-6	$0.51 \pm 0.05^{2,3,4}$	$0.56 \pm 0.02^{3,4,5}$	0.75 ± 0.04^{7}	0.98 ± 0.10^{8}	$0.72 \pm 0.07^{5,6,7}$	0.77 ± 0.12^{7}	$0.67 \pm 0.03^{4,5,6,7}$	$0.60 \pm 0.10^{4,5,6,7}$	$0.61 \pm 0.05^{4,5,6,7}$
Σ n-6 PUFA	$35.29 \pm 0.40^{1,2}$	$35.29 \pm 0.20^{1,2}$	$35.41 \pm 0.45^{1,2}$	$37.61 \pm 1.89^{2,3,4}$	$39.73 \pm 0.48^{4,5,6}$	$38.99 \pm 0.48^{4,5}$	$38.76 \pm 1.28^{4,5}$	$38.14 \pm 1.42^{3,4}$	38.55 ± 0.73^{4}
18:3n-3	0.21 ± 0.04	0.25 ± 0.04	0.34 ± 0.09	0.27 ± 0.17	0.41 ± 0.15	0.39 ± 0.16	0.37 ± 0.18	0.50 ± 0.18	0.41 ± 0.16
20:5n-3*****	0.19 ± 0.02^{a}	0.19 ± 0.01^{a}	0.15 ± 0.02^{ab}	0.15 ± 0.02^{ab}	0.15 ± 0.01^{ab}	0.13 ± 0.01^{b}	0.14 ± 0.01^{bc}	0.15 ± 0.03^{bd}	0.13 ± 0.02^{be}
22:5n-3	2.54 ± 0.26^{a}	4.40 ± 0.14^{b}	4.70 ± 0.23^{b}	3.25 ± 0.39^{c}	2.32 ± 0.18^{ad}	1.88 ± 0.16^{de}	1.72 ± 0.05^{e}	1.80 ± 0.15^{ef}	1.74 ± 0.08^{eg}
22:6n-3*****	6.93 ± 0.67^{a}	7.33 ± 0.30^{a}	9.33 ± 0.79^{ab}	11.01 ± 2.08^{be}	10.48 ± 0.97^{bce}	11.71 ± 0.97^{bde}	12.26 ± 0.85^{e}	11.91 ± 1.15^{ef}	12.81 ± 1.58^{eg}
Σ n-3 PUFA*****	9.92 ± 0.84^{a}	12.22 ± 0.36^{ab}	14.59 ± 0.89^{bh}	14.73 ± 2.38^{bch}	13.39 ± 0.98^{bdh}	14.12 ± 0.94^{beh}	14.51 ± 0.70^{bfh}	14.38 ± 1.02^{bgh}	15.12 ± 1.46^{h}
20:3n-9	0.03 ± 0.01^{a}	0.03 ± 0.01^{ab}	0.04 ± 0.01^{bc}	0.05 ± 0.01^{c}	0.03 ± 0.01^{a}	0.02 ± 0.01^{a}	0.02 ± 0.01^{a}	0.02 ± 0.01^{a}	0.02 ± 0.01^{a}
Σ FA (mg/g)	21 ± 3^{a}	26 ± 2^{b}	27 ± 1^{b}	29 ± 2^{b}	29 ± 2^{b}	26 ± 1^{b}	26 ± 1^{b}	28 ± 1^{b}	27 ± 1^{b}

Mean ± SD, n=5. Different numerical superscripts represent sex x time interactions and different alphabetical superscripts represent differences across time that are significantly different by Tukey's HSD following a significant *F*-value ($p < 0.05$) by two-way ANOVA. *Indicates a significant main effect of sex by two-way ANOVA ($p < 0.05$). SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; FA, fatty acids.

Appendix Table 6.10: Fatty acid composition (weight %) of male heart

	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9
14:0	1.39 ± 0.42^{a}	1.50 ± 1.16^{a}	1.71 ± 0.51^{a}	0.75 ± 0.35^{ab}	0.25 ± 0.17^{b}	0.14 ± 0.11^{bc}	0.34 ± 0.29^{bd}	0.16 ± 0.08^{be}	0.21 ± 0.06^{bf}
16:0	19.45 ± 1.27^{a}	18.91 ± 1.76^{a}	17.64 ± 0.55^{a}	14.34 ± 0.62^{b}	13.44 ± 0.50^{bc}	12.94 ± 1.41^{bc}	13.25 ± 1.41^{bc}	11.88 ± 0.83^{c}	12.12 ± 0.73^{bc}
18:0	19.64 ± 2.30	18.51 ± 2.00	17.70 ± 0.89	18.75 ± 0.87	20.17 ± 0.68	20.78 ± 1.52	19.80 ± 2.40	20.49 ± 1.10	19.86 ± 0.79
22:0	0.19 ± 0.02^{a}	0.20 ± 0.04^{ab}	0.25 ± 0.01^{bcefg}	0.30 ± 0.01^{c}	0.26 ± 0.03^{cdefg}	0.23 ± 0.02^{ae}	0.21 ± 0.04^{af}	0.21 ± 0.01^{ag}	0.10 ± 0.01^{h}
24:0	0.27 ± 0.03^{a}	0.26 ± 0.04^{a}	0.29 ± 0.01^{a}	0.24 ± 0.02^{a}	0.18 ± 0.04^{b}	0.17 ± 0.01^{b}	0.14 ± 0.03^{b}	0.17 ± 0.03^{b}	0.08 ± 0.02^{c}
Σ SFA	44.52 ± 2.17^{5}	43.40 ± 1.41^{4,5}	41.41 ± 0.57^{3,4}	37.94 ± 0.32^{1,2}	37.37 ± 0.38^{1,2}	37.41 ± 0.59^{1,2}	37.30 ± 1.14^{1,2}	36.71 ± 0.58^{1,2}	35.88 ± 0.45^{1}
16:1	0.29 ± 0.03	0.23 ± 0.15	0.20 ± 0.07	0.29 ± 0.11	0.29 ± 0.10	0.37 ± 0.33	0.40 ± 0.30	0.20 ± 0.11	0.28 ± 0.11
18:1n-7	3.27 ± 0.24^{ab}	3.45 ± 0.31^{ab}	3.59 ± 0.15^{a}	3.50 ± 0.18^{ab}	3.61 ± 0.08^{a}	3.40 ± 0.17^{ab}	3.16 ± 0.16^{b}	3.35 ± 0.15^{ab}	3.19 ± 0.10^{bc}
18:1n-9	5.78 ± 0.91	4.28 ± 2.11	4.05 ± 1.11	4.67 ± 0.79	4.51 ± 1.00	4.21 ± 1.91	5.15 ± 2.91	3.73 ± 1.36	4.67 ± 0.85
24:1n-9	0.17 ± 0.01^{a}	0.13 ± 0.01^{b}	0.12 ± 0.01^{bc}	0.10 ± 0.01^{cef}	0.06 ± 0.02^{d}	0.07 ± 0.01^{d}	0.08 ± 0.01^{de}	0.07 ± 0.01^{df}	0.05 ± 0.02^{d}
Σ MUFA	10.25 ± 0.89	8.77 ± 1.89	8.60 ± 1.03	9.18 ± 0.85	9.03 ± 1.09	8.64 ± 2.12	9.52 ± 3.00	8.16 ± 1.39	8.99 ± 0.94
18:2n-6	8.34 ± 1.36^{1}	10.43 ± 1.74^{1}	16.00 ± 0.89^{2}	17.53 ± 1.05^{2,3}	19.55 ± 1.42^{3,4,5}	19.90 ± 0.93^{3,4,5}	21.63 ± 2.92^{4,5,6}	22.07 ± 1.38^{5,6}	23.74 ± 1.22^{6}
18:3n-6	0.04 ± 0.01	0.04 ± 0.02	0.04 ± 0.01	0.04 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.03 ± 0.02	0.02 ± 0.01
20:2n-6*****	0.80 ± 0.13^{a}	0.83 ± 0.04^{a}	0.59 ± 0.01^{b}	0.46 ± 0.03^{c}	0.37 ± 0.03^{cd}	0.36 ± 0.02^{cd}	0.34 ± 0.02^{d}	0.33 ± 0.01^{de}	0.31 ± 0.03^{df}
20:3n-6	1.08 ± 0.09^{a}	1.08 ± 0.08^{a}	0.85 ± 0.02^{b}	0.51 ± 0.02^{c}	0.40 ± 0.02^{d}	0.37 ± 0.02^{d}	0.34 ± 0.01^{d}	0.36 ± 0.02^{d}	0.33 ± 0.02^{d}
20:4n-6	21.68 ± 1.28^{a}	19.75 ± 2.71^{acdefg}	16.04 ± 1.06^{b}	17.16 ± 0.81^{bc}	17.76 ± 0.91^{bd}	17.64 ± 1.54^{be}	16.15 ± 2.46^{b}	17.28 ± 0.97^{bf}	16.44 ± 0.41^{bg}
22:4n-6	2.26 ± 0.20^{7}	1.75 ± 0.19^{6}	1.48 ± 0.05^{5}	1.25 ± 0.08^{4,5}	1.07 ± 0.10^{3,4}	0.94 ± 0.06^{2,3},	0.77 ± 0.05^{1,2}	0.69 ± 0.02^{1,2}	0.66 ± 0.09^{1}
22:5n-6	0.51 ± 0.03^{2,3,4}	0.56 ± 0.09^{3,4,5,6}	0.72 ± 0.04^{5,6,7}	0.97 ± 0.09^{8}	0.74 ± 0.16^{6,7}	0.54 ± 0.03^{2,3,4}	0.42 ± 0.07^{1,2,3}	0.39 ± 0.04^{1,2}	0.34 ± 0.02^{1}
Σ n-6 PUFA	34.75 ± 2.31^{1}	34.48 ± 1.42^{1}	35.76 ± 0.47^{1,2,3}	37.95 ± 1.25^{4,5}	39.93 ± 0.44^{4,5,6}	39.78 ± 1.19^{4,5,6}	39.71 ± 0.47^{4,5,6}	41.16 ± 0.83^{5,6}	41.85 ± 1.03^{6}
18:3n-3	0.20 ± 0.06	0.27 ± 0.20	0.36 ± 0.12	0.38 ± 0.11	0.33 ± 0.11	0.33 ± 0.16	0.48 ± 0.37	0.32 ± 0.15	0.45 ± 0.12
20:5n-3*****	0.21 ± 0.03^{a}	0.20 ± 0.01^{a}	0.16 ± 0.01^{b}	0.16 ± 0.01^{b}	0.14 ± 0.01^{b}	0.14 ± 0.01^{b}	0.15 ± 0.01^{b}	0.15 ± 0.01^{b}	0.15 ± 0.01^{b}
22:5n-3	2.58 ± 0.27^{ac}	4.60 ± 0.75^{b}	4.52 ± 0.50^{b}	3.06 ± 0.24^{a}	2.35 ± 0.22^{ac}	2.10 ± 0.25^{c}	1.94 ± 0.30^{cd}	2.10 ± 0.17^{ce}	1.95 ± 0.19^{cf}
22:6n-3*****	6.28 ± 0.46^{a}	7.98 ± 1.47^{ab}	8.58 ± 1.01^{abf}	10.47 ± 1.15^{bd}	10.59 ± 1.06^{bcd}	11.35 ± 0.97^{d}	10.51 ± 2.17^{bde}	11.03 ± 1.11^{df}	10.46 ± 0.43^{bdg}
Σ n-3 PUFA*****	9.32 ± 0.58^{a}	13.09 ± 2.01^{b}	13.68 ± 1.33^{b}	14.12 ± 1.30^{b}	13.43 ± 1.18^{b}	13.95 ± 1.05^{b}	13.11 ± 2.10^{b}	13.63 ± 1.14^{b}	13.03 ± 0.48^{b}
20:3n-9	0.02 ± 0.01^{ad}	0.03 ± 0.01^{a}	0.04 ± 0.01^{b}	0.05 ± 0.01^{c}	0.02 ± 0.01^{ad}	0.02 ± 0.01^{ad}	0.02 ± 0.01^{ad}	0.01 ± 0.01^{d}	0.02 ± 0.01^{de}
Σ FA (mg/g)	21 ± 2	23 ± 2	27 ± 2	27 ± 1	27 ± 3	26 ± 2	26 ± 6	25 ± 1	27 ± 2

Mean ± SD, n=5. Different numerical superscripts represent sex x time interactions and different alphabetical superscripts represent differences across time that are significantly different by Tukey's HSD following a significant *F*-value ($p< 0.05$) by two-way ANOVA. *Indicates a significant main effect of sex by two-way ANOVA ($p< 0.05$). SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; FA, fatty acids.

7 Examining the Effects of Dietary DHA Supplementation on Spatial Memory and the Hippocampus in Female and Male Rats

7.1 Introduction

Interactions between DHA supplementation and biological sex on episodic and working memory have been reported in young adults [5], but cognitive sexual dimorphisms may begin earlier [20,125]. Modelling these differences in animals could help uncover the mechanisms behind the interactions shown in humans. To date, studies reporting the effects of DHA on spatial memory in animal models have generally included only adult males and n-3 PUFA deficient diets [6,8,9,73]. Additionally, lipidomic analysis on the hippocampus, an area of the brain critical to spatial memory [91], has not been performed in both sexes following dietary DHA supplementation. An animal model of DHA supplementation including both sexes and provided Adequate Fat Western Diets (AFWD), could generate findings more applicable to humans. Combining spatial memory analyses with an examination of the hippocampal lipidome could provide further insight as to how DHA supplementation affects spatial memory in the rat, and examining the erythrocyte lipidome could reveal potential biomarkers for hippocampal DHA status [133]. Therefore, the aim of this study was to determine the effect of DHA supplementation in a rat model using ALA adequate [134,135] AFWDs [100] to provide insight into how sexual dimorphisms affect spatial memory in rats.

7.2 Methods

7.2.1 Animals and Study Design

Six female rats were bred on campus and placed on AIN-93G based AFWDs with or without 2% of total fatty acid mass DHA supplementation (Table 3) after confirmation of

pregnancy by vaginal plug. Diets were purchased from Envigo (Mississauga, Ontario, Canada). The AFWD used in this study is designed to contain a fatty acid profile typically consumed in Western societies, but at an adequate amount of total fat for rodents as outlined in the AIN-93G formulation [136]. This reduction in fat compared to the typical Total Western Diet (TWD) [100] is important, as a high consumption of SFAs is related to mastitis and inflammation during lactation [137]. The 2% DHA supplementation was chosen as this amount has been suggested to maximize brain DHA levels [138]. Rats and their pups were kept in a temperature controlled room with a 12hr light cycle at 10pm-10am. After weaning, pups were placed on the same diets as their mothers, underwent behavioural testing as outlined below at 6 weeks of age, and were sacrificed at 7 weeks of age. Plasma, erythrocytes, whole blood, heart, and liver were collected whole and snap frozen in liquid nitrogen. Brain was separated into brainstem, cerebellum, cortex, hippocampus, striatum, and rest of brain sections over wax sheeting cooled by dry ice, then snap frozen in liquid nitrogen. Tissues were then stored at -80°C until analysis. Culling litters to 10 pups each was considered. However, the reductions in variability from culling on pup weight, nutrient availability, and behaviour are still controversial [128,139,140], and mothers may already have natural adaptations to adjust milk volume to litter sizes [141]. For these reasons, litters were not culled to avoid introducing a confounding factor by reducing perinatal demand for maternal DHA between placental transfer and breast milk production.

7.2.2 Behavioural Testing

During testing, rats were placed in clear, polypropylene cages with heating pads underneath. At the end of testing, rats were dried and returned to their individual cage. The order of testing was chosen at random, and swim patterns were recorded using a Noldus Ethovision XT v8.5 video tracking system (Noldus Information Technology, Leesburg, VA, USA).

The testing protocol began with the learning phase, which consisted of 4 trials per day for 4 days, starting once at each of the 4 starting positions (NW, N, E, SE) with approximately 10 min intertrial intervals. Starting positions were approximately equidistant from the platform and the order of the starting positions was sorted at random. Timers started as the rats were released into the pool facing the wall and stopped when rats touched the platform. Rats had 60s to reach the platform and were allowed to remain on it for 30s to orientate themselves. Unsuccessful rats were guided to the platform after 60s and allowed to remain on it for 30s.

Memory retention was then assessed using a probe test 24h after the last day of the learning phase. After the platform was removed, rats were given 30s to search for the platform and the time spent in the target quadrant was recorded. Finally, a visual test using a styrofoam ball above a platform placed in a different quadrant was performed after the probe test to ensure animals could locate visual cues.

7.2.3 Lipidomic Analyses

Levels of DHA containing lipid species were determined using ultra high pressure liquid chromatography with tandem mass spectrometry (UHPLC-MS/MS). Lipid extracts, spiked with 500pmol 1,2-diheptadecanoyl-*sn*-glycero-3-phosphatidylcholine (17:0/17:0 PC) as the internal standard, were dried under nitrogen gas and resuspended into 100µL of 65:35:5 acetonitrile-isopropanol-water + 0.1% formic acid and stored at 4 ºC until analysis.

The UHPLC-MS/MS system was a Waters Synapt G2S*i* QTOF coupled to a Dionex UltiMate 3000 UHPLC system (Mississauga, ON) equipped with a Waters ACQUITY CSH C18 column with dimensions 1.7µm x 2.1mm x 150mm. A reverse-phase, binary multistep HPLC protocol using A, 60:40 acetonitrile-water + 0.1% formix acid + 10 mM ammonium formate and

B, 90:10 isopropanol-acetonitrile + 0.1% formic acid + 10 mM ammonium formate to separate polar and nonpolar lipids. Column temperature was set at 45 ºC and tray temperature at 4 ºC.

The QTOF (quadrupole time of flight) mass spectrometer was operated under positive electrospray ionization for PC, PE, and TAG analysis. Data dependent acquisition (DDA) for top-5 ions was used with a scan range of m/z 50-1000, a scan frequency of 0.1 sec, and transfer cell collision energy ramps of 20-30V at low mass and 30-50V at high mass. All data were lock-mass corrected using leucine enkephalin (m/z 556.2771) and processed using ProGenesis QI software (Nonlinear Dynamics, Newcastle upon Tyne, UK). Data are expressed as relative abundancies (ratios of analyte/internal standard) with PC data presented as concentrations as the internal standard was 17:0/17:0 PC.

7.2.4 Fatty Acid Analyses

RBC and hippocampal tissues were collected for fatty acid analyses. Lipids were extracted using the methods described in section 4.2, and fatty acids were analysed by gas chromatography as described in section 4.3.

7.2.5 Statistical Analyses

Behavioural data were analysed in two ways: 1) with session data analyzed by pup, or 2) with session data analyzed by litter. When sessions were analyzed by pup, the four trials of each pup in a given session were averaged and analyzed as a function of the overall performance of the individual pups. The data of each pup were then divided into a 2x2 factorial for diet and sex (n=14-16). When sessions were analyzed by litter, the trials of the males and females of each litter were averaged and analyzed as one entity. Groups were then divided into a 2x2 factorial for diet and sex (n=3). No adjustments for sex ratio imbalances outlined in Table 5 were made.

Mean comparisons for behavioural, fatty acid, and identified lipid species analyses were performed using SPSS release 20.0 (IBM, Chicago, IL, USA). The effect of diet, sex, and a diet x sex interaction was examined using a two-way ANOVA. Individual means were compared by Tukey post hoc testing after significant F-value by ANOVA. Fatty acid and lipidomic data are presented as means ± SD with significance accepted when $p<0.05$, and behavioural data are presented as means ± SEM with significance accepted when $p<0.05$.

Analyses to determine significantly different compounds detected by UHPLC-MS prior to manual identification were performed using ProGenesis QI software (Nonlinear Dynamics, Newcastle upon Tyne, UK). Erythrocyte compounds were identified using the ProGenesis q-value system, which adjusted p-values according to multiple testing by adjusting for each individual test, reducing the chances of identifying false positives. Erythrocyte species were identified with a q-value of $q<0.05$, and hippocampal species were identified with a p-value of $p<0.05$. These values were used to reduce the number of detected species into a manageable amount that could be manually identified.

Pearson's correlations were used to examine the association between hippocampal and erythrocyte fatty acids and lipid species. Significance was accepted when $p<0.05$.

7.3 Results

7.3.1 Body Weight and Food Intake

DHA fed rats were significantly heavier than controls from 21 to 47 days of age (Figure 10). Males were heavier than females from 28 to 47 days of age. DHA fed rats consumed more diet at day 21, and males consumed more than females at 28, 35, and 42 days of age (Figure 11).

7.3.2 Behavioural Testing

Statistical analyses for behavioural testing were performed in two ways: with session times analyzed 1) by individual pup, or 2) by litter. *A priori* analyses revealed no differences between litters or treatment groups during the first trial (data not shown). Maulchy's test of sphericity also confirmed that within-subject variances for repeated measures were equal (data not shown). When session times were analyzed by pup, no significant dietary effects or sexual dimorphisms were detected for latency times, distance travelled, or velocity when all learning phase sessions were pooled together. The final session of the learning phase was then analyzed individually to determine if the learning phase concluded differently between groups. This two-way ANOVA anlalysis indicated a significant effect of diet on latency time ($p=0.048$) (Figure 12), but not distance travelled ($p=0.084$) (Figure 13) or velocity ($p=0.622$, data not shown), with the supplemented group taking longer to reach the platform, suggesting greater difficulty memorizing the platform position. For the probe test, no significant dietary effects or sexual dimorphisms were detected for time spent in the correct quadrant, average distance from the platform, or velocity (Figure 14). Conversely, when data were analyzed by litter, no significant effect of diet or sex was detected on latency time (Figure 15), distance travelled (Figure 16), or velocity ($p=0.640$, data not shown) in either the pooled sessions or during the final day of the

learning phase. Similarly, no significant differences were detected during the probe test (Figure 17).

7.3.3 Lipidomic Analyses of the Hippocampus

DHA supplementation reduced ARA-containing lipid species in the hippocampus. Both normalized abundance and concentration measures of PC 18:1_20:4 and PC 18:0_20:4 were lower in the DHA supplemented group (Figure 18). The normalized abundancies of 22:5 containing PE, alkenyl-PE, and a PS species were also lower in the DHA supplemented group (Figure 18). Of note, the normalized abundancies of the 22:5 containing species PE 16:0_22:5 and alkenyl-PE 18:0_22:5 were the most affected, being 2.55 and 2.36 fold higher, respectively, in the control group hippocampi.

7.3.4 Lipidomic Analyses of Erythrocytes

Erythrocytes in DHA supplemented rats also showed reductions in ARA-containing lipid species, and some changes in the hippocampus were mirrored in the erythrocyte fraction (Figure 19). Of the lipid species affected in the hippocampus, PC 18:0_20:4, PC 18:1_20:4, and PE 18:0_22:5 we also reduced in the erythrocytes of the supplemented group but showed an additional sex effect not shown in the hippocampus. In the erythrocytes, the effects of DHA supplementation on fatty acids appeared to have lipid class specificity. Reductions in ARA occurred primarily in the PC fraction, while reductions in 18:2 occurred primarily in PE. Conversely, both PS changes were due to increases in DHA.

Both normalized abundance and concentration of PC 18:0_20:4 was lower in the DHA supplemented group. The normalized abundance of PC 18:1_20:4 was lower in the DHA group

as well, but concentrations were only nearly significant ($p = 0.076$). Interestingly, PC 18:0_20:4 was also higher in females, while PC 18:1_20:4 was higher in males.

DHA containing lipid species also differed by diet and sex. PC 18:0_22:6 was higher in females, and PS 16:0_22:6 in males. Alkenyl-PE 18:0_22:6 was nearly significantly higher ($p = 0.084$) in supplemented females than males, and higher in the DHA group. The normalized abundancies of PS were particularly sensitive to DHA supplementation. Both PS 16:0_22:6 and 18:1_22:6 were higher in the supplemented group. Interestingly, PS 16:0_22:6 was higher in males than females.

Sexual dimorphisms were also present in LA and adrenic acid containing species. In alkenyl-PE, 16:0_22:4 was higher in control males than control females, and lowest in DHA supplemented rats. The ARA precursor LA was also affected by sexual dimorphisms, and PE 16:0_18:2 was higher in males.

7.3.5 Fatty Acid Composition of Hippocampi and Erythrocytes

Fatty acid analyses of total lipid extracts indicated that DHA supplementation increased the relative percentages of all hippocampal n-3 PUFA except 20:3n-3 (Appendix Table 7.5) Interestingly, DHA supplementation also increased hippocampal relative percent and concentrations of ARA precursors, though ARA, adrenic acid, DPAn-6, and total n-6 PUFA concentrations were lower in the DHA supplemented group. The relative percentage of adrenic acid was significant for a sex x diet interaction, being higher in control females than males, and both sexes of the control group being higher than rats fed the supplemented diet. Concentrations and relative levels of hippocampal LA were higher in males.

Some of the differences in the relative percentages of hippocampal fatty acids also occurred in the erythrocytes. Like the hippocampus, the relative percentages of LA, EPA, DPAn-3 and DHA were higher in the supplemented group, while the relative percentages of adrenic acid, DPAn-6, and total n-6 PUFA were lower in the DHA group (Appendix Table 7.6). Males also had higher relative percentages of LA and 18:1n-7 in erythrocytes. The relative percentages of erythrocyte fatty acids also had additional differences as compared with the relative percentages of the hippocampal fatty acids. Females had higher relative percentages of DHA, EPA, and DPAn-6, and males had higher relative percentages of DPAn-3 and total MUFA (Appendix Table 7.6) .

7.3.6 Correlations between Erythrocyte and Hippocampal Fatty acids and Lipid Species

Correlations between the concentrations and relative percentages of erythrocyte and hippocampal ARA, DPAn-6, and DHA were not significant within male or female dietary groups, but relative percentages of adrenic acid was significantly correlated in control males, and concentrations of adrenic acid in control females (Appendix Tables 7.7 and 7.8).

Correlations between lipid species were performed with sex and dietary groups pooled together to compare the results to a previous study[142], and with the groups separated as originally proposed. At the lipidomic level, the acyl lipid species of PE 18:0_22:5, PC 18:0_20:4 and PC 18:1_20:4 in erythrocyets and the hippocampus were correlated. When sex and dietary groups were pooled, all species had significant correlations in normalized abundance or concentration (). When divided by sex or diet only, only nearly significant correlations were observed. In normalized abundance, PC 18:0_20:4 was nearly significantly correlated in the DHA group ($r=0.74, p=0.060$), and in males ($r=0.74$, $p=0.055$), and PE 18:0_22:5 was nearly significant in males ($r=0.72$, $p=0.070$). Some $p<0.10$ correlations were observed in concentration

values as well, with PC 18:1_20:4 in the control group (r=0.65, p=0.082) and in males (r=0.66, p=0.088) (Tables 7 and 8). Finally, when divided by sex and diet as originally proposed, these correlations were no longer significant except for concentrations of PC 18:1_20:4 in DHA males (Table 6).

7.4 Discussion

This study shows that dietary DHA supplementation reduces hippocampal n-6 PUFA containing lipid species and increases latency times of the final day of the learning phase in adolescent female and male rats. This is the first report of adolescent males and females fed AFWDs with dietary DHA supplementation being assessed for spatial memory performance, and changes in hippocampal and erythrocyte lipid species at a medio lipidomic level [143]. No sex differences in behavioural performance were detected, and the DHA supplemented group unexpectedly had significantly longer latency times during the final day of the learning phase. Both diets in this study contained sufficient amounts of ALA [134,135]. This study agrees with reported increases in total fatty acid levels of DHA, but not with reported increases in DHA containing lipid species in the hippocampi of the supplemented group as shown in studies with deficient [78] or unreported [93] amounts of dietary ALA. The findings confirm previous reports of DHA supplementation reducing normalized abundancies of ARA-containing PC lipid species in the hippocampus [78], and also show that these reductions occur in erythrocytes as well. Behavioural results did not support the original hypothesis, and supplemented females did not perform differently than supplemented males, nor did the supplemented group perform better than the unsupplemented group in the MWM. Spatial memory analysis by MWM suggest that the reductions of n-6 PUFA in the hippocampus during DHA supplementation in ALA-sufficient diets could be increasing latency times in female and male adolescent rats. These observations

appear to support recent reports that a balance between ARA and DHA supplementation is most effective when supplementing LCPUFA perinatally[144,145].

The DHA diet used in this model was composed of 2%wt of total fatty acids as DHA. This amount is similar to amounts given to young adults in recent human trials [5] and consumed in populations with regular fish consumption [146]. Compared to previous animal trials examining DHA and spatial memory, the amount of DHA in the supplemented diet was higher than some previously used diets that used 1.1%wt of total fatty acids [6–9,73], but lower than others that used 11.2%wt [78]. This study is unique in that both diets provided to the mothers and dam reared pups were ALA sufficient [134,135]. While DHA supplementation increased normalized abundancies of DHA-containing lipid species in erythrocytes, DHA-containing lipid species in the hippocampus did not change, and ARA- containing lipid species were reduced. The similar brain DHA composition of the control group compared with the supplemented group agrees with previous reports that whole body synthesis rates of ALA are sufficient to supply the brain with adequate DHA [86]. The reductions in DPAn-6 but not DPAn-3 fatty acids in the hippocampus suggest reductions in 22:5-containing lipid species to be DPAn-6. These reductions in the 22:5- and ARA-containing lipid species in the hippocampus agree with previous research reporting a preference in the brain for DHA over DPAn-6 and ARA [147,148]. The reductions of ARA and DPAn-6 in PC, PE, alkenyl-PE, and PS are in agreement with previously reported data [78]. Although no DHA-containing lipid species were significantly increased in the hippocampus following supplementation, the relative percent of DHA in total fatty acids was higher in the supplemented group. These observations suggest that in rats fed adequate amounts of ALA and LA, DHA supplementation does not increase the normalized abundance of specific DHA-

containing lipid species but does reduce DPAn-6 and ARA-containing lipid species in the hippocampus.

Statistical analyses for the behavioural data were run in two ways to explore how the benefits of each would affect the interpretation. The first approach analyzes the data of each rat individually and reduces the impact of litter sex ratio imbalances, which was important for the interpretation of sex effects in this study. The second approach analyzes the data of each litter as a whole, and calls for a balanced subset of multiple litters to be used [125]. The use of a greater number of litters reduces the risk of a model with low genetic variability, and provides greater power as n=15 litters could be more powerful than n=15 individual rats from genetically similar backgrounds. However, the time and financial resources required to perform this method properly could be restrictive, and in this study, only 3 litters were used to meet the power requirements outlined in an *a priori* power analysis performed for ethics clearance, in which studies using similarly small genetic backgrounds were cited [105]. Unfortunately, this study design does not provide the genetic diversity that would provide the additional advantages of testing by litter.

No overall effects of diet or sex were detected in the MWM. However, latency times of the DHA supplemented group during the final day of the learning phase were longer than controls. This may be due to the imbalance between LCPUFA supplementation, and the specificity for DHA over DPAn-6 in the brain [147–149], or due to competition with ARA [150]. A previous study has shown that high consumptions of DHA without ARA leads to reduced water maze performance that is improved with ARA supplementation, but these DHA consumptions were excessive, with DHA composing 5.42-5.45% total energy [150]. However, ARA is essential to water maze performance [145], and the reductions in ARA and 22:5-

containing species in the brain without increases in DHA containing species could be detrimental. PL balance is also critical to brain function [151,152]. The observed reduction of all quantitatively measured PC species without any observed increases in other PC leaves the possibility that the supplementation of DHA without any n-6 LCPUFA supplementation could be altering PC/PE balance, but further research would be necessary.

The correlations between the concentrations of ARA containing PC species in the hippocampus and those in the erythrocytes, and the normalized abundances of 22:5-containing PE species in the hippocampus and erythrocytes are in partial agreement with previous reports of DHA composition by %wt correlating between PE species in erythrocytes and the hippocampus, when animals are not divided by dietary DHA consumption [142]. However, while previous reports only correlated total DHA fatty acids within the PE lipid class, this study demonstrates that reductions in the concentrations of ARA-containing PC, and the normalized abundancies of 22:5-containing PE, can be correlated between erythrocytes and the hippocampus as well in both males and females. Additionally, this study shows that dividing lipidomic data according to the dietary DHA intakes and sex produces some nearly significant correlations, suggesting that lipidomic analyses of human trials with more even distributions of dietary intakes could uncover potential biomarkers.

The lack of a lipidomic and fatty acid analyses of all litters in this study is a limitation. In the interest of time, all litters have completed behavioural assessments, but only 4 rats per diet x sex group have undergone fatty acid and lipidomic analyses. The inclusion of all rats to meet the *a priori* criteria of a 5% type 1 error rate and 80% test power could change the aforementioned results. Future directions should include the addition of a dietary group with a 1:1 ARA:DHA supplementation ratio. Providing rats with this diet would provide further insight into whether

the reduction in ARA is due to an imbalance in dietary LCPUFA, and has implications related to the previously reported hypothesis that in male rats, dietary 18-carbon PUFA consumption plays a larger role in LCPUFA synthesis than gene expression [153]. Erythrocyte fatty acid analysis seems to provide an additional dimension to this hypothesis, as it appears the DHA group had significantly reduced ARA synthesis as compared with controls, which did not have high levels of dietary DHA to impede LA elongation. Lipidomic, genomic, and protein analysis of the collected hepatic tissues and plasma could provide further insight into how DHA supplementation affected ARA synthesis relative to how gene expression affected ARA synthesis. Additionally, kinetic analyses could uncover whether in-vivo incorporation rates of DHA and ARA into the brain differ between diets and sex. These incorporation rates change with availability [148], and data from erythrocytes suggest LCPUFA availability differed between sexes and diets. The balance between PE and PC could be investigated as well. With current lipidomic data suggesting reductions in hippocampal concentrations of PC, a potential imbalance in lipid class homeostasis could be occurring. This could be confirmed by quantitative analysis using a Splash Lipidomix mix containing multiple deuterated phospholipid internal standards [154]. Finally, the 1:1 ARA:DHA diet could provide rats with the spatial memory benefit originally hypothesized. Recent reports in children fed exclusively through infant formula with varying levels of DHA and a consistent amount of ARA during infancy found that those fed formula with either a lower or equal amount of DHA relative to ARA exhibited greater connectivity between frontal and parietal lobes, and greater white matter volume as compared to those fed the higher amount of DHA and controls [144].

7.5 Conclusion

In conclusion, this study demonstrates that dietary DHA supplementation in rats with adequate ALA consumption affects spatial memory and increases latency times during the final day of the learning phase in the MWM. These results were significant for a diet effect. This study also reports that in the hippocampus, DHA supplementation reduced ARA- and 22:5-containing lipid species, but did not increase DHA containing lipid species. However, increases in the relative composition of DHA in total fatty acids of the hippocampus were detected. Finally, significant correlations between 22:5- and ARA-containing PE and PC lipid species were identified between erythrocytes and the hippocampus, but further research into how these findings can translate into identifying biomarkers is required.

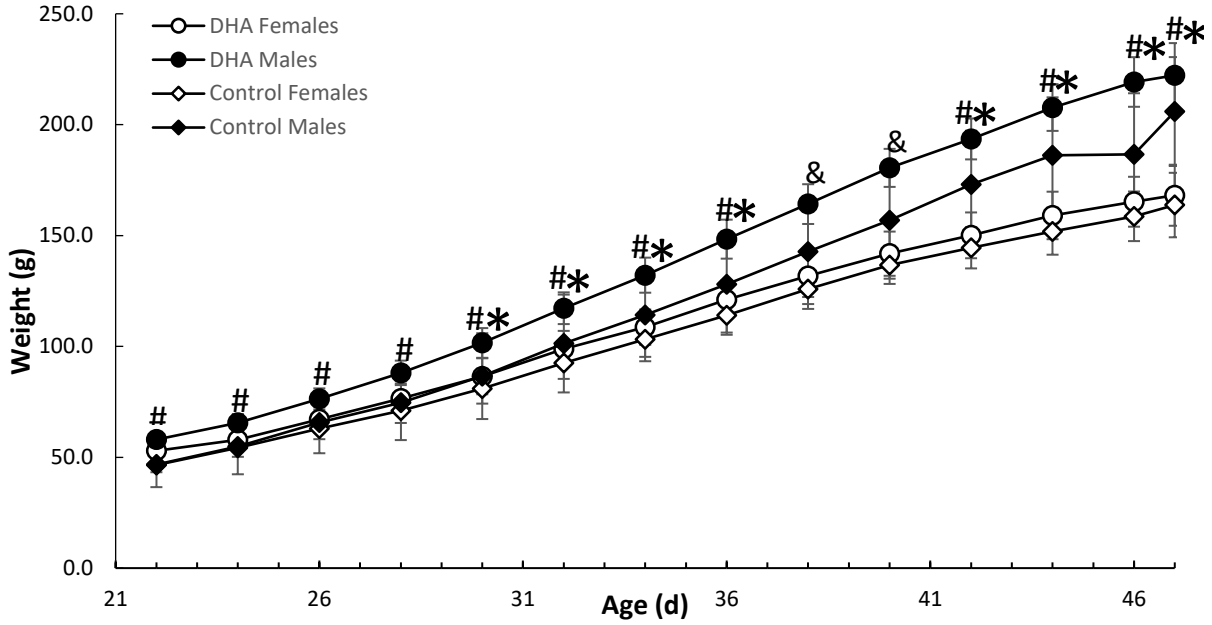

Figure 10: Body weights of male and female pups from weaning until adolescence. & indicates significant interaction between diet and sex, # indicates significant differences between dietary groups, and * indicates significant difference between sexes as determined by two-way ANOVA followed by Tukey post hoc ($p<0.05$). Mean ± SD. DHA females n = 21, DHA males n = 15, Control females n = 20, Control males n = 16.

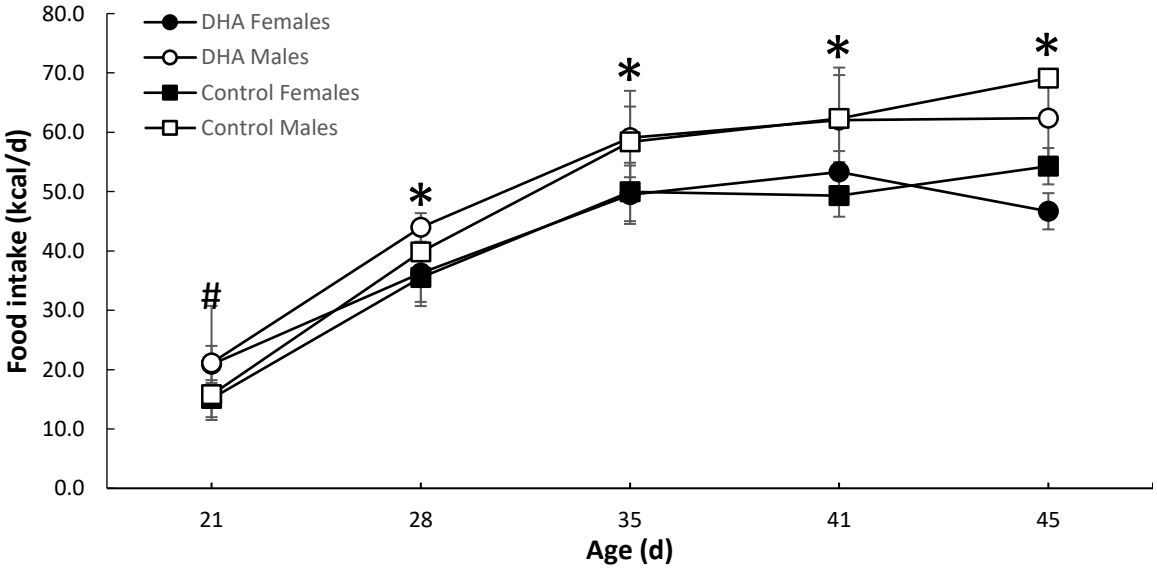

Figure 11: Food intakes of male and female pups from weaning until adolescence. # indicates intakes are significantly different between dietary groups, and * Intakes are significantly different between sexes as determined by two-way ANOVA followed by Tukey post hoc ($p<0.05$). Mean ± SD, n=15-21.

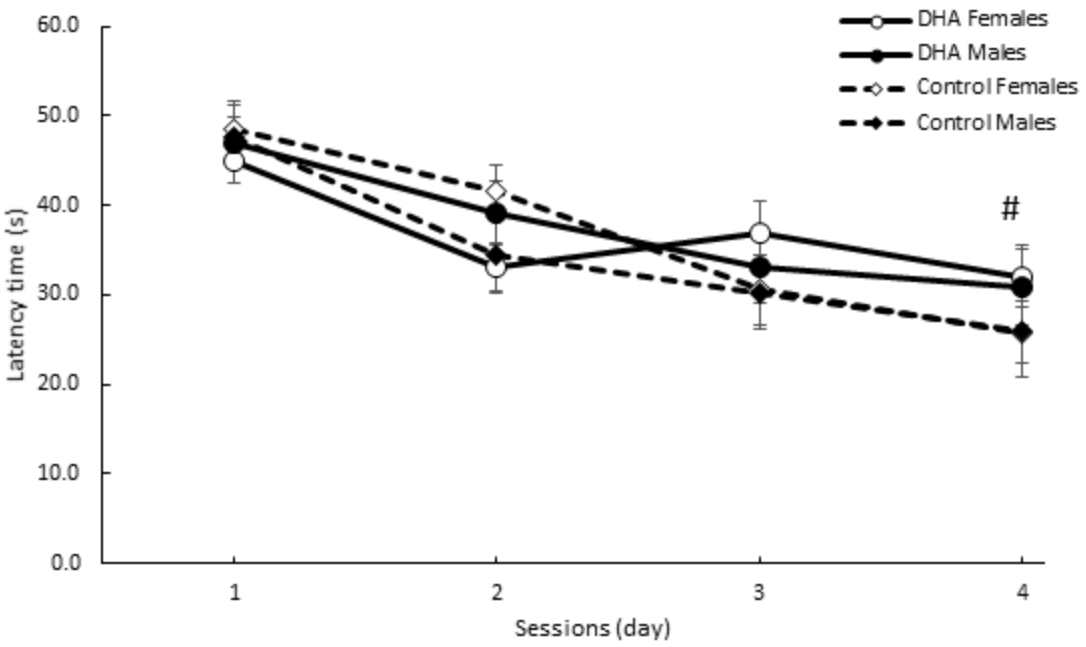

Figure 12: Effect of DHA-supplementation on escape latency in the Morris water maze. The escape latency (time required for a rat to find and climb onto the hidden platform) is presented as the mean of four trials ± SEM, n = 14-16. Significant differences between dietary groups determined by repeated measures one-way ANOVA followed by Tukey post hoc ($p<0.05$).

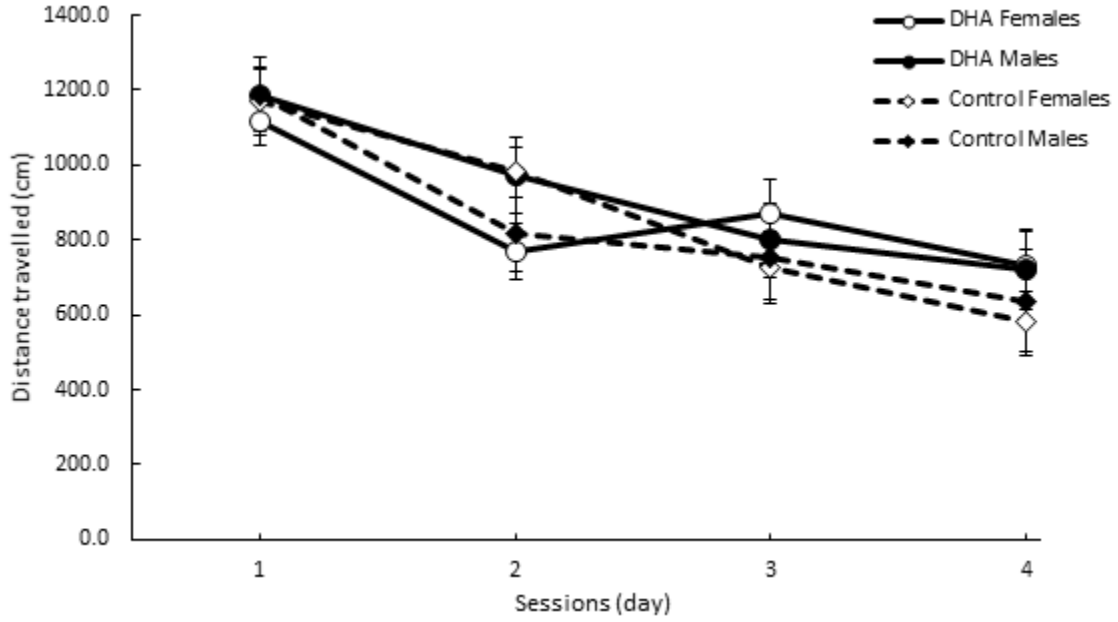

Figure 13: Effect of DHA-supplementation on distance travelled during the Morris water maze The escape latency (time required for a rat to find and climb onto the hidden platform) is presented as the mean of four trials ± SEM, n = 14-16. Significant differences between dietary groups determined by repeated measures one-way ANOVA followed by Tukey post hoc ($p<0.05$).

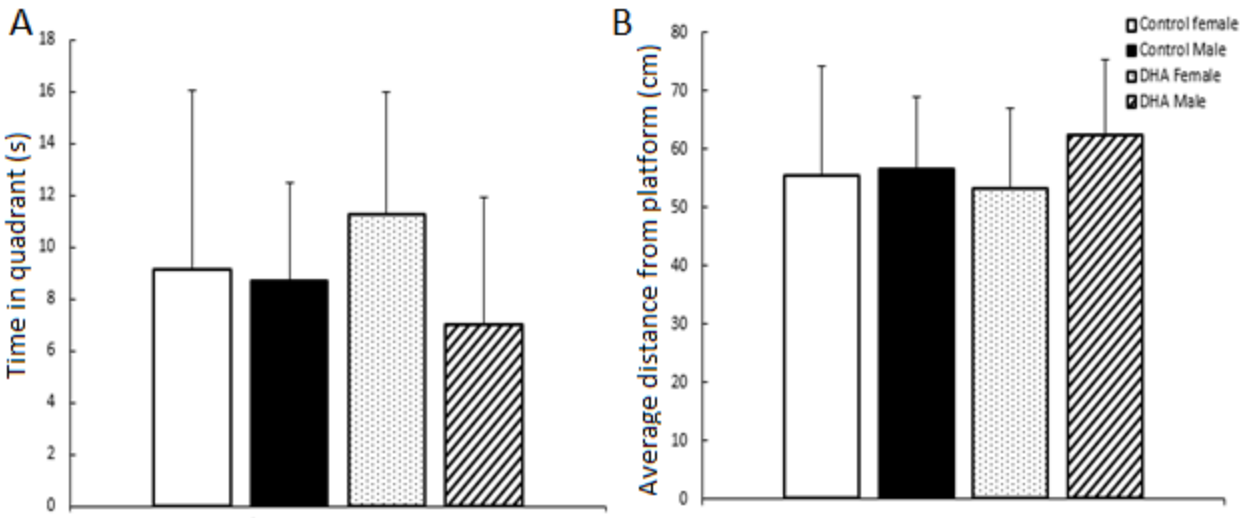

Figure 14: Performance of DHA and Control males and females during the probe test. (A) time in the former platform containing quadrant. (B) average distance from the former platform containing quadrant. No significant differences were detected by F test with ($p>0.05$) two-way ANOVA. n = 14-16 per group.

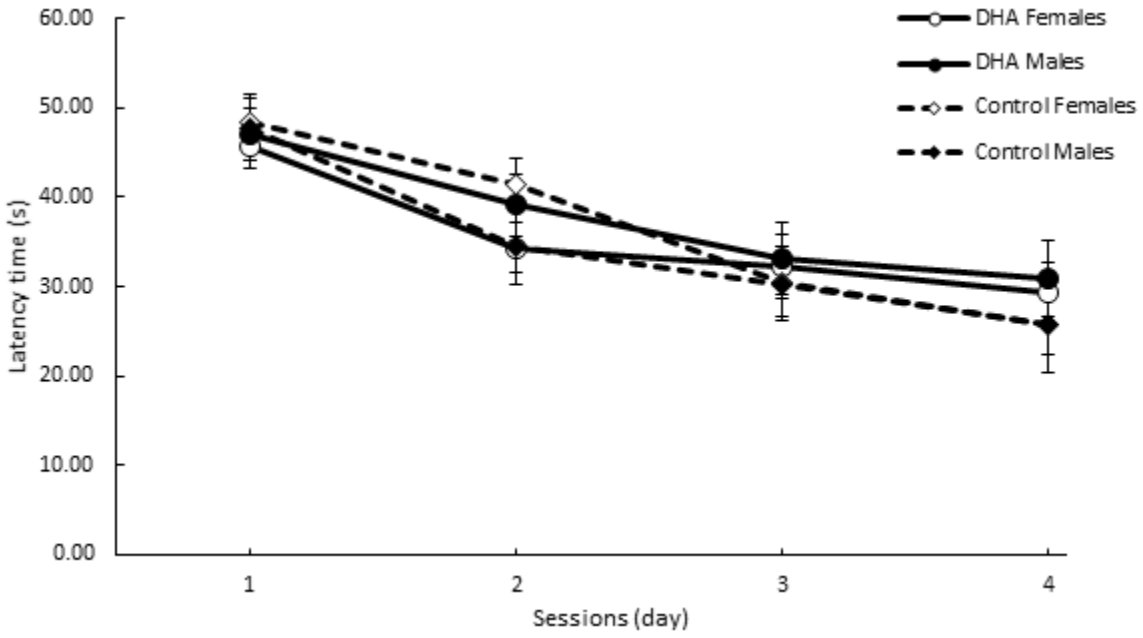

Figure 15: Effect of DHA-supplementation on escape latency in the Morris water maze The time required (escape latency, mean of four trials) for a rat to find and climb onto a hidden platform is presented as the mean ± SEM, n = 3. Significant differences between dietary groups by repeated measures one-way ANOVA followed by Tukey post hoc ($p<0.05$).

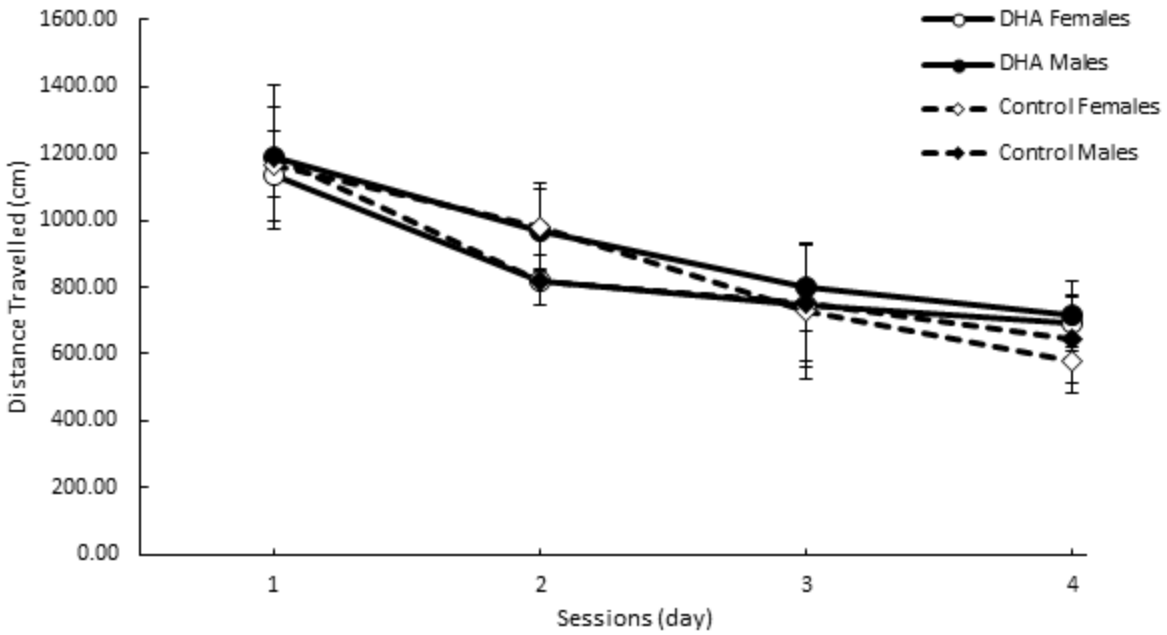

Figure 16: Effect of DHA-supplementation on distance travelled during the Morris water maze The time required (escape latency, mean of four trials) for a rat to find and climb onto a hidden platform is presented as the mean ± SEM, n = 3. Significant differences between dietary groups by repeated measures one-way ANOVA followed by Tukey post hoc ($p<0.05$).

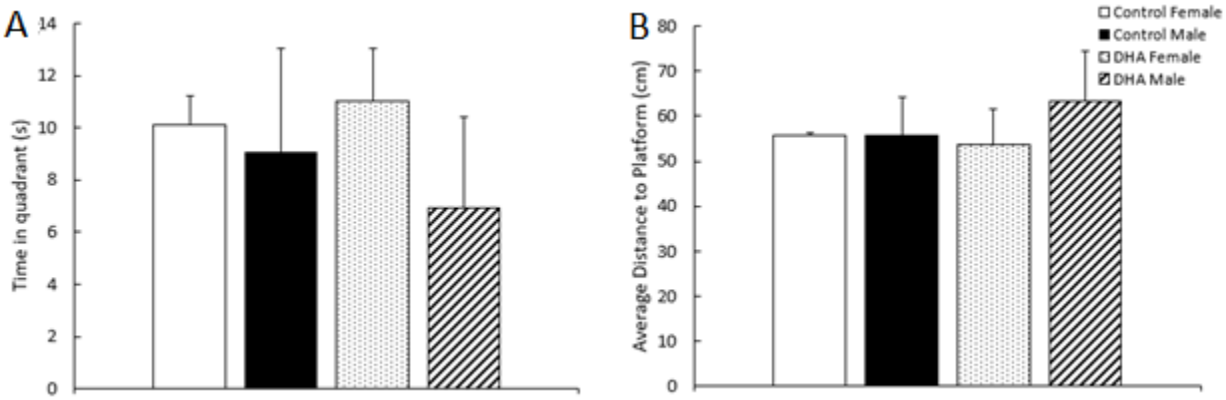

Figure 17: Performance of DHA and Control males and females during the probe test. (A) time in the former platform containing quadrant. (B) average distance from the former platform containing quadrant. No significant differences were detected by F test with ($p>0.05$) two-way ANOVA. n = 3 per group.

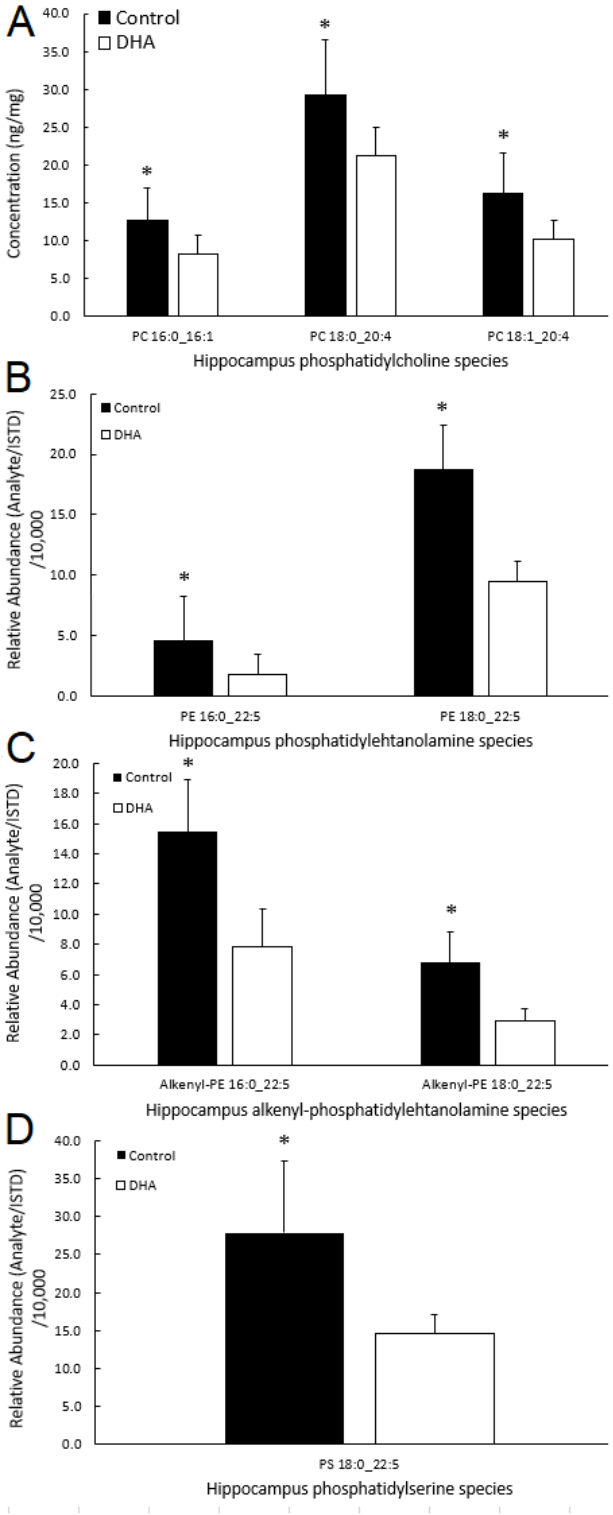

Figure 18: Significant differences in hippocampal PC (A), PE (B), alkenyl-PE (C) and PS (D) concentrations and relative abundancies between DHA and Control groups. Concentrations and relative abundancies are significantly different between diets as determined by two-way ANOVA followed by Tukey post hoc at * being ($p<0.05$), ** being ($p<0.01$). Mean ± SD, n=4.

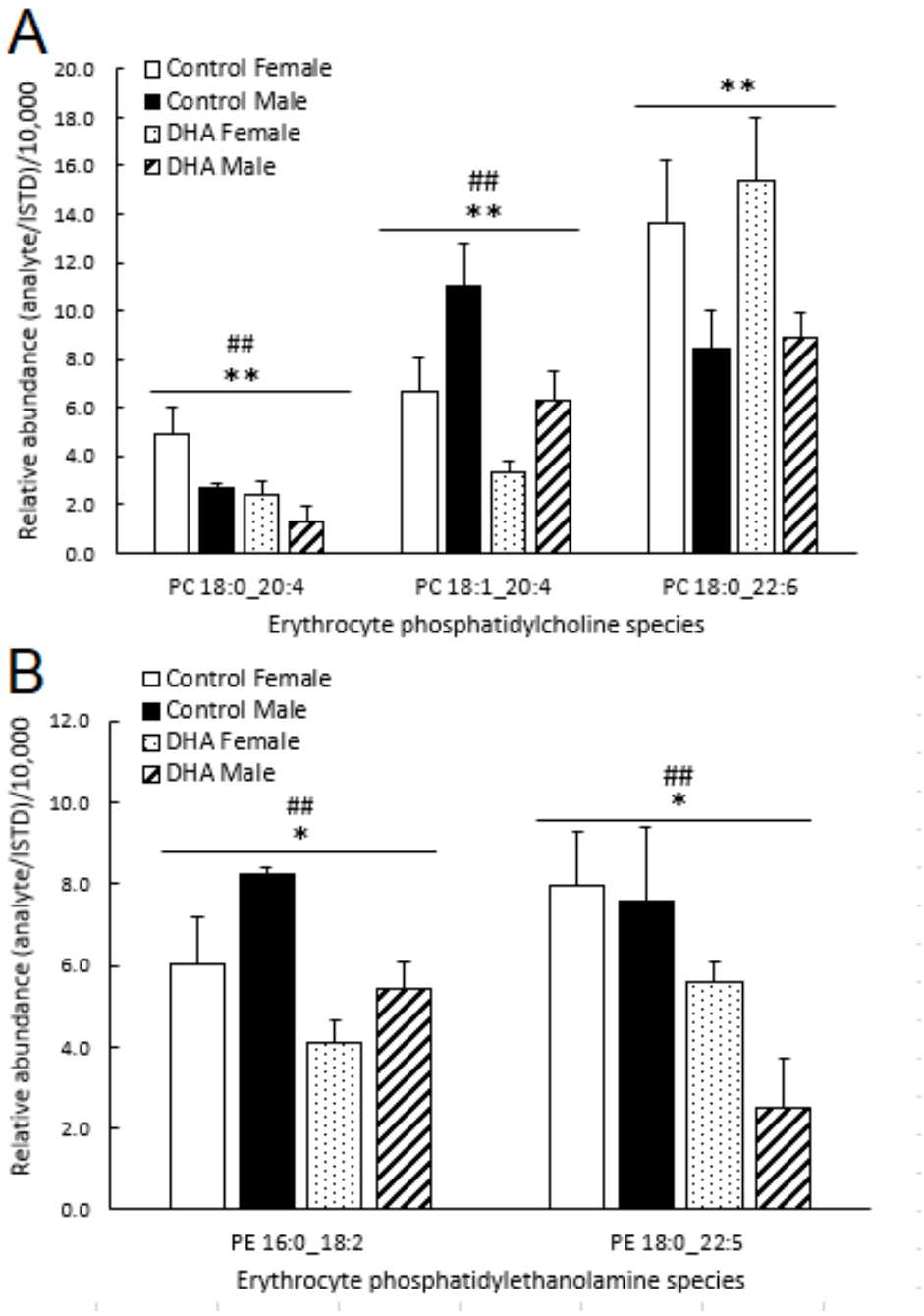

Figure 19: Differences in erythrocyte PC. Relative abundancies between diets (A), sex(B). Relative abundancies are significantly different by as determined by two-way ANOVA followed by Tukey post hoc at by $p<0.05$ signified by * or #, or by $p<0.01$ as signified by ** or ## for sex or diet, respectively. Mean ± SD, n=4.

Table 3: Macronutrient and fatty acid composition of control and DHA supplemented diets used in the behavioural study.

Diet component	Control	DHA+
Macronutrient	*mg/g of diet*	
Protein	17.70	17.70
Carbohydrate	60.10	60.10
Fat	7.20	7.20
Fatty Acid	*% composition*	
16:0	14.60 ± 0.08	14.58 ± 0.18
18:0	7.02 ± 0.05	6.46 ± 0.22
Total SFA	27.95 ± 0.19	24.91 ± 0.16
16:1	0.72 ± 0.01	0.82 ± 0.01
18:1n-7	1.51 ± 0.05	1.41 ± 0.09
18:1n-9	37.13 ± 0.23	38.02 ± 0.14
Total MUFA	39.77 ± 0.21	40.67 ± 0.10
18:2n-6	27.95 ± 0.11	27.92 ± 0.06
20:4n-6	0.05 ± 0.01	0.03 ± 0.01
Total n-6 PUFA	28.07 ± 0.12	28.02 ± 0.06
18:3n-3	3.87 ± 0.01	3.86 ± 0.02
20:3n-3	<0.01	<0.01
20:5n-3	0.01 ± 0.01	0.01 ± 0.01
22:5n-3	0.01 ± 0.01	0.04 ± 0.01
22:6n-3	0.02 ± 0.01	2.10 ± 0.04
Total n-3 PUFA	3.91 ± 0.01	6.01 ± 0.04

Data is mean ± SD from triplicate analysis in our laboratory. SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids. *tr*: trace amounts (<0.01 mg/g)

Table 4: Energy composition of control and DHA supplemented diets used in the behavioural study.

Diet component	Control	DHA+
Energy Density (kcal/g)	3.8	3.8
Macronutrient	% of energy	
Protein	18.8	18.8
Carbohydrate	63.9	63.9
Fat	17.2	17.2
Fatty Acid	% of energy	
16:0	2.51 ± 0.01	2.51 ± 0.03
18:0	1.21 ± 0.01	1.11 ± 0.04
Total SFA	4.81 ± 0.03	4.28 ± 0.03
16:1	0.12 ± 0.01	0.14 ± 0.01
18:1n-7	0.26 ± 0.01	0.24 ± 0.01
18:1n-9	6.39 ± 0.04	6.54 ± 0.02
Total MUFA	6.84 ± 0.04	6.99 ± 0.02
18:2n-6	4.81 ± 0.02	4.80 ± 0.01
20:4n-6	0.01 ± 0.01	0.01 ± 0.01
Total n-6 PUFA	4.83 ± 0.02	4.82 ± 0.01
18:3n-3	0.67 ± 0.01	0.66 ± 0.01
20:3n-3	<0.01	<0.01
20:5n-3	<0.01	<0.01
22:5n-3	<0.01	0.01 ± 0.01
22:6n-3	<0.01	0.36 ± 0.01
Total n-3 PUFA	0.67 ± 0.01	1.03 ± 0.01

Data is mean ± SD from triplicate analysis in our laboratory. SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids.

Table 5: Dam and sire pairings and sex ratios of litters and groups used for behavioural analyses

				Total Number of Pups		Number of Pups Used in MWM	
Litter #	Dam	Sire	Diet	Female	Male	Female	Male
1	1	1	DHA	9	4	6	4
2	2	1	Control	6	4	6	4
3	3	2	Control	7	8	4	6
4	4	2	DHA	6	6	5	5
5	5	3	DHA	6	5	4	6
6	6	3	Control	7	4	6	4
Total:			DHA	21	15	15	15
			Control	20	16	16	14

Each dam was bred once. Each sire was bred with one dam per diet. Total number of pups indicates total litter size, broken down by sex. Number of pups used in MWM (Morris Water Maze) indicates the ratio of female:male pups from the indicated litter used for behavioural analyses.

Table 6: Sex Differences in normalized abundance of erythrocyte lipid species

	Female	Male	P Sex Effect
PC 16:0_20:4	40.80 ± 12.77	40.73 ± 12.11	<0.01
PC 20:4_20:4	2.19 ± 2.00	2.11 ± 1.92	0.90
PE 18:1_18:2	15.20 ± 4.65	16.22 ± 3.66	0.03
PE 18:1_22:6	4.83 ± 2.18	4.31 ± 1.85	0.50
PE 38:6	17.66 ± 7.18	14.17 ± 4.17	0.07
PE 40:8	5.62 ± 1.07	3.61 ± 0.57	<0.01
Alkenyl-PE 16:0_22:4	15.44 ± 6.45	17.26 ± 8.71	0.01
Alkenyl-PE 18:0_22:6	20.58 ± 7.46	19.56 ± 4.35	0.39
Alkenyl-PE 18:1_22:6	12.01 ± 3.34	12.60 ± 3.74	0.52
PS 16:0_22:6	1.38 ± 1.05	2.73 ± 1.79	<0.01
PS 18:1_22:6	6.96 ± 3.74	6.74 ± 3.27	0.80

Abundance represented as analyte abundance divided by ISTD. Mean ± SD. n=4. P values determined by Tukey's HSD following two-way ANOVA.

Table 7: Correlations between hippocampal and erythrocyte lipid species (normalized abundance)

Group	Total		DHA Female		DHA Male		Control Female		Control Male	
n	15		4		3		4		4	
Lipid Species	Pearson's r	p	Pearson's r	p	Pearson's r	p	Pearson's r	p	Pearson's r	p
PC 18:0_20:4	0.39	0.15	-0.90	0.11	0.91	0.27	0.15	0.85	0.69	0.31
PC 18:1_20:4	0.61*	0.02	0.52	0.48	-1.00**	<0.001	-0.03	0.97	-0.25	0.75
PE 18:0_22:5	0.54*	0.04	-0.52	0.48	-0.31	0.80	-0.66	0.34	0.08	0.92

Correlations of lipid species between hippocampi and erythrocytes. Total group includes all rats analyzed by mass spectrometry. Correlations could only be determined for lipid species identified in erythrocytes and the hippocampus. DHA and control groups include rats of both sexes from the respective dietary group. Male and female groups include rats that consumed both diets from the respective sex. Pearson's r: Pearson's correlation coefficient.*p<0.05; **p<0.01

Table 8: Correlations between hippocampal and erythrocyte lipid species (concentrations)

Group	Total		DHA Female		DHA Male		Control Female		Control Male	
n	15		4		3		4		4	
Lipid Species	Pearson's r	p	Pearson's r	p	Pearson's r	p	Pearson's r	p	Pearson's r	p
PC 18:0_20:4	.55*	0.03	0.19	0.81	0.76	0.45	0.10	0.90	0.52	0.48
PC 18:1_20:4	.70**	<0.001	0.83	0.17	-0.20	0.87	-0.77	0.23	0.59	0.41

Correlations of lipid species between hippocampi and erythrocytes. Total group includes all rats analyzed by mass spectrometry. Correlations could only be determined for lipid species identified in erythrocytes and the hippocampus. DHA and control groups include rats of both sexes from the respective dietary group. Male and female groups include rats that consumed both diets from the respective sex. Pearson's r: Pearson's correlation coefficient;**p<0.01; *p<0.05

Appendix Table 7.1: Normalized abundancies of hippocampal lipid species in all groups

	DHA Male	DHA Female	Control Male	Control Female	P Sex x time Interaction	P Sex Effect	P Diet Effect
PC 16:0_16:1	5.95 ± 1.84	6.55 ± 1.42	10.40 ± 2.31	8.54 ± 0.77	0.06	0.18	>0.01
PC 18:0_20:4	15.51 ± 2.89	18.67 ± 0.74	25.33 ± 8.51	22.14 ± 4.14	0.23	1.00	0.02
PC 18:1_20:4	7.37 ± 1.12	8.18 ± 0.85	14.46 ± 4.21	10.21 ± 1.96	0.62	0.85	0.03
PC 38:5	0.05 ± 0.06	0.11 ± 0.12	1.03 ± 0.70	1.22 ± 0.50	0.64	0.44	>0.01
PE 16:0_22:5	1.89 ± 0.60	1.73 ± 0.20	5.53 ± 2.94	3.70 ± 1.65	0.42	0.73	>0.01
PE 18:0_22:5	10.40 ± 1.28	8.62 ± 1.61	18.35 ± 4.57	19.07 ± 3.18	0.35	0.27	0.01
Alkenyl-PE 16:0_22:5	8.18 ± 3.56	7.47 ± 1.51	15.62 ± 4.49	15.34 ± 2.73	0.28	0.94	>0.01
Alkenyl-PE 18:0_22:5	2.71 ± 0.73	3.07 ± 1.08	7.14 ± 2.05	6.47 ± 2.16	0.86	0.78	>0.01
PS 18:0_22:5	15.49 ± 0.89	13.78 ± 3.21	27.78 ± 10.63	28.04 ± 9.81	0.80	0.85	>0.01
PG 18:1_16:0	2.31 ± 0.15	3.82 ± 0.59	5.63 ± 1.59	4.40 ± 1.02	0.01	-	-

Abundance represented as analyte abundance divided by ISTD. Mean ± SD. n=4. P values determined by Tukey's HSD following two-way ANOVA.

Appendix Table 7.2: Normalized abundancies of erythrocyte lipid species in all groups

	DHA male	DHA female	Control Male	Control Female	P Sex x time Interaction	P Sex Effect	P Diet Effect
PC 16:0_20:4	30.22 ± 2.36	29.88 ± 5.17	51.24 ± 6.11	51.73 ± 5.98	0.60	<0.01	0.34
PC 18:0_20:4	1.25 ± 0.66	2.37 ± 0.56	2.69 ± 0.17	4.88 ± 1.18	0.88	0.98	<0.01
PC 18:0_22:6	8.91 ± 1.00	15.38 ± 2.61	8.39 ± 1.63	13.67 ± 2.54	0.19	<0.01	<0.01
PC 18:1_20:4	6.29 ± 1.19	3.30 ± 0.49	11.01 ± 1.79	6.70 ± 1.36	0.35	<0.01	<0.01
PC 20:4_20:4	0.36 ± 0.18	0.79 ± 0.68	3.86 ± 0.64	3.59 ± 1.90	0.56	0.90	<0.01
PE 18:1_22:6	5.69 ± 1.92	6.56 ± 1.35	2.93 ± 0.25	3.09 ± 1.13	0.60	0.44	<0.01
PE 16:0_18:2	5.41 ± 0.33	4.09 ± 0.56	8.24 ± 2.00	6.04 ± 0.60	0.47	0.01	<0.01
PE 18:0_22:5	2.51 ± 0.97	5.61 ± 1.28	7.59 ± 1.60	7.94 ± 1.24	0.07	0.03	<0.01
PE 18:1_18:2	13.34 ± 1.03	11.88 ± 1.28	19.10 ± 2.67	18.51 ± 4.41	0.77	0.50	<0.01
PE 38:6	17.61 ± 1.36	23.41 ± 2.59	10.73 ± 2.53	11.92 ± 5.03	0.21	0.07	<0.01
PE 40:8	3.31 ± 0.37	5.16 ± 0.97	3.91 ± 0.59	6.09 ± 1.08	0.71	<0.01	0.10
Alkenyl-PE 16:0_22:4	9.16 ± 0.13	9.52 ± 1.48	25.37 ± 1.22	21.36 ± 1.20	0.01	0.01	<0.01
Alkenyl-PE 18:0_22:6	22.96 ± 3.88	27.38 ± 1.97	16.15 ± 1.15	13.79 ± 1.71	0.01	0.39	<0.01
Alkenyl-PE 18:1_22:6	15.71 ± 1.75	14.82 ± 0.95	9.49 ± 1.96	9.19 ± 2.01	0.75	0.52	<0.01
PS 16:0_22:6	4.29 ± 0.76	2.28 ± 0.46	1.18 ± 0.69	0.48 ± 0.47	0.06	<0.01	<0.01
PS 18:1_22:6	9.21 ± 2.66	10.30 ± 1.14	4.27 ± 1.65	3.62 ± 1.27	0.34	0.80	<0.01

Abundance represented as analyte abundance divided by ISTD. Mean ± SD. n=4. P values determined by Tukey's HSD following two-way ANOVA.

Appendix Table 7.3: Concentrations of hippocampal phosphatidylcholine species in all groups

	DHA male	DHA female	Control Male	Control Female	P Sex x time Interaction	P Sex Effect	P Diet Effect
PC 18:1_20:4	10.19 ± 2.29	10.13 ± 3.01	18.64 ± 6.96	13.69 ± 2.24	0.26	0.25	0.01
PC 18:0_20:4	21.02 ± 1.11	23.07 ± 5.39	31.53 ± 9.78	29.76 ± 5.11	0.59	0.97	0.03
PC 16:0_16:1	8.06 ± 2.05	8.25 ± 3.37	13.70 ± 5.36	11.75 ± 3.11	0.57	0.64	0.03
PC 38:5	0.07 ± 0.08	0.12 ± 0.15	1.16 ± 0.69	1.57 ± 0.37	0.38	0.27	>0.01

Concentration reported in ng/mg. Mean ± SD. n=4. P values determined by Tukey's HSD following two-way ANOVA.

Appendix Table 7.4: Concentrations of erythrocyte phosphatidylcholine species in all groups

	DHA male	DHA female	Control Male	Control Female	P Sex x time Interaction	P Sex Effect	P Diet Effect
PC 16:0_20:4	5.54 ± 0.81	5.12 ± 2.07	10.08 ± 5.87	7.41 ± 1.45	0.36	0.11	0.44
PC 18:0_20:4	0.22 ± 0.11	0.41 ± 0.18	0.51 ± 0.23	0.68 ± 0.10	0.53	0.40	0.08
PC 18:0_22:6	1.63 ± 0.24	2.52 ± 0.46	1.68 ± 1.09	1.93 ± 0.28	0.95	0.07	0.01
PC 18:1_20:4	1.15 ± 0.24	0.57 ± 0.21	2.18 ± 1.31	0.95 ± 0.21	0.40	0.03	0.08
PC 20:4_20:4	0.07 ± 0.03	0.13 ± 0.11	0.73 ± 0.35	0.47 ± 0.18	0.17	0.38	<0.01

Concentration reported in ng/mg. Mean ± SD. n=4. P values determined by Tukey's HSD following two-way ANOVA.

Appendix Table 7.5: Fatty acid composition (weight %) of hippocampi

	DHA Female	DHA Male	Control Female	Control Male	P Sex x time Interaction	P Sex Effect	P Diet Effect
14:0	0.30 ± 0.01	0.37 ± 0.05	0.29 ± 0.01	0.31 ± 0.03	0.13	<0.01	0.04
16:0	21.19 ± 1.26	22.15 ± 0.49	20.95 ± 1.42	20.66 ± 1.16	0.29	0.57	0.16
18:0	20.66 ± 0.23	20.89 ± 0.30	20.99 ± 0.15	20.43 ± 0.42	0.02	-	-
20:0	0.38 ± 0.10	0.41 ± 0.08	0.40 ± 0.05	0.46 ± 0.12	0.75	0.33	0.48
22:0	0.46 ± 0.15	0.48 ± 0.08	0.48 ± 0.11	0.60 ± 0.19	0.49	0.31	0.32
24:0	0.69 ± 0.32	0.66 ± 0.02	0.78 ± 0.29	0.94 ± 0.34	0.49	0.63	0.21
SFAs	48.13 ± 0.61	49.11 ± 0.79	48.11 ± 0.68	47.79 ± 1.15	0.14	0.45	0.13
16:1	0.38 ± 0.03	0.43 ± 0.02	0.36 ± 0.04	0.36 ± 0.05	0.35	0.18	0.04
18:1n-7	2.71 ± 0.10	2.56 ± 0.12	2.72 ± 0.11	2.95 ± 0.20	0.02	-	-
18:1n-9	13.56 ± 1.12	12.45 ± 0.86	13.28 ± 1.86	14.35 ± 1.63	0.15	0.98	0.28
20:1n-9	0.46 ± 0.12	0.40 ± 0.08	0.44 ± 0.15	0.54 ± 0.17	0.25	0.77	0.37
22:1n-9	0.34 ± 0.09	0.43 ± 0.15	0.28 ± 0.14	0.20 ± 0.09	0.20	0.94	0.03
24:1n-9	0.47 ± 0.21	0.32 ± 0.13	0.45 ± 0.29	0.63 ± 0.33	0.21	0.95	0.28
MUFAs	18.84 ± 1.79	17.33 ± 1.39	18.46 ± 2.63	20.14 ± 2.64	0.17	0.94	0.29
18:2n-6	0.68 ± 0.05	0.78 ± 0.06	0.53 ± 0.04	0.58 ± 0.01	0.27	<0.01	<0.01
18:3n-6	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	<0.01	0.16	0.68	0.08
20:2n-6	0.09 ± 0.01	0.11 ± 0.01	0.07 ± 0.02	0.09 ± 0.01	0.69	<0.01	0.03
20:3n-6	0.49 ± 0.01	0.51 ± 0.04	0.34 ± 0.05	0.35 ± 0.04	0.67	0.44	<0.01
20:4n-6	11.17 ± 0.81	11.41 ± 0.53	12.31 ± 1.16	11.50 ± 0.59	0.22	0.50	0.16
22:2n-6	0.03 ± 0.02	0.03 ± 0.01	0.04 ± 0.01	0.03 ± 0.01	0.67	0.60	0.95
22:4n-6	2.52 ± 0.03	2.64 ± 0.08	3.06 ± 0.11	2.85 ± 0.14	<0.01	-	-
22:5n-6	0.34 ± 0.04	0.38 ± 0.03	0.95 ± 0.17	0.91 ± 0.13	0.50	0.92	<0.01
N-6	15.31 ± 0.88	15.88 ± 0.60	17.30 ± 1.34	16.31 ± 0.61	0.11	0.65	0.02
18:3n-3	0.02 ± 0.01	0.03 ± 0.02	0.01 ± 0.01	0.02 ± 0.01	0.44	0.08	0.03
20:3n-3	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.79	0.11	0.42
20:5n-3	0.04 ± 0.01	0.04 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	0.50	0.29	<0.01
22:5n-3	0.18 ± 0.01	0.19 ± 0.01	0.16 ± 0.02	0.18 ± 0.02	0.49	0.14	0.01
22:6n-3	14.37 ± 0.68	14.77 ± 1.02	12.93 ± 1.02	12.32 ± 1.08	0.32	0.84	<0.01
N-3	14.62 ± 0.68	15.05 ± 1.02	13.14 ± 1.00	12.55 ± 1.06	0.31	0.87	<0.01
20:3n-9	0.10 ± 0.02	0.08 ± 0.01	0.09 ± 0.02	0.10 ± 0.03	0.19	0.68	0.70
PUFAs	30.03 ± 1.54	31.00 ± 0.98	30.53 ± 2.32	28.96 ± 1.64	0.16	0.73	0.38
N-6/N-3	1.05 ± 0.02	1.06 ± 0.09	1.32 ± 0.02	1.30 ± 0.06	0.66	0.99	<0.01
Total (mg/g)	38.2 ± 5.1	38.6 ± 3.2	40.4 ± 7.6	41.4 ± 3.4	0.90	0.80	0.35

Mean ± SD, n=4. P values determined by Tukey's HSD following two-way ANOVA. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; FA, fatty acids

Appendix Table 7.6: Fatty acid composition (weight %) of erythrocytes

	DHA Female	DHA Male	Control Female	Control Male	P Sex x time Interaction	P Sex Effect	P Diet Effect
14:0	0.45 ± 0.02	0.48 ± 0.03	0.42 ± 0.02	0.46 ± 0.01	0.41	0.01	0.04
16:0	24.88 ± 0.30	27.98 ± 1.22	24.37 ± 0.42	26.84 ± 0.58	0.40	<0.01	0.04
18:0	14.17 ± 0.62	11.11 ± 0.59	15.03 ± 0.55	11.89 ± 0.73	0.91	<0.01	0.02
20:0	0.17 ± 0.02	0.22 ± 0.10	0.15 ± 0.01	0.16 ± 0.03	0.58	0.33	0.17
22:0	0.46 ± 0.03	0.48 ± 0.15	0.40 ± 0.03	0.42 ± 0.03	0.93	0.63	0.16
24:0	1.14 ± 0.08	1.10 ± 0.22	1.18 ± 0.04	1.14 ± 0.06	0.98	0.51	0.58
SFAs	45.89 ± 1.02	44.79 ± 1.25	45.75 ± 1.35	45.27 ± 0.38	0.57	0.17	0.76
16:1	0.30 ± 0.05	0.35 ± 0.04	0.31 ± 0.04	0.40 ± 0.04	0.30	<0.01	0.17
18:1n-7	2.11 ± 0.06	2.39 ± 0.08	2.19 ± 0.02	2.61 ± 0.11	0.09	<0.01	<0.01
18:1n-9	7.91 ± 0.14	8.25 ± 0.18	8.05 ± 0.15	8.58 ± 0.17	0.27	<0.01	0.01
20:1n-9	0.12 ± 0.01	0.14 ± 0.01	0.12 ± 0.01	0.14 ± 0.01	0.98	0.01	0.48
22:1n-9	0.60 ± 0.40	0.78 ± 0.62	0.28 ± 0.31	0.49 ± 0.61	0.95	0.46	0.25
24:1n-9	0.77 ± 0.51	0.94 ± 0.10	1.02 ± 0.06	1.16 ± 0.11	0.93	0.28	0.10
MUFAs	13.50 ± 0.84	14.60 ± 0.28	13.68 ± 0.36	15.19 ± 0.57	0.46	<0.01	0.19
18:2n-6	7.47 ± 0.21	8.92 ± 0.18	6.91 ± 0.38	8.00 ± 0.29	0.22	<0.01	<0.01
18:3n-6	0.06 ± 0.01	0.04 ± 0.01	0.08 ± 0.01	0.05 ± 0.01	0.59	<0.01	0.01
20:2n-6	0.25 ± 0.02	0.29 ± 0.01	0.24 ± 0.01	0.30 ± 0.02	0.35	<0.01	0.87
20:3n-6	0.49 ± 0.01	0.58 ± 0.01	0.40 ± 0.01	0.47 ± 0.03	0.20	<0.01	<0.01
20:4n-6	21.89 ± 0.71	20.00 ± 0.23	24.92 ± 0.24	23.37 ± 0.50	0.49	<0.01	<0.01
22:2n-6	0.05 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.02	0.49	0.58	0.62
22:4n-6	0.77 ± 0.02	0.72 ± 0.05	1.47 ± 0.13	1.47 ± 0.10	0.64	0.62	<0.01
22:5n-6	0.24 ± 0.02	0.21 ± 0.02	0.60 ± 0.05	0.55 ± 0.03	0.65	0.04	<0.01
N-6	31.21 ± 0.67	30.81 ± 0.25	34.66 ± 0.16	34.25 ± 0.62	1.00	0.12	<0.01
18:3n-3	0.32 ± 0.48	0.08 ± 0.01	0.08 ± 0.01	0.09 ± 0.02	0.33	0.36	0.36
20:3n-3	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.02	0.73	0.72	0.78
20:5n-3	0.71 ± 0.04	0.65 ± 0.04	0.32 ± 0.02	0.30 ± 0.02	0.16	0.03	<0.01
22:5n-3	1.64 ± 0.03	1.74 ± 0.04	1.80 ± 0.07	1.84 ± 0.06	0.28	0.03	<0.01
22:6n-3	6.04 ± 0.21	5.53 ± 0.17	3.01 ± 0.12	2.77 ± 0.12	0.12	<0.01	<0.01
N-3	8.74 ± 0.55	8.03 ± 0.14	5.24 ± 0.15	5.03 ± 0.19	0.13	0.01	<0.01
20:3n-9	0.05 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	0.07 ± 0.01	0.25	0.12	<0.01
PUFAs	40.00 ± 0.91	38.89 ± 0.36	39.96 ± 0.19	39.35 ± 0.57	0.39	0.01	0.48
N-6/N-3	3.58 ± 0.22	3.84 ± 0.05	6.62 ± 0.20	6.82 ± 0.32	0.81	0.06	<0.01
Total (mg/g)	4.24 ± 0.44	4.26 ± 0.30	4.74 ± 2.11	5.01 ± 0.45	0.82	0.79	0.28

Mean ± SD, n=4. P values determined by Tukey's HSD following two-way ANOVA. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; FA, fatty acids

Appendix Table 7.7: Correlations between hippocampal and erythrocyte fatty acid concentrations (mg/g)

Group	Total	DHA Female	DHA Male	Control Female	Control Male
n	16	4	4	4	4
Fatty Acid	Pearson's r	Pearson's r	Pearson's r	Pearson's r	Pearson's r
20:4n-6	0.679**	-0.089	-0.772	0.841	0.531
22:4n-6	0.832**	-0.446	-0.743	0.979*	0.875
22:5n-6	0.874**	0.630	-0.720	0.475	-0.822
22:6n-3	0.484	-0.384	-0.844	0.897	-0.513

Correlations of fatty acid concentrations (mg/g) between hippocampi and erythrocytes. Total group includes all rats analyzed by GC-FID. Pearson's r: Pearson's correlation coefficient.*p<0.05; **p<0.01

Appendix Table 7.8: Correlations between hippocampal and erythrocyte fatty acid compositions (wt %)

Group	Total	DHA Female	DHA Male	Control Female	Control Male
n	16	4	4	4	4
Fatty Acid	Pearson's r	Pearson's r	Pearson's r	Pearson's r	Pearson's r
20:4n-6	0.306	-0.940	0.414	-0.124	-0.374
22:4n-6	0.849**	-0.479	-0.541	0.276	0.967*
22:5n-6	0.932**	-0.434	-0.075	0.268	-0.249
22:6n-3	0.714**	-0.950	-0.467	-0.166	0.465

Correlations of fatty acid compositions (wt %)between hippocampi and erythrocytes. Total group includes all rats analyzed by GC-FID. Pearson's r: Pearson's correlation coefficient.*p<0.05; **p<0.01

8 General Discussion

The main objectives of this thesis were to assess the effects of DHA supplementation in the context of Western diets on maternal tissue stores and the spatial memory of female and male pups at adolescence. These objectives were approached through a series of three experiments: the first to identify the effect of DHA supplementation on pups and mothers in mothers fed high fat TWDs compared to controls; the second to examine when sexual dimorphisms in LCPUFA begin in selected tissues of pups fed chow diets; and the third to determine the effect of DHA supplementation in AFWDs on spatial memory in adolescent rats. Altogether, these studies aimed to address the gap between recommendations and actual intakes of DHA during pregnancy, and better understand the effects of dietary supplementation on spatial memory in males and females during adolescence.

The hypotheses and limitations of individual studies are discussed within their individual chapters, however there are general hypotheses and limitations to this thesis. Currently, recommendations for omega-3 PUFA intakes, and more specifically DHA intakes during pregnancy, are broad and are not tailored to individual dietary habits. For example, Health Canada and the IOM recommend 150g of fish per week to women of childbearing age [26], and the ISSFAL recommends at least 200mg of DHA/d for pregnant women [25]. However, the combined findings of studies 1 and 3 suggest recommendations for dietary n-3 PUFA could be more effective if they were provided relative to total fat or caloric intakes, such as the general recommendations provided by the Brazilian Dietary Guide [155]. Current ISSFAL recommendations to pregnant women suggest that increased DHA consumption is unlikely to affect maternal plasma and tissue contents of ARA [25]. However, because fetal ARA accretion predominantly occurs postnatally[156], potential changes to maternal ARA status during

lactation could affect infant ARA status. Further research on the effect of increased dietary DHA consumption on perinatal ARA status should be considered.

DHA accounted for between 0.3 and 0.5% of total caloric intakes in the supplemented diets used in studies 1 and 3. However, 34.5% of total caloric intakes in study 1 were from fat, while in study 3 only 17.2% of total caloric intakes were from fat. In these studies, DHA appeared to be beneficial to the mothers in study 1, as those on the supplemented diets maintained or improved tissue DHA status during pregnancy and postpartum. However, it may have been harmful to adolescent pups in study 3, as those from the DHA group had longer latency times during the final day of the learning phase during the MWM as compared with controls. Providing consumers with the tools necessary to contextualize DHA recommendations to their daily fat intakes could improve their DHA status and optimize their health benefits from DHA consumption.

Although spatial memory outcomes were only measured in study 3, diet significantly affected the fatty acid composition of arachidonic acid in both studies 1 and 3. In the pregnancy study, mothers fed TWD+ and TWD- had significantly lower ARA in maternal livers and whole body fetuses than mothers fed chow. Additionally, ARA was significantly lower in plasma at postpartum compared to baseline in all diets, but ARA in maternal liver and adipose was lower during the postpartum period in both the TWD groups. These findings of low tissue ARA levels despite high LA intakes are in accordance with previous research [157], and suggest that the consumption of the high amounts of SFAs and MUFAs in high fat diets could be displacing ARA, much like how DHA supplementation appeared to displace ARA in the spatial memory study.

The TWDs and AFWDs used in the pregnancy and spatial memory studies are worth discussing. The fatty acid composition of the diets used in these studies are based on fatty acid intakes reported in the 2005 NHANES study [100]. These data, however, are based on USDA standard references that have been shown in the past to under-report ARA content, and over-report n-3 PUFA content in red meat and poultry [158]. Since ARA is predominantly consumed in meat, confirming the ARA and n-3 PUFA content in red meat and poultry for recent USDA standard references may be important. Regardless, low levels of ARA consumption are also reported in studies using a mix of the French food consumption table and USDA standard references [159], in pregnant African-Americans[160], and in developing countries [161]. The results of the spatial memory study suggest this prevalent low consumption of ARA is detrimental when combined with long term DHA supplementation. A 1:1 ratio of ARA:DHA appears to be optimal when supplementing the LCPUFA perinatally [144], and further research into using this ratio of fatty acids should be conducted.

Limitations in this thesis include the differences between puberty in humans and in rats. In rats, females begin puberty earlier than males [24,114,115]. This complicates translating findings of adolescence in rats studied at a single time point to stages of adolescence in humans between both sexes, as female rats may be more developed than males during a single time point, whereas humans would be similar. Additionally, the estrous cycle has been reported to affect fatty acid status [162] and Morris water maze performance in rats [163]. Measuring estradiol levels in females and males could assist in contextualizing the findings between animal and human studies, and between stages of the estrous cycle in animal studies.

The interpretation of this thesis is also limited by differences in lipoproteins and fatty acid metabolism between rodents and humans. Although lipoprotein profiles of rats and humans

are similar, rats can have higher amounts of circulating TAG and VLDL compared to healthy humans [164]. Additionally, the elongation enzyme ELOVL2 which can elongate both 18 and 20-carbon PUFA to 22-carbon PUFA in rodents, appears to only operate on 20-carbon PUFA in humans [165]. The potential interaction of these lipoprotein profiles and enzymes with sex, diet, or pregnancy, and the differences they may have between rodents and humans should be taken into consideration when interpreting these findings.

The aim of this thesis was to determine the effects of DHA supplementation in the context of western diets on maternal tissue status and spatial memory in female and male pups during adolescence. Examination of maternal tissues during pregnancy and the postpartum period revealed that maternal adaptations to meet lipid and fatty acid requirements change in the transition from pregnancy to lactation with the pup requirement for DHA during lactation being particularly detrimental to maternal tissue levels when dietary DHA levels are low. These findings can be used to guide examinations of the kinetics of a potential DHA mobilization from maternal tissues, or as a guide to examine previously understudied candidates for maternal stores of DHA that could be mobilized to meet fetal demand. Furthermore, the examination of DHA supplementation from the confirmation of pregnancy to adolescence on spatial memory in males and females revealed that DHA supplementation in diets adequate for LA and ALA displaces ARA and 22:5n-6 containing lipid species in the hippocampus, and increases latency times in the Morris Water Maze. These findings corroborate recent suggestions that a 1:1 ratio of ARA:DHA supplementation is optimal for cognitive benefits [144], and could be used to guide future recommendations for dietary LCPUFA recommendations. Altogether, these findings show that further research is needed in determining appropriate LCPUFA recommendations for the early stages of life. Considering the impact of dietary DHA supplementation in the context of Western

diets in future study designs could provide valuable insight into its effects on maternal and perinatal health.

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