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Concentrations of docosahexaenoic acid are reduced in maternal liver, adipose, and heart in rats fed high-fat diets without docosahexaenoic acid throughout pregnancy

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Highlights

- DHA supplementation is examined in rats during pregnancy with a background diet that resembles human Western diets and a standard rodent chow.
- DHA concentrations are lower in maternal adipose during pregnancy and postpartum in the Western diet without DHA.
- At postpartum DHA concentrations decrease below baseline levels in maternal heart and liver of the dams fed Western diets without DHA and in liver in the chow diet.
- Low maternal DHA intakes was associated with lower DHA concentrations in the 7d old pups but not the fetuses

**Concentrations of docosahexaenoic acid are reduced in maternal liver, adipose, and heart
in rats fed high-fat diets without docosahexaenoic acid throughout pregnancy**

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Running Head: Maternal Tissues as a Source of Fetal DHA

Abstract

Fetal accretion for DHA is high during late pregnancy due to the brain growth spurt. Prior evidence suggests that DHA is mobilized from maternal liver and adipose to meet fetal accretion and physiological requirements. However, changes in the DHA levels of various maternal tissues throughout pregnancy and into lactation of mothers on diets with and without dietary DHA, and with a background dietary fatty acid profile that resembles human intake has not been examined. Sprague Dawley rats were fed a total western diet with (TWD+) or without DHA (TWD-) along with a commercial rodent chow control (Chow) throughout pregnancy and postpartum. The fatty acid compositions of adipose, brain, heart, liver, erythrocytes, and plasma were determined before pregnancy, at 15 and 20 days of pregnancy, and 7 days postpartum. The placenta, fetuses, and pups were also examined when available. Maternal DHA concentrations were increased in plasma at 20 days pregnancy in all the diets with TWD+>Chow>TWD-. Maternal DHA concentrations in the TWD- group were lower in adipose throughout pregnancy as compared with the other diets. At postpartum, DHA concentrations decreased below baseline levels in the heart of the TWD- and Chow dams and the liver of the TWD- dams. Whole body DHA concentrations of the fetuses did not differ but there was evidence of decreased DHA in the whole body and tissues of the TWD- and Chow 7d old pups. In conclusion, it appears that in this rodent model of pregnancy, maternal adaptations were made to meet fetal DHA requirements, but they may compromise maternal DHA status and the ability to deliver DHA during lactation.

Keywords

Docosahexaenoic acid, omega-3, pregnancy, lactation, postpartum, heart, plasma, fetus, pup, infant, fatty acid composition

Abbreviations

ALA, alpha-linolenic acid; ARA: arachidonic acid; LA, linoleic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; LC, long chain; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; BHT, Butylated hydroxytoluene.

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1. Introduction

Docosahexaenoic acid (DHA) has been shown to be essential for neurodevelopment and infant health [1, 2]. Levels of DHA in plasma increase approaching parturition in both humans [3-5] and rats [6, 7], likely as an adaptation to meet fetal accretion 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 [2, 6, 8-10]. Maternal intake of DHA during pregnancy is low in North America [11], therefore fetal DHA requirements for accretion likely exceeds maternal DHA intakes near parturition [12] for a considerable proportion of the population. Although *de novo* synthesis of DHA appears to be upregulated during pregnancy [8], the rate of DHA conversion from alpha-linolenic acid (ALA) in adults is generally considered to be less than 1% [13]. Evidence suggests DHA is mobilized from maternal liver and adipose depots during pregnancy and lactation [9, 10, 14, 15]. It is possible that DHA could also be mobilized from other maternal tissues, particularly those demonstrated to be rich in DHA [16] to help meet fetal and infant tissue accretion requirements.

General lipid mobilization from maternal tissues during pregnancy and lactation could assist in providing a stable and constant supply of fatty acids and prevent diurnal and dietary intake-based fluctuations that impact fetal and infant development. Dependency on dietary intake would be subject to postprandial fluctuations in lipid circulation [17], and considerable variation in the fatty acid composition of individual meals. DHA intake by humans can be quite sporadic, especially in Western populations [18, 19]. While *de novo* biosynthesis from ALA could provide a stable supply of DHA, high dietary intakes of n-6 polyunsaturated fatty acids (PUFA) [20] and monounsaturated fatty acids (MUFA) [21] in most human diets [22, 23] could limit hepatic elongation and desaturation rates. Therefore, mobilization of DHA that has been accrued in

maternal tissues over time could be an adaptation to ensure that the fetus and infant have a stable supply of a minimal amount of DHA during critical periods of development regardless of dietary intake during pregnancy. Balance studies of rats fed diets without eicosapentaenoic acid (EPA) and DHA show a net loss of DHA in adipose during pregnancy [15, 24]. Maternal DHA status has also been linked to DHA levels in breast milk, and mobilization of DHA from maternal stores may be critical to support lactation [25].

DHA may be mobilized from various maternal tissues in addition to adipose during pregnancy and postpartum. We hypothesized that DHA in different maternal tissues will change differently during pregnancy, and that these changes would be influenced by maternal dietary DHA intakes. This was examined with a background rodent diet designed to emulate a typical human Western diet, including percentages of saturated fatty acids (SFA), MUFA and PUFA [26] and compared to a common rodent chow diet as an additional reference 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.

2. Materials and methods

2.1 Study design

All animal experiments were performed in agreement with the policies of the Canadian Council on Animal Care and approved by the University of Waterloo Animal Care Committee. Female Sprague-Dawley rats (7 weeks of age) and diets were purchased from Envigo (Mississauga, Ontario, Canada). Baseline rats were not bred and fed either a fixed-formula 8640 Teklad 22/5 Rodent Diet (Chow), a TD.110424 total western diet without DHA (TWD-), or the total western diet with DHA added (TWD+) (Table 1) for 7 days prior to being sacrificed (n=6 per diet). The chow diet was a natural-ingredient diet consisting of dehulled soybean meal, ground corn, wheat middlings, flaked corn, fish meal, cane molasses, soybean oil, ground wheat, and dried whey with additional vitamins and minerals. The formulation of the TWD was designed to mimic the dietary intake of Americans rather than rodent growth and fertility [26]. Briefly, it has more energy from fat (Supplemental Table 1) and composed of purified ingredients with protein from casein with additional cystine, carbohydrates from corn starch, maltodextrin and sucrose, and fat from a mixture of olive oil, soybean oil, corn oil, lard, beef tallow and milkfat. The TWD diet is also lower in micronutrient content with the exception of sodium and other phytochemicals such as isoflavones were likely lower in the TWD diet due to the use of purified ingredients, but these differences were not confirmed biochemically. In the TWD+ diet, 4.85 g/kg of DHASCO (DSM Nutritional Products Inc., Columbia, MD, USA) replaced a proportion of the fat mixture to achieve a DHA composition of 1.21% total fatty acids to mimic levels consumed by Japanese women [27].

Rats assigned to the pregnancy groups were housed with male breeders and fed chow diets until confirmation of pregnancy by vaginal plug. Upon confirmation of pregnancy, female

rats were immediately assigned to chow, TWD+, or TWD- diets for the remainder of the experiment. Following an overnight fast, rats were sacrificed at day 15 of pregnancy, day 20 of pregnancy, or 7 days postpartum (Fig. 1) by exsanguination after anesthesia using isoflurane (n=6 per group). Food intake and body weight were measured at baseline, day 15 and 20 of pregnancy, and postpartum. Exsanguinated blood was collected in the presence of EDTA and plasma was isolated by centrifugation at 1,500 g and 4°C. Brain, liver, heart, white adipose (perirenal), and placenta were excised, washed in saline (0.9% v/v), weighed, and flash-frozen in liquid nitrogen. Fetuses were sacrificed by decapitation, washed in saline, weighed, and snap frozen in liquid nitrogen. Pups sacrificed by exsanguination following anesthesia using isoflurane were used to collect heart, brain, and liver or preserved for whole body analyses. After collection, samples were stored at -80°C until analysis.

2.2 Fatty acid analyses

The lipids from pulverized tissue samples and plasma were extracted using 2:1 chloroform:methanol [28] 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) as internal standard. Aqueous and organic phases were separated by the addition of 0.2M sodium phosphate buffer, and the organic layer was collected. Erythrocyte lipids were isolated using similar reagents but with a double extraction protocol [29]. The collected organic phases were dried under nitrogen and fatty acid methyl esters were generated with the addition of 1mL of 14% boron trifluoride in methanol and 0.3mL hexane, and heating on a heating block at 95°C for 1h [30]. Fatty acid methyl esters were then washed in 1mL hexane and 1mL water and the hexane layer was collected for analysis by gas chromatography with flame ionization detection. Briefly, a fast gas chromatography protocol

[31] was followed using a Varian 3900 gas chromatograph equipped with a DB-FFAP 15m x 0.10mm internal diameter x 0.10 μ m film thickness nitroterephthalic acid-modified polyethylene glycol capillary column (J&W Scientific/ Agilent Technologies, Mississauga, Ontario, Canada). Hydrogen was used as the carrier gas and temperature ramping was designed to maximize peak resolution [32] with 1 μ L samples injected at a 100:1 split ratio. Individual fatty acid peaks were identified by comparison to a reference mixture of fatty acids (GLC-462; Nu-Chek Prep Inc.).

2.3 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 \pm SD with significance accepted when $p < 0.05$.

3. Results

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. Although there was evidence of increased consumption by the chow group throughout, the mass intake by the chow dams was only significantly higher at 7 days postpartum (47.5 ± 4.37 g) as compared with TWD+ dams (34.0 ± 10.0 g) and TWD- dams (32.33 ± 12.45 g). This coincided with a significant increase in maternal energy intakes in all diets at 7 days postpartum as compared with baseline and gestation across all diets (Fig. 2). 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 [6]. Maternal and fetal body masses did not differ between

diets (data not shown). However, the body mass of 7 day old pups from TWD+ dams ($14.16 \pm 2.99\text{g}$) and TWD- dams ($14.14 \pm 2.63\text{g}$) were significantly higher than those from chow dams ($12.63 \pm 1.68\text{g}$).

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 (Fig.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 (Fig. 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 (Supplemental Table 2). The TWD+ and TWD- diets also resulted in higher percentages of saturated and monounsaturated and lower percentages of n-6 polyunsaturated fatty acids as compared with plasma from the chow diet. Arachidonic acid (ARA) concentrations in plasma at day 20 of pregnancy did not differ from baseline (data not shown), but the percentages were decreased at day 20 of pregnancy in all diets and remained low in TWD+ and TWD- (Supplemental Table 2) In erythrocytes, the increase in DHA concentrations at day 20 was also observed in all the diets, but the increase was more gradual, and very subtle in the chow and TWD-, and extended into postpartum for the TWD+ group (Fig 3 B). In contrast to plasma, the total fatty acid concentrations of erythrocytes did not increase at day 20 of pregnancy. The concentration of ARA in erythrocytes was decreased with chow at day 20 of pregnancy and 7 days postpartum. The percentage of ARA

tended to decrease in late pregnancy and the postpartum of all diets, with the TWD- diet having a slightly higher percentage of ARA at these time points (Supplemental Table 3).

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 (Fig. 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 , Supplemental Table 4). 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 7 days postpartum (2.5 – 2.7 fold, Supplemental Table 4). 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. Liver ARA concentrations did not change from baseline except for a decrease at day 20 in the chow dams (data not shown). The percentage of hepatic ARA tended to be higher in the chow diet with decreased percentages at 7 days postpartum in the TWD diets.

Adipose DHA concentrations were decreased throughout pregnancy in the TWD- group (Fig 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 (Supplemental Table 5). ARA concentrations (data not shown) and percentages decreased in both TWD diets from baseline to postpartum with no differences in the chow rats. In the heart, DHA concentrations increased in the TWD+ at day 20 of pregnancy and then returned to baseline levels (Fig 3E). In the TWD- and chow groups, heart DHA

concentrations did not increase at day 20 and significantly decreased at 7 days postpartum (Fig 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 (Supplemental Table 6). The concentrations and percentages of ARA in heart tended to be increased above baseline at day 20 of pregnancy in all diets with TWD- concentrations and percentages tending to be higher than the other diets. 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 (Fig 3F) and the percentage of brain DHA and ARA did not differ in any of the groups (Supplemental Table 7).

3.3 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 (Fig 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 (Supplemental Table 8). ARA concentrations did not differ in the placenta, but the percentages at day 20 decreased significantly in chow, and tended to be decreased in TWD. In the fetus, whole body DHA concentrations did not differ (Fig 4B) although the percentage of DHA increased in the TWD+ and chow fetuses from day 15 to day 20 (Supplemental Table 9). 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, Fig 4B). After birth, total fatty acid concentrations increased significantly in all the pups with large increases in 18:2n-6, 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, which also

had increases in 18:1n-9 (Supplemental Table 9). The concentration of ARA in whole body pups was higher than the fetuses, while the percentages of ARA were lower.

3.4 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 (Fig. 5). DHA concentrations in the brain and heart of TWD- group were also significantly lower than the chow group. ARA concentrations were higher in heart with chow and in liver with TWD- and no differences in brain. 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 (Supplemental Table 10). 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 (Supplemental Table 10). The percentage of ARA in the brain of TWD- pups was higher than pups from the other diets. 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 ARA (Supplemental Table 10).

4. Discussion

Our results 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 [33], 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 [34].

An increase in DHA concentrations in maternal plasma has been observed previously [3, 6, 9, 14, 15, 35]. We also confirm 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 palmitoyldocosahexaenoyl phosphatidylcholine for incorporation into lipoproteins during pregnancy induced hyperlipidemia [6]. In the present study, we demonstrate 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 increase DHA in 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 [6] and in humans [3, 5]. This rise and fall is associated with changes in lipoprotein levels, but also DHA availability for incorporation into phosphatidylcholine [6, 36]. 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 and percentage of DHA [16] and a high fatty acid turnover rate [37] with a potential specificity for lipolysis of long chain polyunsaturated fatty acid such as DHA [38, 39]. Fatty acid β -oxidation can provide 50-70% of the adult heart's energy requirement, however this is under complex control [40]. Under certain conditions, including perfusion with insulin, cardiomyocytes can engage in reverse fatty acid transport [41]. Insulin levels are elevated during pregnancy [42]. It is possible that DHA could have been released from the heart to the circulation in the present study but additional studies are required to confirm.

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 decrease but that perirenal adipose do not change at 18 days of pregnancy [15]. We

observed the increase in DHA in perirenal adipose in the chow and TWD+ diets at day 20 but not day 15 of pregnancy.

The lower levels of DHA in the adipose of the TWD- group throughout pregnancy and postpartum suggest that the fetal/pup requirements was not being met by the intake of dietary DHA and that adaptations to increase DHA biosynthesis during pregnancy [6, 43-45] could not meet the requirements 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 LA to ALA and the energy % of over 50 diets [46], 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 our study included different levels of preformed DHA in the diets. In addition, high MUFA intakes can reduce ALA metabolism [21] 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.

Placental and whole body pup DHA concentrations from our model are consistent with previous reports of maternal DHA status affecting fetal and infant DHA status through both placental transfer [12] and lactation [47]. 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 accretion requirements, but that this did not extend into

postpartum lactation. 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, 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 [48]. 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 physiological requirements by the pup via lactation.

The changes in ARA levels differed from DHA. With a standard rodent chow diet, the percentage of ARA has been shown to decrease in plasma phosphatidylcholine and liver phosphatidylcholine and phosphatidylethanolamine during pregnancy and it was speculated that fetal uptake of ARA may be met through transfer of ARA from abundant maternal tissue stores rather than new synthesis [6]. The present study appears to confirm this response in the chow rats as well as the TWD diets and ARA tended to decrease or remain the same in plasma and liver. ARA and DHA in adipose, heart and brain did have relatively similar responses throughout pregnancy, but DHA tended to increase in placenta while ARA decreased. In the fetuses/pups, the percentage of ARA tended to decrease slightly during pregnancy followed by a large

decrease postpartum especially in the TWD diets. In contrast, DHA percentages increased in fetal whole bodies at day 20 of pregnancy and then decreased postpartum.

This study has several limitations. Maternal DHA status was examined by manipulating the DHA content of the TWD diet that is designed to mimic the human diet of Americans. The TWD has higher fat content but also differences in sucrose and fiber as well as micronutrients when compared to the rodent chow. We included a natural ingredient chow diet with most of the fat provided by soybean oil to allow for crude comparison to the existing literature that is predominantly based on rodent chow recommendations. However, it appears that low fat diets that reflect human fat consumption while controlling for micronutrient content should be considered in future studies. While we screened several maternal and fetal/pup tissues across various time points to identify decreases in tissue levels as evidence of maternal mobilization of DHA for fetal/pup transfer, only static measures of tissue fatty acid levels were completed. Also, while we did examine several maternal tissues, our own 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 [15]. We hope that the present findings can guide targeted kinetic studies using fatty acid tracers to assess true DHA mobilization in the future. While our 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 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 [49]. 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 we only measured one postpartum time point, the duration of this response and the consequences of these diets on maternal and pup health need to be examined in more detail.

In conclusion, we provide 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. Our 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.

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Author Contributions

KDS, and AC conceived the animal experiment. AC conducted the animal trial. DMLK and JJAH analyzed the fatty acid composition of maternal tissues and JJAH, SJH, and DMLK analyzed fetal/pup tissues. DMLK, SJH and KDS completed statistical analyses. DMLK and KDS wrote the first draft; all authors approved the final draft.

Conflict of Interest

All authors declare no conflict of interest

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Table 1

Nutrient and fatty acid composition of chow versus DHA supplemented (TWD+) and unsupplemented (TWD-) Total Western Diets.

Diet component	Chow	TWD+	TWD-
Energy Density (kcal/g)	4.4	4.4	3.0
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 (weight %)			
16:0	13.24 ± 0.11	18.73 ± 0.08	18.81 ± 0.13
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
16:1	0.77 ± 0.01	1.18 ± 0.01	1.14 ± 0.01
18:1n-7	1.31 ± 0.01	1.49 ± 0.02	1.52 ± 0.06
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
18:2n-6	48.51 ± 0.05	20.21 ± 0.04	20.86 ± 0.03
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
18:3n-3	4.78 ± 0.01	1.83 ± 0.01	1.90 ± 0.01
20:3n-3	0.01 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
20:5n-3	0.42 ± 0.01	0.01 ± 0.01	0.01 ± 0.01
22:5n-3	0.08 ± 0.01	0.05 ± 0.01	0.03 ± 0.01
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 for fatty acid composition (% wt) is mean ± SD from triplicate analysis in our laboratory. SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids.

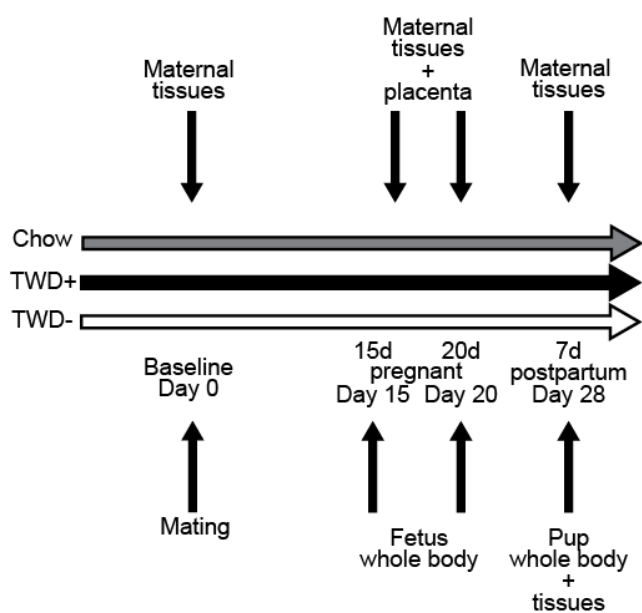


Fig 1. Study design flowchart. Female Sprague Dawley rats were fed either a fixed formula 8640 Teklad 22/5 Rodent Diet (Chow), a TD.110424 total western diet with DHA (TWD+) or a total western diet without DHA (TWD-). Baseline rats were sacrificed at 9 weeks of age after one week on the diets. Rats assigned 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).

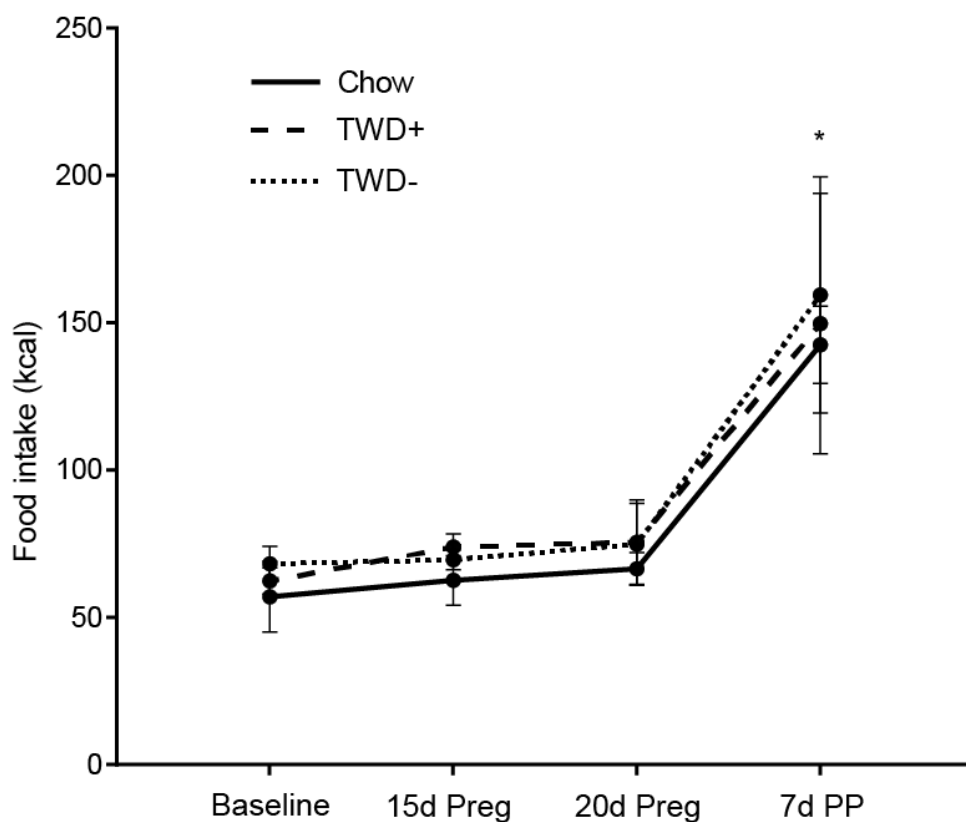


Fig. 2. Food intake across pregnancy and postpartum. *Significantly higher than intakes at other time points for all diets (main effect of time by two-way ANOVA followed by Tukey post hoc ($p < 0.05$)). Mean \pm SD, $n = 6$ for each point.

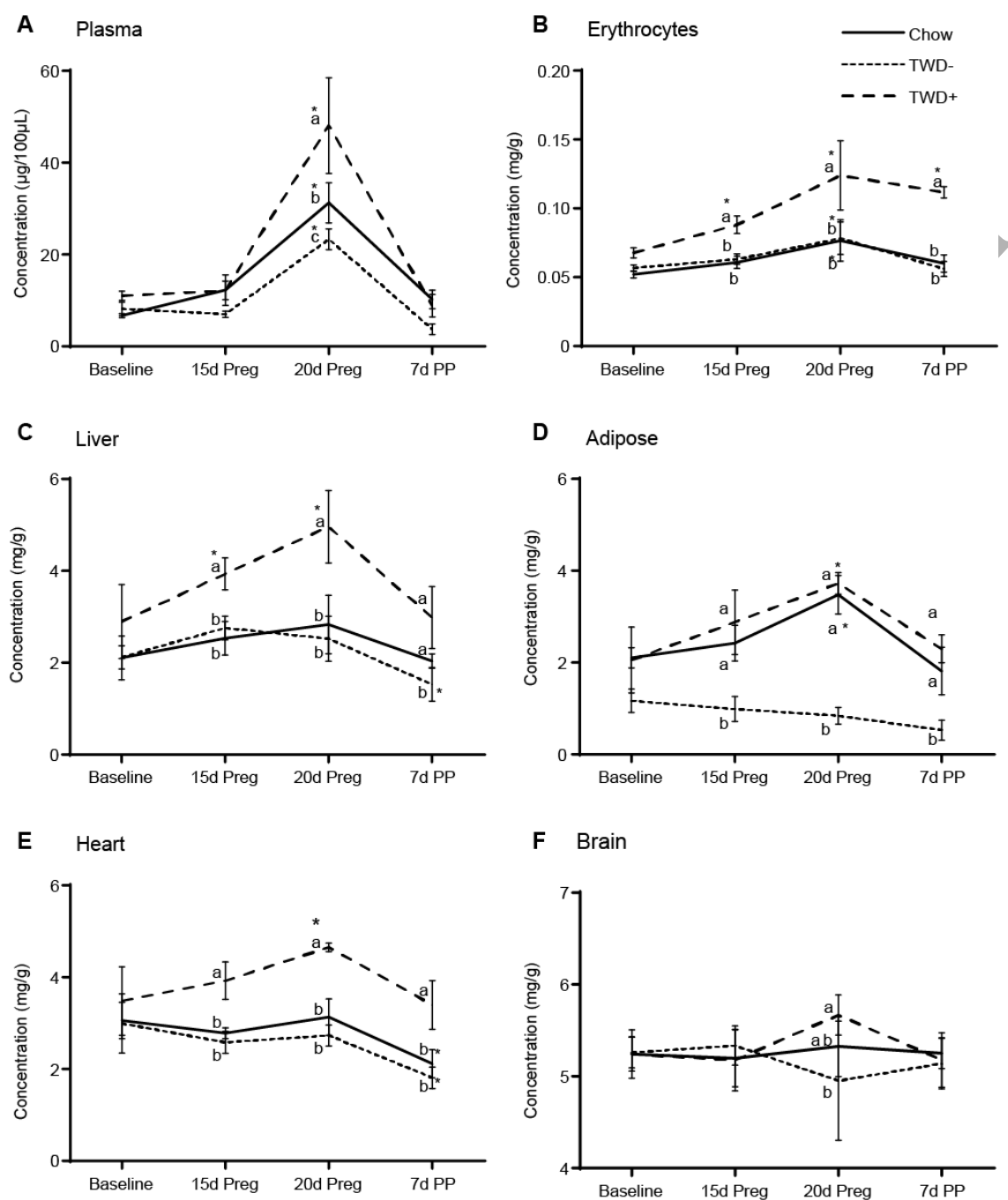


Fig. 3. Effects of pregnancy and diet on DHA concentrations of maternal tissues. Adipose is white perirenal adipose tissue. Different letters indicate diet differences within a time point, and * indicate differences within a diet from baseline. All differences were determined by Tukey's post hoc following significant F-value by two-way ANOVA ($p < 0.05$). Mean \pm SD, $n = 6$ for each point.

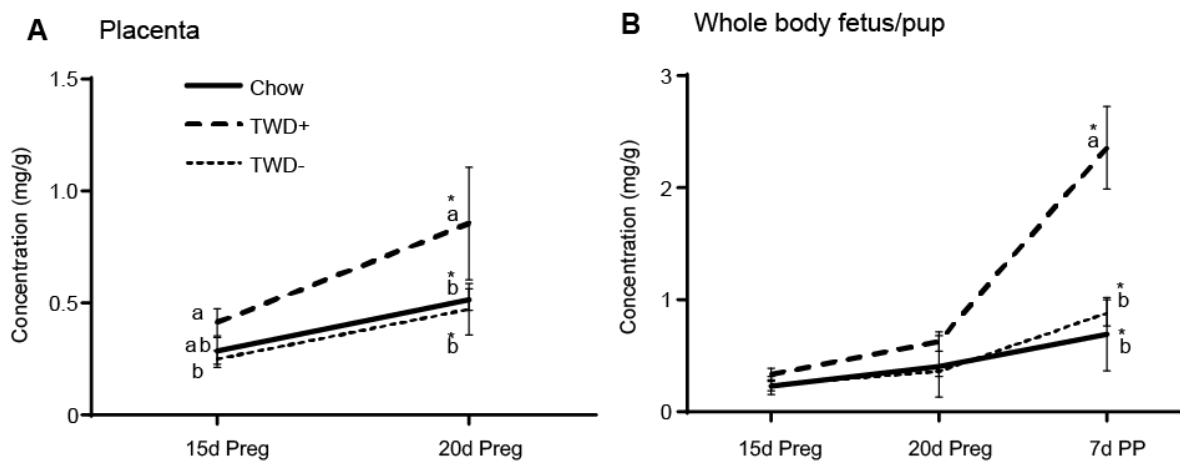


Fig. 4. Effects of pregnancy and diet on placental, and whole body fetus/pup DHA concentrations. Different letters indicate diet differences within a time point, and * indicate within diet differences from baseline. All differences are as determined by Tukey's post hoc following significant F-value by two-way ANOVA ($p < 0.05$). Mean \pm SD, $n = 6$ for each point.

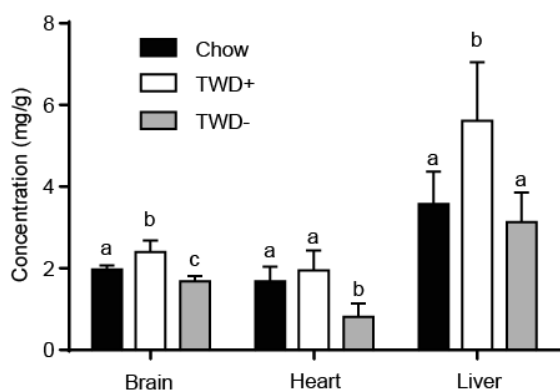


Fig. 5. Effect of maternal diet on DHA concentrations of brain, heart, and liver of 7-day old pups. Different letters indicate differences between diets as determined by Tukey's post hoc following significant F-value by one-way ANOVA ($p < 0.05$).