INTRAUTERINE GROWTH RESTRICTION ALTERS ESSENTIAL FATTY ACID

RATIOS AND ESSENTIAL FATTY ACID CONVERSION ENZYMES IN A

SEX-SPECIFIC MANNER IN THE RAT

by

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STATEMENT OF THESIS APPROVAL

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ABSTRACT

Intrauterine growth restriction (IUGR) increases the risk of chronic lung disease (CLD), and males have worse outcomes. Altered ratios of omega-6 and omega-3 essential fatty acids (EFA) in preterm infants are associated with increased risk of CLD. Conversion of omega-6 and omega-3 EFAs require the same set of EFA conversion enzymes. We hypothesized that IUGR would alter EFA ratios and EFA conversion enzymes in a sex-specific manner in fetal serum, liver and lung. Because maternal docosahexanoic acid (DHA) supplementation has been shown to ameliorate lung dysfunction in IUGR rats, we further hypothesized that alterations in EFA ratios and EFA conversion enzymes would be ameliorated with maternal DHA supplementation. We found that IUGR altered EFA ratios and EFA conversion enzymes in a sex-specific manner. IUGR induced changes in male circulating EFAs that correlate with EFA ratios seen in male preterm infants at increased risk for CLD. Maternal DHA supplementation ameliorated the altered circulating EFAs in male IUGR rats. IUGR induced changes in liver and lung EFA conversion enzyme mRNA and protein did not explain alterations in circulating and lung EFA ratios. We conclude that IUGR induces sex-specific changes in circulating EFA ratios and liver EFA conversion enzymes in newborn IUGR rat pups. We speculate that IUGR induced changes in EFA levels may occur as a result of alterations in placental EFA transfer, and that IUGR impacts placental transfer of EFAs in a sex-specific manner.

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INTRODUCTION

<u>Intrauterine Growth Restriction</u>

Intrauterine growth restriction (IUGR) occurs when a fetus is prevented from reaching its full growth potential in utero. IUGR is defined as a birth weight that falls below the 10th percentile for gestational age [1, 2], and affects up to 10% of pregnancies annually in the United States. The most common cause of IUGR in developed countries is preeclampsia, which limits oxygen and nutrient delivery to the developing fetus by restricting uteroplacental blood flow [1, 3]. This restriction in oxygen and nutrient delivery has many consequences on the developing fetus including increased perinatal morbidity and mortality [4], with male infants having worse outcomes than female infants . Specifically, IUGR infants, especially those born prematurely, have an increased risk of developing chronic lung disease (CLD) [5, 6]. In addition, male IUGR infants have worse respiratory outcomes and higher rates of CLD compared to female IUGR infants [7]. A mechanism that may contribute to lung disease seen in IUGR infants involves circulating essential fatty acids (EFA).

<u>Serum Essential Fatty Acid Levels and CLD in Premature Infants</u>

Essential fatty acids (EFA) are modulators of inflammation, and play key roles in membrane fluidity, cell signaling and gene transcription [8]. The omega-6 and omega-3 fatty acids are essential. In the omega-6 pathway, linoleic acid (LA) is ultimately

converted to arachadonic acid (AA). In the omega-3 pathway, linolenic acid (ALA) is converted to eicosapentaenoic acid (EPA) and docosahexanoic acid (DHA). In general, the omega-6 fatty acids are pro-inflammatory, while the omega-3 fatty acids are anti-inflammatory. Alterations in the relative amounts of omega-6 to omega-3 fatty acids have been associated with neonatal morbidities [9].

A recent study showed that altered serum EFA levels in premature infants are associated with increased risk of developing CLD [9]. CLD have increased levels of total omega-6 relative to omega-3 EFA's [9]. Furthermore, the ratios of EFA within the omega-6 and omega-3 pathway appear to be more important than the overall amounts. An increased ratio of AA to DHA and decreased ratio of DHA to ALA is associated with CLD in premature infants [9]. An increased ratio of AA to LA represents an increase in the conversion along the omega-6 pathway [9]. Similarly, a decreased ratio of DHA to ALA represents a decrease in conversion along the omega-3 pathway [9]. Changes in EFA ratios in newborn infants may result from either altered placental transport of EFA, or from changes in the local conversion of EFA along the omega-6 or omega-3 pathways.

Essential Fatty Acid Metabolism

Conversion of fatty acids along the omega-6 and omega-3 pathways utilizes the same groups of elongase and desaturase enzymes (Figure 1). Δ5-desaturase and Δ6-desaturase introduce double bonds into the growing fatty acid chain, and elongase 2 and elongase 5 are responsible for the addition of 2 carbon molecules to the fatty acid chain. Levels of Δ5-desaturase, Δ6-desaturase, elongase 2 and elongase 5 affect the relative concentrations of the EFAs along the omega-6 and omega-3 pathway.

Changes in liver activity of Δ5-desaturase, Δ6-desaturase, elongase 2 and elongase 5 may account for altered serum EFA levels in IUGR infants. In the fetus, EFAs are processed in the liver before being transported in the serum to various tissues where Δ5-desaturase, Δ6-desaturase, elongase 2 and elongase 5 can act at the local level to further alter EFA profiles.

IUGR in the Rat Model

Our lab previously showed that surgically induced IUGR in the rat predisposes the rat to lung dysfunction, and male IUGR rats have worse outcomes than female IUGR rats [10]. We have shown that rats born with IUGR have delayed alveolar formation, stemming from reduced elastin fiber deposition and improper thinning of lung mesenchyme at birth [10, 11]. IUGR alters surfactant in rat lung [11], and rats born with IUGR have increased lung compliance [10]. Interestingly, the molecular, structural and functional lung changes observed in our IUGR rat model are ameliorated with maternal supplementation of the EFA DHA [12].

How EFA and Δ5-desaturase, Δ6-desaturase, elongase 2 and elongase 5 levels are affected by IUGR, and how maternal DHA supplementation impacts EFA and Δ5-desaturase, Δ6-desaturase, elongase 2 and elongase 5 levels in IUGR offspring is unknown. We hypothesize that IUGR will alter EFA ratios as well as Δ5-desaturase, Δ6-desaturase, elongase 2 and elongase 5 levels in a sex-specific manner in fetal serum, liver and lung. We further hypothesize that alterations in EFA ratios and Δ5-desaturase, Δ6-desaturase, elongase 2 and elongase 5 levels will be ameliorated with maternal DHA supplementation.
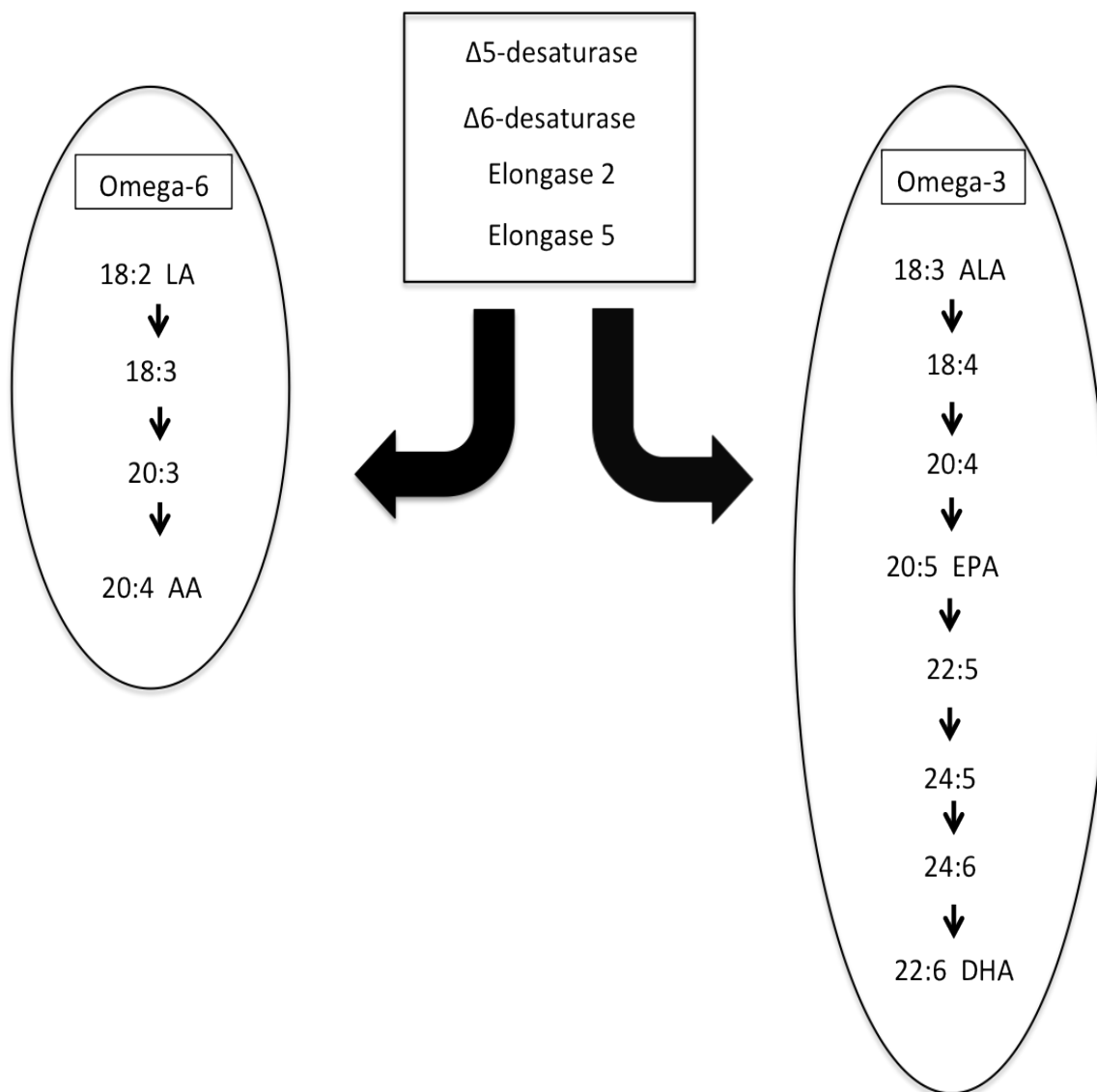
Figure 1: Pathways of omega-6 and omega-3 metabolism. LA, linoleic acid; AA, arachidonic acid; ALA, α-linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid. Both the omega-3 and omega-6 pathways are processed by the same group of enzymes, shown in the box.

METHODS

Rat Model

This project utilized a rat model of IUGR induced by uteroplacental insufficiency (UPI). Pregnant dams were assigned to standard rat chow, or to rat chow with DHA, and to either the control group or the IUGR group. To induce IUGR, pregnant rats were anesthetized with ketamine and zylazine and the inferior uterine arteries were ligated at day 19 of gestation. Rats in the control group were subjected to identical anesthesia procedures. At term gestation (day 21.5) fetal rat pups were delivered via caesarean section. Rat pups were killed by decapitation, and blood, lung and liver tissues were harvested and flash frozen in liquid nitrogen. Tissues were stored at -80C until use.

Maternal DHA Supplementation

DHA was provided via a custom diet containing either 0.1% DHA or 1% DHA. Diets were constructed by replacing 0.1% or 1% of the soybean oil in standard rat chow with purified DHA. The 0.1% and 1% DHA diets maintained the macronutrient content of the standard rat chow diet (21.8% protein, 40.8% carbohydrate and 5.4% fat with a caloric density of 3 Kcal/g). One percent DHA diet was used for serum and lung EFA. All other experiments used 0.1% DHA diet. Pregnant rats were fed regular diet, 0.1% DHA diet, or 1% DHA diet ad libitum from day 13 of gestation to term.

Study Groups

Six sex-matched nonsibling rat pups were assigned to each study group. The study groups were control, IUGR, DHA-control, and DHA-IUGR. A sample size of 6 in each group was used, because we have shown that this size sample is sufficient to show a statistically significant difference with a 10% change.

Serum and Lung EFA Analysis

Prior to the start of this study, serum and lung EFA levels of fetal rat pups from dams fed either a standard rat chow diet or a rat chow diet with 1% DHA were analyzed via gas chromatography by a collaborator. The lung and serum EFA raw data were analyzed for this study.

Conversion along the omega-6 and omega-3 pathway was determined by calculating the ratio of AA to LA and the ratio of DHA to ALA, respectively. The ratios of the total omega-6 and omega-3, as well as end point fatty acids AA and DHA, were also calculated.

Real-Time Reverse Transcriptase PCR

Real-time reverse transcriptase PCR was used to quantify mRNA levels of Δ5 desaturase, Δ6 desaturase, elongase 2 and elongase 5 within lung and liver samples. The genes that code for the EFA conversion enzymes are as follows: FADS1 codes for Δ5-desaturase, FADS2 codes for Δ6-desaturase, ELOVL2 codes for elongase 2, and ELOVL5 codes for elongase 5. Total mRNA was extracted from lung and liver using a "NucleoSpin RNA II" RNA extraction kit according to manufacturer protocol. Total

RNA was quantified and 1 µg was used to synthesize cDNA with the "High Capacity cDNA Reverse Transcription Kit" (Applied Biosystems). The following assay-on-demand primer/probe sets were used: FADS1-Rn00584915_m1, FADS2-Rn00580220_m1, ELOVL2-Rn01450663_m1, ELOVL5-Rn00592812_m1. GAPDH primer and probe sequences; Forward: CAAGATGGTGAAGGTCGGTGT; Reverse: CAAGAGAAGGCAGCCCTGGT; Probe: GCGTCCGATACGGCCAAATCCG.

Western Blotting

Western blotting was used to determine relative abundance of lung and liver Δ5 desaturase, Δ6 desaturase, elongase 2 and elongase 5 protein. Lung and liver proteins were isolated by homogenizing in RIPA buffer with a protease inhibitor, followed by centrifuging at 10,000 rpm at $4^{o}C$ for 10 minutes. Proteins were quantified using the Bicinchoninic Acid Protein Assay Kit (Pierce). Immunoblotting was performed using standard procedures. The following primary antibodies were used: Anti-FADS1 antibody [EPR6898] (abcam); Anti-FADS2 (H-40): sc-98480 (Santa Cruz Biotechnology); Anti-ELOVL2 (S-16): sc-54874 (Santa Cruz Biotechnology, Inc.); and Anti-ELOVL5 (G-18): sc-54882 (Santa Cruz Biotechnology). Following incubation with the appropriate secondary antibodies, enhanced chemiluminesence was used to determine signaling.

Statistical Analysis

Statistical analysis was performed using a Mann-Whitney U test in Statview software. Two way ANOVA was performed to determine the independent effect of IUGR, diet and sex. P-values <0.05 were considered significant

RESULTS

Effect of IUGR on Circulating EFAs

For each result the effect of IUGR alone (IUGR), DHA alone (DHA-control) and

the combination of DHA and IUGR (DHA-IUGR) will be presented. In male serum,

IUGR increased the ratio of total omega-6 to total omega-3 (p=0.0062) compared to

control. In contrast, DHA-control decreased the ratio of total omega-6 to total omega-3

(p=0.0039) compared to control. DHA-IUGR decreased the ratio of total omega-6 to total

omega-3 (p=0.0039) compared to control, and increased the ratio of total omega-6 to total

omega-3 (p=0.0374) compared to DHA-control (Figure 2A). A significant IUGR-DHA

interaction (p=0.0001) was observed in male serum.

In female serum, IUGR did not significantly alter the ratio of total omega-6 to

total omega-3 (p=0.157) compared to control. DHA-control decreased the ratio of total

omega-6 to total omega-3 (p=0.0105) compared to control. DHA-IUGR decreased the

ratio of total omega-6 to total omega-3 (p=0.0209) compared to control, and increased the

ratio of total omega-6 to total omega-3 (p=0.0105) compared to DHA-control (Figure

2B). A significant DHA-sex interaction (p=0.0001) and a significant IUGR-sex

interaction (p=0.0008) was observed

In male serum, IUGR increased the ratio of AA to LA (p=0.0062) compared to

control. Similarly, DHA-control increased the ratio of AA to LA (p=0.0039) compared to

control. DHA-IUGR increased the ratio of AA to LA (p=0.0062) compared to control (Figure 3A). A significant DHA-IUGR interaction (p=0.0001) was observed.

In female serum, IUGR increased the ratio of AA to LA (p=0.05) compared to control. DHA-control increased the ratio of AA to LA (p=0.009) compared to control. DHA-IUGR increased the ratio of AA to LA (p=0.0143) compared to control (Figure 3B). A significant DHA-sex interaction (p=0.0002) and a significant IUGR-sex interaction (p=0.0022) was observed.

In male serum, IUGR decreased the ratio of DHA to ALA (p=0.0065) compared to control. In contrast, DHA-control increased the ratio of DHA to ALA (p=0.0039) compared to control. DHA-IUGR decreased the ratio of DHA to ALA (p=0.0039) compared to DHA-IUGR (Figure 4A). In male serum, a significant DHA-IUGR interaction (p=0.0002) was observed.

In female serum, IUGR did not alter the ratio of DHA to ALA (p=0.83) compared to control. DHA-control increased the ratio of DHA to ALA (p=0.0039) compared to control. Similarly, DHA-IUGR increased the ratio of DHA to ALA (p=0.0062) compared to control (Figure 4B). Additionally, a significant DHA-sex interaction (p=.0096) was observed.

In male serum, IUGR increased the ratio of AA to DHA (p=0.0062) compared to control. DHA-control decreased the ratio of AA to DHA (p=0.0039) compared to control. DHA-IUGR decreased the ratio of AA to DHA (p=0.0039) compared to control, and increased the ratio of AA to DHA (p=0.0039) compared to DHA-control (Figure 5A). In male serum, a significant DHA-IUGR interaction (p=0.0001) was observed.

In female serum, IUGR with regular diet did not alter the ratio of AA to DHA (p=0.85) compared to control. DHA-control decreased the ratio of AA to DHA (p=0.0039) compared to control. Similarly, DHA-IUGR decreased the ratio of AA to DHA (p=0.0062) compared to control, and increased the ratio of AA to DHA (p=0.0176) compared to DHA-control (Figure 5B). In female serum, a significant DHA-IUGR interaction (p=0.0305) was observed. Additionally, a significant DHA-sex interaction (p=0.0029) was observed.

<p style="text-align:center;">Effect of IUGR on Liver EFA Conversion Enzymes</p>

In male liver, IUGR decreased $\Delta5$ desaturase mRNA (p=0.0374) compared to control. Similarly, DHA-IUGR decreased $\Delta5$ desaturase mRNA (p=0.0104) compared to control (Figure 6A).

In female liver, IUGR, DHA-control, and DHA-IUGR did not alter $\Delta5$ desaturase mRNA compared to control (Figure 6B). In female liver, a significant IUGR-DHA interaction (p=0.017) was observed. Additionally, a significant IUGR-sex interaction (p=0.0339) was observed.

In male liver, IUGR decreased $\Delta5$ desaturase protein (p=0.0105) compared to control. Similarly, DHA-control decreased $\Delta5$ desaturase protein (p=0.0209) compared to control (Figure 7A). In male liver, a significant DHA-IUGR interaction (p=0.0066) was observed. In female liver, IUGR, DHA-con and DHA-IUGR did not significantly alter $\Delta5$ desaturase protein compared to control (Figure 7B).

In male liver, IUGR decreased $\Delta6$ desaturase mRNA (p=0.025) compared to control. DHA-control decreased $\Delta6$ desaturase mRNA (p=0.0039) compared to control.

DHA-IUGR decreased Δ6 desaturase mRNA (p=0.0039) compared to control, and decreased Δ6 desaturase mRNA (p=0.0374) compared to DHA-control (Figure 8A).

In female liver, IUGR did not alter Δ6 desaturase mRNA (p=0.5218) compared to control. In contrast, DHA-IUGR decreased Δ6 desaturase mRNA (p=0.0106) compared to control (Figure 8B).

In male liver, IUGR did not alter Δ6 desaturase protein (p=0.17) compared to control. DHA-control increased Δ6 desaturase protein (p=0.0106) compared to control. In contrast, DHA-IUGR decreased Δ6 desaturase protein (p=0.0176) compared to DHA-control (Figure 9).

In female liver, IUGR did not alter Δ6 desaturase protein (p=0.5218) compared to control. In contrast, DHA-control increased Δ6 desaturase protein (p=0.0039) compared to control. DHA-IUGR decreased Δ6 protein (p=0.0062) compared to control (Figure 9B). Additionally, in female liver a significant IUGR-DHA interaction (p=0.0353) was observed.

In male liver, IUGR did not alter elongase 2 mRNA (p=0.3785) compared to control. DHA-control decreased elongase 2 mRNA (p=0.0374) compared to control. Similarly, DHA-IUGR decreased elongase 2 mRNA (p=0.0039) compared to control (Figure 10A).

In female liver, IUGR did not alter elongase 2 mRNA (p=0.465) compared to control. DHA-control decreased elongase 2 mRNA (p=0.0065) compared to control. DHA-IUGR decreased elongase 2 mRNA (p=0.0062) compared to control (Figure 10B).

Elongase 2 protein could not be measured because the antibody did not work, potentially because liver elongase 2 expression is too low to measure in the newborn rat.

In male liver, IUGR did not alter elongase 5 mRNA (p=0.0656) compared to control. DHA-control decreased elongase 5 mRNA (p=0.0039) compared to control. DHA-IUGR decreased elongase 5 mRNA (p=0.0039) compared to control (Figure 11A).

In female liver, IUGR did not alter elongase 5 mRNA (p=0.298) compared to control. DHA-control decreased elongase 5 mRNA (p=0.0453) compared to control. DHA-IUGR did not alter elongase 5 mRNA (p=0.078) (Figure 11B). Additionally, a significant IUGR-sex interaction (p=0.0341) was observed. Elongase 5 protein could not be measured because the antibody did not work, potentially because liver elongase 5 expression is too low in the newborn rat.

Effect of IUGR on Lung EFA Levels

DHA-control and DHA-IUGR EFA levels in the lung were not measured for this study. In male lung, IUGR did not alter the ratio of total omega-6 to total omega-3 (p=0.1495) compared to control (Figure 12A). In contrast, in female lung IUGR increased the ratio of total omega-6 to total omega-3 (p=0.0105) compared to control (Figure 12B). Additionally, a significant IUGR-sex interaction (p=0.0459) was observed

In male lung, IUGR decreased the ratio of AA to LA (p=0.0163) compared to control (Figure 13A). In contrast, in female lung IUGR did not alter the ratio of AA to LA (p=0.7488) compared to control (Figure 13B).

In male lung, IUGR did not alter the ratio of DHA to ALA (p=0.873) compared to control (Figure 14A). Similarly, in female lung IUGR did not alter the ratio of DHA to ALA (p=0.831) compared to control (Figure 14B).

In male lung, IUGR did not significantly alter the ratio of AA to DHA (p=0.7488) compared to control (Figure 15A). Similarly, in female lung IUGR did not significantly alter the ratio of AA to DHA (p=0.2012) compared to control (Figure 15B).

<div align="center">Effect of IUGR on Lung EFA Conversion Enzyme Levels</div>

In male lung, IUGR, DHA-control, and DHA-IUGR did not alter FADS1 mRNA (Figure 16A). Similarly, in female lung IUGR, DHA-control, and DHA-IUGR did not alter FADS1 mRNA (Figure 16B).

In male lung, IUGR did not alter $\Delta 5$ desaturase protein compared to control (Figure 17A). Male lung DHA-control and DHA-IUGR blots were unsuccessful due to the protein being degraded in the available samples.

In contrast, in female lung IUGR increased $\Delta 5$ desaturase protein (p=0.006) compared to control, DHA-IUGR increased $\Delta 5$ desaturase protein (p=0.0039) compared to control, and increased $\Delta 5$ desaturase protein (p=0.0039) compared to DHA-control (Figure 17B).

In male lung, IUGR increased $\Delta 6$ desaturase mRNA (p=0.0285) compared to control. DHA-IUGR decreased $\Delta 6$ desaturase mRNA (p=0.0283) compared to control (Figure 18A). Additionally, a significant DHA-IUGR interaction (p=0.0221) was observed. Male lung DHA-control and DHA-IUGR blots were unsuccessful due to the protein being degraded in the available samples. In female lung, IUGR, DHA-control, and DHA-IUGR did not alter $\Delta 6$ desaturase mRNA (Figure 18B).

In male lung, IUGR did not alter $\Delta 6$ desaturase protein compared to control (Figure 19A). In contrast, in female lung IUGR increased $\Delta 6$ desaturase protein

(p=0.001) compared to control. DHA-IUGR increased Δ6 desaturase protein (p=0.003) compared to control, and increased Δ6 desaturase protein (p=0.003) compared to DHA-control (Figure 19B).

In male lung, IUGR did not alter elongase 2 mRNA (p=0.0833) compared to control. DHA-control decreased elongase 2 mRNA (p=0.05) compared to control. DHA-IUGR increased elongase 2 mRNA (p=0.0446) compared to DHA-control (Figure 20A).

In female lung, IUGR did not alter elongase 2 mRNA (p=0.2733) compared to control. DHA-control decreased elongase 2 mRNA (p=0.0176) compared to control (Figure 20B).

Elongase 2 protein could not be measured because the antibody did not work, potentially because lung elongase 2 expression is too low to measure in the newborn rat.

In male lung, IUGR did not alter elongase 5 mRNA (p=0.715) compared to control. DHA-control increased elongase 5 mRNA (p=0.009) compared to control. DHA-IUGR increased elongase 5 mRNA (p=.00283) compared to control, and decreased elongase 5 mRNA (p=0.0472) compared to DHA-IUGR (Figure 21A).

In female lung, IUGR did not alter elongase 5 mRNA (p=0.2002) compared to control. DHA-control increased elongase 5 mRNA (p=0.2002) compared to control. DHA-IUGR increased elongase 5 mRNA (p=0.0106) compared to control (Figure 21B). Additionally, a significant DHA-IUGR interaction (p=0.0134) was observed.

Elongase 5 protein could not be measured because the antibody did not work, potentially because lung elongase 5 expression is too low to measure in the newborn rat.
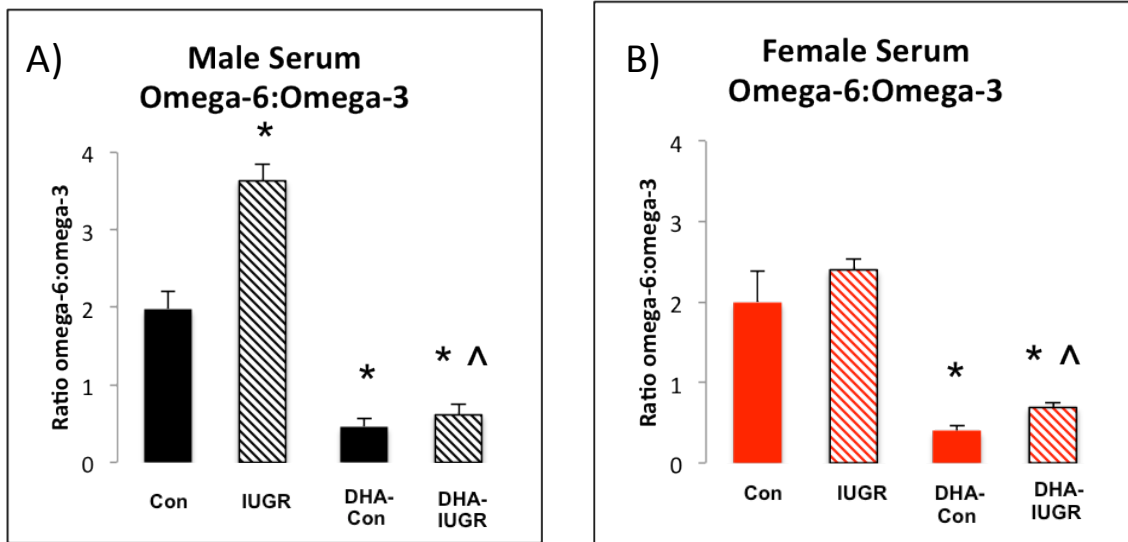
Figure 2. Ratio of total omega-6 to total omega-3 in male and female serum. A) Male serum ratio of total omega-6 to total omega-3 EFA. B) Female serum ratio of total omega-6 to total omega-3 EFA. * different from regular diet control (Mann-Whitney U). ^ different from DHA control (Mann-Whitney U).
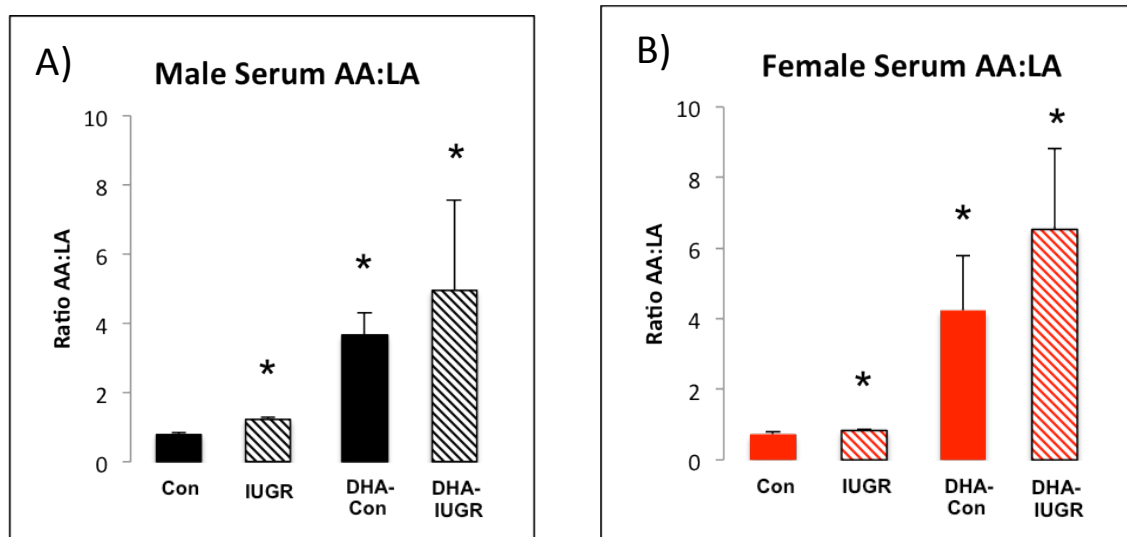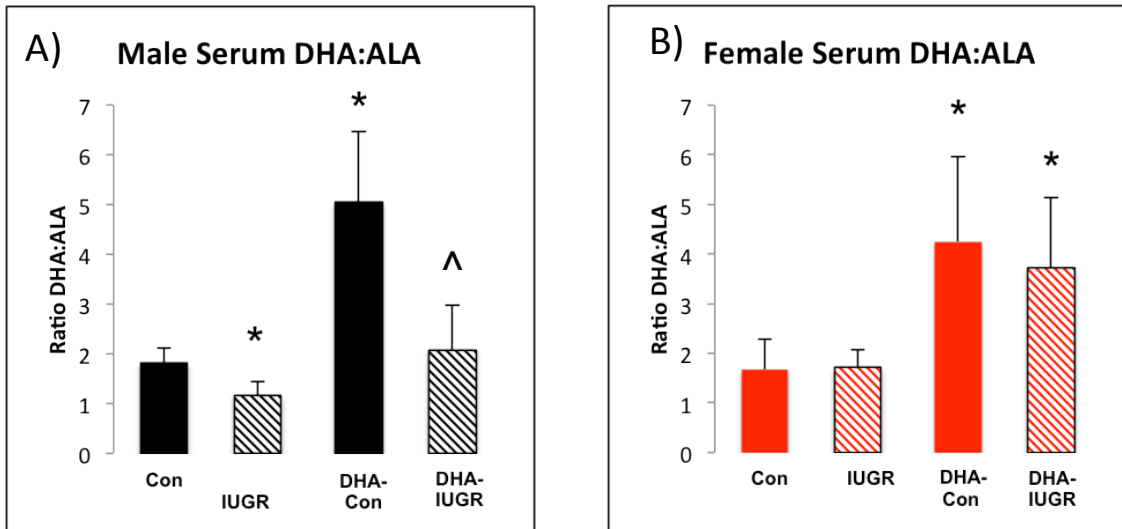

Figure 3. Ratio of AA to LA in male and female serum. A) Male serum ratio of AA to LA. B) Female serum ratio of AA to LA. * different from regular diet control (Mann-Whitney U). ^ different from DHA control (Mann-Whitney U).

Figure 4. Ratio of DHA to ALA in male and female serum. A) Male serum ratio of DHA to ALA. B) Female serum ratio of DHA to ALA. * different from regular diet control (Mann-Whitney U). ^ different from DHA control (Mann-Whitney U).
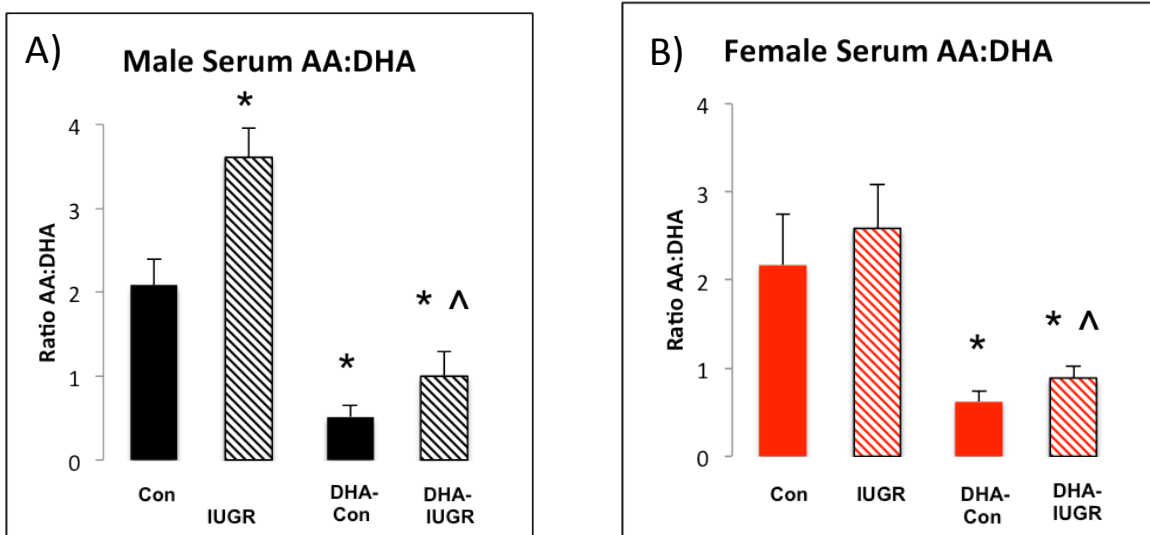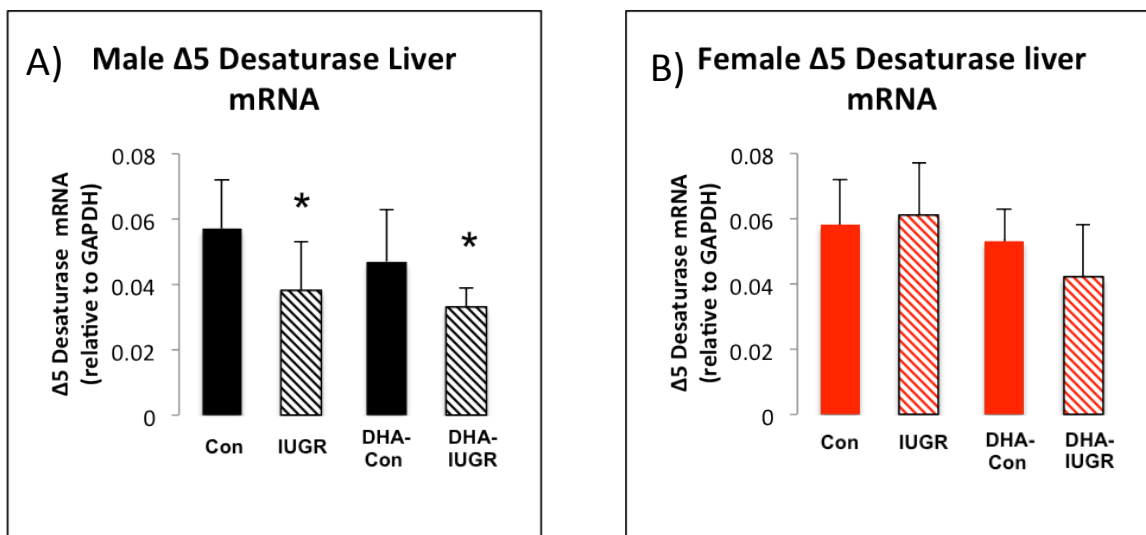

Figure 5. Ratio of AA to DHA in male and female serum. A) Male serum ratio of AA to DHA. B) Female serum ratio of AA to DHA. * different from regular diet control (Mann-Whitney U). ^ different from DHA control (Mann-Whitney U).

Figure 6. Δ5 desaturase mRNA in male and female liver. A) Male liver Δ5 desaturase mRNA. B) Female liver Δ5 desaturase mRNA. * different from regular diet control (Mann-Whitney U). ^ different from DHA control (Mann-Whitney U).
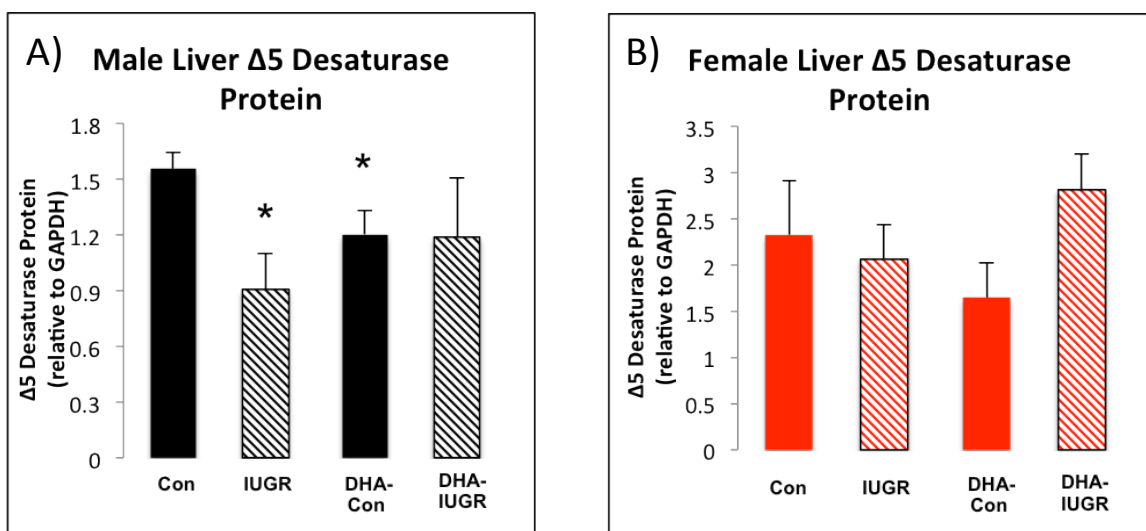

Figure 7. Δ5 desaturase protein in male and female liver. A) Male liver Δ5 desaturase protein. B) Female liver Δ5 desaturase protein. * different from regular diet control (Mann-Whitney U). ^ different from DHA control (Mann-Whitney U).
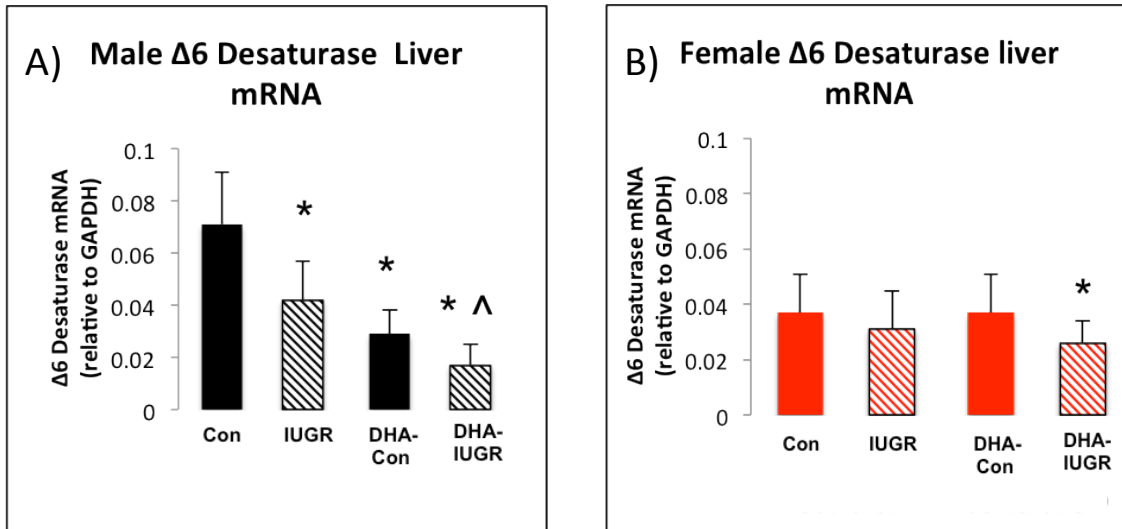
Figure 8. Δ6 desaturase mRNA in male and female liver. A) Male liver Δ6 desaturase mRNA. B) Female liver Δ6 desaturase mRNA. * different from regular diet control (Mann-Whitney U). ^ different from DHA control (Mann-Whitney U).

Figure 9. Δ6 desaturase protein in male and female liver. A) Male liver Δ6 desaturase protein. B) Female liver Δ6 desaturase protein. * different from regular diet control (Mann-Whitney U). ^ different from DHA control (Mann-Whitney U).
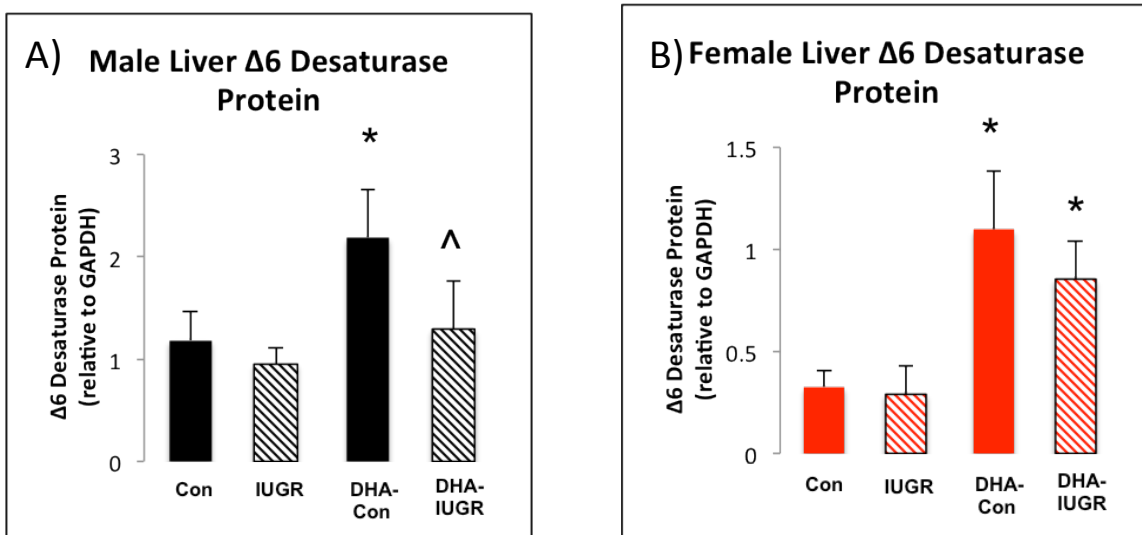
Figure 10. Elongase 2 mRNA in male and female liver. A) Male liver elongase 2 mRNA. B) Female liver elongase 2 mRNA. * different from regular diet control (Mann-Whitney U). ^ different from DHA control (Mann-Whitney U).
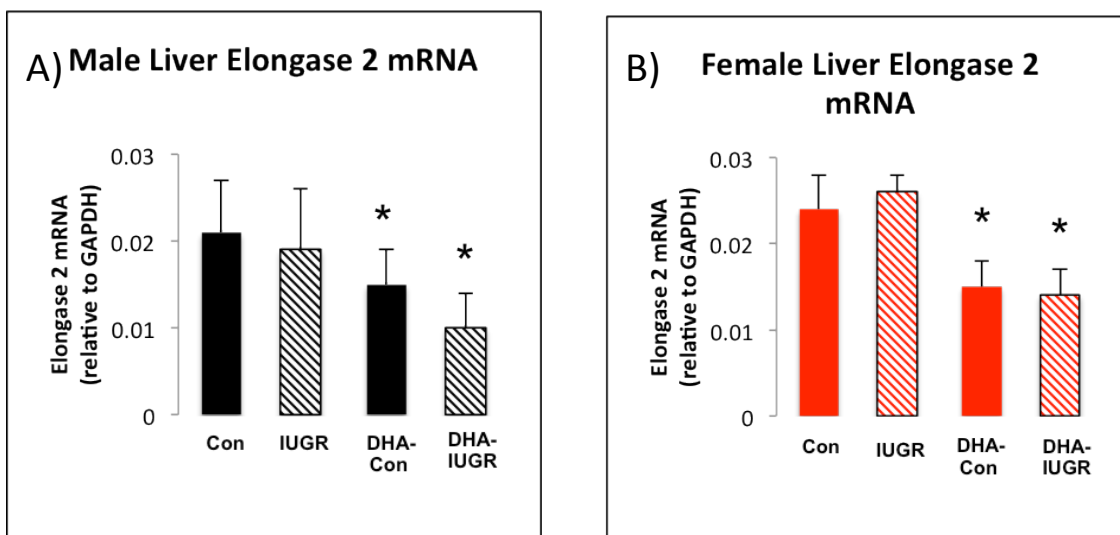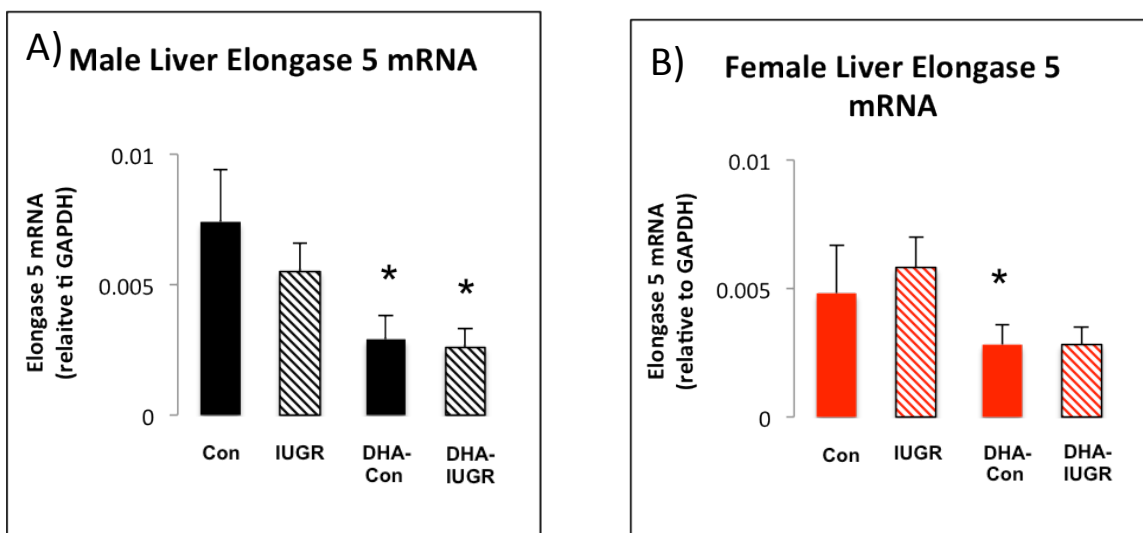

Figure 11. Elongase 5 mRNA in male and female liver. A) Male liver elongase 5 mRNA. B) Female liver elongase 5 mRNA. * different from regular diet control (Mann-Whitney U). ^ different from DHA control (Mann-Whitney U
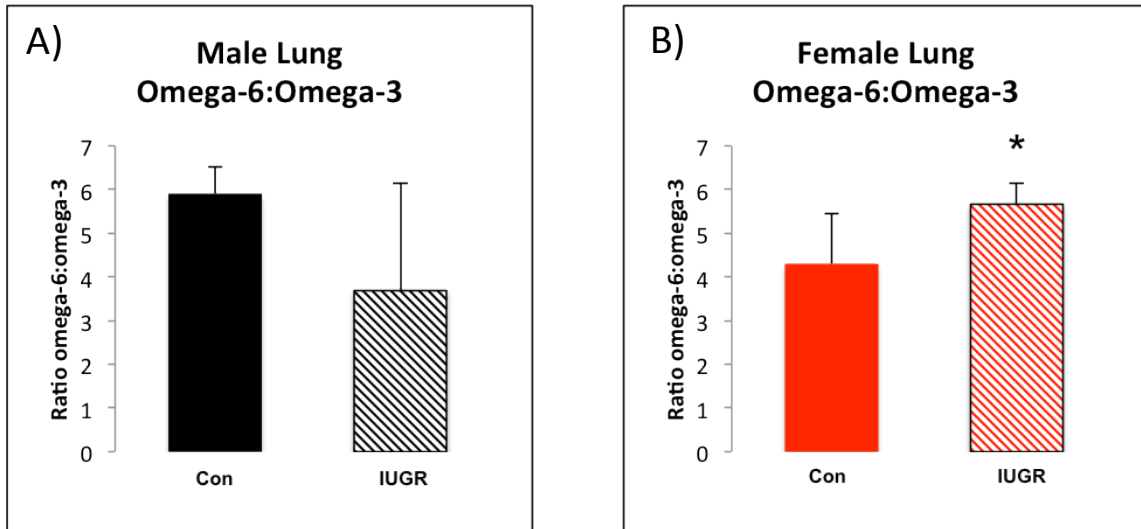
Figure 12. Ratio of total omega-6 to total omega-3 in male and female lung. A) Male lung ratio of total omega-6 to total omega-3 EFA. B) Female lung ratio of total omega-6 to total omega-3 EFA. * different from regular diet control (Mann-Whitney U). ^ different from DHA control (Mann-Whitney U).
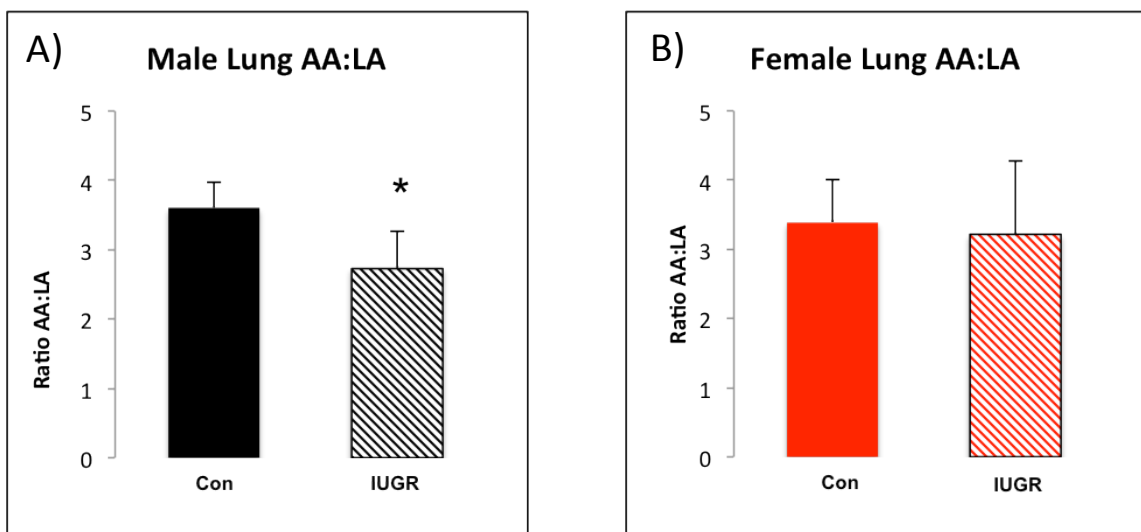

Figure 13. Ratio of AA to LA in male and female lung. A) Male lung ratio AA to LA. B) Female lung ratio AA to LA. * different from regular diet control (Mann-Whitney U). ^ different from DHA control (Mann-Whitney U)

Figure 14. Ratio of DHA to ALA in male and female lung. A) Male lung ratio DHA to ALA. B) Female lung ratio DHA to ALA. * different from regular diet control (Mann-Whitney U). ^ different from DHA control (Mann-Whitney U).

Figure 15. Ratio of AA to DHA in male and female lung. A) Male lung ratio AA to DHA. B) Female lung ratio AA to DHA. * different from regular diet control (Mann-Whitney U). ^ different from DHA control (Mann-Whitney U).
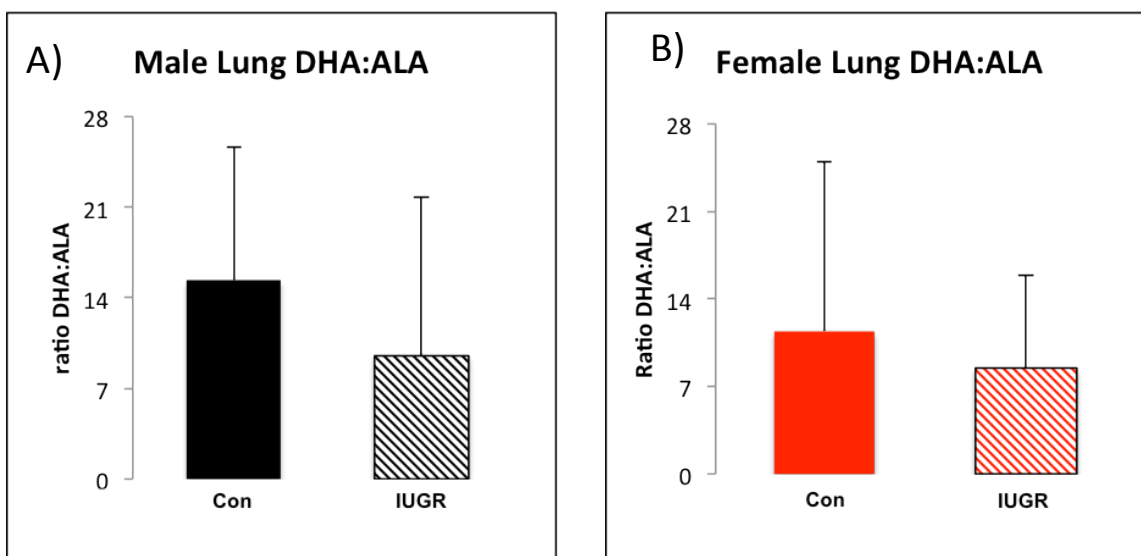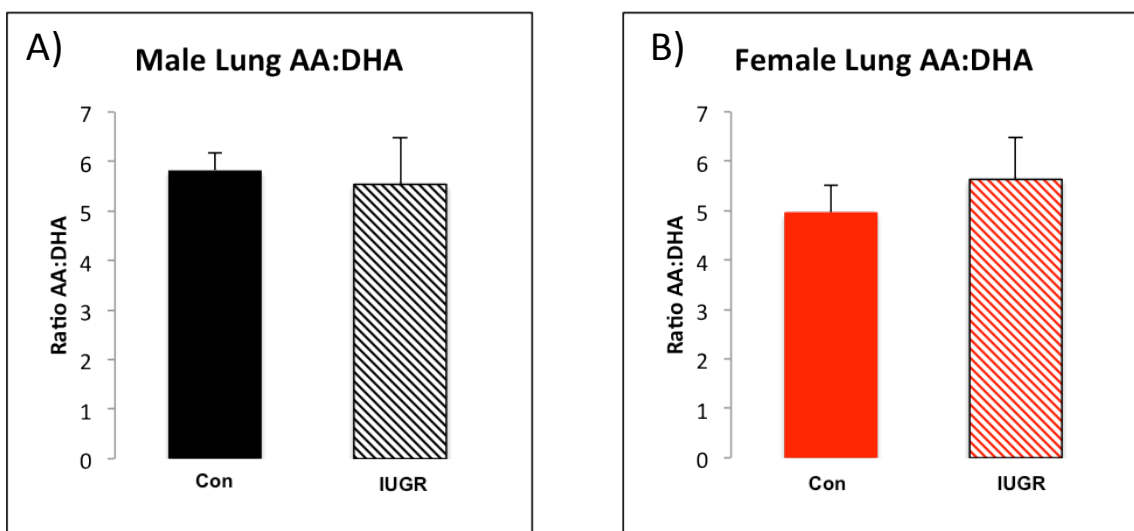
Figure 16. Δ5 desaturase mRNA in male and female lung. A) Male lung Δ5 desaturase mRNA. B) Female lung Δ5 desaturase mRNA. * different from regular diet control (Mann-Whitney U). ^ different from DHA control (Mann-Whitney U).

Figure 17. Δ5 desaturase protein in male and female lung. A) Male lung Δ5 desaturase protein. B) Female lung Δ5 desaturase protein. * different from regular diet control (Mann-Whitney U). ^ different from DHA control (Mann-Whitney U).
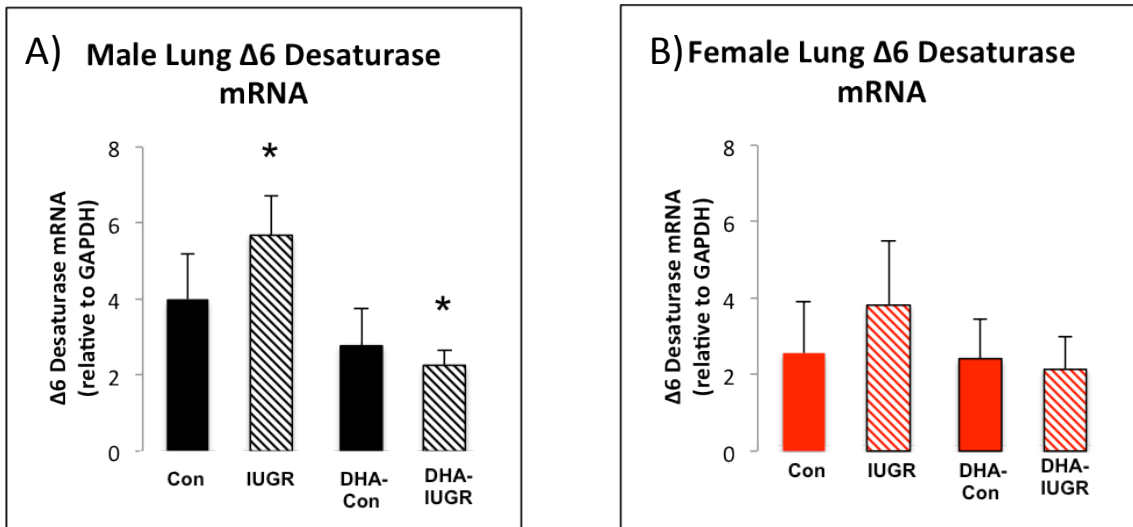
Figure 18. Δ6 desaturase mRNA in male and female lung. A) Male lung Δ6 desaturase mRNA. B) Female lung Δ6 desaturase mRNA* different from regular diet control (Mann-Whitney U). ^ different from DHA control (Mann-Whitney U).

Figure 19. Δ6 desaturase protein in male and female lung. A) Male lung Δ6 desaturase protein. B) Female lung Δ6 desaturase protein. * different from regular diet control (Mann-Whitney U). ^ different from DHA control (Mann-Whitney U).

Figure 20. Elongase 2 mRNA in male and female lung. A) Male lung elongase 2 mRNA. B) Female lung elongase 2 mRNA. * different from regular diet control (Mann-Whitney U). ^ different from DHA control (Mann-Whitney U).
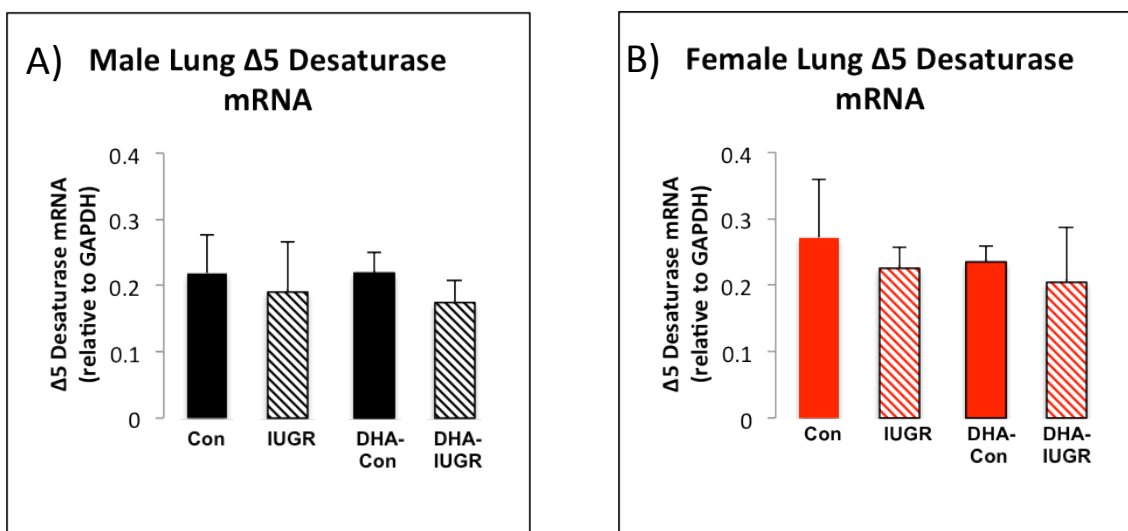

Figure 21. Elongase 5 mRNA in male and female lung. A) Male lung elongase 5 mRNA. B) Female lung elongase 5 mRNA. * different from regular diet control (Mann-Whitney U). ^ different from DHA control (Mann-Whitney U).
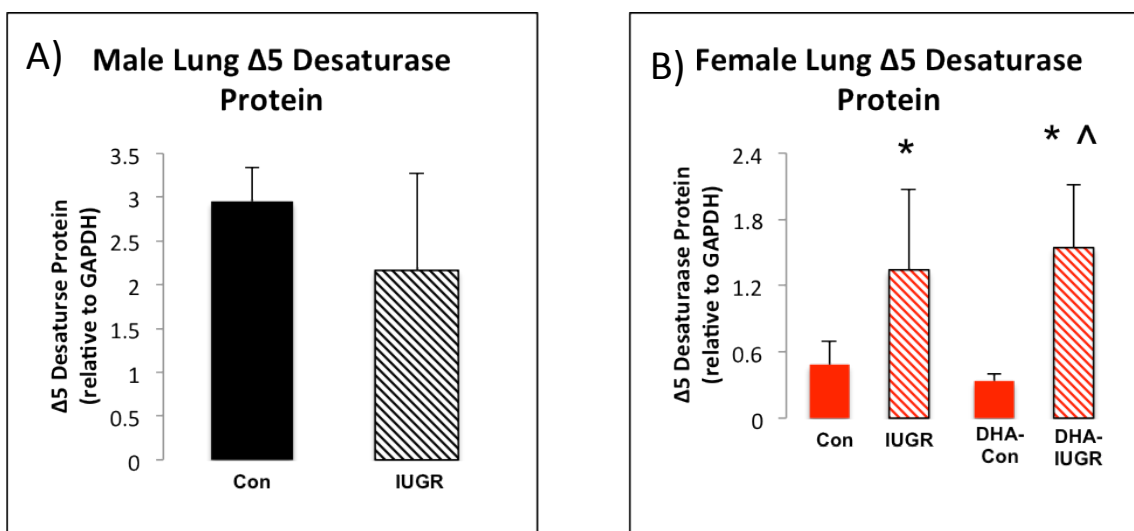
DISCUSSION
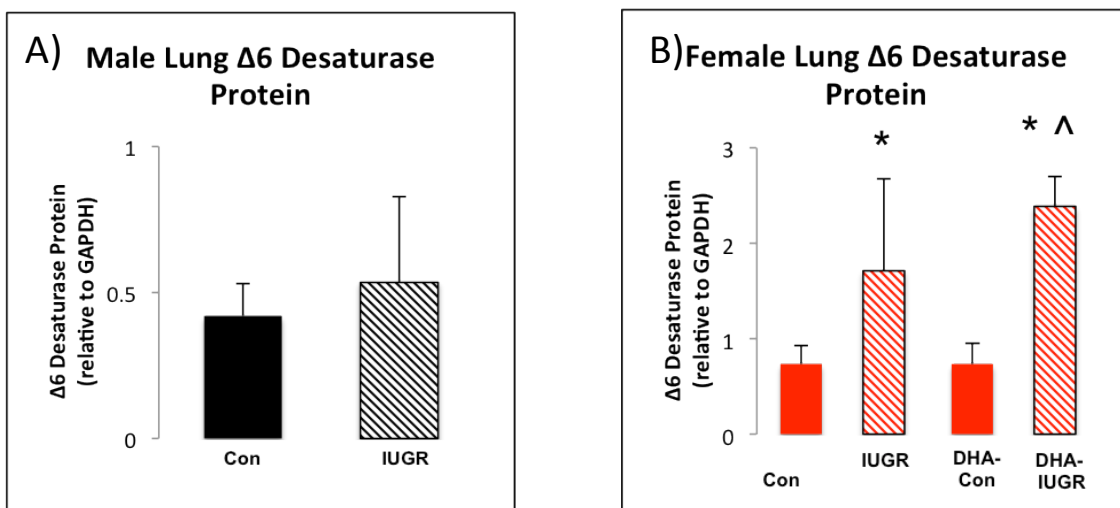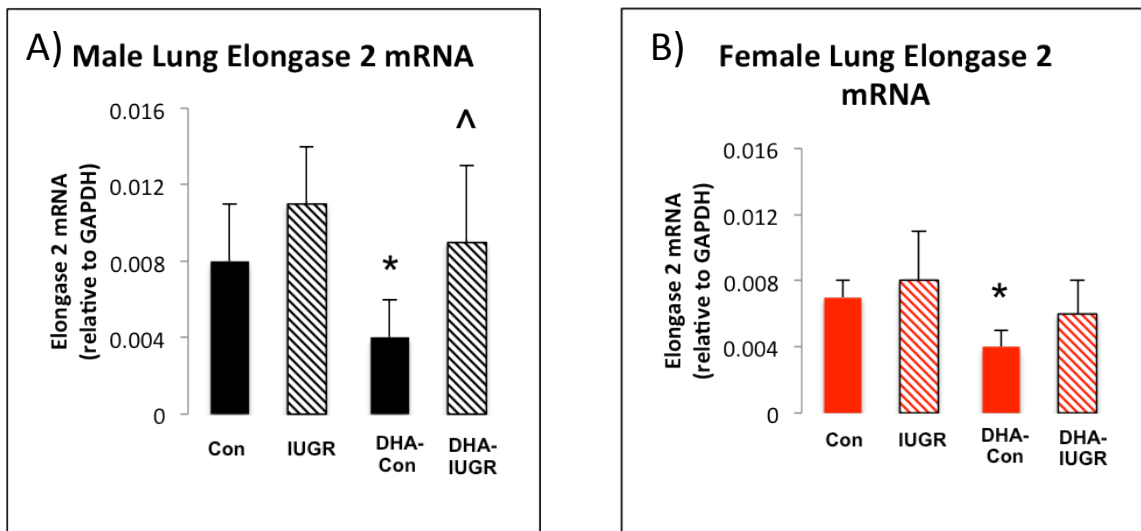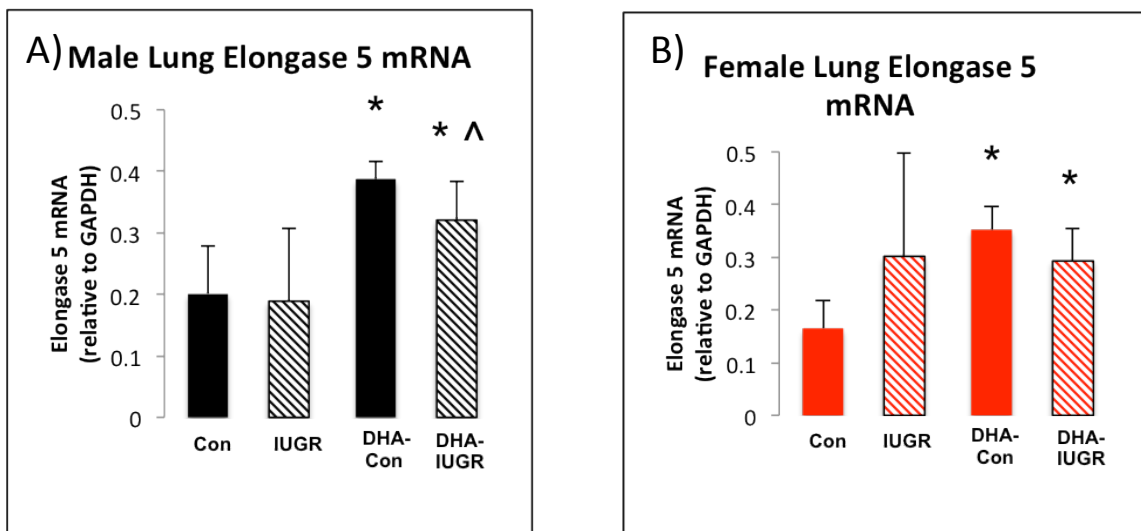

We hypothesized that IUGR would alter EFA and Δ5-desaturase, Δ6-desaturase, elongase 2 and elongase 5 levels in a sex-specific manner, and that alterations in EFA and Δ5-desaturase, Δ6-desaturase, elongase 2 and elongase 5 levels would be ameliorated with maternal DHA supplementation. This study shows, for the first time, that EFA ratios and EFA conversion enzymes are altered in IUGR. In addition, this study shows that IUGR impacts EFA ratios and EFA conversion enzymes in a sex-specific manner. It has been shown that IUGR affects males more severely than females [7], and the results of this study elucidate a potential mechanism behind the sex-specific impact of IUGR.

Our results showed that IUGR induced sex-specific changes in circulating EFAs. Sex-specific differences in circulating EFAs have previously been shown in premature human infants. Male preterm infants have a decreased DHA to ALA ratio, and an increased AA to LA and total omega-6 to omega-3 ratio [9]. In our study, IUGR induced changes in male circulating EFAs that correlate with the altered EFA ratios seen in male preterm infants.

Interestingly, DHA supplementation ameliorated the altered circulating EFAs in male IUGR rats. Several studies have looked at the effect of maternal fat intake on circulating fetal fatty acid levels. In rats, a maternal high-fat diet rich in DHA from salmon oil increases downstream omega-3 EFAs in the newborn plasma [13]. A similar

study showed that maternal DHA supplementation from fish oil resulted in increased downstream omega-3 EFAs, and decreased downstream omega-6 EFAs in fetal plasma [14]. These results are consistent with the effect of maternal DHA supplementation on circulating EFAs seen in our study.

In male circulation, but not female circulation, IUGR induced changes in the omega-3 pathway that were independent of diet. IUGR in males resulted in a reduction in the circulating ratio of DHA to ALA in both standard diet and 1% DHA diet. Despite exposure to the same intrauterine environment, IUGR has a sex-specific impact on the circulating ratio of DHA to ALA.

We speculate two mechanisms may be responsible for the altered circulating omega-3 profiles seen in male IUGR rats. First, sex hormones may play a role in regulating omega-3 fatty acid levels in IUGR offspring. Estradiol and progesterone have been positively associated with circulating and tissue levels of omega-3 fatty acids, while testosterone has been negatively associated with circulating and tissue levels of omega-3 fatty acids [15]. Our lab has shown that testosterone is increased and estrogen is decreased in male IUGR rats (unpublished). Second, circulating omega-3 EFA profiles in IUGR offspring may be a result of altered placental transfer of omega-3 EFAs. The changes seen in circulating omega-3 EFAs in male IUGR rats may be a result of IUGR altering the transport of longer and more desaturated EFAs in male placentas but not female placentas. At this time, no studies have looked at how IUGR impacts EFA transfer at the placental level.

The potential role of altered placental EFA transfer on circulating EFA levels in IUGR infants is supported by our liver EFA conversion enzyme results. IUGR induced

changes in liver EFA conversion enzymes, and these changes did not fully explain alterations in circulating EFAs. Liver EFA conversion enzyme mRNA and protein was correlated with circulating levels of the omega-3 EFAs, but not with circulating levels of omega-6 EFAs. Additionally, the changes seen in circulating EFAs with DHA supplementation were not explained by altered liver EFA conversion enzymes. At this time, no other studies have correlated liver EFA conversion enzyme levels with circulating EFAs in IUGR offspring.

From our results, we speculate that IUGR induced changes in circulating EFA levels may be a result of altered placental EFA transfer. The developing fetus must rely on placental transfer to receive EFAs [16]. In healthy pregnancies, there is preferential transfer of AA and DHA from the mother to the developing fetus [17]. A study of human IUGR infants found that IUGR decreases maternal-fetal transfer of DHA and AA [4]. The mechanisms for preferential transfer of EFAs across the placenta are not fully understood [18]. The placenta uptakes fatty acids from circulation by placental lipases, including lipoprotein lipase (LPL) and endothelial lipase [18], and by triglyceride hydrolase [17]. Additionally, fatty acid binding proteins play an important role in placental fatty acid uptake [17]. The placenta lacks the ability to modify EFAs because the placenta does not express $\Delta 5$ and $\Delta 6$ desaturase enzymes [19]. It was previously shown that the placenta in pregnancies complicated by IUGR have decreased placental LPL activity and decreased lipid deposition [17].

In conjunction with selective placental uptake and transfer of EFAs, selective uptake of EFAs may occur in fetal tissues. Our results showed that differences in fetal EFA levels between circulation and the lung are not explained by alterations in lung EFA

conversion enzyme mRNA or protein. The ability of the lung to alter levels of EFAs from the circulation may be a result of selective uptake of EFAs by the lung. Tissues contain varying amounts of fatty acids, and tissue levels of fatty acids are often independent of circulating fatty acids [13]. Previous studies have demonstrated preferential uptake of EFAs into tissue, particularly selective uptake of DHA into the retina and brain [20, 21]. This suggests that tissue-specific selective transport of fatty acids occurs. Plasma fatty acid transport proteins are responsible for the uptake of polyunsaturated fatty acids into cells [22]. While the mechanisms for selective uptake of fatty acids into cells are not fully understood, carbon number and degree of desaturation is thought to be a determinant for selective uptake of EFAs into cells [21].

This study is not without limitations. In this study, we utilized an animal model to study a human condition. Our rat model of IUGR was induced surgically, and thus does not mimic a human maternal condition. However, the uteroplacental insufficiency (UPI)-induced IUGR rat model used is well characterized and does mimic the effects of UPI-induced IUGR in human infants. Additionally, our research did not address how placental transfer of EFAs impact EFA levels in the IUGR fetus. The rat model we used in this study is not appropriate to study the effect of IUGR on the placenta. However, we have a rat model of IUGR induced by maternal tobacco smoke that does mimic the maternal effects of IUGR seen in humans that will be appropriate for placental study. Finally, in this study we were not able to obtain serum or lung EFA levels in newborn rat pups born to dams fed a diet with 0.1% DHA. The 0.1% DHA diet is a more biologically relevant dose than the 1% DHA diet. We are not able to infer if the changes we saw in serum or lung EFA ratios with the 1% DHA diet would have been seen with a 0.1% DHA diet.

Additionally, we are not able infer if the changes we saw in liver and lung EFA conversion enzymes with 0.1% DHA diet would have correlated with 0.1% DHA diet serum or lung EFA ratios.

In conclusion, IUGR induced sex-specific changes in circulating EFA ratios and liver EFA conversion enzyme mRNA in newborn IUGR rat pups. Overall, changes in EFA conversion enzyme mRNA and protein did not correlate with EFA levels in circulation or the lung. We speculate that IUGR induced changes in EFA levels may occur as a result of alterations in placental EFA transfer, and that IUGR impacts placental transfer of EFAs in a sex-specific manner.

REFERENCES

1.   Pike, K., J. Jane Pillow, and J.S. Lucas, Long term respiratory consequences of intrauterine growth restriction. *Semin Fetal Neonatal Med*. 2012;17(2):92-8.

2.   Peleg, D., C.M. Kennedy, and S.K. Hunter, Intrauterine growth restriction: identification and management. *Am Fam Physician*. 1998;58(2):453-60, 466-7.

3.   Cox, P. and T. Marton, Pathological assessment of intrauterine growth restriction. *Best Pract Res Clin Obstet Gynaecol*. 2009;23(6):751-64.

4.   Cetin, I., et al., Intrauterine growth restriction is associated with changes in polyunsaturated fatty acid fetal-maternal relationships. *Pediatr Res*. 2002;52(5):750-5.

5.   Reiss, I., et al., Increased risk of bronchopulmonary dysplasia and increased mortality in very preterm infants being small for gestational age. *Arch Gynec Obstet*. 2003;269(1):40-4.

6.   Rozance, P.J., et al., Intrauterine growth restriction decreases pulmonary alveolar and vessel growth and causes pulmonary artery endothelial cell dysfunction in vitro in fetal sheep. *Am J Physiol-Lung C*. 2011;301(6):L860-71.

7.   Torrance, H.L., et al., Predictors of outcome at 2 years of age after early intrauterine growth restriction. *Ultrasound Obstet Gynecol*. 2010;36(2):171-7.

8.   Deckelbaum, R.J., T.S. Worgall, and T. Seo, n-3 fatty acids and gene expression. *Am J Clin Nutr*. 2006;83(6 Suppl):1520S-1525S.

9.   Martin, C.R., et al., Decreased postnatal docosahexaenoic and arachidonic acid blood levels in premature infants are associated with neonatal morbidities. *J Pediatr*. 2011;159(5):743-749 e1-2.

10.   Joss-Moore, L.A., et al., IUGR decreases elastin mRNA expression in the developing rat lung and alters elastin content and lung compliance in the mature rat lung. *Physiol Genomics*. 2011;43(9):499-505

11.   O'Brien, E.A., et al., Uteroplacental insufficiency decreases p53 serine-15 phosphorylation in term IUGR rat lungs. *Am J Physiol-Reg I*. 2007;293(1):R314-22.

12.   Joss-Moore, L.A., et al., IUGR decreases PPARgamma and SETD8 Expression in neonatal rat lung and these effects are ameliorated by maternal DHA supplementation. *Early Hum Dev*. 2010;86(12):785-91.

13.   Childs, C.E., et al., Maternal diet during pregnancy has tissue-specific effects upon fetal fatty acid composition and alters fetal immune parameters. *Prostag Leukotr Ess*. 2010;83(4-6):179-84.

14.   Amusquivar, E. and E. Herrera, Influence of changes in dietary fatty acids during pregnancy on placental and fetal fatty acid profile in the rat. *Biol Neonate*. 2003;83(2):136-45

15.   Childs, C.E., et al., Gender differences in the n-3 fatty acid content of tissues. *P Nutr Soc*. 2008;67(1):19-27.

16.   Karadag, A., et al., Effect of maternal food restriction on fetal rat lung lipid differentiation program. *Pediatr Pulm*. 2009;44(7):635-44.

17.   Hanebutt, F.L., et al., Long-chain polyunsaturated fatty acid (LC-PUFA) transfer across the placenta. *Clin Nutr*. 2008;27(5):685-93.

18.   Gil-Sanchez, A., et al., Mechanisms involved in the selective transfer of long chain polyunsaturated fatty acids to the fetus. *Front Genet*. 2011;2:57.

19.   Dutta-Roy, A.K., Cellular uptake of long-chain fatty acids: role of membrane-associated fatty-acid-binding/transport proteins. *Cell Mol Life Sci*. 2000;57(10):1360-72.

20.   Li, J., M.G. Wetzel, and P.J. O'Brien, Transport of n-3 fatty acids from the intestine to the retina in rats. *J Lipid Res*. 1992;33(4):539-48.

21.   Wang, N., R.D. Wiegand, and R.E. Anderson, Uptake of 22-carbon fatty acids into rat retina and brain. *Exp Eye Res*. 1992;54(6):933-9.

22.   Schwenk, R.W., et al., Fatty acid transport across the cell membrane: regulation by fatty acid transporters. *Prostag Leukotr Ess*. 2010;82(4-6):149-54.