Published in final edited form as:

Placenta. 2018 September ; 69: 96–101. doi:10.1016/j.placenta.2018.07.016.

Maternal obesity is not associated with placental lipid accumulation in women with high omega-3 fatty acid levels

Fernanda L. Alvarado¹, Virtu Calabuig-Navarro^{1,2}, Maricela Haghiac¹, Michelle Puchowicz³, Pai-Jong S Tsai⁴, Perrie O'Tierney-Ginn^{1,2,*}

¹Center for Reproductive Health, MetroHealth Medical Center, Cleveland, OH, United States;

²Department of Reproductive Biology, Case Western Reserve University, Cleveland, OH, United States;

³Department of Nutrition, Case Western Reserve University, Cleveland, OH, United States;

⁴Department of Obstetrics, Gynecology, and Women's Health, John A. Burns School of Medicine, University of Hawaii, Honolulu, HI, United States

Abstract

Introduction—Placentas of obese women have higher lipid content compared to lean women. We have previously shown that supplementation of overweight and obese women with omega-3 fatty acids decreases placental esterification pathways and total lipid content in a mid-western population (Ohio). We hypothesized that placental lipid accumulation and inflammation would be similar between lean and obese women living in a region of high omega-3 intake, such as Hawaii.

Methods—Fifty-five healthy, normal glucose tolerant women from Honolulu Hawaii, dichotomized based on pre-pregnancy BMI into lean (BMI <25 kg/m², n=29) and obese (BMI >30 kg/m², n=26), were recruited at scheduled term cesarean delivery. Maternal plasma DHA levels were analyzed by mass spectrometry. Expression of key genes involved in fatty acid oxidation and esterification were measured in placental tissue using qPCR. Total lipids were extracted from placental tissue via the Folch method. TNF- α concentration was measured by enzyme-linked immunosorbent assay in placental lysates.

Results—DHA levels were higher in lean women compared to obese women ($P=0.02$). However, DHA levels in obese women in Hawaii were eight times higher compared to obese Ohioan women ($P<0.0001$). Placental lipid content and expression of key genes involved in fatty acid oxidation and esterification were similar ($P>0.05$) between lean and obese women in Hawaii. Furthermore, TNF- α placental lysates were not different between lean and obese women.

Conclusions—Though obese women in Hawaii have lower DHA levels compared to their lean counterparts, these levels remain over eight times as high as obese Ohioan women. These relatively high plasma omega-3 levels in obese women in Hawaii may suppress placental lipid esterification/storage and inflammation to the same levels of lean women, as seen previously in vitro.

*Corresponding Author: Perrie O'Tierney-Ginn, PhD, Mother Infant Research Institute, Tufts Medical Center, Boston, MA, 02111, potierneyginn@tuftsmedicalcenter.org.

Keywords

placenta; omega-3 fatty acids; inflammation; esterification; obesity

Introduction

Obesity prevalence among women of childbearing age has increased to 36% in the last 20 years [1]. Obesity during pregnancy increases the risk for perinatal and long term maternal complications [2–5]. Moreover, offspring of obese women have higher adiposity predisposing them to cardiovascular and metabolic complications [5–7].

High fatty acids (FA) and/or the pro-inflammatory cytokine environment in obese women impair placental metabolic homeostasis and increase placental lipid accumulation, inflammation, and oxidative stress [8–10]. The mechanism involved in increased placental lipid accumulation may be mediated by the increase in FA esterification/storage and a decrease in FA β -oxidation [7].

Omega-3 (n-3) long chain polyunsaturated fatty acids (LCPUFA), such as docosahexaenoic acid (DHA; 22:6 N-3) and eicosapentanoic acid (EPA; 20:5 N-3) have anti-inflammatory and antioxidant properties in adults [11,12]. Haghiac *et al.* showed that n-3 LCPUFA supplementation in obese pregnant women decreased inflammation in adipose and placental tissue [13]. Our group also showed (in a secondary analysis of a randomized controlled trial in a U.S. mid-western population) that n-3 LCPUFA supplementation during pregnancy, compared to placebo, was associated with a 30% decrease in placental lipid accumulation and a decrease in FA storage gene expression. Further, when we quantified the *in-vitro* effect of DHA and EPA in primary trophoblast cells isolated from obese women, we found that both combined and separately, DHA and EPA inhibited esterification of [3 H]-palmitate [7].

Omega-3 LCPUFA consumption varies among different populations [14]. DHA and EPA are found in significant amounts in fish and other seafoods. A single lean fish meal accounts for 0.2 to 0.3 g of marine omega-3 LCPUFA, while a single oily fish meal accounts for 1.5 to 3.0 g of marine omega-3 LCPUFA [15]. Greenland Eskimos and Japanese have a lower incidence of coronary heart disease, where inflammation is thought to play an important role. In addition, they have a diet high in omega-3 LCPUFA. Given the anti-inflammatory properties of omega-3 LCPUFA [11], it is suggested this may be the most important factor that accounts for their decreased cardiovascular risk [16–19]. Within the United States, there are large regional differences in fish intake. Based on the United States Environmental Protection Agency (EPA) Reports and Fact Sheets about Fish Consumption and Human Health, coastal regions such as Hawaii have twice the total fish consumption of a population living in the mainland Great Lakes region, such as Ohio [14]. We hypothesized that obese women living in a high fish intake (high omega-3 LCPUFA) region will not exhibit higher placental lipid accumulation and inflammation compared to their lean counterparts, contrary to what was shown in obese women in a lower fish intake region such as Ohio [14]. To test this hypothesis, we measured placental lipid content, FA esterification pathways, and the inflammatory cytokine TNF α , in a cohort of healthy lean and obese women living in Hawaii.

Materials and Methods

Study Design

We performed a cross sectional analysis of a cohort of healthy women recruited at term (>37 weeks gestation) who delivered by elective pre-labor cesarean section at Kapiolani Medical Center for Women and Children (Honolulu, Hawaii), previously described by Tsai et al. [20,21]. Women were divided according to their pre-pregnancy BMI: (lean <25 kg/m² (n=29), obese >30 kg/m² (n=26).]. Subjects with multiple gestations, pre-eclampsia, diabetes (pre-existing and gestational), uterine growth restriction, shoulder dystocia and macrosomia (birth weight >4500 grams) were excluded. Placenta tissue was collected at the time of delivery from the maternal face of the placenta, halfway between the cord insertion and margins. Four full-thickness samples of the placenta from different quadrants (north, south, east, west from the center, excluding basal plate) were collected within 15 minutes of delivery. Tissue from each location was combined for lipid and RNA extractions. Maternal and cord blood were collected and processed as previously described [20,21]. Written and informed consent was obtained prior to participation and the study was approved by the Western Institutional Review Board and by the Institutional Review Board of MetroHealth Medical Center/Case Western Reserve University (IRB 14-00751)

Maternal and Cord Plasma assay

Non-esterified fatty acids (mEq/L) were measured in maternal and cord plasma using a colorimetric assay (HR Series NEFA-HR (2); Wako Diagnostics, Richmond, VA) as per manufacturer's directions. Cord plasma leptin was analyzed using the Quantikine enzyme-linked immunosorbent assay kits (R&D systems, Minneapolis, Minnesota) as previously described and published by Tsai *et al* [20]. All samples were run in duplicate.

Gas Chromatography Mass Spectrometry Assay for Maternal DHA Measurements

A known quantity of plasma (100 µl) was hydrolyzed (alkaline conditions using KOH/ ethanol solvent mixture and heated for 3 hrs at 85°C) and extracted with hexane, after adding a known amount of [²H₅]DHA as internal standard. DHA and [²H₅]DHA were analyzed as their trimethylsilyl derivatives (TBDMS) using gas chromatography-electron impact ionization mass spectrometry (GC-MS), as previously described but modified [22]. Briefly, a mass selective detector (model 5973N, Agilent) equipped with a gas chromatography system (GC-MS; model 6890, Agilent), coupled to a ZB-5MS capillary column (30 m _0.25 mm_ 0.25 µl) with a helium flow of 1.5 ml/min, was used. The starting oven temperature was 80°C and increased linearly to 220°C and held for 1 min. Derivatized samples were injected in splitless mode and analyzed by selected ion monitoring in EI mode. The m/z ions reflecting fragments for the GC/MS of the TBDMS derivatives of DHA and [²H₅]-DHA were m/z 385 and m/z 390, respectively. DHA concentrations (mM) for each sample were quantified against the [²H₅] -DHA internal standard using standard curve and regression analysis.

Placental lipid analysis

Total lipids were extracted from 80 to 100 mg frozen placental tissue with chloroform:methanol [2:1 volume-to-volume ratio(v/v)], as previously described [7,23], allowed to dry under SpeedVac (Thermo Scientific), and normalized to tissue weight. Data were expressed as total extractable lipids/g tissue.

Separation of phospholipids (PL) and neutral lipid species was performed on thin layer precoated silica gel chromatography (TLC) plates (Millipore, Billerica, MA). TLC plates were prewashed by an ascending development up to 1 cm from the top in a clean tank containing a mixture of chloroform:methanol (1:1, v/v). Plates were air dried in a fume hood for 30 minutes to remove any material interfering with quantitative and qualitative analyses. Before use, plates were completely wetted with 2.3% boric acid, drained for 5 minutes in a fume hood, and dried at 100 C for 15 minutes. A total of 2 µl each sample and 1 µl standard in chloroform was spotted 1 cm from the edge of the plate in 1cm bands. Plates were developed in a stepwise fashion in chambers saturated with: 1) chloroform-methanol-water 60:30:5 (v/v/v) up to the middle of the plate; and 2) hexane-diethyl ether-acetic acid 80:20:1.5 (v/v) up to 1 cm from the top. After separation, lipids were charred by spraying the plate with phosphomolybdic acid solution (Sigma-Aldrich, St. Louis, MO), thoroughly air dried in a fume hood, and immediately heated at 200°C for 2 to 4 minutes. An image of the plate was acquired with ChemiDoc-It TS2 810 imager (UVP, Upland CA). Lipid spots were quantified using UVP VisionWorksLS software. Total of lipid fractions per sample was considered 100%, and each lipid fraction was calculated based on amount of lipid applied and normalized to grams of starting tissue. Standards for each of the lipid classes were applied to every plate [cholesteryl oleate, triolein, oleic acid, free cholesterol, and 1,2 distearoyl, (18–5a standard from Nu-chek Prep, Elysian, MN); phosphatidylethanolamine (PE), phosphatidylcholine (PC), lysophosphatidylcholine, all from egg yolk; phosphatidylinositol (PI) from soybean, and cardiolipin (CL) from bovine heart (Sigma-Aldrich, St. Louis, MO) [10].

Placenta gene expression analysis by quantitative polymerase chain reaction

Total RNA was obtained following homogenization of 50mg placenta tissue in TRIzol reagent (Invitrogen) following the manufacturer's guidelines. RNA integrity was assessed for each sample by visualizing ribosomal RNA via gel electrophoresis. Reverse transcription of 2 µg RNA to complementary DNA was performed using MultiScribe reverse transcription with random primers following manufacturer's guidelines and cycling conditions (high-capacity complementary DNA reverse-transcription kit; Applied Biosystems, Carlsbad, CA). Gene expression was monitored by real-time polymerase chain reaction (PCR) using a Roche thermal cycler (Roche Applied Science, Indianapolis, IN) with Lightcycler Fast-Start DNA Sybr Green 1 master mix (Roche). Gene-specific primers were designed to analyze the expression of genes involved in *FA accumulation/esterification*: FA synthase, acetyl-CoA carboxylase (ACCa), peroxisome proliferator-activated receptor (PPAR) γ , diacylglycerol O-acyltransferase-1 (DGAT1); *FA oxidation (FAO) (mitochondrial)*: CPT1b, PPAR α , carnitine/acylcarnitine translocase (CACT), carnitine O-palmitoyltransferase 2, organic cation/carnitine transporter 2; *FAO (peroxisomal)*: peroxisomal carnitine O-octanoyltransferase (COT). Placenta mitochondrial biogenesis and number were assessed by

messenger RNA (mRNA) expression of PPAR γ coactivator 1 α (PGC1- α , a transcriptional coactivator required for mitochondrial biogenesis). L19 was used as a reference gene, as previously described [10]. For the quantitative determination of mitochondrial DNA (mtDNA) content of cytochrome B (CytB) relative to nuclear (β -actin) DNA [24]. Primer sequences are shown in Supplemental Table 1.

Placenta TNF- α detection by enzyme-linked immunosorbent assay

Total tissue lysates were prepared by homogenizing weighed placental tissue in TRIzol (Invitrogen), and centrifuging at 13 000g for 10 min at 4°C. TNF- α concentration was measured by enzyme-linked immunosorbent assay (ELISA) (R&D System (HSTA00D), Minneapolis, USA) as per manufacturer's instructions. Data was normalized to weight of placental tissue homogenized.

Statistics

All data in tables and text are presented as mean $\pm$ standard deviation. Figures are presented as mean $\pm$ standard error of the mean. The results of the mRNA analyses are expressed in arbitrary units, normalized (by natural log transformation) prior to analysis. Demographic data was tested for normality via Shapiro-Wilk's test and was normalized using z-scores. Differences between groups were analyzed using Student's T-test or Mann-Whitney U test when appropriate. Frequency data was analyzed by Chi-square test. Correlation between total lipid content and mRNA expression was assessed using Pearson's correlation. All analyses were performed using R studio [25] with R version 3.3.0 [26], gridExtra [27], and ggplot2 [28]. P values $\leq$ 0.05 were considered statistically significant.

Results

Demographic data is summarized in Table 1. Lean women were on average three years older than obese women ($P=0.03$). There were not significant differences in maternal gestational age at delivery, gestational weight gain, or plasma free FA acids between lean and obese women. Birth weight and placental weight were significantly higher in the offspring of obese women than their lean counterparts. However, placenta efficiency was significantly higher in the offspring of lean women. Placenta efficiency was defined as grams of fetus produced per gram of placenta (birth weight (g)/placental weight (g)) [29].

Maternal plasma DHA levels in lean and obese women

Plasma DHA levels were higher in the lean compared to the obese women (453 ± 187 vs 347 ± 120 $\mu\text{mol/L}$; $P=0.02$) (Figure 1). Using the same methodology, we measured plasma DHA levels in ten obese mothers (pre-gravid BMI: 33.7 ± 2.7 kg/m^2 , maternal and neonatal characteristics shown in Supplementary Table 2) delivering in Ohio (41 ± 28 $\mu\text{mol/L}$). This confirms that obese women in Hawaii have higher levels of DHA compared with similarly obese women in Ohio.

Placental lipid accumulation in obese and lean women in a region of high omega-3 intake

To establish the effect of long term, high omega-3 intake on placental lipid storage, total placental lipid content was extracted. There was no difference in the total lipid content

between the placentas of lean women compared to placentas of obese women (Figure 2). Total placental lipid content was not associated with birth weight, plasma free fatty acids, or plasma leptin in the cord.

TLC was used to separate placental lipid into PL and neutral lipid components. There were no significant differences in the esterified lipids (cholesterol esters, triglycerides, PE, and PC) between placentas from lean and obese women (Table 2). Placental free FA content was not statistically different between the two groups.

Effect of high maternal omega-3 on placental FA esterification pathway gene expression

Placental genes involved in FA accumulation/esterification (PPAR- γ , ACCa, DGAT1, FAS) (Figure 3) were not differentially expressed between obese and lean women.

Effect of high maternal omega-3 on placental FA oxidation gene expression

We estimated placental mitochondrial biogenesis and number by measuring PGC1- α mRNA expression, and mtDNA relative to nuclear DNA (Cytb/ β -actin), respectively. Based on these markers, maternal obesity was associated with a reduced placental mitochondrial number ($P < 0.05$). We did not find a difference in placental mitochondrial biogenesis between the placentas of lean and obese women (Figure 4). Mitochondrial FAO (PPAR α , OCTN2, CPT1b, CACT, CPT2) and Peroxisomal FAO (COT) did not differ between the placentas of obese and lean women (Figure 4). However, placental mRNA expression of PPAR α was negatively correlated with placental lipid content ($r = -0.309$, 95% CI: -0.530 , -0.047 , $P = 0.021$).

Effect of high omega-3 on levels of TNF- α in placental tissue lysate in lean and obese women

Placental TNF- α lysate levels were similar in the lean compared to the obese women (10 ± 8 vs 11 ± 17 ng/mg tissue; $P = 0.794$). Using the same method, we measured placental TNF- α lysate levels in obese mothers (pre-gravid BMI 35.6 ± 4.4 kg/m², maternal and neonatal characteristics shown in Supplementary Table 1) delivering in Ohio (24 ± 22 ng/mg tissue, $N = 23$), and found the levels were significantly higher than those of obese women in Hawaii (95% CI: -25.2 , -1.5 , $P = 0.02$), (Supplementary Figure 1).

Effect of high omega-3 on fetal leptin levels in offspring of lean and obese women.

In a secondary analysis of data previously published by Tsai *et al* [20], we found no difference in cord leptin between offspring of lean and obese women (5.9 ± 3.6 vs 7.2 ± 4.7 ng/mL). Using the same method, we measured cord leptin in offspring of obese mothers (pre-gravid BMI 39 ± 6.8 kg/m², maternal and neonatal characteristics shown in Supplementary Table 1) delivering in Ohio (18.9 ± 3 ng/mL, $N = 31$), and found they were significantly higher than those of offspring born to obese mothers in Hawaii (95% CI: -13.8 , -0.50 , $P = 0.02$), (Supplementary Figure 2).

Discussion

Placentas from obese women living in Hawaii have similar lipid content, and expression of FA esterification/storage and mitochondrial FAO genes, as their lean counterparts. We found that the neutral lipid fraction (triglycerides, cholesterol esters) and PL (PE, PC) concentrations were also similar in placentas of these women. Moreover, when we measured placental TNF α concentration, we found no difference between lean and obese women. This is in contrast with findings from cohorts recruited in mainland US showing that placentas from obese women have higher lipid and inflammatory cytokine concentration when compared to their lean counterparts [8,10,30,31]. We hypothesize that the absence of high lipid content and inflammatory marker levels in placentas from obese women in Hawaii is due to their comparatively high plasma DHA levels. Indeed, we found that obese women from Hawaii have plasma DHA levels 8 times higher than obese women from Ohio. Thus, we propose that high omega-3 LCPUFA intake may prevent lipotoxicity and inflammation in placentas of obese women.

Our group and others have previously shown that obese women have higher placental lipid content than lean women [8,10,30]. This finding is supported by increased expression of genes involved in FA esterification and storage pathways [10]. Placental lipid concentration may be elevated in obese women due to increased maternal supply, secondary to maternal insulin resistance and elevated lipolysis [10,32]. Animal and human studies show that omega-3 intake regulates lipid metabolism in different tissues including adipose [34], hepatic, and placental tissue [13,35]. The DHA/ EPA mechanism involved in the reduction of placental lipid accumulation may be explained by diverse means: 1) DHA/EPA act as ligands at several nuclear receptors/transcriptional factors (such as the PPARs) affecting FAO and FA esterification/storage pathways [7,33,36,37]. Calabuig et al. showed that 20 weeks of n-3 LCPUFA supplementation in obese and overweight pregnant women lowered placental lipid concentration by inhibiting the lipid esterification pathway (PPAR γ) [7]. As opposed to hepatocytes [33,37], placental FAO pathways (PPAR α , CPT1b), and lipases were not affected by n-3 supplementation [7]. 2) DHA/EPA may also decrease serum levels of free FA, lowering their placental delivery [37]. We found that women in Hawaii had similar plasma levels of free FA regardless of their weight. Conversely, obese Ohioan women have significantly higher free FA levels [10]. Taken together, our results are consistent with both pathways, where high omega-3 intake decreased systemic and placental lipid concentration in obese women.

If there is indeed a causal relationship, our study would suggest that there is a threshold level of placental lipid content/esterification required for normal placental function, as evidenced by the lack of differences among women in Hawaii, despite lower DHA levels in obese compared to lean women. To support this, previous *in vitro* work by our group found that exposure to DHA for 24 hours reduced the ability of trophoblast cells isolated from obese women to esterify and store lipids to levels similar to lean women. Conversely, DHA did not lower esterification in trophoblast cells isolated from lean women [38].

Obese pregnant women have higher plasma saturated FA, which can increase tissue accumulation of lipids, leading to oxidative stress and stimulation, synthesis and release of

inflammatory cytokines (TNF- α , IL-6) [7,31,39–41]. Interestingly, we found no difference in TNF- α concentration in placental lysates from obese and lean women in Hawaii. However, placentas from obese women in Hawaii have lower TNF- α levels than those from Ohio. N-3 LCPUFA phospholipid membrane incorporation may serve as substrate for eicosanoid products and synthesize inflammatory mediators that are less potent than those synthesized from n-6 LCPUFA [11,15,42]. Moreover, DHA/EPA is a substrate for lipid anti-inflammatory mediators (protectines, resolvines), and it has been shown to decrease the TLR4-innate immune response in trophoblast tissue [13,15], supporting a potential role for high maternal plasma DHA in suppression of placental inflammation [13].

Omega-3 supplementation in obese women during pregnancy increases birth weight and length [43–45]. Obese women in Hawaii have heavier babies than those of obese women from Ohio [10]. Larger babies have an increased risk of childhood obesity and metabolic syndrome later in life [46]. However, birth weight alone is a crude estimate of adiposity [47,48]. On the other hand, fetal leptin is a predictor of regional neonatal adiposity [48]. Furthermore, it is involved in early hypothalamic programming in the fetus, which is thought to play an important role in the development of metabolic syndrome later in life [49]. To that effect, Tsai et al measured cord leptin in these newborns [20]. In a secondary analysis of these data, we found no difference in cord leptin between offspring of lean and obese women in Hawaii. Interestingly, obese Ohioan offspring have double the cord leptin concentrations of offspring of obese mothers in Hawaii, suggesting that, though lighter, they may have greater fat accrual. Further investigation using direct measures of adiposity are needed.

Our study is based on observational differences and therefore does not prove causation. However, as stated above, there are well-established biological mechanisms that could explain these findings. We did not have a nutritional intake index, though we directly measured maternal plasma DHA levels at delivery. Our cross-sectional design at time of delivery impairs our ability to draw conclusions throughout pregnancy or to measure the long-term effects on the children. However, we speculate that long term omega-3 LCPUFA intake modifies the early pregnancy environment, when many of these placental pathways are established.

In summary, we found no difference in placental lipid content, TNF- α levels, or cord leptin concentrations between obese and lean women in Hawaii, a region with high fish intake in pregnancy. Based on comparisons with similarly obese women from Ohio, we speculate that high maternal DHA levels suppress placental lipid accumulation, and subsequent lipotoxicity in obese women from Hawaii. Though we cannot conclude a causal relationship, the lower cord leptin levels in offspring of obese women in Hawaii as compared to offspring of obese Ohioan women, suggests that high fetal fat accrual may be associated with high placental lipid accumulation. Further studies are necessary to determine how these changes affect long-term metabolic outcomes for the offspring.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgement

We would like to thank Ankita Shukla for her assistance with sample preparation for lipid extraction.

Funding

This work was supported by the National Institutes of Health (R00HD062841).

Bibliography

- [1]. Hales CM, Carroll MD, Fryar CD, Ogden CL, Prevalence of obesity among adults and youth: United States, 2015 – 2016, National Center for Health Statistics, 2017. <https://www.cdc.gov/nchs/data/databriefs/db288.pdf> (accessed December 22, 2017).
- [2]. Antza C, Cifkova R, Kotsis V, Hypertensive complications of pregnancy: A clinical overview, *Metabolism*. (2017). doi:10.1016/j.metabol.2017.11.011.
- [3]. Haslam DW, James WPT, Obesity, *The Lancet*. 366 (2005) 1197–1209. doi:10.1016/S0140-6736(05)67483-1.
- [4]. Ritchey MD, Loustalot F, Bowman BA, Hong Y, Trends in Mortality Rates by Subtypes of Heart Disease in the United States, 2000–2010, *JAMA*. 312 (2014) 2037–2039. doi:10.1001/jama.2014.11344. [PubMed: 25399281]
- [5]. Catalano PM, Shankar K, Obesity and pregnancy: mechanisms of short term and long term adverse consequences for mother and child, *BMJ*. 356 (2017) j1. doi:10.1136/bmj.j1. [PubMed: 28179267]
- [6]. Pantham P, Aye ILMH, Powell TL, Inflammation in Maternal Obesity and Gestational Diabetes Mellitus, *Placenta*. 36 (2015) 709–715. doi:10.1016/j.placenta.2015.04.006. [PubMed: 25972077]
- [7]. Calabuig-Navarro V, Puchowicz M, Glazebrook P, Haghiac M, Minium J, Catalano P, Hauguel deMouzon S, O'Tierney-Ginn P, Effect of ω -3 supplementation on placental lipid metabolism in overweight and obese women¹², *Am. J. Clin. Nutr* 103 (2016) 1064–1072. doi:10.3945/ajcn.115.124651. [PubMed: 26961929]
- [8]. Saben J, Lindsey F, Zhong Y, Thakali K, Badger TM, Andres A, Gomez-Acevedo H, Shankar K, Maternal Obesity is Associated with a Lipotoxic Placental Environment, *Placenta*. 35 (2014) 171–177. doi:10.1016/j.placenta.2014.01.003. [PubMed: 24484739]
- [9]. Lewis RM, Desoye G, Placental Lipid and Fatty Acid Transfer in Maternal Overnutrition, *Ann. Nutr. Metab* 70 (2017) 228–231. doi:10.1159/000463397. [PubMed: 28301855]
- [10]. Calabuig-Navarro V, Haghiac M, Minium J, Glazebrook P, Ranasinghe GC, Hoppel C, Hauguel de-Mouzon S, Catalano P, O'Tierney-Ginn P, Effect of Maternal Obesity on Placental Lipid Metabolism, *Endocrinology*. 158 (2017) 2543–2555. doi:10.1210/en.2017-00152. [PubMed: 28541534]
- [11]. Calder PC, n-3 Polyunsaturated fatty acids, inflammation, and inflammatory diseases, *Am. J. Clin. Nutr* 83 (2006) S1505–S1519S.
- [12]. Leghi GE, Muhlhauser BS, The effect of n-3 LCPUFA supplementation on oxidative stress and inflammation in the placenta and maternal plasma during pregnancy, *Prostaglandins Leukot. Essent. Fat. Acids* PLEFA113 (2016) 33–39. doi:10.1016/j.plefa.2016.08.010.
- [13]. Haghiac M, Yang X, Presley L, Smith S, Dettelback S, Minium J, Belury MA, Catalano PM, Mouzon SH, Dietary Omega-3 Fatty Acid Supplementation Reduces Inflammation in Obese Pregnant Women: A Randomized Double-Blind Controlled Clinical Trial, *PLOS ONE*. 10 (2015) e0137309. doi:10.1371/journal.pone.0137309. [PubMed: 26340264]
- [14]. O. US EPA, Estimated Fish Consumption Rates Reports, US EPA. (2015). <https://www.epa.gov/fish-tech/estimated-fish-consumption-rates-reports> (accessed December 28, 2017).
- [15]. Calder PC, Marine omega-3 fatty acids and inflammatory processes: Effects, mechanisms and clinical relevance, *Biochim. Biophys. Acta BBA - Mol. Cell Biol. Lipids* 1851 (2015) 469–484. doi:10.1016/j.bbalip.2014.08.010.

- [16]. Bang HO, Dyerberg J, Plasma lipids and lipoproteins in Greenlandic west coast Eskimos, *Acta Med. Scand*192 (1972) 85–94. [PubMed: 5052396]
- [17]. Iso H, Kobayashi M, Ishihara J, Sasaki S, Okada K, Kita Y, Kokubo Y, Tsugane S, Intake of Fish and n3 Fatty Acids and Risk of Coronary Heart Disease Among Japanese: The Japan Public Health Center-Based (JPHC) Study Cohort I, *Circulation*. 113 (2006) 195–202. doi:10.1161/CIRCULATIONAHA.105.581355. [PubMed: 16401768]
- [18]. Watters CA, Edmonds CM, Rosner LS, Sloss KP, Leung P, A Cost Analysis of EPA and DHA in Fish, Supplements, and Foods, *J. Nutr. Food Sci*2 (2012). doi:10.4172/2155-9600.1000159.
- [19]. Okuda N, Ueshima H, Okayama A, Saitoh S, Nakagawa H, Rodriguez BL, Sakata K, Choudhury SR, Curb JD, Stamler J, Relation of long chain n-3 polyunsaturated fatty acid intake to serum high density lipoprotein cholesterol among Japanese men in Japan and Japanese–American men in Hawaii: the INTERLIPID study, *Atherosclerosis*. 178 (2005) 371–379. doi:10.1016/j.atherosclerosis.2004.09.007. [PubMed: 15694947]
- [20]. Tsai P-JS, Davis J, Bryant-Greenwood G, Systemic and Placental Leptin and Its Receptors in Pregnancies Associated With Obesity, *Reprod. Sci*22 (2015) 189–197. doi:10.1177/1933719114537718. [PubMed: 24899470]
- [21]. Tsai P-JS, Davis J, Thompson K, Bryant-Greenwood G, Visfatin/Nampt and SIRT1 Roles in Postterm Delivery in Pregnancies Associated With Obesity, *Reprod. Sci*22 (2015) 1028–1036. doi:10.1177/1933719115570908. [PubMed: 25670718]
- [22]. Brunengraber DZ, McCabe BJ, Kasumov T, Alexander JC, Chandramouli V, Previs SF, Influence of diet on the modeling of adipose tissue triglycerides during growth, *Am. J. Physiol. Endocrinol. Metab*285 (2003) E917–E925. doi:10.1152/ajpendo.00128.2003. [PubMed: 12799315]
- [23]. Folch J, Lees M, Stanley GHS, A Simple Method for the Isolation and Purification of Total Lipides from Animal Tissues, *J. Biol. Chem*226 (1957) 497–509. [PubMed: 13428781]
- [24]. Lassance L, Haghiac M, Leahy P, Basu S, Minium J, Zhou J, Reider M, Catalano PM, Mouzon SH, Identification of early transcriptome signatures in placenta exposed to insulin and obesity, *Am. J. Obstet. Gynecol*212 (2015) 647.e1–647.11. doi:10.1016/j.ajog.2015.02.026. [PubMed: 25731694]
- [25]. RStudio Team, RStudio: Integrated Development Environment for R, RStudio, Inc., Boston, MA, 2016. <http://www.rstudio.com/>.
- [26]. R Core Team, R: A Language and Environment for Statistical Computing, R Foundation for Statistical Computing, Vienna, Austria, 2016. <https://www.R-project.org/>.
- [27]. Auguie B, gridExtra: Miscellaneous Functions for “Grid” Graphics, 2016. <https://CRAN.R-project.org/package=gridExtra>.
- [28]. Wickham H, ggplot2: elegant graphics for data analysis, Springer, New York, 2009. <http://had.co.nz/ggplot2/book>.
- [29]. Fowden AL, Sferruzzi-Perri AN, Coan PM, Constancia M, Burton GJ, Placental efficiency and adaptation: endocrine regulation, *J. Physiol*587 (2009) 3459–3472. doi:10.1113/jphysiol.2009.173013. [PubMed: 19451204]
- [30]. Hirschmugl B, Desoye G, Catalano P, Klymiuk I, Scharnagl H, Payr S, Kitzinger E, Schlieffsteiner C, Lang U, Wadsack C, Hauguel-de Mouzon S, Maternal obesity modulates intracellular lipid turnover in the human term placenta, *Int. J. Obes*2005.41 (2017) 317–323. doi:10.1038/ijo.2016.188.
- [31]. Challier J, Basu S, Bintein T, Hotmire K, Minium J, Catalano P, Hauguel-de Mouzon S, Obesity in pregnancy stimulates macrophage accumulation and inflammation in the placenta, *Placenta*. 29 (2008) 274–281. doi:10.1016/j.placenta.2007.12.010. [PubMed: 18262644]
- [32]. Lindsay KL, Hellmuth C, Uhl O, Buss C, Wadhwa PD, Koletzko B, Entringer S, Longitudinal Metabolomic Profiling of Amino Acids and Lipids across Healthy Pregnancy, *PLoS ONE*. 10 (2015). doi:10.1371/journal.pone.0145794.
- [33]. Gillies PJ, Bhatia SK, Belcher LA, Hannon DB, Thompson JT, Heuvel JPV, Regulation of inflammatory and lipid metabolism genes by eicosapentaenoic acid-rich oil, *J. Lipid Res*53 (2012) 1679–1689. doi:10.1194/jlr.M022657. [PubMed: 22556214]

- [34]. Effect of body fat distribution on the transcription response to dietary fat interventions | Genes & Nutrition | Full Text, (n.d.). <https://genesandnutrition.biomedcentral.com/articles/10.1007/s12263-009-0122-9> (accessed January 11, 2018).
- [35]. Keelan JA, Mas E, D'Vaz N, Dunstan JA, Li S, Barden AE, Mark PJ, Waddell BJ, Prescott SL, Mori TA, Effects of maternal n-3 fatty acid supplementation on placental cytokines, pro-resolving lipid mediators and their precursors, *Reproduction*. 149 (2015) 171–178. doi:10.1530/REP-14-0549. [PubMed: 25504868]
- [36]. Skulas-Ray AC, West SG, Davidson MH, Kris-Etherton PM, Omega-3 fatty acid concentrates in the treatment of moderate hypertriglyceridemia, *Expert Opin. Pharmacother*9 (2008) 1237–1248. doi:10.1517/14656566.9.7.1237. [PubMed: 18422480]
- [37]. Duda MK, O'Shea KM, Stanley WC, ω -3 polyunsaturated fatty acid supplementation for the treatment of heart failure: mechanisms and clinical potential, *Cardiovasc. Res*84 (2009) 33–41. doi:10.1093/cvr/cvp169. [PubMed: 19474179]
- [38]. Calabuig-Navarro et al., Scientific Abstracts, *Reprod. Sci*23 (2016) 239A. doi:10.1177/1933719117699773. [PubMed: 26342054]
- [39]. Christian LM, Porter K, Longitudinal Changes in Serum Proinflammatory Markers across Pregnancy and Postpartum: Effects of Maternal Body Mass Index, *Cytokine*. 70 (2014) 134–140. doi:10.1016/j.cyto.2014.06.018. [PubMed: 25082648]
- [40]. Yang X, Li M, Haghiac M, Catalano PM, O'Tierney-Ginn P, Mouzon SH, Causal relationship between obesity-related traits and TLR4-driven responses at the maternal–fetal interface, *Diabetologia*. 59 (2016) 2459–2466. doi:10.1007/s00125-016-4073-6. [PubMed: 27535280]
- [41]. Yang X, Haghiac M, Glazebrook P, Minium J, Catalano PM, Hauguel-de Mouzon S, Saturated fatty acids enhance TLR4 immune pathways in human trophoblasts, *Hum. Reprod. Oxf. Engl*30 (2015) 2152–2159. doi:10.1093/humrep/dev173.
- [42]. Burke MF, Burke FM, Soffer DE, Review of Cardiometabolic Effects of Prescription Omega-3 Fatty Acids, *Curr. Atheroscler. Rep*19 (2017) 60. doi:10.1007/s11883-017-0700-z. [PubMed: 29116404]
- [43]. Olafsdottir AS, Magnusardottir AR, Thorgeirdottir H, Hauksson A, Skuladottir GV, Steingrimsdottir L, Relationship between dietary intake of cod liver oil in early pregnancy and birthweight, *BJOG Int. J. Obstet. Gynaecol*112 (2005) 424–429. doi:10.1111/j.1471-0528.2005.00477.x.
- [44]. Li G-L, Chen H-J, Zhang W-X, Tong Q, Yan Y-E, Effects of maternal omega-3 fatty acids supplementation during pregnancy/lactation on body composition of the offspring: A systematic review and meta-analysis, *Clin. Nutr. Edinb. Scotl* (2017). doi:10.1016/j.clnu.2017.08.002.
- [45]. Bernard JY, Tint M-T, Aris IM, Chen L-W, Quah PL, Tan KH, Yeo GS-H, Fortier MV, Yap F, Shek L, Chong Y-S, Gluckman PD, Godfrey KM, Calder PC, Chong MFF, Kramer MS, Botton J, Lee YS, Maternal plasma phosphatidylcholine polyunsaturated fatty acids during pregnancy and offspring growth and adiposity, *Prostaglandins Leukot. Essent. Fatty Acids*121 (2017) 21–29. doi:10.1016/j.plefa.2017.05.006. [PubMed: 28651694]
- [46]. Ornoy A, Prenatal origin of obesity and their complications: Gestational diabetes, maternal overweight and the paradoxical effects of fetal growth restriction and macrosomia, *Reprod. Toxicol. Elmsford N32* (2011) 205–212. doi:10.1016/j.reprotox.2011.05.002.
- [47]. Muthayya S, Dwarkanath P, Thomas T, Vaz M, Mhaskar A, Mhaskar R, Thomas A, Bhat S, Kurpad AV, Anthropometry and body composition of south Indian babies at birth, *Public Health Nutr.* 9 (2006) 896–903. doi:10.1017/PHN2006943. [PubMed: 17010256]
- [48]. Donnelly JM, Lindsay KL, Walsh JM, Horan M, Molloy EJ, McAuliffe FM, Fetal metabolic influences of neonatal anthropometry and adiposity, *BMC Pediatr.* 15 (2015). doi:10.1186/s12887-015-0499-0.
- [49]. Bouret SG, Early Life Origins of Obesity: Role of Hypothalamic Programming, *J. Pediatr. Gastroenterol. Nutr*48 (2009). doi:10.1097/MPG.0b013e3181977375.

Figure 1. Maternal plasma DHA levels in lean and obese women

DHA concentrations were analyzed by gas chromatography/mass spectrometry and expressed as $\mu\text{mol/L}$. Data are mean $\pm$ standard error of the mean. * $P < 0.05$ obese vs lean by Student's t-test. ^This line represents the mean maternal DHA of 10 obese women from Ohio ($41 \pm 28 \mu\text{mol/L}$).

Figure 2. Placenta lipid content in lean and obese women.

Total lipid extracted using the Folch method and normalized to tissue weight. Data are mean $\pm$ standard error of the mean.

Figure 3. Effect of high omega-3 on placental FA esterification pathway gene expression. mRNA expression of placental genes involved in FA esterification from 29 lean and 26 obese placentas. Data (mean $\pm$ standard error of the mean) are expressed as the ratio of gene of interest: reference gene (L19).

Figure 4. Effect of high omega-3 on placental FA oxidation gene expression

mRNA expression of placental genes involved in FA oxidation from 29 lean and 26 obese placentas. Data (mean $\pm$ standard error of the mean) are expressed as the ratio of gene of interest: reference gene (L19). Mitochondrial number was indicated by the ratio of CytB/ β -actin DNA. *P = 0.05 obese vs lean by Student's t-test.

Table 1.**Maternal and Neonatal Characteristics of the Study Population**

	Lean (n=29)	Obese (n=26)	P value
Maternal			
Race (cauc/ph/ea/others [§])	4 / 5 / 7 / 13	5 / 3 / 2 / 16	0.314 [#]
Parity (previous live births)	1.1 ± 0.9	1.6 ± 1.1	0.097
Maternal Age (y)	33.3 ± 5.6	30.3 ± 4.8	0.032
Gestational Age at delivery (wks)	38.8 ± 0.6	39.1 ± 0.4	0.088
Pre-Pregnancy BMI (kg/m ²)	21.1 ± 2.2	36.8 ± 6.9	<0.0001
Gestational Weight Gain (kg)	13.9 ± 5.3	13.4 ± 7.2	0.741
Free fatty acids (mEq/L)	0.52 ± 0.28	0.58 ± 0.20	0.422
Currently smoking/non-smoking (%)	1/28 (3%)	1/25 (4%)	0.999
Neonatal			
Birth Weight (kg)	3.3 ± 0.4	3.5 ± 0.4	0.039
Baby's Sex (M/F)	11/18	15/11	0.959
Placental Weight (g)	472 ± 88	552 ± 95	0.002
Placental Efficiency (g) [*]	7.1 ± 0.9	6.5 ± 1.0	0.022
Cord-free fatty acids (mEq/L)	0.12 ± 0.08	0.13 ± 0.05	0.814

Values are mean ± SD. Abbreviations: cauc/ph/ea/others, Caucasians,Philippines,East Asia,

[§]Others include groups from Micronesia, Polynesia (Part Hawaiian, Samoan/Togan), Other Mixes and others.

^{*} Placenta Efficiency: Birth Weight (g)/Placental Weight (g).

[#] P value calculated via Chi-squared test. P values calculated by Student's t-test.

Table 2.

Placenta Lipid Profile

$\mu\text{g Lipid/g Tissue}$	Lean (n=29)	Obese (n=26)	P value
Free Cholesterol	44 ± 15	43 ± 10	0.76
Cholesterol esters	46 ± 16	45 ± 10	0.56
Free fatty acids	29 ± 9	27 ± 8	0.35
Triglyceride	33 ± 11	30 ± 7	0.26
Cardiolipin	26 ± 9	23 ± 5	0.09
Phosphatidylethanolamine	38 ± 14	36 ± 8	0.50
Phosphatidylinositol	32 ± 11	29 ± 5	0.24
Phosphatidylcholine	40 ± 14	39 ± 9	0.67

Data are mean $\pm$ standard deviation. P value calculated via Mann-Whitney U test