Placenta 69 (2018) 96–101

Contents lists available at ScienceDirect

Placenta

journal homepage: www.elsevier.com/locate/placenta

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

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ARTICLE INFO

Keywords:
Placenta
Omega-3 fatty acids
Inflammation
Esterification
Obesity

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/m^2, n = 29) and obese (BMI > 30 kg/m^2, 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*.

1. 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

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https://doi.org/10.1016/j.placenta.2018.07.016
Received 1 June 2018; Received in revised form 10 July 2018; Accepted 28 July 2018

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 0.2–0.3 g of marine omega-3 LCPUFA, while a single oily fish meal accounts for 1.5–3.0 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 TNFα, in a cohort of healthy lean and obese women living in Hawaii.

2. Materials and methods

2.1. 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/m^2 (n = 29), obese > 30 kg/m^2 (n = 26)]. Subjects with multiple gestations, pre-eclampsia, diabetes (pre-existing and gestational), uterine growth restriction, shoulder dystocia and macrosomia (birth weight > 4500 g) 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 min 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).

2.2. 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.

2.3. 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 h at 85 °C) and extracted with hexane, after adding a known amount of [$^2H_5$]DHA as internal standard. DHA and [$^2H_5$]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 5973 N, 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 [$^2H_5$]-DHA were *m/z* 385 and *m/z* 390, respectively. DHA concentrations (mM) for each sample were quantified against the [$^2H_5$] -DHA internal standard using standard curve and regression analysis.

2.4. 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 min to remove any material interfering with quantitative and qualitative analyses. Before use, plates were completely wetted with 2.3% boric acid, drained for 5 min in a fume hood, and dried at 100C for 15 min. A total of 2 µl each sample and 1 µl standard in chloroform was spotted 1 cm from the edge of the plate in 1 cm 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 2–4 min. 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 distearolyl, (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].

2.5. Placenta gene expression analysis by quantitative polymerase chain reaction

Total RNA was obtained following homogenization of 50 mg 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-