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Protein expression of fatty acid transporter 2 is polarized to the trophoblast basal plasma membrane and increased in placentas from overweight/obese women

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ABSTRACT

Background: Obese and overweight women are more likely to deliver a large infant or an infant with increased adiposity, however the underlying mechanisms are not well established. We tested the hypothesis that placental capacity to transport fatty acid is increased in overweight/obese women.
Methods: Pregnant women with body mass index (BMI) ranging from 18.4 to 54.3 kg/m^2 and with uncomplicated term pregnancies were recruited for collection of blood samples and placental tissue. Maternal and fetal levels of non-esterified fatty acids (NEFAs) were measured in plasma. The expression and localization of CD36/fatty acid translocase (FAT), fatty acid transport protein (FATP)2, and FATP4 was determined in fixed placental tissue and in isolated syncytiotrophoblast plasma membranes from normal and high BMI mothers.
Results: Maternal and fetal plasma NEFA levels did not correlate ($n = 42$). FATP2 and FATP4 expressions were higher in the basal plasma membrane (BPM) compared to the microvillous membrane ($P < 0.001$; $n = 7$) per unit membrane protein. BPM expression of FATP2 correlated with maternal BMI ($P < 0.01$; $n = 30$); there was no association between CD36/FAT or FATP4 expression and maternal BMI.
Conclusion: The polarization of FATPs to the BPM will facilitate fatty acid transfer across the placenta. In overweight/obese pregnancies, the increased FATP2 expression could contribute to increased fatty acid delivery to the fetus and while we have no direct data we speculate that this could lead accelerated fetal growth or increased fat deposition.

1. Introduction

Large numbers of women in developed countries like the USA are overweight or obese when they become pregnant [1,2], and therefore they are more likely to deliver a large for gestational age infant [3,4] or an infant with increased adiposity [5]. Large size at birth is associated with perinatal complications [6]. Furthermore, children of obese mothers are 2–3 times more likely to become obese themselves, an effect largely independent of birth weight [7]. Although the adverse short- and long-term consequences of maternal obesity on her offspring are well established [6], the underlying mechanisms are largely unknown.

Increased fetal lipid availability may contribute to fetal overgrowth and/or increased fat accumulation in fetuses of obese mothers. First, circulating levels of lipids [8,9] are elevated in obese

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pregnant women as compared to normal weight pregnant women, which could enhance fatty acid transfer across the placenta. Second, some indirect evidence suggests that maternal obesity may increase placental capacity to transport lipids. In animal models of maternal obesity, the expression of several placental fatty acid transporters is increased [10,11], consistent with an elevated capacity to transfer fatty acids. In human placental homogenates, maternal obesity has been shown to decrease the expression of fatty acid transporter protein (FATP)4, while increasing the expression of CD36/fatty acid translocase (FAT) [12]. However, the expression of these membrane-bound proteins in placental homogenates may not be representative of their expression in the syncytiotrophoblasts plasma membranes, the cell-layer considered the primary barrier for placental nutrient transport [13].

Numerous proteins involved in fatty acid transfer are expressed in the placenta, including cytosolic fatty acid binding proteins and membrane-bound FATPs [13]. The FATPs are important for cellular uptake of long-chain fatty acids [14]. The mammalian placenta expresses mRNA for five of the six known FATP isoforms: FATP1-4 and FATP6 [15]. FATP1 and FATP4, as well as CD36/FAT, have been demonstrated in both the basal plasma membrane (BPM) and the microvillous membrane (MVM) of the syncytiotrophoblast of human placenta [12,16,17].

Regulation of placental expression of FATPs has been studied in cultured human primary trophoblasts. These studies have identified the peroxisome proliferator-activated receptor (PPAR)γ/RXR signaling pathway as a differential regulator of FATP2 and FATP4 mRNA expression [18]. Other factors affecting trophoblast fatty acid transporter expression are interleukin (IL)-6 which reduces FATP4 mRNA expression [19] and leptin which is a positive regulator of CD36/FAT [20]. We have recently shown maternal circulating levels of leptin, but not IL-6, are increased in a population of high BMI predominantly Hispanic women [21].

In this study we tested the hypothesis that the protein expression of placental fatty acid transporters is up-regulated in response to maternal overweight and obesity. To this effect, we determined the protein expression of FATP2, FATP4, and CD36/FAT in isolated syncytiotrophoblast plasma membranes from term placentas of women with varying BMI.

2. Patients and methods

2.1. Study subjects

Human placental tissue and blood samples were collected with informed written consent. The protocol was approved by the Institutional Review Board at University of Texas Health Science Center, San Antonio (HSC20100262H), and blood and placental samples obtained at delivery were added to a tissue repository. From this tissue repository, 58 women who delivered at term (≥37 weeks gestation) with no major pregnancy complications were selected and the repository provided coded plasma and placental tissue samples and de-identified relevant medical information. The women were grouped into either "normal BMI" (<25.0 kg/m^2, n = 22) or "high BMI" (>25.0 kg/m^2, n = 36) based on their pre-pregnancy or early pregnancy BMI. Only deliveries by Caesarean section prior to the onset of labor were included in this study. Additional samples from normal BMI women were used for localization studies.

2.2. Plasma NEFA analysis

Fasting maternal blood samples (n = 42) were collected prior to Caesarean section and fetal venous cord blood was obtained immediately after delivery. Plasma concentrations of non-esterified fatty acids (NEFAs) were determined using an enzymatic colorimetric assay (Wako Diagnostics, Richmond, VA).

2.3. Immunofluorescence assay

Placenta (n = 5) were obtained immediately after delivery and several small villous tissue pieces were rinsed in cold physiological saline before placed in formalin fixation solution. The fixed tissue was embedded in paraffin and was cut into (3 μm) sections. Paraffin was removed and antigen retrieval performed by submerging tissue sections in sodium citrate buffer (10 mM sodium citrate, 0.025% Tween 80, pH 6) at 95C for 10 min. Sections were blocked in 5% BSA for 30 min before incubation with primary antibodies, polyclonal rabbit anti-human CD36/FAT (Abcam, ab78054), polyclonal rabbit anti-human FATP2 (Abcam, ab85801), polyclonal rabbit anti-human FATP4 (Sigma-Aldrich, HPA007293), and polyclonal sheep anti-human Anti-Placental Alkaline Phosphatase (Abcam, ab64671) for 60 min at RT. In negative controls, the primary antibody was omitted. Sections were then incubated with a mixture of secondary antibodies Alexa Fluor 555 donkey anti-rabbit (Molecular Probes, A31572) and Alexa Fluor 488 donkey anti-sheep (Molecular Probes, A11015) for 60 min RT in a dark chamber. All slides were mounted with ProLong Gold anti-fade reagent with DAPI (Molecular Probes, P36935). Images were viewed at 63x magnification with oil immersion using Zeiss LSM 780 confocal microscope with Zen Black 2.1 software.

2.4. Preparation of placental homogenates

Placentas (n = 37) were obtained immediately after delivery. After removing decidua basalis and chorionic plate, approximately 100 g of villous tissue was collected and rinsed in cold physiological saline. The villous tissue was transferred to ice-cold buffer D (250 mM sucrose, 10 mM hepes, pH 7.4) containing protease and phosphatase inhibitors (Sigma-Aldrich, St. Louis, MO) and homogenized on ice. Placental homogenates were frozen in liquid nitrogen and stored at −80 °C until further processing for isolation of plasma membranes.

2.5. Isolation of microvillous and basal plasma membranes

Syncytiotrophoblast BPM and MVM were isolated according to a previously described protocol [23,24] with some modifications. Thawed villous tissue was re-homogenized and centrifuged for 15 min (10,000*g*, 4 °C). The supernatant was collected, the pellet was re-suspended in buffer D, homogenized, and centrifuged (10 min at 10,000*g*, 4 °C). The supernatants from both spins were combined and centrifuged for 30 min (125,000*g*, 4 °C). The resulting pellet was re-suspended in buffer D, $MgCl_2$ was added to a final concentration of 12 mM, and the mixture was stirred on ice for 20 min and subsequently centrifuged for 15 min (2,500*g*, 4 °C). The MVM containing supernatant was centrifuged for 30 min (125,000*g*, 4 °C), while the BPM containing pellet was re-suspended, homogenized, and layered on a sucrose gradient. The BPM was separated by centrifugation for 60 min (144,000*g*, 4 °C). The collected MVM and BPM were centrifuged for 30 min (125,000*g*, 4 °C), resulting pellets re-suspended in buffer D containing protease and phosphatase inhibitors, frozen in liquid nitrogen, and stored at −80 °C.

As a marker for MVM enrichment, alkaline phosphatase activity was measured in isolated MVM-vesicles and placental homogenates. Alkaline phosphatase activity was 15.3 ± 0.8 fold higher in MVM-vesicles compared to placental homogenates (n = 30), and did not significantly differ between the groups (normal BMI, 14.9 ± 1.5; high BMI, 15.6 ± 1.1). Enrichment of BPM was verified