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Fatty acid transporter expression and regulation is impaired in placental macrovascular endothelial cells in obese women

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Abstract

Objective: Fetal fatty acid (FA) delivery is ultimately controlled by placental transport. Focus has been the maternal-placental interface, but regulation at the feto-placental interface is unknown.

Methods: Placental macrovascular endothelial cells (EC) (n=4/group) and trophoblasts (TB) (n=5/group) were isolated from lean (pregravid BMI <25 kg/m²) and obese (BMI>30) women. Fatty acid transporters FAT/CD36, FABPpm, FATP4, FABP 3, 4 and 5, PLIN2 and PPAR α , δ , γ expression, was measured in EC and TB. Transporter response to 24h palmitate (PA) was assessed.

Results: mRNA expression of FABP3, 4, 5 and PPAR γ was 2–3 fold reduced in EC of obese vs lean women (p<0.03), but not in TB. Protein level of FABPpm was 20% lower in obese (p<0.05). PA up-regulated CD36, FABP3, FABP4 and PLIN2 gene expression by 3–4 fold in lean but not obese EC (p<0.05), while PA increased FABP4 and PLIN2 in lean and obese TB, and FABP5 in lean (p<0.05) EC. PA exposure up-regulated PPARs 2-fold in lean and obese EC (p<0.05), but not in TB.

Conclusions: In obese women, FA transporter expression is lower in placental EC, but not TB, and less sensitive to saturated FA, compared to lean women. FA transport may be regulated at the feto-placental interface.

Keywords

fatty acid transporters; placenta; trophoblasts; endothelial cells; obesity

Introduction

Infants of obese women (pregravid body mass index (BMI) >30 kg/m²) may have increased fat mass at birth [1], and a greater risk of developing obesity, diabetes and cardiovascular disease in later life [2, 3], as compared to offspring of lean women (BMI<25 kg/m²). Variations in nutrient supply during development, such as excess lipid delivery, or, conversely, impairments in long-chain polyunsaturated fatty acid (e.g. omega-3) delivery, are

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Declaration of Interest

The authors report no conflicts of interest.

also associated with these poor outcomes [3, 4]. Fetal fatty acid (FA) delivery is ultimately controlled by placental transport, and previous studies have shown that long-chain FA uptake is decreased in placentas of obese women [5, 6], which may be related to changes in FA transporter and binding protein expression [5, 6, 7]. However, the regulation of placental FA transporters and fetal lipid delivery remains poorly understood.

Proteins involved in FA transport across the cell membrane, including fatty acid translocase (FAT/CD36), fatty acid transport proteins (FATP1–6), and plasma membrane fatty acid binding protein (FABPpm), have been localized to either the maternal-facing or basal membrane of the syncytiotrophoblast layer [8, 9], though their expression in other placental cell types is unknown. Fatty acid binding proteins (FABP 1, 3–5), located within the cytoplasm of the syncytiotrophoblast, are responsible for trafficking of FA to sites for esterification, β -oxidation, and subsequent transfer across the basal membrane [8, 9]. Peroxisome proliferator activated receptors (PPARs) are important regulators of these FA handling proteins [10, 11]. In the rodent placenta, stimulation of PPAR γ is associated with upregulation of FABPs, increased lipid storage, and decreased FA transport to the fetus [12, 13], suggesting that PPARs in the placenta play an important role in lipid metabolism, storage and transport.

To date, previous work has generally utilized whole placental tissue, with the assumption that regulation of transport was occurring at the maternal-placental interface, or syncytiotrophoblast layer. To reach the fetal circulation, FAs must also cross the endothelial cells lining the placental vasculature, which form the feto-placental interface. FA transporter expression and regulation at this site is unknown. We investigated the localization of FA transporters within the term placenta, and determined how maternal obesity affects the expression of FA handling proteins and their lipid-stimulated regulation at the maternal-placental (trophoblast) and feto-placental (endothelium) interfaces.

Methods

Study participants

Eighteen healthy, non-laboring women scheduled for Caesarean section, were recruited at term. Those with multiple gestation, fetal anomalies, intrauterine growth restriction, or diabetes (pre-existing or gestational) were excluded. Obesity was defined as pre-gravid BMI $> 30 \text{ kg/m}^2$ (N=9) and lean body type as BMI $< 25 \text{ kg/m}^2$ (N=9).

Tissue collection

Only placentas delivered via Caesarean were used to avoid the inflammatory effects of labor, variation introduced by length of labor, and to maintain sterility of the tissue for cell isolation and culture. Placental villous tissue was fixed in zinc formalin (Z-fix, Anatech, MI) and processed for immunohistochemistry. Placental fragments (total 50 g) from lean and obese women (n=5/group) were immediately processed for isolation of trophoblast cells as described below. Placental macrovascular veins from other lean and obese women (n=4/group), were dissected within 15 min for endothelial cell isolation.

Immunohistochemistry

Paraffin sections, 6 μm thick, were processed as described [14]. Antigen retrieval was undertaken at 100°C in 10mM Sodium Citrate pH6.0 with 0.05% Tween20 for 25 minutes. Primary antibodies were incubated with sections overnight at 4°C: FAT/CD36 (PA1-16813, 1:500; Thermo Fisher Scientific, Waltham, MA, USA), FABPpm (ab93928, 1:800; Abcam, Cambridge, MA, USA), FABP4 (sc-18661, 1:100; Santa Cruz, Dallas, TX, USA), FATP4 (Sc-25670, 1:200; Santa Cruz). Vector Elite ABC Kit was used according to directions apart from 3h incubations. Slides were incubated with DAB for visualization, and counterstained with Hematoxylin QS for 20 seconds (Vector Laboratories). Slides were imaged on a Nikon E600 with Spot RT camera. All antibodies were diluted in PBS with 1% BSA and 0.3% TX-100 and validated by negative control (absence of primary antibody).

Placental cell isolation and culture

Trophoblast cells were isolated by sequential trypsin and DNase (Sigma-Aldrich, St Louis, MO) digestion followed by gradient centrifugation [15]. Cells were placed into 12-well plates at a density of 1.5×10^6 cells/well and cultured overnight in Iscoves's modified DMEM culture medium supplemented with 10% (vol./vol.) FBS (Gibco, Grand island, NY, USA) and 1% (vol./vol.) penicillin/streptomycin at 37°C under 5% CO_2 . The medium was replaced with 1 ml DMEM with 1% (vol./vol.) FBS 2 h before addition of 400 μM palmitate (PA, Sigma-Aldrich; pre-conjugated 1:1 with fatty-acid free BSA) for 24 h. Medium with BSA alone was used as control.

For human placental macrovascular endothelial cell (HPVEC) isolation, macrovascular veins from the placental chorionic plate of obese women were cut out in lengths of 3–5 cm and washed with Hank's Balanced Salt Solution (HBSS) with 2.5% (vol./vol.) penicillin/streptomycin. Cells were isolated via collagenase/dispase digestion as previously described [16]. Isolated HPVECs were cultured at 37°C under 5% CO_2 in 12-well plates that had been pre-coated with 1% (vol./vol.) gelatin (Sigma Aldrich) in HBSS for 1 h at 37°C. After reaching 80% confluence, cells were transferred to 25 cm^2 flasks and expanded for determination of purity [17, 18]. The identity of the cells was confirmed by immunohistochemical staining with the endothelial markers anti-von Willebrand factor (A0082, 1:2000; Dako, Carpinteria, CA, USA), Vimentin (M0725, 1:100; Dako) and Desmin (M0760, 1:200; Dako). Lack of immunoreactivity with markers for smooth muscle cells (smooth muscle actin, M0851, 1:100; Dako), and fibroblasts (CD90, 1:100; Dianova) confirmed cell purity (Supplemental Figure 1).

For FA stimulation experiments, HPVECs were incubated in 12-well plates with 5% (vol./vol.) FBS + EBM + EGM-MV BulletKit culture medium (Lonza, Allendale, NJ, USA). After reaching 75% confluence, the medium was replaced with serum-free media 2h before adding 100 μM palmitate (PA; pre-conjugated 1:1 with fatty-acid free BSA) for 24 h. BSA alone was used as control. MTT was used to determine the cytotoxicity of 50, 100, 200 and 500 μM PA at 6h, 24h and 48h (Supplemental Figure 2) in HPVECs, as was previously done in TB [15]. HPVECs were exposed to 4-fold lower concentration of PA than TB (100 μM vs 400 μM , respectively) to mimic the 3.8-fold lower PA concentration in cord vs maternal blood [19].

Quantitative real-time PCR

Total RNA was extracted from trophoblast cells and endothelial cells using TRIzol (Invitrogen, Carlsbad, CA, USA). Gene expression was measured in duplicate by quantitative real-time PCR using a Roche Lightcycler (Roche Applied Science, Indianapolis, IN, USA) as previously described[15]. Specific primers are shown in Supplemental Table 1. Results were normalized for *β-actin*. Data were quantified as fold change of lean and obese groups calculated against the value of standard sample for trophoblasts or endothelial cells cDNA. Standard sample is a mixture of multiple cDNAs from the specific cell type and is run on each qPCR plate for each target gene. For PA treatment groups, data were calculated as fold change of PA vs BSA control.

Protein detection

Total protein was extracted from 1.5×10^6 cultured trophoblast cells or endothelial cells. The protein lysates were prepared as previously described [15]. Proteins were electrophoresed on a 4%–15% SDS gel (Bio-Rad, Hercules, CA, USA), loaded with 100 µg total protein per well, and then transferred to a nitrocellulose filter (Invitrogen). The membrane was blocked with 5% non-fat milk for 1h, incubated with Mouse monoclonal FABPpm (ab93928, 1:1000; Abcam) and *β-actin* (1:2000; Abcam) at 4°C overnight, then secondary antibodies for 1h. Amersham ECL Plus Western blotting Reagents (GE Healthcare, Aurora, OH, USA) was used for detection. All antibodies were diluted in 5% non-fat milk and size validated by protein standard (BIO-RAD). Densitometric data from autoradiograms were quantified by Image J (<https://imagej.nih.gov/ij/download.html>).

Statistical analysis

Values in figures are presented as means $\pm$ standard error of the mean (SEM). Differences in gene expressions between lean and obese groups were analysed by Student's *t* test. Effect of treatments between groups were calculated by Two-way ANOVA. Statistical significance was set at $p < 0.05$.

Results

Fatty acid transporter expression at the maternal–fetal interface

Immunohistochemical analysis showed FAT/CD36, FABPpm, FATP4, and FABP4 localized in both the syncytiotrophoblast layer and endothelial layer of the intravillous vasculature (Figure 1). FABPpm protein levels were lower in both trophoblasts and endothelial cells of obese compared to lean women (Figure 2). Gene expression of the FA binding proteins, *FABP3*, *4*, and *5* were reduced 2–6 fold in HPVECs, but not trophoblasts, of obese compared to lean women (Figure 2A&B). *FATP4* expression was also reduced in HPVECs of obese women, but did not reach statistical significance ($p = 0.056$). *FAT/CD36* and *PLIN2* showed no difference between groups in either cell type (Figure 2).

Regulation of placental fatty acid transporter expression

Palmitate (PA), the most abundant FA in maternal and cord blood at delivery [19], was used to evaluate FA-stimulated transporter regulation in trophoblasts and HPVEC isolated from

lean and obese women (Figure 3). mRNA expression of *FABP4*, and *PLIN2* was increased by 3–5 fold ($p<0.05$) vs control (BSA) after 24h treatment with PA in trophoblasts isolated from both lean and obese women. *FABP5* expression was only upregulated in trophoblasts of lean women after PA treatment ($p<0.01$).

Under similar stimulation conditions, the expression of *FAT/CD36*, *FABP3*, *FABP4* and *PLIN2* was increased 3-fold in HPVECs isolated from lean women ($p<0.05$), while endothelial *FATP4* was significantly upregulated in both lean and obese women by 1.5-fold ($p<0.05$).

Regulation of placental PPAR signalling pathway

The mRNA expression of *PPAR α* , *PPAR δ* , and *PPAR γ* was measured in trophoblasts and HPVECs to determine the role of these lipid-activated transcription factors in the changes in FA transporter expression. There was no difference in trophoblast PPAR expression between lean and obese women at baseline (Figure 2C). In contrast, *PPAR γ* was 2-fold lower in HPVECs from obese women (Figure 2D; $p<0.05$). Trophoblast PPAR expression was not altered by PA exposure (Figure 3C). In contrast, PPAR expression in HPVECs was 1.5–2 fold upregulated in response to 24h PA in both lean and obese women (Figure 3D; $p<0.05$).

Discussion

The regulation of FA delivery to the fetus remains poorly understood. The maternal-placental interface (trophoblast) has been the focus of prior investigations on this topic [5, 20]. This is the first study, to our knowledge, that has examined FA transporter expression and regulation at the feto-placental interface (HPVEC). Our key findings were that 1) placental endothelial cells express FA transporters and binding proteins, 2) these are differentially expressed in lean and obese women, and 3) are regulated by palmitate. Furthermore, compared to trophoblasts, transporters within HPVECs are more sensitive to regulation by lipids.

Fatty acid transporters CD36, FABPpm, and FATP4 were immunolocalized to both the syncytiotrophoblast layer, and placental endothelial cells (capillary and macrovessels). The sheer number of transport proteins at the maternal-placenta interface suggests that this site is critical for maintaining high rates of FA uptake into the placenta. Once inside the trophoblast, FA may be metabolized (a potential point of regulation[21]), and then must traverse the vascular endothelium to reach the fetal circulation. The presence of transport proteins at the endothelial layer provides another opportunity for regulation of fetal FA delivery. Consistent with findings in mice [22], FABP4 was highly expressed within the endothelium of the placenta as compared to trophoblasts, signifying an important role for this binding protein at the feto-placental interface. Endothelial FABP4 and FABP5 facilitate FA uptake, storage and cell proliferation [23, 24], and are thought to be an important link between lipid metabolism and angiogenesis [25]. Thus, regulation of FABP expression in HPVECs may be important for both fetal lipid delivery and placental growth.

FABPpm staining was abundant in trophoblast and endothelium, and protein expression was decreased to a similar degree in both cell types isolated from obese, compared to

lean, women. FABPpm FA uptake activity is regulated by membrane trafficking[26], and although this decrease in expression is consistent with reports that FA uptake is decreased in placentas of obese women[5], we did not measure membrane FABPpm content to confirm that trafficking was also decreased. These results suggest that FA transporters are regulated by maternal obesity on both the maternal- and feto-placental interfaces.

At the transcriptional level, all FA binding proteins measured (FABP3/4/5) were downregulated in HPVECs isolated from obese women, while we were unable to detect differences in gene expression between trophoblasts isolated from lean and obese women. Dube *et al.* reported that maternal obesity was associated with higher expression of placental FAT/CD36 but lower FATP4, FABP1 and FABP3 protein levels[5]. Brass *et al.* found lower FAT/CD36 and FABP5, but no difference in FABP4 or FATP4 mRNA expression in placentas of obese mothers with high adiposity male offspring [6]. Conversely, Scifres *et al.* reported that placental FABP4 and FABP5 mRNA and FABP4 protein expression are higher in obese-diabetic women, but not obese non-diabetic, compared to lean women [7]. Altogether, studies using whole placental tissue find that placental FA transporter and binding protein expression is affected by the obese environment, however differences may depend on maternal insulin sensitivity and fetal sex. Furthermore, it is unclear how or if these changes in expression alter transport, and what cell population is affected. Our data suggests that these transporters are more likely to be regulated on the feto-placental interface at the transcriptional level.

In order to determine the environmental factors that may regulate these transporters, we exposed trophoblasts and HPVECs to palmitate, at doses reflecting the physiological concentration differences in maternal and fetal circulations [19]. PLIN2, a component of lipid droplets, was upregulated by 24hr palmitate exposure in both cell types. Palmitate (C16:0) did not alter transporter expression in trophoblasts. In HPVECs, however, FAT/CD36 mRNA and FATP4 were upregulated in lean women. Fatty acid binding proteins in both cell types were more responsive than transporters to palmitate levels, reflecting their role in cellular lipid metabolism [25]. Others have reported a negative response of trophoblast FABP4 mRNA expression to three days of linoleic acid (C18:2) exposure [27]. This downregulation may be due to the difference in saturation, or the longer period of exposure (72 vs 24 hrs), which is consistent with our findings that FABPs were decreased in cells isolated from obese women. Cells isolated from obese women appeared to be less responsive to lipid exposure as compared to lean women, which may reflect a “desensitization” following an *in vivo* hyperlipidemic environment, or inhibition of FA transporters by inflammatory cytokines [20]. Our group has recently reported that HPVECs isolated from obese women have a robust inflammatory response to lipopolysaccharide stimulation [16], thus an inflammatory milieu as found in obese women [28], may downregulate FA transporters and subdue the response to lipids. We speculate that in endothelial cells, the lack of response of FAT/CD36 to palmitate exposure in obese compared to lean women, may impair FA uptake, explaining the lower sensitivity of intracellular FABPs and PLIN2 to lipid exposure, in the obese group.

Palmitate, and other FA, may regulate their transporter and binding protein expression via interaction with PPARs, the lipid-sensitive nuclear transcription factors that are involved in

FA metabolism and transport within the placenta [11]. We found that trophoblast PPAR α , β , and γ mRNA expression was not different between lean and obese women, and was not sensitive to palmitate exposure. Consistent with these trophoblast data, Dube *et al.* reported that whole tissue placental PPAR α , PPAR δ and PPAR γ mRNA expression did not differ in obese compared to lean women[5]. On the other hand, we found that HPVEC PPAR γ was lower in obese women, consistent with the lower expression of the FABPs, which are responsive to PPAR γ stimulation [29]. Interestingly, all PPAR isoforms in HPVECs were stimulated by palmitate exposure, regardless of maternal phenotype. Though consistent with the notion that HPVECs are more sensitive to lipid exposure, these data do not support a causative role for PPARs in the differences detected in lean and obese HPVECs in response to palmitate.

HPVECs used in this study were isolated from the chorionic vessels of the placenta. Placental micro- and macrovascular endothelium are morphologically similar [30], and the presence of FA transport machinery suggests these macrovascular HPVECs are involved in fetal lipid delivery. However it is possible that macrovascular cells do not exactly reflect the function of microvascular endothelial cells from the capillaries. Functional uptake data, particularly exploring directionality of uptake, will be necessary to determine the significance of these findings for fetal lipid delivery.

In summary, our data show that FA transporters and binding proteins are localized to both the maternal- and feto-placental interfaces, suggesting two points of regulation of FA delivery to the fetus. However, only at the feto-placental interface, was mRNA expression of transporters and binding proteins affected by maternal obesity. Furthermore, HPVEC FA transporter and binding proteins were transcriptionally regulated in response to lipid exposure. We conclude that FA handling proteins are transcriptionally regulated at the feto-placental interface in lean and obese women. We speculate that regulation of fetal FA delivery occurs at this interface, and that these proteins important for long-chain FA transport are responsive to fetal lipid levels. Further studies will be necessary to determine whether the lower expression of FA handling proteins described here is associated with impairments in long-chain FA delivery to offspring of obese women.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Sewell MF, Huston-Presley L, Super DM, Catalano P. Increased neonatal fat mass, not lean body mass, is associated with maternal obesity. *American journal of obstetrics and gynecology*. 2006;195:1100–3. [PubMed: 16875645]
2. Reynolds RM, Allan KM, Raja EA, Bhattacharya S, McNeill G, Hannaford PC, Sarwar N, Lee AJ, Bhattacharya S, Norman JE. Maternal obesity during pregnancy and premature mortality from cardiovascular event in adult offspring: follow-up of 1 323 275 person years. *Bmj*. 2013;347:f4539. [PubMed: 23943697]
3. Boney CM, Verma A, Tucker R, Vohr BR. Metabolic syndrome in childhood: association with birth weight, maternal obesity, and gestational diabetes mellitus. *Pediatrics*. 2005;115:e290–6. [PubMed: 15741354]
4. Weisinger HS, Armitage JA, Sinclair AJ, Vingrys AJ, Burns PL, Weisinger RS. Perinatal omega-3 fatty acid deficiency affects blood pressure later in life. *Nature medicine*. 2001;7:258–9.
5. Dube E, Gravel A, Martin C, Desparois G, Moussa I, Ethier-Chiasson M, Forest JC, Giguere Y, Masse A, Lafond J. Modulation of fatty acid transport and metabolism by maternal obesity in the human full-term placenta. *Biology of reproduction*. 2012;87:14, 1–1. [PubMed: 22553224]
6. Brass E, Hanson E, O'Tierney-Ginn PF. Placental oleic acid uptake is lower in male offspring of obese women. *Placenta*. 2013;34:503–9. [PubMed: 23602336]
7. Scifres CM, Chen B, Nelson DM, Sadovsky Y. Fatty acid binding protein 4 regulates intracellular lipid accumulation in human trophoblasts. *The Journal of clinical endocrinology and metabolism*. 2011;96:E1083–91. [PubMed: 21525163]
8. Kazantzis M, Stahl A. Fatty acid transport proteins, implications in physiology and disease. *Biochimica et biophysica acta*. 2012;1821:852–7. [PubMed: 21979150]
9. Campbell FM, Bush PG, Veerkamp JH, Dutta-Roy AK. Detection and cellular localization of plasma membrane-associated and cytoplasmic fatty acid-binding proteins in human placenta. *Placenta*. 1998;19:409–15. [PubMed: 9699962]
10. Wierzbicki M, Chabowski A, Zendzian-Piotrowska M, Gorski J. Differential effects of in vivo PPAR alpha and gamma activation on fatty acid transport proteins expression and lipid content in rat liver. *Journal of physiology and pharmacology : an official journal of the Polish Physiological Society*. 2009;60:99–106.
11. Schaiff WT, Bildirici I, Cheong M, Chern PL, Nelson DM, Sadovsky Y. Peroxisome proliferator-activated receptor-gamma and retinoid X receptor signaling regulate fatty acid uptake by primary human placental trophoblasts. *The Journal of clinical endocrinology and metabolism*. 2005;90:4267–75. [PubMed: 15827101]
12. Schaiff WT, Knapp FF Jr., Barak Y, Biron-Shental T, Nelson DM, Sadovsky Y. Ligand-activated peroxisome proliferator activated receptor gamma alters placental morphology and placental fatty acid uptake in mice. *Endocrinology*. 2007;148:3625–34. [PubMed: 17463056]
13. Xu Y, Agrawal S, Cook TJ, Knipp GT. Maternal di-(2-ethylhexyl)-phthalate exposure influences essential fatty acid homeostasis in rat placenta. *Placenta*. 2008;29:962–9. [PubMed: 18829104]
14. O'Tierney-Ginn P, Roberts V, Gillingham M, Walker J, Glazebrook PA, Thornburg KL, Grove K, Frias AE. Influence of high fat diet and resveratrol supplementation on placental fatty acid uptake in the Japanese macaque. *Placenta*. 2015;36:903–10. [PubMed: 26145226]
15. Yang X, Haghiac M, Glazebrook P, Minium J, Catalano PM, Hauguel-de Mouzon S. Saturated fatty acids enhance TLR4 immune pathways in human trophoblasts. *Human reproduction*. 2015;30:2152–9. [PubMed: 26202921]
16. Yang X, Li M, Haghiac M, Catalano PM, O'Tierney-Ginn P, Hauguel-de Mouzon S. Causal relationship between obesity-related traits and TLR4-driven responses at the maternal-fetal interface. *Diabetologia*. 2016;59:2459–66. [PubMed: 27535280]
17. Lang I, Schweizer A, Hiden U, Ghaffari-Tabrizi N, Hagedorfer G, Bilban M, Pabst MA, Korgun ET, Dohr G, Desoye G. Human fetal placental endothelial cells have a mature arterial and a juvenile venous phenotype with adipogenic and osteogenic differentiation potential. *Differentiation; research in biological diversity*. 2008;76:1031–43. [PubMed: 18673379]

18. Lang I, Pabst MA, Hiden U, Blaschitz A, Dohr G, Hahn T, Desoye G. Heterogeneity of microvascular endothelial cells isolated from human term placenta and macrovascular umbilical vein endothelial cells. *European journal of cell biology*. 2003;82:163–73. [PubMed: 12751902]
19. O'Tierney-Ginn PF, Gillingham M, Fowler J, Brass E, Marshall NE, Thornburg KL. Maternal Weight Gain Regulates Omega-3 Fatty Acids in Male, Not Female, Neonates: A Cross-Sectional Study. *Reproductive sciences*. 2016.
20. Lager S, Jansson N, Olsson AL, Wennergren M, Jansson T, Powell TL. Effect of IL-6 and TNF-alpha on fatty acid uptake in cultured human primary trophoblast cells. *Placenta*. 2011;32:121–7. [PubMed: 21144584]
21. Perazzolo S, Hirschmugl B, Wadsack C, Desoye G, Lewis RM, Sengers BG. The influence of placental metabolism on fatty acid transfer to the fetus. *Journal of lipid research*. 2017;58:443–54. [PubMed: 27913585]
22. Makkar A, Mishima T, Chang G, Scifres C, Sadovsky Y. Fatty acid binding protein-4 is expressed in the mouse placental labyrinth, yet is dispensable for placental triglyceride accumulation and fetal growth. *Placenta*. 2014;35:802–7. [PubMed: 25096952]
23. Iso T, Maeda K, Hanaoka H, Suga T, Goto K, Syamsunarno MR, Hishiki T, Nagahata Y, Matsui H, Arai M, Yamaguchi A, Abumrad NA, Sano M, Suematsu M, Endo K, Hotamisligil GS, Kurabayashi M. Capillary endothelial fatty acid binding proteins 4 and 5 play a critical role in fatty acid uptake in heart and skeletal muscle. *Arteriosclerosis, thrombosis, and vascular biology*. 2013;33:2549–57.
24. Basak S, Das MK, Duttaroy AK. Fatty acid-induced angiogenesis in first trimester placental trophoblast cells: possible roles of cellular fatty acid-binding proteins. *Life sciences*. 2013;93:755–62. [PubMed: 24095946]
25. Yu CW, Liang X, Lipsky S, Karaaslan C, Kozakewich H, Hotamisligil GS, Bischoff J, Cataltepe S. Dual role of fatty acid-binding protein 5 on endothelial cell fate: a potential link between lipid metabolism and angiogenic responses. *Angiogenesis*. 2016;19:95–106. [PubMed: 26625874]
26. Chabowski A, Coort SL, Calles-Escandon J, Tandon NN, Glatz JF, Luiken JJ, Bonen A. The subcellular compartmentation of fatty acid transporters is regulated differently by insulin and by AICAR. *FEBS letters*. 2005;579:2428–32. [PubMed: 15848183]
27. Daoud G, Simoneau L, Masse A, Rassart E, Lafond J. Expression of cFABP and PPAR in trophoblast cells: effect of PPAR ligands on linoleic acid uptake and differentiation. *Biochimica et biophysica acta*. 2005;1687:181–94. [PubMed: 15708366]
28. Basu S, Haghiac M, Surace P, Challier JC, Guerre-Millo M, Singh K, Waters T, Minium J, Presley L, Catalano PM, Hauguel-de Mouzon S. Pregravid obesity associates with increased maternal endotoxemia and metabolic inflammation. *Obesity*. 2011;19:476–82. [PubMed: 20930711]
29. Sun X, Tang Y, Tan X, Li Q, Zhong W, Sun X, Jia W, McClain CJ, Zhou Z. Activation of peroxisome proliferator-activated receptor-gamma by rosiglitazone improves lipid homeostasis at the adipose tissue-liver axis in ethanol-fed mice. *American journal of physiology Gastrointestinal and liver physiology*. 2012;302:G548–57. [PubMed: 22173916]
30. Leach L, Firth JA. Structure and permeability of human placental microvasculature. *Microscopy research and technique*. 1997;38:137–44. [PubMed: 9260844]

Figure 1: Localization of fatty acid transporters in human term placenta

Immunohistochemical analysis showed fatty acid translocase (FAT/CD36), plasma membrane associated fatty acid binding protein (FABPpm), fatty acid transport protein 4 (FATP4) and fatty acid binding protein (FABP4) localized in the syncytiotrophoblast layer (large arrows) and endothelial layer of the placental vasculature (small arrows). Scale bars = 10 μm.

Figure 2: Expression of fatty acid transporters and PPARs in placentas of lean and obese women *FAT/CD36*, *FABPpm*, *FATP4* and *FABP3,4,5*, *PLIN2*, *PPARα*, *PPARδ*, and *PPARγ* mRNA expression in trophoblasts (A, C), and HPVECs (B, D) from lean and obese women (n=5/group or n=4/group, respectively). For each primary endothelial cell line, 4 samples from passage 5 to passage 9 were tested. Results were normalized to β -actin. FABPpm (47KD) protein expression in trophoblasts (A inset) and HPVECs (B inset) was measured by western blot. Results were normalized to β -actin. n=4 in each group. Data are shown as mean $\pm$ standard error of the mean (SEM). *p<0.05 vs lean group by Student's t-test. AU: arbitrary units.

Figure 3: Regulation of fatty acid transporters and PPARs by palmitate (PA) in human trophoblasts and placental venous endothelial cells from lean and obese women

Primary human trophoblast cells (A, C) and HPVECs (B, D) from lean and obese placentas were treated with 400μM (A, C) or 100μM (B, D) PA (1:1 with BSA), or BSA alone, for 24h. *FAT/CD36*, *FABPpm*, *FATP4*, *FABP3*, *FABP4*, *FABP5*, *PLIN2*, *PPARα*, *PPARδ*, and *PPARγ* mRNA expression was detected by RT-PCR. Results were normalized to β -actin and expressed as fold change vs. BSA control. Data are shown as mean $\pm$ standard errors of the mean (SEM) for 5 independent experiments and analyzed by Two-way ANOVA. * $p < 0.05$ vs BSA; ## $p < 0.05$ for effect of PA.