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Fatty Acid Binding Protein-4 is expressed in the mouse placental labyrinth, yet is dispensable for placental triglyceride accumulation and fetal growth

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Abstract

Introduction—Fatty Acid Binding Protein-4 (FABP4) is a member of a family of FABP proteins that regulate intracellular lipid trafficking in diverse tissues. We recently showed that FABP4 regulates triglyceride accumulation in primary human trophoblasts. To assess the function of placental FABP4 *in vivo*, we tested the hypothesis that FABP4 is expressed in the murine placenta, and regulates placenta triglyceride accumulation.

Methods—C57Bl/6 wild type or *Fabp4*-null mice were time-bred, and fetuses and placentas harvested at different time points during pregnancy. Placental FABP4 expression was assessed at different gestational ages, using quantitative PCR, immunohistochemistry, immunofluorescence and western immunoblotting. FABPs expression was examined by RT-qPCR. Placental lipids were extracted using the Folch method and triglyceride levels determined using a colorimetric quantification kit.

Results—Using immunohistochemistry, we found that FABP4 was expressed in the placental labyrinthine layer, predominantly in endothelial cells in association with CD31 positive fetal capillaries. The level of placental FABP4 mRNA and protein increased from E12.5 to E16.5 and slightly decreased at E18.5. Breeding of *Fabp4* heterozygous mice resulted in embryonic genotypes that followed a Mendelian distribution and exhibited normal weight and morphology,

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triglyceride content, and expression of other FABP family members. Exposure to hypoxia ($O_2=12\%$) between E12.5–E18.5 did not uncover a difference between wild type and *Fabp4*-null mice.

Conclusions—FABP4 is expressed in the mouse placental labyrinth, with highest expression at E16.5. FABP4 is dispensable for feto-placental growth and placental lipid accumulation.

Keywords

FABP4; trophoblast; fetus; fatty acids; triglycerides

Introduction

The trans-placental maternal-to-fetal transport of nutrients is essential for normal growth and development of the eutherian embryo. Fetal growth restriction that results from fetal hypotrition has been associated with intrauterine fetal death, neonatal and childhood morbidity, and the adult metabolic syndrome with its consequences [1]. Among nutrients that support intrauterine growth, fatty acids are germane, particularly during the second half of pregnancy, when fetal growth accelerates and fat accretion increases exponentially [2–5]. Fatty acids are also essential for organ development—for instance, in the brain, retina, heart and lungs [6–11]—where they serve as a source of calories and membrane building blocks and also as precursors for the production of eicosanoids, phospholipids, and their derivatives [12, 13].

The major fraction of fatty acids that circulate in the maternal blood is covalently bound by triglycerides and lipoproteins, with a smaller fraction circulating as free fatty acids bound by albumin, VLDL, or chylomicron. Fatty acids are released for placental uptake by the action of triglyceride hydrolases and transported across the trophoblastic microvillous membrane, with kinetics that depend on biochemical characteristics such as chain length, lipid solubility, and affinity to carrier proteins [14–19]. Within cells, cytoplasmic fatty acids are bound by fatty acid binding proteins (FABPs). These small (132 aa) proteins mediate intracellular fatty acid transport to lipid droplets for storage, to mitochondria or peroxisomes for oxidation and energy production, to the endoplasmic reticulum for membrane synthesis and cell signaling, and to the nucleus for regulation of gene expression [20].

We have previously determined that FABP1, FABP3, FABP4, FABP5, and FABPpm are the dominant FABP forms expressed in human placental trophoblasts [21, 22]. Importantly, we found that hypoxia selectively upregulated the expression of FABP1, FABP3 and FABP4 and that fatty acids enhance the expression of FABP4. These changes were associated with increased lipid droplet and triglyceride accumulation in primary human trophoblasts (PHT) and were attenuated by inhibition of FABP4 [21, 22]. Coupled with the known function of FABP4 (also known as adipocyte FABP4, AFABP4, adipocyte protein 2, and aP2) as a fatty acid chaperone [23–25], our findings led us to propose the hypothesis that FABP4 is a key regulator of trophoblastic lipid transport and accumulation. To assess the function of FABP4 *in vivo*, we sought to determine the expression of FABP4 in the mouse placenta, and determine the impact of *Fabp4* ablation in mice on feto-placental development and triglyceride accumulation.

Methods

Animals and breeding

Our experiments were performed using either wild type (wt) C57Bl/6 mice, obtained from Jackson Laboratory, or C57Bl/6 mice that harbor a targeted mutation in the *Fabp4* gene, which were provided by Dr. G. Hotamisligil (Harvard University) [26]. Our experimental protocols were approved by the Institutional Animal Care and Use Committee of the University of Pittsburgh. We performed timed breeding by pairing heterozygous males and females overnight, with the morning after mating labeled as embryonic day 0.5 (E0.5) after verifying the presence of a vaginal plug. Pregnancy was confirmed by a 10% weight gain on E10.5–11.5. Pregnant dams were kept under standard conditions of 12:12 h light-dark cycle and provided standard rodent chow and water *ad libitum* until E18.5. Dams were euthanized by CO₂ asphyxiation at the time points indicated (E12.5–E18.5), fetuses and placentas delivered trans-abdominally and assessed for weight and morphology. For analysis, we included dams carrying 5–10 embryos. Mouse genotyping was performed as previously described [26].

Exposure of pregnant dams to hypoxia, where relevant, was initiated on E12.5, when the mice were exposed to either normobaric hypoxia (O₂ = 12%) between E12.5–E18.5 (hypoxia group) or standard atmospheric conditions. For exposure to normobaric hypoxia we used a polymer hypoxic airlock system [27] that is specifically designed for hypoxia experiments in live rodents (Coy Laboratory Products, Grass Lake, MI).

Placental histology, immunohistochemistry, and immunofluorescence

For histomorphology, placentas were fixed in 4% fresh paraformaldehyde in PBS overnight, and embedded in paraffin, using standard procedures. Paraffin sections (5 µm thickness) were cut using a microtome (Leica RM2255, Wetzlar, Germany), mounted on glass slides, and stained with SelectTech Hematoxylin and Eosin (Surgipath, Richmond, IL). For immunohistochemistry, paraffin sections (5 µm thickness) were processed by a microwave in 10 mM citrate buffer (pH 6) for heat induced epitope retrieval, and immunohistochemistry was performed using goat anti-FABP4 primary antibody (4 µg/mL, #sc-18661, Santa Cruz Biotechnology, Dallas, TX). We ensured signal specificity by pre-incubating the primary antibody with a blocking peptide (#sc-18661P, Santa Cruz Biotechnology). We detected the primary antibody using a biotinylated anti-goat secondary antibody (Vector Laboratories, Burlingame, CA), followed by incubation with avidin and horseradish peroxidase-conjugated biotin (Vectastain Standard ABC Elite kit; Vector). The color reaction was performed with diaminobenzidine tetrahydrochloride. The sections were analyzed using a Nikon 90i microscope (Nikon, Tokyo, Japan) equipped with DS-Ri1 CCD camera (Nikon). For immunofluorescence we used an anti-PECAM-1 polyclonal rabbit IgG2 antibody (abcam, 1:50, Cambridge MA, #ab-28364) or anti-cytokeratin-8 monoclonal rat IgG (TROMA1, 1:100, Developmental Studies Hybridoma Bank, Iowa City, IA), in combination with goat anti-FABP4 antibody (4 µg/mL, Santa Cruz Biotechnology). Sections were incubated with anti-rabbit Alexa Fluor 488 secondary antibodies (for PECAM-1, Molecular Probes, Eugene, OR), anti-goat Alexa Fluor 555 or anti-goat Alexa Fluor 488 secondary antibodies (for FABP4, Molecular Probes, Eugene, OR), or anti-rat Alexa Fluor

594 secondary antibody (for cytokeratin-8, Molecular Probes, Eugene, OR). Signals were visualized with an inverted microscope (Axiovert 40, Carl Zeiss, Germany) equipped with a cooled digital camera system (DS-Qi1Mc, Nikon, Japan), and with Nikon90i equipped with Qimaging retiga 2000R cooled digital camera system (Surrey, BC, Canada).

Reverse transcription and quantitative polymerase chain reaction (RT-qPCR)

Total RNA was extracted from placental tissue, using TRI Reagent (Molecular Research Center, Cincinnati, OH) according to the manufacturer's instructions. RNA quality and quantity were determined and cDNA prepared by reverse transcription as we previously described [27]. We used a total of 8–12 mouse placental cDNA samples for each experiment. Primer sequences have been previously described by our lab [21, 22]. qPCR was carried out in GeneAmp 7900 (Applied Biosystems, Foster City, CA). The specificity of amplification was confirmed using a dissociation curve of the PCR product. Detection of L32 for mouse was used as a normalization control and relative expression calculated as described [27].

Western blot analysis

We performed western immunoblotting as we previously described [27], using 12% sodium dodecyl sulfate–polyacrylamide gel at electrophoresis at 100 V for 1 h, subsequently transferred to polyvinylidene difluoride membranes (Bio-Rad, Hercules, CA) overnight. After blocking with 5% non-fat dried milk in TBST, the membranes were incubated overnight with goat anti-FABP4 Antibody (0.4 µg/mL, Santa Cruz Biotechnology) or mouse anti-actin antibody (MAB1501, Millipore, Bedford, MA) at 4°C. After washing with TBST, the membranes were incubated with donkey anti-goat IgG–horseradish peroxidase conjugated antibody (0.4 µg/mL #sc-2020, Santa Cruz Biotechnology) for 2 h at room temperature. Detection was performed with Western Lightning enhanced chemiluminescence kit (PerkinElmer, Waltham, MA) and signals quantified using UVP Biospectrum 500 Imaging System (UVP, Upland, CA).

Triglyceride Assay

Lipids were extracted from homogenized placentas or fetal liver (10–20 µg each, normalized by weight) by the Folch method [28]. Chloroform/methanol mix based on the Folch method was also used to extract lipids from the maternal serum. Triglyceride concentration was determined using a triglyceride quantification kit (BioVision, Milpitas, CA) and detected using a VersaMax spectrometry microplate reader (Molecular Devices, Sunnyvale, CA) as previously described [22].

Statistics

Statistical analysis was performed using Student's t-test, or ANOVA for multiple comparisons. A Kruskal-Wallis test was used for multiple comparisons of FABP levels by RT-qPCR. Significance was determined at $p < 0.05$.

Results

To gain insight into the role of placental FABP4 *in vivo*, we initially sought to examine the expression of FABP4 in placentas of wild type C57Bl/6 mice. Using immunohistochemistry (Fig. 1), we confirmed that FABP4 is highly expressed in the maternal decidua, as previously shown [29]. Importantly, within the placenta proper, we found that FABP4 is primarily expressed in the labyrinthine layer and not in the junctional zone (Fig. 1, 2I–J). Using immunofluorescent co-staining for the endothelial cell marker PECAM-1 (Fig 2, A–D) or for the trophoblast cell marker cytokeratin-8 (Fig. 2, E–J) we confirmed that FABP4 is expressed predominantly in the labyrinthine endothelial cells.

Our immunohistochemistry data suggested a change in FABP4 expression as pregnancy progressed. We therefore used western immunoblotting and RT-qPCR to quantitatively assess gestational age-dependent changes in FABP4 expression. We found that the expression of FABP4 was maximal at E14.5–16.5 and declined near term (Fig. 3A–B), suggesting a time-dependent regulation of placental FABP4 expression during murine pregnancy.

We next sought to assess the impact of FABP4 deficiency on feto-placental growth, and confirmed the effect of *Fabp4* gene ablation on FABP4 expression at the RNA (Fig. 5b) and protein level (not shown), as previously reported [26]. Crossbreeding of heterozygous *Fabp4* males and females revealed a normal Mendelian distribution of embryonic genotypes. We also found that *Fabp4*-null fetuses were indistinguishable from their wt littermates with respect to weight and morphology (not shown). Because we previously showed that human trophoblastic FABP4 is upregulated in hypoxia *in vitro* [21], we assessed the impact of hypoxia (O₂=12%, E12.5–18.5) on fetal growth, using protocols previously described in our lab [30, 31]. Except for the predicted growth restriction by 10–20% of all mouse fetuses exposed to hypoxia [30, 31], there were no differences among littermates representing the different genotypes.

Because FABP4 plays a role in fatty acid uptake by PHT cells [22], we examined the accumulation of triglycerides in the placenta of wt and *Fabp4*-null mice in standard or hypoxic conditions. As shown in Fig. 4A, maternal serum triglyceride levels were similar between the genotypes. Notably, the concentration of placental triglycerides was similar among littermates (Fig. 4B). Placental Oil Red O staining was also comparable between the genotypes (not shown). Similarly, we found no difference in fetal hepatic triglyceride levels (Fig. 4C), suggesting that FABP4 deficiency did not alter the supply of triglycerides to the embryo.

To assess whether the lack of a phenotypic difference in *Fabp4*-null fetuses and placentas reflected a compensatory upregulation of another *Fabp* member, we determined the expression of *Fabp* transcripts in placentas from wt or *Fabp4*-null fetuses. As we previously showed, using cultured PHT cells [21], the expression of *Fabp1* and *Fabp4* was upregulated by hypoxia (Fig. 5A). Unlike our findings in human trophoblasts, the expression of murine *Fabp3* was not increased in hypoxia *in vivo*. As expected, the expression of *Fabp4* was reduced in the *Fabp4*-null placenta (Fig. 5A). Importantly, ablation of *Fabp4* did not lead to

increased expression of other *Fabp* family members in either standard conditions or in hypoxia (Fig. 5B), suggesting that the basal expression of FABP family members was sufficient to compensate for FABP4 deficiency in the mouse placenta.

Discussion

We previously found that FABP4 is expressed in human placental trophoblasts. We also found that exposure of PHT cells to either fatty acids or hypoxia *in vitro* enhanced the expression of FABP4 and that pharmacological or siRNA-mediated inhibition of FABP4 markedly reduced the accumulation of triglycerides in trophoblasts [21, 22]. Notably, FABP4 was previously shown to be highly expressed in the mouse decidua as early as E5, with a major increase in expression on E7–8 [29], yet the expression of FABP4 in the murine placenta proper has not been hitherto reported. In the present work, we first examined the spatial and temporal expression of FABP4 in the mouse placenta. We found that FABP4 is selectively expressed within the placental labyrinthine, predominantly in the fetal endothelial layer, supporting a role for FABP4 in placental trafficking of lipids at the maternal-fetal interface. Interestingly, the expression of FABP4 was regulated during pregnancy, with the highest level of FABP4 expression in E14.5–E16.5, consistent with a period of accelerated fetal growth.

In contrast to our observations using PHT cells *in vitro*, we found that *Fabp4* ablation in the mouse had no effect on placental or fetal triglyceride accumulation *in vivo*. Thus, our findings extend previous observations, which reported normal reproductive outcome and Mendelian distribution of pups born after *Fabp4* heterozygous mating [26, 32]. Furthermore, although *Fabp4* was significantly upregulated in response to hypoxia *in vivo*, *Fabp4*-null embryos, exposed to hypoxia from E12.5–E18.5, were not different from their wt littermates with respect to their viability, size, and triglyceride levels. At this point we cannot rule out the possibility that the predominant FABP4 expression in murine placental endothelial cells (unlike the expression of FABP4 in human trophoblasts) contribute to our results. Similarly, infiltration of the placenta by FABP4-expressing maternal cells (e.g., macrophages) may have further complicated the interpretation of the *Fabp4* mouse phenotype.

Other functions of FABP4 can be substituted by FABP5 (also known as *Mal2*, epidermal FABP). For example, FABP4 deficiency results in upregulation of expression of FABP5 in adipose tissue, probably compensating for deficiency of FABP4 for many, but not all, FABP4 functions [26, 32] (and reviewed in [25]). We found no change in FABP5 or in any other of the FABPs that are co-expressed with FABP4 in the mouse placenta. Consistent with our findings, there was no increase in FABP5 in adipocytes after RNAi-mediated knock-down of FABP4 [33]. Taken together, our data suggest that, while FABP4 is expressed in the placenta, and is likely to play a role in placental lipid transport and accumulation, its expression in the placenta is not indispensable, and basal expression of other FABP family members is sufficient to offset FABP4 deficiency in the mouse placenta *in vivo*. This metabolic redundancy might provide an evolutionary advantage to sustain critical nutrient delivery to the fetus.

FABP4 deficiency promotes insulin sensitivity and reduces the release of triglycerides, probably reflecting a reduction in obesity-induced TNF α production in adipocytes [26] (and reviewed in [25]). Thus, to uncover the consequences of *Fabp4* ablation in mouse placenta, additional physiological challenges may be required, such as induced obesity during pregnancy, or cAMP-mediated lipolysis [24]. Alternatively, a combined ablation of more than one FABP family member [25] may be necessary in order to fully appreciate the role of FABPs in placental fat mobilization and storage in mice *in vivo*.

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References

1. Barker DJ, Lampl M, Roseboom T, Winder N. Resource allocation in utero and health in later life. *Placenta*. 2012; 33 (Suppl 2):e30–4. [PubMed: 22809673]
2. Al MD, van Houwelingen AC, Hornstra G. Long-chain polyunsaturated fatty acids, pregnancy, and pregnancy outcome. *Am J Clin Nutr*. 2000; 71:285S–91S. [PubMed: 10617984]
3. Haggarty P. Fatty acid supply to the human fetus. *Annu Rev Nutr*. 2010; 30:237–55. [PubMed: 20438366]
4. Haggarty P. Meeting the fetal requirement for polyunsaturated fatty acids in pregnancy. *Curr Opin Clin Nutr Metab Care*. 2014; 17:151–5. [PubMed: 24468708]
5. Herrera E. Lipid metabolism in pregnancy and its consequences in the fetus and newborn. *Endocrine*. 2002; 19:43–55. [PubMed: 12583601]
6. Dutta-Roy AK. Transport mechanisms for long-chain polyunsaturated fatty acids in the human placenta. *Am J Clin Nutr*. 2000; 71:315S–22S. [PubMed: 10617989]
7. Neuringer M, Connor WE, Lin DS, Barstad L, Luck S. Biochemical and functional effects of prenatal and postnatal omega 3 fatty acid deficiency on retina and brain in rhesus monkeys. *Proc Natl Acad Sci U S A*. 1986; 83:4021–5. [PubMed: 3459166]
8. Uauy R, Treen M, Hoffman DR. Essential fatty acid metabolism and requirements during development. *Semin Perinatol*. 1989; 13:118–30. [PubMed: 2662412]
9. Larque E, Demmelmair H, Koletzko B. Perinatal supply and metabolism of long-chain polyunsaturated fatty acids: importance for the early development of the nervous system. *Ann N Y Acad Sci*. 2002; 967:299–310. [PubMed: 12079857]
10. Lauritzen L, Hansen HS, Jorgensen MH, Michaelsen KF. The essentiality of long chain n-3 fatty acids in relation to development and function of the brain and retina. *Prog Lipid Res*. 2001; 40:1–94. [PubMed: 11137568]
11. Herrera E, Amusquivar E. Lipid metabolism in the fetus and the newborn. *Diabetes Metab Res Rev*. 2000; 16:202–10. [PubMed: 10867720]
12. Hornstra G, Al MD, van Houwelingen AC, Foreman-van Drongelen MM. Essential fatty acids in pregnancy and early human development. *Eur J Obstet Gynecol Reprod Biol*. 1995; 61:57–62. [PubMed: 8549848]
13. Kuipers RS, Luxwolda MF, Offringa PJ, Boersma ER, Dijck-Brouwer DA, Muskiet FA. Fetal intrauterine whole body linoleic, arachidonic and docosahexaenoic acid contents and accretion rates. *Prostaglandins Leukot Essent Fatty Acids*. 2012; 86:13–20. [PubMed: 22115845]
14. Kuipers RS, Luxwolda MF, Offringa PJ, Martini IA, Boersma ER, Dijck-Brouwer DA, et al. Gestational age dependent content, composition and intrauterine accretion rates of fatty acids in fetal white adipose tissue. *Prostaglandins Leukot Essent Fatty Acids*. 2012; 86:39–49. [PubMed: 22093549]

15. Duttaroy AK. Transport of fatty acids across the human placenta: a review. *Prog Lipid Res.* 2009; 48:52–61. [PubMed: 19041341]
16. Gil-Sanchez A, Demmelmair H, Parrilla JJ, Koletzko B, Larque E. Mechanisms involved in the selective transfer of long chain polyunsaturated Fatty acids to the fetus. *Front Genet.* 2011; 2:57. [PubMed: 22303352]
17. Larque E, Demmelmair H, Gil-Sanchez A, Prieto-Sanchez MT, Blanco JE, Pagan A, et al. Placental transfer of fatty acids and fetal implications. *Am J Clin Nutr.* 2011; 94:1908S–13S. [PubMed: 21562082]
18. Gil-Sanchez A, Larque E, Demmelmair H, Acien MI, Faber FL, Parrilla JJ, et al. Maternal-fetal in vivo transfer of [¹³C]docosahexaenoic and other fatty acids across the human placenta 12 h after maternal oral intake. *Am J Clin Nutr.* 2010; 92:115–22. [PubMed: 20444955]
19. Magnusson AL, Waterman IJ, Wennergren M, Jansson T, Powell TL. Triglyceride hydrolase activities and expression of fatty acid binding proteins in the human placenta in pregnancies complicated by intrauterine growth restriction and diabetes. *J Clin Endocrinol Metab.* 2004; 89:4607–14. [PubMed: 15356070]
20. Makowski L, Hotamisligil GS. Fatty acid binding proteins--the evolutionary crossroads of inflammatory and metabolic responses. *J Nutr.* 2004; 134:2464S–8S. [PubMed: 15333743]
21. Biron-Shental T, Schaiff WT, Ratajczak CK, Bildirici I, Nelson DM, Sadovsky Y. Hypoxia regulates the expression of fatty acid-binding proteins in primary term human trophoblasts. *Am J Obstet Gynecol.* 2007; 197:516.e1–6. [PubMed: 17826730]
22. Scifres CM, Chen B, Nelson DM, Sadovsky Y. Fatty acid binding protein 4 regulates intracellular lipid accumulation in human trophoblasts. *J Clin Endocrinol Metab.* 2011; 96:E1083–91. [PubMed: 21525163]
23. Bernlohr DA, Bolanowski MA, Kelly TJ Jr, Lane MD. Evidence for an increase in transcription of specific mRNAs during differentiation of 3T3-L1 preadipocytes. *J Biol Chem.* 1985; 260:5563–7. [PubMed: 3988766]
24. Coe NR, Simpson MA, Bernlohr DA. Targeted disruption of the adipocyte lipid-binding protein (aP2 protein) gene impairs fat cell lipolysis and increases cellular fatty acid levels. *J Lipid Res.* 1999; 40:967–72. [PubMed: 10224167]
25. Kralisch S, Fasshauer M. Adipocyte fatty acid binding protein: a novel adipokine involved in the pathogenesis of metabolic and vascular disease? *Diabetologia.* 2013; 56:10–21. [PubMed: 23052058]
26. Hotamisligil GS, Johnson RS, Distel RJ, Ellis R, Papaioannou VE, Spiegelman BM. Uncoupling of obesity from insulin resistance through a targeted mutation in aP2, the adipocyte fatty acid binding protein. *Science.* 1996; 274:1377–9. [PubMed: 8910278]
27. Mishima T, Miner JH, Morizane M, Stahl A, Sadovsky Y. The expression and function of fatty acid transport protein-2 and -4 in the murine placenta. *PLoS One.* 2011; 6:e25865. [PubMed: 22028793]
28. Folch J, Lees M, Sloane Stanley GH. A simple method for the isolation and purification of total lipides from animal tissues. *J Biol Chem.* 1957; 226:497–509. [PubMed: 13428781]
29. Tian Z, Zhao ZA, Liang XH, Zhang XH, Sha AG, Zhang ZR, et al. Expression and function of fatty acid-binding protein 4 during mouse decidualization. *Fertil Steril.* 2011; 95:2749–52e1. [PubMed: 21704217]
30. Tomlinson TM, Garbow JR, Anderson JR, Engelbach JA, Nelson DM, Sadovsky Y. Magnetic resonance imaging of hypoxic injury to the murine placenta. *Am J Physiol Regul Integr Comp Physiol.* 2010; 298:R312–9. [PubMed: 19923363]
31. Larkin J, Chen B, Shi XH, Mishima T, Kokame K, Barak Y, et al. NDRG1 deficiency attenuates fetal growth and the intrauterine response to hypoxic injury. *Endocrinology.* 2014; 155:1099–106. [PubMed: 24424031]
32. Shaughnessy S, Smith ER, Kodukula S, Storch J, Fried SK. Adipocyte metabolism in adipocyte fatty acid binding protein knockout mice (aP2^{-/-}) after short-term high-fat feeding: functional compensation by the keratinocyte [correction of keratinocyte] fatty acid binding protein. *Diabetes.* 2000; 49:904–11. [PubMed: 10866041]

33. Yang R, Castriota G, Chen Y, Cleary MA, Ellsworth K, Shin MK, et al. RNAi-mediated germline knockdown of FABP4 increases body weight but does not improve the deranged nutrient metabolism of diet-induced obese mice. *Int J Obes (Lond)*. 2011; 35:217–25. [PubMed: 20603627]

Highlights

- FABP4 regulates lipid trafficking in human trophoblasts. Its role in the placenta *in vivo* is unknown.
- FABP4 is expressed in the mouse placental labyrinthine layer, mainly in endothelial cells.
- The level of mouse placental FABP4 is highest at E16.5.
- FABP4 is dispensable for murine feto-placental growth and placental lipid accumulation.

Fig. 1. The expression of FABP4 in the mouse placenta

FABP4 expression was determined using immunohistochemistry in placental specimens obtained between E12.5–18.5, as described in Methods. The control panel on the right side represents a parallel procedure performed using an antibody pre-incubation with a blocking peptide. The panel is a representative experiment, performed three times as described in Methods.

Fig. 2. FABP4 expression in the placental labyrinthine

Images depict high magnification immunofluorescence staining, showing labyrinthine localization of FABP4 (red, A), PECAM1 (marking endothelial cells in green, CD31, B), nuclei (DAPI, C) FABP4/PECAM colocalization image (D). Images also depict a mostly distinct expression of FABP4 (green, E) from the trophoblastic marker cytokeratin-8 (red, F). Nuclei (DAPI, G), FABP4/cytokeratin-8 colocalization image (H). Also shown are low magnification immunofluorescence staining, depicting labyrinthine localization of FABP4 (green, I), and FABP4/cytokeratin-8 colocalization image (J). Bar=100 μ m for high magnifications, and Bar=1 mm for low magnifications. The panel is a representative experiment, performed three times as described in Methods.

Fig. 3. The expression of placental FABP4 during murine pregnancy

(A) A representative western blot analysis, performed three times in duplicate, as described in Methods. Bands were quantified by densitometry, shown in the graph below the image. * denotes $p < 0.01$ (ANOVA), compared to the band from E12.5. The gel represents three independent experiments. (B) RT-qPCR analysis of *Fabp4* mRNA expression ($n=3$). * denotes $p < 0.05$ (ANOVA), compared to the expression at E12.5.

Fig. 4. Triglyceride levels in mouse tissues during pregnancy
 (A) maternal serum, (B) placenta, (C) fetal liver. None of the differences between genotypes, exposed to standard or hypoxic atmosphere, were significant (n=10–20 fetuses for each paradigm).

Fig. 5. The expression of *Fabp* transcripts in *Fabp4*-null placentas in standard or hypoxic conditions

The expression of *Fabp* members was determined by RT-qPCR. (A) The effect of hypoxia on the expression of *Fabp* members in wt placentas. (B) The effect of *Fabp4* deficiency on placental expression of *Fabp* members in standard or hypoxic conditions. Only *Fabp* members that we previously showed to be expressed in the mouse placenta were tested. * denotes $p < 0.05$ ($n = 10-20$ fetuses for each paradigm).