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Effects of Labor on Placental Fatty Acid Beta Oxidation

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Abstract

OBJECTIVE—To measure the effect labor exerts on fatty acid (FA) oxidation in term human placentas, and to compare enzymes expression and activity between placenta and liver.

METHODS—Placental samples were collected: a) scheduled non-labored cesarean section and b) normal vaginal delivery at or beyond 37 weeks. Long and medium-chain FA oxidation were measured using ³H-labeled FA, ATP concentration was measured via commercial kit. Activity and expression levels of 11 FA enzymes were measured and results compared to both human and mouse liver.

RESULTS—Placentas undergoing labor had significantly decreased palmitate oxidation and ATP levels. Octanoic acid oxidation was 10-fold higher than palmitic acid oxidation. No difference in expression or activity level was detected between the groups.

CONCLUSION—Term human placentas express all the enzymes required to oxidize FA, at a rate 20-fold lower than liver. FA Oxidation is not likely an important placental energy source during labor. Further work is needed to determine the functionality of this pathway in placenta.

Keywords

Fatty acid metabolism; placental metabolism; parturition

INTRODUCTION

Mitochondrial fatty acid (FA) oxidation is an important energy-producing pathway in tissues such as heart, liver, and exercising skeletal muscle. However, little is known about the role of FA oxidation in placental energy metabolism. It is generally believed that glucose is the major energy source for fetal tissue [1, 2] and that FAs are used for anabolism rather than energy [3, 4]. Yet the placenta, which is mostly fetal in origin, expresses the enzymatic machinery required for mitochondrial FA oxidation [5-7]. This has led to speculation that placenta catabolizes FA for energy. Interestingly, women carrying fetuses with genetic FA oxidation disorders are more likely to develop acute fatty liver disease (AFLP) or the syndrome of Hemolysis, Elevated Liver enzymes and Low Platelets (HELLP) [8]. While the cause of these diseases remains obscure, it has been proposed that genetic defects in

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placental FA oxidation lead to an accumulation of toxic FA intermediates which in turn trigger maternal liver disease [6, 7].

Previous studies have suggested several possible physiological roles for placental FA oxidation pathway. First, it has been shown to directly support the early energy-requiring mitochondrial steps in the pathway of progesterone synthesis [9]. Second, based on low enzyme activity of medium-chain FA oxidation enzymes relative to long-chain enzymes, it has been suggested that the placenta may partially chain-shorten long-chain FA down to medium-chain FA which can diffuse across membranes and be readily metabolized by the fetus [7, 10, 11]. Such mitochondrial partial chain-shortening of FA has not been described in other tissue types and may be unique to placental energy metabolism. Finally, evidence suggests that FA may be an important energy source for the placenta during labor, known to be a catabolic state. Normal human labor is associated with a high maternal energy expenditure leading to the depletion of glucose [12] and perhaps a possible decrease in substrate availability to the fetus. Changes in placental gene expression as measured by microarray suggest increased uptake and trafficking of FA during parturition [13]. PPAR α and PPAR β/δ , which are known to regulate many genes in FA oxidation pathways, are similarly increased [14].

Despite these proposed roles for FA oxidation in placenta, the actual rate of oxidation has not been measured and partial chain-shortening has not been verified. Previous studies measuring mRNA levels, protein levels, and enzymatic activities indicate that placenta has the capacity for FA oxidation. However, the rate of FA oxidation is strictly regulated at the point of entry into mitochondria via carnitine palmitoyltransferase-1 (CPT1), and thus the physiological significance of the pathway can only be determined by following the flux of substrate to product. The present study was designed to test the hypothesis that FA oxidation would be higher in placenta from laboring women versus women undergoing cesarean section. We further hypothesized that if placenta engages in partial chain-shortening of long-chain FA, this would manifest as a low ratio of medium chain (octanoate) FA oxidation to long-chain (palmitate) FA-oxidation. Therefore, we directly measured FA oxidation using radiolabeled FA in fresh placental explants. As a yardstick by which to evaluate placental FA oxidation we used mouse liver, a readily available source of fresh tissue in which lipid metabolism has been extensively studied. Finally, frozen human liver specimens were used for comparison studies of enzyme activity and protein levels.

METHODS

Sample collection

The study was approved by the Institutional Review Board at Women & Infants' Hospital (protocol #10-0122). The use of human liver (protocol HSTB #0506140) and mouse liver (protocol IACUC #0910603) samples as controls was approved by the University of Pittsburgh Medical Center (UPMC). All placenta samples were taken from pregnancies at or beyond 37 weeks. Pregnancies complicated by a multiple gestation, pre-gestational or gestational diabetes mellitus, HIV/Hepatitis B or C infection, chronic hypertension, AFLP, Pre-eclampsia/Eclampsia/HELLP, chorioamnionitis, known fetal aneuploidy/anomalies, intrauterine growth restriction or intrahepatic cholestasis of pregnancy were excluded. Nine samples were harvested from patients undergoing both induced or spontaneous vaginal delivery (labor group) and another nine from patients presenting for scheduled cesarean section (non-labor group). A sample approximately 2-grams in weight was collected from a central cotyledon free of any visible placental lesion close to the fetal side avoiding maternal decidual contamination. The samples were collected within 10 minutes of delivery and washed thoroughly in phosphate buffered saline (PBS). A portion of each sample was flash-frozen in liquid nitrogen and stored at -80°C for later measurement of enzyme activities,

ATP concentration, and protein expression by western blot. A portion was immediately used for measuring rates of FA oxidation with ^3H -labeled substrates as follows.

Tritium release assay

Approximately 100 mg of freshly sampled villous tissue was minced and placed into tissue-culture plates pre-filled with culture medium containing 5mM glucose to simulate in-vivo fasting blood glucose levels. ^3H -palmitic acid (Cat # NET043001MC, Perkin Elmer®, Waltham, MA) bound to BSA or unbound ^3H -octanoic acid (Cat # ART 1295, ARC, Inc.®, St. Louis, MO) were added to a final concentration of 125 μM and the samples incubated for 1 hour at 37°C. All assays were run in duplicate and averaged. Blank wells were treated identically but without added placental tissue. The reactions were stopped with 20% trichloroacetic acid (TCA) and placed on ice. The samples were then centrifuged and the supernatant collected. The acid was neutralized with potassium hydroxide. Excess unmetabolized ^3H -fatty acids were removed using Dowex ion-exchange resin. Released $^3\text{H}_2\text{O}$ was measured by liquid scintillation counting, and the rates of FA oxidation were calculated as described by Narayan et al [15].

Acyl-CoA dehydrogenase (ACAD) enzyme activity

ACAD activities were measured in placental tissue extracts with the anaerobic electron transferring flavoprotein (ETF) fluorescence reduction assay as previously described [16] using a Jasco fluorescence spectrophotometer. The reaction was started by the addition of either butyryl (C_4)-CoA for short-chain acyl-CoA dehydrogenase (SCAD), octanoyl (C_8)-CoA for medium-chain acyl-CoA dehydrogenase (MCAD), or palmitoyl (C_{16})-CoA for the combined activities of long-chain acyl-CoA dehydrogenase (LCAD), very long-chain acyl-CoA dehydrogenase (VLCAD), and acyl-CoA dehydrogenase-9 (ACAD9). All CoA substrates were from Sigma-Aldrich (Cat #'s B1508, O6877, P9716, St. Louis, MO) and used at a final concentration of 25 μM . Activities were calculated as mU of activity per mg of total placental protein.

Complex I activity

NADH oxidase activity of the electron transport chain complex I was measured in placenta homogenates using the Complex I “dipstick assay” (Mitosciences® Eugene, Oregon) [17]. Briefly, this immunocapture assay uses monoclonal antibodies located on the dipstick to trap Complex I from homogenized placental tissue. The dipstick is then submerged in NADH-rich buffer solution containing a dye-labeled electron acceptor. Following color development, the dipsticks were scanned and the bands quantified by densitometry.

Western blotting

Frozen tissue was homogenized in 50 mM Tris-HCl (pH 8.0), 2 mM EDTA, and 0.25% lubrol detergent pH 8.0 with protease inhibitors and incubated on ice. The placental lysate was sonicated and homogenates centrifuged. The supernatants were analyzed for protein concentration in triplicate using the Bradford method (Bio-Rad® Hercules, CA). Rabbit polyclonal antisera used to detect long-chain hydroxyacyl CoA dehydrogenase (LCHAD) (1:3,000), long-chain 3-ketoacyl CoA thiolase, (LKAT) (1:2500) and short-chain hydroxyacyl CoA dehydrogenase (SCHAD) (1:5,000) (Courtesy of Dr. Arnold W. Strauss, Cincinnati Children's Hospital) [6]. Rabbit polyclonal antisera were raised against SCAD, MCAD, LCAD, VLCAD, ACAD9, and ETF (all used 1:1000) (Courtesy of Dr. Jerry Vockley, Children's Hospital of Pittsburgh). Antibodies against complexes II and V of the electron transport chain (Cat #'s MS201c, MS501c, Mitosciences® Eugene, Oregon) were used at a 1:1000 dilution. Following incubation with secondary antibody (donkey anti-rabbit, 1:10,000 dilution - Cat # 31238, Thermo Scientific, Rockford, IL.) the protein bands

were visualized and normalized by densitometry to either the expression of β -actin (Cat # SC-130300, Santa Cruz Biotechnology® Santa Cruz, CA, used at 1:1000) or glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (Cat # AB8245, Abcam® Cambridge, MA dilution 1:2,500).

ATP concentration

ATP concentration was measured using a colorimetric microplate assay (BioVision®, Mountain View, California) [18]. ATP was extracted from approximately 60 mg of frozen placenta by homogenization in perchloric acid. Following centrifugation, the supernatants were neutralized with KOH and ATP was measured following the manufacturer's instructions.

Statistical analyses

Statistical analysis was performed using Sigma Stat v3.5 (Aspire Software International, Ashburn, VA). All continuous variables underwent statistical comparisons using the Student t-test. We conducted a two-sided hypothesis test with a p value less than 0.05 considered statistically significant.

RESULTS

Comparison of long and medium chain FA oxidation

FA oxidation rates were evaluated in fresh placental samples collected from laboring women (n=9), cesarean section women (n=9) and non-fasted mouse liver (n=3) (fresh human liver was not available). In order to determine if FA oxidation was occurring primarily in peroxisomes or mitochondria, placental explant samples were evaluated in the presence or absence of etomoxir, a potent inhibitor of CPT-1. FA oxidation in placenta was found to be almost exclusively mitochondrial based on >95% inhibition of ^3H -palmitate oxidation by etomoxir, (data not shown).

Contrary to our hypothesis, FA oxidation was not increased by labor, but rather was significantly decreased compared to placentas from cesarean section (Figure 1A). Overall, the rate of placental palmitate oxidation was very low relative to mouse liver. The rate of octanoate oxidation in placenta was expected to be lower than that for palmitate based on the hypothesis of partial chain shortening. However, ^3H -octanoic acid oxidation was approximately 10-fold higher than ^3H -palmitic acid oxidation (Figure 1B). This demonstrated that placental FA oxidation of medium chain FA was greater than long chain FA in placental tissue; suggesting CPT-1 limits long chain FA oxidation. ^3H -octanoic acid oxidation in mouse liver was about 10-fold higher than ^3H -palmitic acid oxidation. For both FA substrates, the rate of oxidation was about 20-fold lower in human placenta compared to mouse liver.

In an attempt to explain the overall decreased fatty acid oxidation rate, placental ATP concentration was measured (Figure 1B). ATP is also required for activation of FA to acyl-CoAs by the acyl-CoA synthases. Using ATP as a marker of the overall energetic state, levels of this substrate were found to be significantly lower in tissues from patients undergoing labor (n=9) compared to those from elective cesarean section not in labor (n=9).

ACAD enzyme activity and expression

To further investigate the difference in long-chain FA-oxidation seen between placentas from laboring and cesarean section patients, activity of the ACAD enzymes was measured in placental extracts. Five chain-length specific ACAD enzymes (VLCAD, LCAD, ACAD9, MCAD, and SCAD) catalyze the first step in the mitochondrial FA-oxidation pathway.

Assays with octanoyl-CoA and butyryl-CoA as substrates primarily reflect the activities of MCAD and SCAD, respectively. Because the long-chain enzymes LCAD, VLCAD, and ACAD9 are all active against palmitoyl-CoA [19], activity in tissue extracts determined with this substrate represents their combined activities. Activities measured with all three acyl-CoA substrates were similar between the two groups of placental samples (n=5) (Table 1). As a measure of comparison, activity level of the ACADs were measured and contrasted in both placenta and human liver. Activity with palmitoyl-CoA (LCAD/VLCAD/ACAD9) was 2-fold lower in placenta compared to human liver, and SCAD activity (butyryl-CoA) about 3-fold lower. In contrast, MCAD activity (octanoyl-CoA) was much lower (12-fold) in placenta than in liver.

Bases on our previous observation that ATP concentrations were reduced in labored placental samples, we sought to determine the functionality of the electron transport system. Activity was measured for complex I of the electron transport chain in placental tissue extracts (n=5). Mean enzymatic activity in placentas exposed to labor was 1.73 U/mg and comparable to a mean of 1.0 U/mg seen in placentas from scheduled cesarean section (p=0.15). Activity of complex I was not measured in liver.

To determine if other enzymes might be down regulated causing the decrease in FA oxidation associated with labor, we evaluated the pattern of protein expression of 11 different enzymes involved in the FA oxidation pathway and the electron transport chain by western blot. No difference in expression levels were seen between cesarean section and vaginally-delivered placentas with regards to the 5 ACAD enzymes, the ACAD electron acceptor ETF, the FA oxidation enzymes downstream of the ACADs (LCHAD, LKAT, SHCAD), or for subunits of electron transport chain complexes II and V (Figure 2).

Finally, we compared the expression level of all five ACADs between placental tissue and human liver (Figure 3). Surprisingly, VLCAD protein levels are as high as or even higher in placenta than in human liver. The other four ACAD enzymes measured were all several-fold lower in placenta. LCAD expression in particular was very low in placenta relative to liver. Based on the blot in Figure 3, which compares 5 μ g of liver extract to 50 μ g of placental extract, LCAD expression is estimated to be 35-fold lower in placenta than in human liver. Thus, in placenta, VLCAD is clearly the dominant long chain ACAD enzyme.

CONCLUSIONS

In contrast to our hypothesis we found that placental metabolism of FA decreased with labor and is unlikely to be a primary energy-source for the placenta and fetus [13, 14]. Our data indicates that at term, fresh explants of fetal placenta have low FA oxidation rates. Various factors can certainly contribute to this overall reduced metabolic rate. Previous studies have revealed that the placenta plays a major role in both fatty acid storage and shuttling towards the fetus, both of which could contribute to the overall low mitochondrial oxidation observed. Wolfe et al showed that FA levels in cord blood of neonates paralleled maternal levels [20], indicating the active role of placental FA trafficking. Ramsey et al evaluated porcine placental [21] explants taken from the maternal side; the authors suggested that about 50% of long-chain FA taken up underwent FA oxidation and the remaining 50% was stored. Explants from the fetal side, however, oxidized only 25-30% with esterification being highly favored. Thus, fetal placenta may serve as a temporary warehouse for FA that is destined for the fetal compartment, and only a small fraction of this FA is partitioned for energy via oxidation. A similar mechanism has been demonstrated in adipose tissue [22]. Interestingly, PPAR γ , the master lipogenic transcription factor, is critical for development of both adipose tissue and placenta [23]. This mutual regulatory mechanism could be a factor in determining the role in both tissues of FA storage rather than metabolism. These

studies support the hypothesis that longer chain FAs are not used as an energy source by the placenta but are transported to the fetal compartment for fetal growth.

Previous studies had demonstrated that the human placenta possesses the enzymatic machinery necessary to oxidize FA for energy [5-7]. Our results strengthen those findings and extend them by comparing placental enzyme expression and activity as well as the rate of FA oxidation to liver. We demonstrated that labor depleted cellular energy stores reflected by lower levels of ATP. This reduction did not result in compensation by increased FA oxidation since palmitate oxidation was not induced. Rather, labor reduced the rate of palmitate oxidation which may have in turn contributed to the decline in ATP levels. The abundance and activities of FA oxidation enzymes were not altered by labor, suggesting that the decreased palmitate oxidation was due to other mechanisms that include a decreased cellular uptake or decreased transport across the mitochondrial membrane via CPT-1.

While intracellular trafficking of long-chain FA and entry into the mitochondria are likely major reasons for the low rates of FA oxidation in placenta, the pattern of distribution of FA oxidation enzymes may also be a contributing factor. It is possible that the regulatory mechanism for the β -oxidation pathway within the placenta is different from the arrangement observed in other tissues. The relative abundance of the intramitochondrial long-chain FA oxidation enzymes indicate that the machinery for shortening long chain FA is certainly present. The present study and that of Oey et al [7] indicate that VLCAD is highly expressed in placenta making the first step of mitochondrial β -oxidation (ACAD step) unlikely to be a rate-limiting step. It could also explain why patients with this enzymatic defect tend to improve clinically during pregnancy [24]. The remaining three enzymatic reactions involved in chain-shortening long-chain FA are catalyzed by the octameric trifunctional protein (TFP). Oey et al [7] demonstrated that in human liver the ratio of TFP:ACAD activity is at least 30, while in placenta this ratio is reduced to only 3. It is therefore possible that the low concentration and functionality of TFP observed within placental tissue may be explained by its likely role as a rate-limiting step in placental metabolism. The potential vital role TFP has within FAO metabolism in placenta is further evidenced when cases of deficiency in LCHAD, a major component of TFP, are evaluated. Fetuses carrying this defect in FA metabolism tend to develop AFLP/HELLP during pregnancy at a rate higher than any other FAO enzymatic defect [8]. Fetal LCHAD deficiency could contribute to an increase in fatty acid concentration in addition to intermediates of FAO metabolism building up leading to maternal overload and fat deposit in the liver.

Furthermore, no evidence was found to support the concept of partial FA shortening in placental mitochondria with the purpose of exporting to fetus as an energy source. Our explant octanoate oxidation studies indicate that MCAD is not limiting within placenta, as the explants oxidized octanoate at a high rate. The ratio of octanoate: palmitate oxidation was similar between placenta and mouse liver with both tissue types metabolizing octanoate about 10 times faster than palmitate. In our comparison to human liver, the expression and activity of MCAD, the rate-limiting enzyme for medium-chain FA oxidation, were 10-12-fold lower in placenta. Oey et al [7] reported that MCAD activity is about 50-fold lower in placenta compared to human liver. The difference between our result and that of Oey et al may be due to a difference in methodology. Oey et al used phenylpropionyl-CoA as substrate and HPLC as the method of detection whereas we used octanoyl-CoA and the ETF fluorescence-based assay. Overall, it appears that the FA enzymatic machinery present in placenta is not intended to shorten FA acids for fetal consumption and its true role needs to be further investigated.

Although our study seems to indicate a secondary role of fatty acid oxidation in placental energy generation, our evaluation was limited to term placentas from normal pregnancies. Obstetrical complications such as intrauterine growth restriction and gestational diabetes could theoretically cause alterations in the metabolic rate of fatty acid by the placenta as both an adaptive and compensatory mechanism. The impact of these pathologies on the pathway as well as the clinical relevance should be evaluated in further studies. In conclusion, we found that long-chain FA oxidation was decreased in placentas from laboring women while no difference was noted in medium chain FA oxidation. The association between fetal defects in FA oxidation enzymes and maternal liver diseases requires further investigation.

REFERENCES

1. Illsley NP. Placental glucose transport in diabetic pregnancy. *Clin Obstet Gynecol.* 2000; 43(1): 116–26. [PubMed: 10694994]
2. Jones CT, Rolph TP. Metabolism during fetal life: a functional assessment of metabolic development. *Physiol Rev.* 1985; 65(2):357–430. [PubMed: 3885272]
3. Larque E, Demmelmair H, Gil-Sanchez A, Prieto-Sanchez MT, Blanco JE, Pagan A, Faber FL, Zamora S, Parrilla JJ, Koletzko B. Placental transfer of fatty acids and fetal implications. *Am J Clin Nutr.* 2011
4. Roux JF, Yoshioka T. Lipid metabolism in the fetus during development. *Clin Obstet Gynecol.* 1970; 13(3):595–620. [PubMed: 4992814]
5. Rakheja D, Bennett MJ, Foster BM, Domiati-Saad R, Rogers BB. Evidence for fatty acid oxidation in human placenta, and the relationship of fatty acid oxidation enzyme activities with gestational age. *Placenta.* 2002; 23(5):447–50. [PubMed: 12061861]
6. Shekhawat P, Bennett MJ, Sadovsky Y, Nelson DM, Rakheja D, Strauss AW. Human placenta metabolizes fatty acids: implications for fetal fatty acid oxidation disorders and maternal liver diseases. *Am J Physiol Endocrinol Metab.* 2003; 284(6):E1098–105. [PubMed: 12582009]
7. Oey NA, den Boer ME, Ruiter JP, Wanders RJ, Duran M, Waterham HR, Boer K, van der Post JA, Wijburg FA. High activity of fatty acid oxidation enzymes in human placenta: implications for fetal-maternal disease. *J Inher Metab Dis.* 2003; 26(4):385–92. [PubMed: 12971426]
8. Browning MF, Levy HL, Wilkins-Haug LE, Larson C, Shih VE. Fetal fatty acid oxidation defects and maternal liver disease in pregnancy. *Obstet Gynecol.* 2006; 107(1):115–20. [PubMed: 16394048]
9. Tialowska B, Klimek J, Zelewski L. Progesterone biosynthesis supported by fatty acid oxidation in the mitochondrial fraction of human term placenta. *Acta Biochim Pol.* 1983; 30(1):11–21. [PubMed: 6868904]
10. Prip-Buus C, Pegorier JP, Duee PH, Kohl C, Girard J. Evidence that the sensitivity of carnitine palmitoyltransferase I to inhibition by malonyl-CoA is an important site of regulation of hepatic fatty acid oxidation in the fetal and newborn rabbit. Perinatal development and effects of pancreatic hormones in cultured rabbit hepatocytes. *Biochem J.* 1990; 269(2):409–15. [PubMed: 2167069]
11. Saggerson ED, Carpenter CA. Regulation of hepatic carnitine palmitoyltransferase activity during the foetal-neonatal transition. *FEBS Lett.* 1982; 150(1):177–80. [PubMed: 7160469]
12. Scheepers HC, de Jong PA, Essed GG, Kanhai HH. Fetal and maternal energy metabolism during labor in relation to the available caloric substrate. *J Perinat Med.* 2001; 29(6):457–64. [PubMed: 11776675]
13. Lee KJ, Shim SH, Kang KM, Kang JH, Park DY, Kim SH, Farina A, Shim SS, Cha DH. Global gene expression changes induced in the human placenta during labor. *Placenta.* 31(8):698–704. [PubMed: 20554320]
14. Xu Y, Wang Q, Cook TJ, Knipp GT. Effect of placental fatty acid metabolism and regulation by peroxisome proliferator activated receptor on pregnancy and fetal outcomes. *J Pharm Sci.* 2007; 96(10):2582–606. [PubMed: 17549724]

15. Narayan SB, Boriack RL, Messmer B, Bennett MJ. Establishing a reference interval for measurement of flux through the mitochondrial fatty acid oxidation pathway in cultured skin fibroblasts. *Clin Chem*. 2005; 51(3):644–6. [PubMed: 15738519]
16. Goetzman ES. The regulation of acyl-CoA dehydrogenases in adipose tissue by rosiglitazone. *Obesity (Silver Spring)*. 2009; 17(1):196–8. [PubMed: 18948967]
17. Marusich MF, Murray J, Xie J, Capaldi RA. Novel antibody-based strategies for the rapid diagnosis of mitochondrial disease and dysfunction. *Int J Biochem Cell Biol*. 2009; 41(10):2081–8. [PubMed: 19460456]
18. Li C, Numata M, Takeuchi M, Shinkai S. A sensitive colorimetric and fluorescent probe based on a polythiophene derivative for the detection of ATP. *Angew Chem Int Ed Engl*. 2005; 44(39):6371–4. [PubMed: 16173002]
19. He M, Rutledge SL, Kelly DR, Palmer CA, Murdoch G, Majumder N, Nicholls RD, Pei Z, Watkins PA, Vockley J. A new genetic disorder in mitochondrial fatty acid beta-oxidation: ACAD9 deficiency. *Am J Hum Genet*. 2007; 81(1):87–103. [PubMed: 17564966]
20. Wolfe MD, Chuang LT, Rayburn WF, Wen PC, Vanderjagt DJ, Glew RH. Low fatty acid concentrations in neonatal cord serum correlate with maternal serum. *J Matern Fetal Neonatal Med*.
21. Ramsay TG, Karousis J, White ME, Wolverson CK. Fatty acid metabolism by the porcine placenta. *J Anim Sci*. 1991; 69(9):3645–54. [PubMed: 1938647]
22. Wang T, Zang Y, Ling W, Corkey BE, Guo W. Metabolic partitioning of endogenous fatty acid in adipocytes. *Obes Res*. 2003; 11(7):880–7. [PubMed: 12855758]
23. Fournier T, Therond P, Handschuh K, Tsatsaris V, Evain-Brion D. PPARgamma and early human placental development. *Curr Med Chem*. 2008; 15(28):3011–24. [PubMed: 19075649]
24. Mendez-Figueroa H, Shchelochkov OA, Shaibani A, Aagaard-Tillery K, Shinawi MS. Clinical and biochemical improvement of very long-chain acyl-CoA dehydrogenase deficiency in pregnancy. *J Perinatol*. 2010; 30(8):558–62. [PubMed: 20668464]

Figure 1. Graphic representations of ATP concentration and C₁₆ and C₈ metabolism in placenta

Figure A. Measurement of C₁₆ and C₈ metabolism in all 18 samples expressed in pmole/mg/hr (y-axis). The Y-axis has been transformed to logarithmic scale in order to allow better comparison among groups. Metabolism of long chain FA (C₁₆) is significantly decreased with labor (t-test, $p = 0.016$). In general, placental metabolism is 20X less than mouse liver (black column). Error bars represent standard error.

Figure B. ATP concentration measured in all 18 samples expressed in nmoles ATP/mg of tissue (y-axis). There is a statistically significant difference in ATP concentration among both groups (t-test, $p=0.027$). Error bars represent standard error.

Figure 2. Enzymatic expression

Densitometric analysis of protein expression corrected for the presence of Actin or GAPDH. There was no statistically significant difference between both groups (n=9 for each group). Y-axis represents the relative densitometric units. All error bars represent standard error. All values analyzed using student t-test.

Figure 3. Representative Western blots comparing placental samples to human liver

Comparison of human liver and placental samples. All samples were loaded on the same gel using the same protocol while using β -actin as an internal control. All wells were loaded with 25 μ g of total protein (except for the LCAD blot which required loading of 5 μ g of human liver vs. 50 μ g of placenta in order to obtain a linear range).

Table 1

Enzymatic activity

Enzyme Activity Assay (units)	Substrate	C/S (non-laboring placenta) Mean (SD)	Vaginal Mean (SD)	Human Liver Mean (SD)
LCAD/VLCAD/ACAD9 (mU/mg)	C16-CoA	10.0 (3.4)	8.2 (3.6)	20.0 (0.4)
MCAD (mU/mg)	C ₈ -CoA	3.3 (1.1)	3.7 (1.8)	39.1 (6.1)
SCAD (mU/mg)	C ₄ -CoA	2.9 (1.7)	3.4 (1.9)	10.4 (3.1)
Complex I (U/mg)	NADH	1.0 (0.59)	1.73 (0.84)	--

C/S= cesarean section, SD = standard deviation, FA = fatty acid, VLCAD = Very long chain Acyl-CoA Dehydrogenase, LCAD = Long chain Acyl-CoA Dehydrogenase, MCAD = Medium chain Acyl-CoA Dehydrogenase, SCAD = Short chain Acyl-CoA Dehydrogenase, ACAD9 = Acyl-CoA Dehydrogenase-9, m = milli, U = units, mg = milligrams tissue, hr = hour, pmole = picomoles, CoA = Coenzyme A.