Am J Physiol Endocrinol Metab 284: E1098–E1105, 2003.
First published February 11, 2003; 10.1152/ajpendo.00481.2002.

Human placenta metabolizes fatty acids: implications for fetal fatty acid oxidation disorders and maternal liver diseases

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Submitted 5 November 2002; accepted in final form 6 February 2003

Shekhawat, Prem, Michael J. Bennett, Yoel Sadovsky, D. Michael Nelson, Dinesh Rakheja, and Arnold W. Strauss. Human placenta metabolizes fatty acids: implications for fetal fatty acid oxidation disorders and maternal liver diseases. *Am J Physiol Endocrinol Metab* 284: E1098–E1105, 2003. First published February 11, 2003; 10.1152/ajpendo.00481.2002.—The role of fat metabolism during human pregnancy and in placental growth and function is poorly understood. Mitochondrial fatty acid oxidation disorders in an affected fetus are associated with maternal diseases of pregnancy, including preeclampsia, acute fatty liver of pregnancy, and the hemolysis, elevated liver enzymes, and low platelets syndrome called HELLP. We have investigated the developmental expression and activity of six fatty acid β-oxidation enzymes at various gestational-age human placentas. Placental specimens exhibited abundant expression of all six enzymes, as assessed by immunohistochemical and immunoblot analyses, with greater staining in syncytiotrophoblasts compared with other placental cell types. β-Oxidation enzyme activities in placental tissues were higher early in gestation and lower near term. Trophoblast cells in culture oxidized tritium-labeled palmitate and myristate in substantial amounts, indicating that the human placenta utilizes fatty acids as a significant metabolic fuel. Thus human placenta derives energy from fatty acid oxidation, providing a potential explanation for the association of fetal fatty acid oxidation disorders with maternal liver diseases in pregnancy.

acute fatty liver of pregnancy; mitochondria; hemolysis, elevated liver enzymes, and low platelets syndrome

BECAUSE THE PLACENTA PROVIDES THE FETUS with nutrients needed for growth and serves as an excretory organ to eliminate wastes from fetal metabolism, placental pathology profoundly affects the developing fetus. The placenta grows exponentially during gestation, from an average of 6 g at 3 wk of gestation to ~470 g at term. Moreover, the villous surface increases from 830 cm^2 at 3 wk of gestation to ~125,000 cm^2 at term, and the maternal-fetal diffusion distance decreases from 55 to 4.8 mm (16). The placenta requires a constant and abundant source of energy to supply the needs for its own rapid growth and maturation and to transport the nutrients, ions, vitamins, waste, and other molecules required for fetal growth and homeostasis from the maternal to the fetal circulation and vice versa.

A common belief among fetal physiologists (14, 16, 18, 20, 26) is that glucose transported to and across the placenta from the maternal circulation provides all placental and fetal energy needs via glycolysis and the citric acid cycle. Because this supply of glucose is constant, consistent, and reliable, it has been suggested that the placenta and fetus do not need to regulate energy-producing metabolic pathways. The focus of most research has been on transplacental passage of nutrients, including both amino acids and fatty acids, but the metabolic fuel required by the human placenta has not been determined conclusively. The presence of multiple glucose transporters and enzymes of glycolysis and the citric acid cycle in the placenta is consistent with glucose being a major energy source (14). It has been postulated that adequate glucose supply, conversion of acetyl-CoA to malonyl-CoA by acetyl-CoA carboxylase, and the resultant inhibition of carnitine palmitoyltransferase I (CPT I) (26) inhibit fatty acid uptake and oxidation by placental mitochondria in utero (20). Although fatty acids are actively transported across the placenta to the fetus, there are scant data to assess the role of lipids as a metabolic fuel for placental growth and development (18).

Fatty acid oxidation (FAO) defects are autosomal recessive and potentially fatal disorders that are now diagnosed with increased frequency in the perinatal and infantile periods. Uniquely among inherited metabolic defects, FAO enzyme disorders in the affected fetus may cause significant maternal morbidity and mortality (11, 13, 15, 23, 27, 32, 33, 39). We (11, 13, 23) and others (15, 27, 32, 33, 39) have recently shown that maternal acute fatty liver of pregnancy (AFLP), the HELLP syndrome (hemolysis, elevated liver enzymes, and low platelets), placental floor infarction, and preeclampsia are associated with defects in FAO in the fetus. The majority of neonates born after such preg-

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nancies are premature, exhibit growth restriction, and present in the newborn period or early infancy with fasting-induced hypoketotic hypoglycemia and hepatic encephalopathy, which may progress to coma and death (11, 33).

Because FAO disorders in the fetus are associated with maternal complications and because the human placenta is mostly of fetal origin, we hypothesized that energy supplied from fatty acid β-oxidation in the placenta could be an important metabolic energy source for survival, growth, and function in both the placenta and the fetus. As a corollary, if FAO were active in the placenta and because late-gestation placenta is of fetal origin, fetal defects in this pathway would generate long-chain fatty acids that could enter the maternal circulation in levels toxic to the mother. We report here the developmental expression and activity of six different FAO enzymes of the mitochondrial β-oxidation spiral in human placenta (Fig. 1): medium-chain acyl-CoA dehydrogenase (MCAD), long-chain acyl-CoA dehydrogenase (LCAD), very-long-chain acyl-CoA dehydrogenase (VLCAD), short-chain L-3-hydroxyacyl-CoA dehydrogenase (SCHAD), long-chain L-3-hydroxyacyl-CoA dehydrogenase (LCHAD), and long-chain 3-ketoacyl-CoA thiolase (LKAT). We used immunohistochemistry, Western blot analysis, enzyme activity measurements, and metabolic flux studies to demonstrate substantial FAO expression and function of these enzymes in the human placenta, consistent with our hypotheses.

METHODS

Placental tissues and cells. This study was approved by the Human Studies Committee of Washington University School of Medicine. Placental specimens were collected at gestational ages ranging from 12 to 43 wk. Tissue was placed in chilled, buffered saline to remove maternal blood and then snap-frozen in liquid nitrogen and stored at −80°C or fixed in 10% neutral buffered formalin solution at 4°C for 24 h before being processed for paraffin embedding and immunohistochemical studies. Twenty-eight placental specimens were used for enzyme activity and Western blot studies. Specimens were divided for analysis into the following gestational age groups: 12–19 (n = 3), 20–28 (n = 3), 29–34 (n = 5), 35–37 (n = 4), 38 wk (n = 9), and >40 wk (n = 4). All samples were utilized to measure enzyme activity, and nine of these samples from 12 to 43 wk of gestation were used to run Western blots for the six FAO enzymes.

For enzyme activity and metabolic flux studies, primary human cytotrophoblasts were isolated from normal-term human placentas (n = 4) by use of the trypsin-DNAse-dispase/Percoll method, as described (25). Cultures were plated at a density of 350,000 cells/cm^2 and maintained in Earle's medium 199 (M199) containing fetal bovine serum, 20 mM HEPES, pH 7.4, 0.5 mM L-glutamine, penicillin (10 U/ml), streptomycin (10 mg/ml), and fungizone (0.25 mg/ml). All cultures were maintained at 37°C in a 5% CO_2 atmosphere, and the medium was changed every 24 h. Our trophoblast cell isolates were 95–97% cytokeratin positive, which indicates that they were trophoblast cells (25).

Immunohistochemistry. Term placentas from uncomplicated pregnancies were collected, and 5-μm-thick sections of paraffin-embedded tissue were cut, applied to glass slides, deparaffinized in xylene, and rehydrated in an ethanol gradient. Endogenous peroxidase activity was quenched by incubating the specimens in 3% H_2O_2 in methanol for 30 min. After equilibration for 5 min in distilled water, the samples were subjected to heat antigen retrieval using citrate buffer (pH 6.0). The samples were heated at maximum power in a microwave for 5 min, cooled for 5 min, reheated for 5 min, and allowed to stand at room temperature for 20 min.

The slides were then washed and blocked using an avidin-biotin blocking kit (Vector Labs, Burlingame, CA) for 30 min

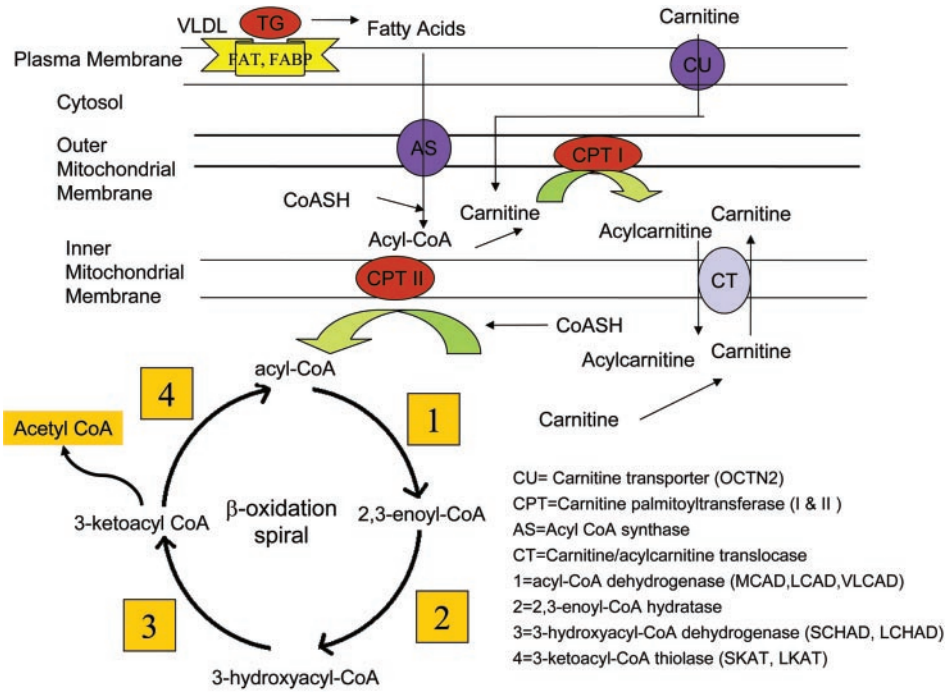

Fig. 1. The mitochondrial fatty acid oxidation (FAO) pathway. This schematic representation shows the uptake of fatty acids and carnitine into the placental cell, transfer of fatty acid from the cytosol into mitochondria, and the fatty acid β-oxidation spiral. See *key* for definitions of terms. Medium- and short-chain fatty acids are transported directly into the cytosol, but long-chain fatty acids and carnitine are transported by specific plasma membrane transporters [fatty acid transporter (FAT), fatty acid-binding protein (FABP)]. Fatty acids utilize carnitine acyltransferases CPT I and II and CT to enter mitochondria. The initial step in the FAO spiral (labeled *1*) is the acyl-CoA dehydrogenase reaction that is catalyzed by the homologous enzymes MCAD, LCAD, and VLCAD and leads to formation of 2,3-enoyl-acyl-CoA. The second step (labeled *2*) is conversion of of 2,3-enoyl-acyl-CoA to 3-hydroxyacyl-CoA catalyzed by 2,3-enoyl-CoA hydratase. The 3rd step of the spiral (labeled *3*) is conversion of 3-hydroxyacyl-CoA to 3-ketoacyl-CoA catalyzed by the 2 homologous enzymes SCHAD and LCHAD, and the final step (labeled *4*) is removal of an acetyl-CoA from 3-ketoacyl-CoA by the 2 homologous enzymes SKAT and LKAT, respectively.

followed by a blocking buffer (NEN-Life Sciences, Boston, MA) for 30 min. The blocking buffer was removed, and the sections were exposed to primary rabbit polyclonal antisera raised against one of the following enzymes at the indicated dilution: MCAD (1:200), LCAD (1:400), VLCAD (1:200), SCHAD (1:200), LCHAD (1:400), LKAT (1:400), or human β-chorionic gonadotropin (β-HCG; Dako, Carpinteria, CA). The primary antibody was applied with 0.3% Triton X-100 in phosphate-buffered saline (PBS) overnight at 4°C. After two washes with PBS the next day, secondary goat anti-rabbit biotinylated antibody (NEN-Life Sciences) was applied at a concentration of 1:800 for 1 h at room temperature. The tertiary reagent was streptavidin-horseradish peroxidase (Dako) at a concentration of 1:1,000 for 1 h at room temperature followed by application of 3,3-diaminobenzidine substrate for 1–5 min. The slides were rinsed, counterstained with Mayer's hematoxylin, dehydrated in ethanol, cleared with xylene, and mounted with glass coverslips using Histomount (Zymed Laboratories). Two to five sets of placental tissue from term pregnancies were stained for all six FAO enzymes.

Western blot analyses. Placental tissue freed of maternal blood (100–250 mg) was lysed in a buffer containing 0.1 M sodium phosphate, 0.5 mM EDTA, and 0.5% Triton with protease inhibitors by means of a polytron. The placental lysate was sonicated three times for 10 s each on ice. The lysates were subjected to centrifugation at 3,000 *g* for 5 min, and the protein concentration of the supernatant was measured by the Bradford method. Fifty micrograms of protein were analyzed by immunoblotting with rabbit polyclonal antisera raised against one of the six different FAO spiral enzymes at the following dilutions: MCAD (1:1,000), LCAD (1:5,000), VLCAD (1:500), SCHAD (1:5,000), LCHAD (1:3,000), and LKAT (1:2,000). Incubation with secondary antibody (goat anti-rabbit, 1:1,000 dilution) and visualization with diaminobenzidine reagent were done until the protein bands were visible. Two to five blots were prepared for each enzyme, and a representative immunoblot was analyzed with an AlphaImager 3400 (Alpha Innotech, San Leandro, CA) using its AlphaEase image analysis software for densitometry. Densitometry data were subjected to statistical analysis to determine any relationship of gestational age to antigen expression.

Enzyme kinetics and metabolic flux studies. The activities of SCHAD, LCHAD, and LKAT in placental homogenates were measured as described (3, 35, 38). Trophoblast cells were harvested from four uncomplicated term pregnancies, and three 75-cm^2 flasks from each placenta were used for experiments. Metabolic flux studies were performed using tritiated water released from [9,10-^{3}H]palmitate and [9,10-^{3}H]-myristate in 24-well microplates (22, 24). The cells were grown for 20 h while being incubated with 22 μM [9,10-^{3}H]palmitate or 110 μM [9,10-^{3}H]myristate, respectively. Each experiment was run in triplicate, and in all assays, palmitic or myristic acids were complexed with defatted bovine serum albumin (0.45 mg/ml). By use of these tritium-labeled substrates, where ^{3}H is distributed equally between two adjacent carbon atoms, 75–100% of the label is converted to ^3H_2O during complete β-oxidation cycle in intact cytotrophoblast cells (22, 24, 36).

Statistical analyses. We made three enzyme activity measurements from each sample and compared a total of 28 samples with one another after categorizing them into gestational age groups; we used multivariate analysis of variance (MANOVA) with calculation of Wilk's λ and *P* values. Data were later subjected to Student-Newman-Keuls post hoc analysis for multiple comparisons using statistical software SPSS for PC, version 11.01.

We carried out univariate and multivariate regression analyses on densitometric data for the six FAO enzymes to assess the significance of regression, i.e., the relationship between gestational age and densitometric readings. The slope of regression was compared with zero; an R^2 value, *F* statistic, and significance (*P*) value were calculated for each slope. Data are presented as means ± SD unless stated otherwise; statistical significance was set at $P < 0.05$.

RESULTS

FAO pathway. Figure 1 shows the metabolic pathway of entry of fatty acids into the cell and their breakdown through the mitochondrial β-oxidation spiral. Among the six enzymes of the spiral studied here, the highly homologous enzymes MCAD, LCAD, and VLCAD catalyze the first step by using substrates of differing chain lengths. SCHAD and LCHAD, which are also highly homologous, catalyze the second and third reaction of the spiral, and LKAT performs the final cleavage step in this pathway.

Immunohistochemistry. We examined expression of these six enzymes by immunohistochemistry in 2–5 term human placentas (Fig. 2). Minimal nonspecific background staining was observed in control sections processed without primary antibody (Fig. 2*A*). After incubation with β-HCG antibody as a positive control (Fig. 2*B*), intense staining of the syncytiotrophoblast layer was observed and, as expected, there was no reaction in cells from the rest of chorionic villi. Figure 2, *C-H*, shows immunoreactivity for MCAD, SCHAD, LKAT, VLCAD, LCAD and LCHAD, respectively. The intensity of staining for all six enzymes was highest in syncytiotrophoblast and similar to that of β-HCG, suggesting abundant expression of all FAO enzymes. No specific immunoreactivity for any FAO enzyme was detected in the chorionic villous vessels or connective tissue. Although the highest levels of FAO enzyme immunoreactivity were in the syncytiotrophoblast layer of the chorionic villi, there was also detectable expression of all FAO enzymes in the villous cytotrophoblasts. These results show abundant and cell type-specific expression of the FAO enzymes in both syncytiotrophoblast and cytotrophoblast but not in core cells of the villi.

Expression and activity of FAO enzymes during human placental development. Figure 3 is a composite immunoblot to analyze expression of the six FAO enzyme antigens in placental villi by use of specimens from between 12 and 43 wk of gestation. Densitometric analysis of immunoblots indicated that expression of LCHAD, VLCAD, and SCHAD was 2- to 2.5-fold higher at the lower gestational ages of 12 and 17 wk compared with term placenta. LKAT expression was four- and threefold higher at 12 and 17 wk of gestation, respectively, compared with term placenta. Our multivariate regression analysis found an inverse correlation between expression of LCHAD, SCHAD, VLCAD, and LKAT with gestational age (R^2 = 0.65, 0.75, 0.51, and 0.73 for LCHAD, SCHAD, VLCAD, and LKAT, respectively). The slope of regression was significantly different from zero for LCHAD, SCHAD, VLCAD, and

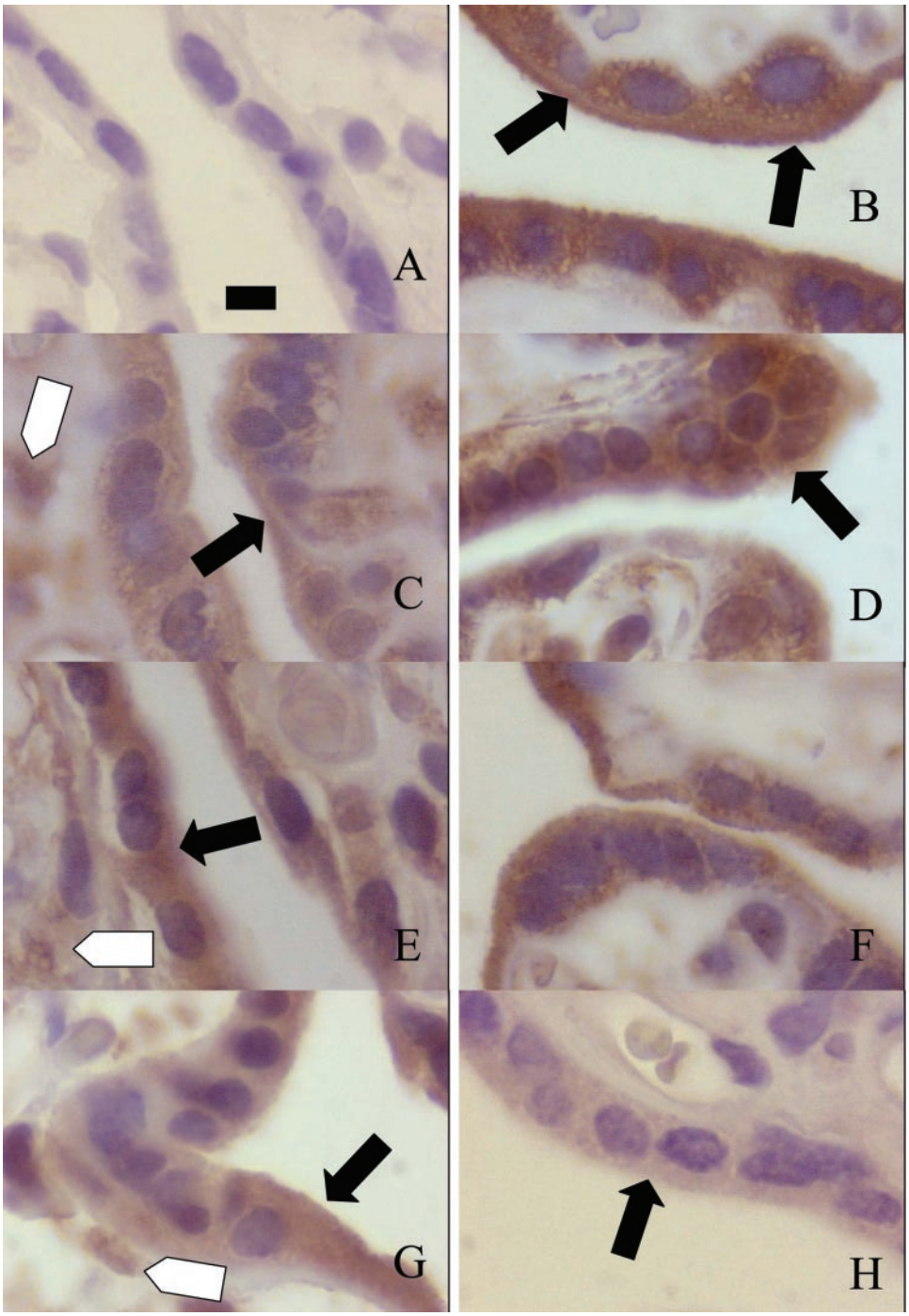

Fig. 2. Photomicrographs of FAO enzyme expression in term human placenta. Shown are immunohistochemical slides of placental villi from term human placenta (originally at ×100) stained by the peroxidase-diaminobenzidine method. *A*: negative control with no primary antibody. Bar, 20 μm. *B*: positive control stained for human β-chorionic gonadotropin (β-HCG) with anti-human monoclonal antibody demonstrating expression in the synctiotrophoblast layer (black arrows). C-H: staining for each of the 6 FAO enzymes. Localization of these 6 FAO enzymes in term villi is similar to that of β-HCG. FAO enzymes are expressed mainly in the synctiotrophoblast layer (black arrows), but there is detectable, lower expression in cytotrophoblasts (white arrows). *C*: incubation with rabbit anti-human MCAD polyclonal antibody; *D*: with LCAD antibody; *E*: with VLCAD antibody; *F*: with SCHAD antibody; *G*: with LCHAD antibody; *H*: with LKAT antibody.

LKAT, with *P* values of 0.008, 0.002, 0.02, and 0.002, respectively. There was no measurable difference among various gestational ages for MCAD and LCAD expression. Figure 4 shows LCHAD, SCHAD, and LKAT enzyme activities measured from extracts of 28 placental samples. The specific activities (nmol·min^{-1}·mg $tissue^{-1}$) were significantly higher for all three enzymes at lesser gestational ages (12–28 wk) compared with term placentas with MANOVA, with Wilk's λ of 0.290 and *P* value of 0.02. Groupwise comparisons using the Student-Newman-Keuls test showed a significantly higher activity of LCHAD and LKAT at 12–28 wk of gestation compared with term and postterm placentas and a significantly higher activity of SCHAD at 12–19 wk compared with term placentas. These results show that enzymatic activities are regulated during the course of placental development.

Tissue-specific activities of FAO enzymes. We compared the enzyme activities of LCHAD, SCHAD, LKAT, and CPT II in fresh, crude, placental tissue extracts with our previously published data of activities in fresh human liver and skeletal muscle extracts and from cultured fibroblasts from normal individuals

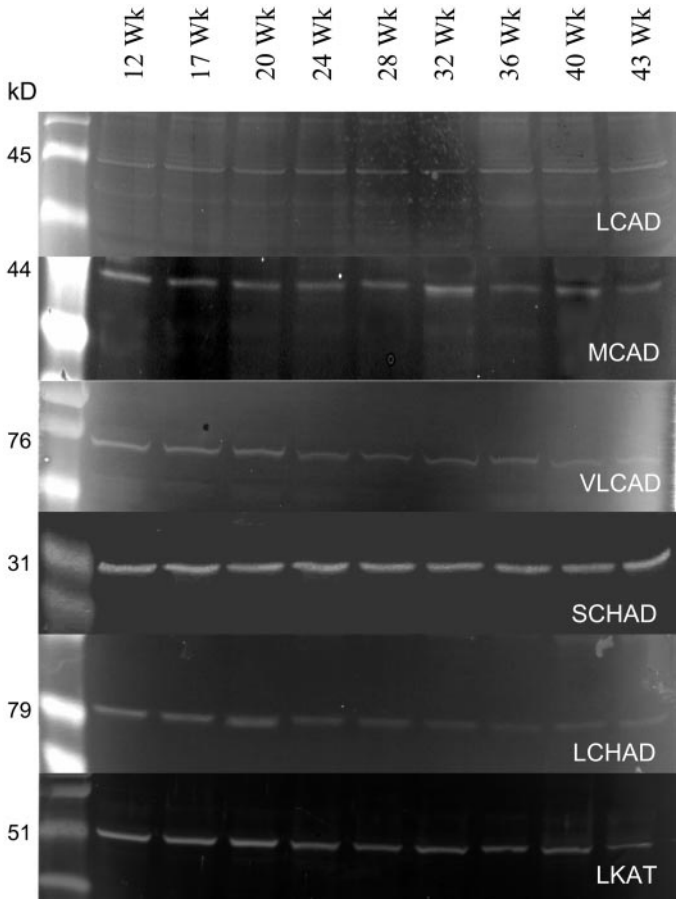

Fig. 3. Expression of FAO enzyme antigens in placentas of various gestational ages. Composite Western blots were generated to assess tissue expression of 6 FAO enzymes at gestational ages between 12 and 43 wk. Gestational ages of placentas from which protein extracts were prepared are shown above each lane at *top*. Positions of migration and mass (in kDa) of protein size markers are depicted at *left*. Each of the 6 panels was analyzed with antibody to the FAO enzyme indicated at *bottom right*. There was expression of all 6 enzymes throughout gestation, with greater expression at lower gestational age for LCHAD, SCHAD, VLCAD, and LKAT.

(2, 3, 38). The enzyme activities in placenta were two- to fivefold less than in liver throughout gestation. Compared with skeletal muscle, both LCHAD and LKAT, components of trifunctional protein that utilize long-chain substrates, were similar or higher in placental extracts from 12- to 19-wk-gestation pregnancies. FAO enzyme activities in crude placental extract were comparable to those in cultured human fibroblasts, but freshly isolated cytotrophoblasts from term pregnancies had substantially greater (2- to 8-fold) LCHAD, SCHAD, and LKAT activities than cultured fibroblasts. The enzyme activities in term trophoblast cells in primary culture were two- to threefold greater than those in fresh placental tissue from term pregnancies. These data show that placenta contains levels of FAO enzymes comparable to those present in mature, fatty acid-dependent tissues such as skeletal muscle, especially between 12 and 19 wk of gestation, and that cytotrophoblasts contain long-chain activities (LKAT and LCHAD) comparable to those of liver.

Fatty acid flux in term human cytotrophoblast cells in culture. Table 1 shows the results of metabolic flux studies in cytotrophoblasts with tritiated palmitic acid or myristic acid as substrates. Primary trophoblast cells cultured for 36–48 h used the two fatty acids as metabolic fuel, with overall oxidation of fatty acids 30–100% greater than fibroblasts in culture ($P < 0.05$).

DISCUSSION

Our results show that expression and activities of FAO enzymes as well as overall FAO of palmitate and myristate are substantial in human placental villi during gestation and identify trophoblast components as

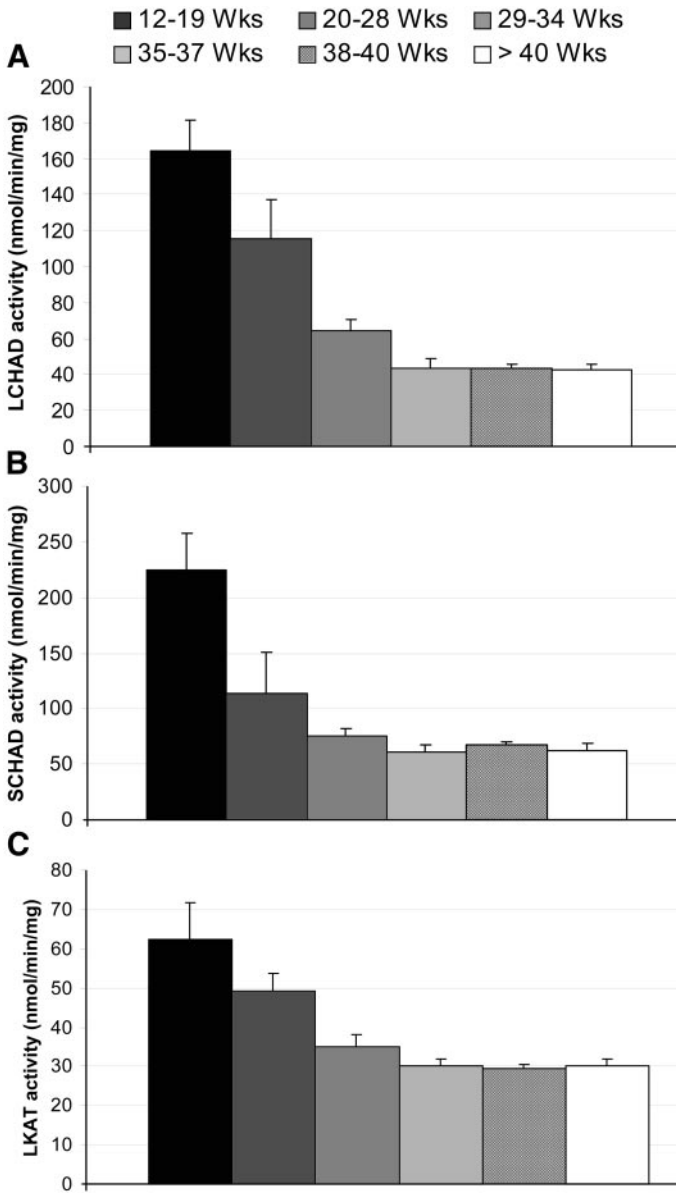

Fig. 4. FAO enzyme activities in human placentas of various gestational ages. Pooled placentas of the gestational ages indicated in the boxes were assayed for the 3 enzymes LCHAD (*A*), SCHAD (*B*), and LKAT (*C*) indicated on the abscissas. There was a higher activity of SCHAD, LCHAD, and LKAT at earlier gestational ages (12–28 wk) compared with term ($P < 0.024$).

Table 1. *Metabolic flux studies in human cytotrophoblast cells and fibroblasts in culture*

Tissue	Palmitic Acid	Myristic Acid
Fibroblast ($n = 34$)[3,35,38]	23.8 ± 3.0	19.0 ± 2.3
Cytotrophoblast ($n = 4$)	30.8 ± 29.7	38.3 ± 26.9

Data are means ± SD and are expressed as pmol·min^{-1}·mg protein^{-1} ($P < 0.05$ for myristic acid). References for normative data are shown in superscript nos.

the primary FAO sites in the term placenta. Expression of FAO enzymes and measured activities at lower gestational ages of 12–28 wk are comparable to that in mature slow skeletal muscle, a tissue that uses fatty acids as a substrate to satisfy high energy requirements. The data provide direct evidence that fatty acids undergo extensive mitochondrial β-oxidation in the placenta. Our major conclusion is that fatty acids are utilized as a significant metabolic fuel and energy source in this organ, consistent with our hypothesis. This overall conclusion is novel and at variance with the current concept that glucose is the sole energy source in the placenta (14, 16). On the basis of this conclusion, we speculate that human placental mitochondrial FAO is critical for normal growth and maturation of the placenta and for fueling the energy-consuming functions of ion, nutrient, and waste transplacental transport.

Our study's limitations include a lack of correlation between our in vitro data and in vivo FAO in the fetal-placental unit and some discrepancy between our Western blot and enzyme activity data. We are also limited by the fact that we used term normal placenta to extract trophoblast cells, and our metabolic flux data cannot be applied directly to trophoblast cells from earlier gestations. Thus our in vitro data need to be correlated with in vivo experiments, preferably in human subjects. FAO has been studied in humans by use of ^{13}C-labeled stable isotope technology, and a similar study model can be applied to pregnant women to elucidate our hypothesis more conclusively.

Our metabolic flux data for palmitate and myristate in Table 1 have high standard deviations, indicating variability in results. Variability among primary cultures is not uncommon, and trophoblast cells are no exception. Our comparison between fibroblasts, a well-studied approach to quantification of fatty acid oxidation in humans with suspected FAO disorders, and trophoblasts showed no statistical difference; the major conclusion from these studies is that trophoblasts indeed oxidize fatty acids. Because all cell lines were cultured in media containing glucose and not in physiological conditions present in vivo during fasting, we have shown that significant metabolic flux through the FAO pathway occurs even when glucose is the available energy substrate source. Our previous data in fibroblasts show that, when fatty acids are supplied as substrate, expression of FAO enzymes and flux through the β-oxidation pathway increases. Thus the flux measurement presented in Table 1 represents minimal values.

Previous studies are consistent with our conclusion that fatty acid uptake and metabolism are prominent in placenta. Lipoprotein lipase is highly expressed on the maternal surface of the syncytiotrophoblast and hydrolyzes maternal plasma triacylglycerol (17, 29). This enzyme activity would make long-chain free fatty acids available for uptake. Maternal triglyceride levels rise two- to threefold in late gestation, thereby increasing availability of fatty acids for uptake and metabolism (10, 17). Fatty acid-binding proteins that are critical for uptake of free long-chain fatty acids are also located on the microvillous membranes of the syncytiotrophoblasts facing the maternal circulation (1, 4, 5). This location favors unidirectional flow of maternal fatty acids into the placenta. Furthermore, the VLDL/apolipoprotein E receptor is positioned on the microvillous surface in human placental trophoblast cells, consistent with a role in placental lipid uptake and transport (40). Perhaps most importantly, carnitine, an essential factor for transfer of long-chain fatty acids from the cytosol to mitochondria for subsequent β-oxidation, is actively transported across the placenta by an organic cation/carnitine, sodium-dependent transporter (OCTN2) that is highly expressed in placental tissue (37). Thus previously reported results, in conjunction with our data, strongly suggest that the human placenta is capable not only of transporting fatty acids to the fetus but also of using them as a metabolic fuel.

Our major conclusion has important implications for understanding the known association of maternal liver diseases of pregnancy, AFLP, HELLP syndrome, placental floor infarction, and preeclampsia, with LCHAD or complete trifunctional protein deficiency in the fetus (11, 13, 15, 23, 27, 32, 33, 39). We (11, 13, 23) and others (33, 34) have shown that pregnancies carrying an affected fetus with the missense mutation G1528C in the α-subunit of mitochondrial trifunctional protein, a mutation that occurs in the active site of the LCHAD enzyme, have one of these maternal complications 75% of the time. This mutation is relatively common among individuals of northern European ancestry (11, 33), with a carrier frequency of 1 in 175 in the United States and 1 in 680 in the Netherlands (8). In LCHAD deficiency, accumulation of the long-chain hydroxyacylcarnitines, free plasma hydroxy-long-chain fatty acids, and dicarboxylic acids occurs. These metabolites are cytotoxic because they inhibit mitochondrial FAO enzymes, uncouple oxidative phosphorylation, and impair ATP production (7, 28, 30, 31). Long-chain acylcarnitines are also known to damage isolated canine myocyte sarcolemmal membranes and potentiate free-radical-induced lipid membrane peroxidative injury in ischemia (21).

We previously postulated (11) that long-chain fatty acids and their metabolites cause maternal liver damage in AFLP and other maternal liver diseases. However, we were puzzled, because the source of the postulated toxic metabolites was unclear given that the fetus does not utilize fatty acids for energy production. The data presented here provide a likely explanation of

the seemingly conflicting clinical findings, that is, that the LCHAD-deficient fetal-derived placenta may be the source of these harmful metabolites, particularly as the placental mass and energy requirements increase substantially during the third trimester. In addition to direct toxicity, contributing factors to maternal liver disease might be the 50% decrease in maternal hepatic long-chain fatty acid oxidation capacity related to maternal heterozygosity for LCHAD mutations and the increased liberation of fatty acids during the latter half of pregnancy due to increased lipoprotein lipase and increased reliance on fat as an energy source by the mother late in gestation. Thus we suggest that the placental FAO defect causes maternal liver disease in families with LCHAD or trifunctional protein (TFP) mutations.

A second implication of our findings is that placental insufficiency due to lack of energy production in pregnancies with FAO-deficient fetuses may occur. We (11, 13) and others (15, 27, 32, 33, 39) have noted that fetal growth restriction and prematurity are common among LCHAD-affected fetuses. In addition, we have shown that ablation of the trifunctional protein α-subunit in mice causes intrauterine growth restriction and perinatal lethality (12). Moreover, in VLCAD- and LCAD-deficient mice, late-gestation prenatal fetal death is common, despite the fact that the fetus does not rely on β-oxidation for energy (Exil VJ, Sims HF, Qin W, Roberts R, Rinaldo P, Zimmerman F, and Strauss AW, unpublished observations; 19). These results are all consistent with the hypothesis we posed: that placental FAO is critical for the health of the fetal-placental-maternal unit.

Other FAO defects may also be associated with maternal liver disease but only rarely. Single case reports of maternal liver diseases occurring during pregnancies with fetuses affected by CPT I and SCAD deficiency exist (15, 28). Three cases of maternal liver disease during pregnancies carrying fetuses with complete trifunctional protein deficiency have also been published (6). However, among families with MCAD deficiency, the commonest FAO disorder, and VLCAD deficiency, maternal liver diseases of pregnancy are extremely rare. This raises the possibility that 3-hydroxy- and other long-chain fatty acids that must accumulate in isolated LCHAD or complete TFP deficiencies are particularly toxic.

A second conclusion from our results is that FAO enzymes are expressed in a cell-specific manner within the placenta and that there is some developmental regulation of expression during gestation. Trophoblast cells from term placenta express key enzymes of the β-oxidation spiral, and expression was higher in the synctiotrophoblast layer than in the cytotrophoblasts (Fig. 2). The syncytiotrophoblast layer of chorionic villi plays an important role in the uptake of lipids, ions, and glucose into the placenta and their transfer to the fetus (17, 29), functions consistent with a large energy requirement. Our measurements revealed modestly higher enzyme activities at lower gestational ages (Fig. 4), emphasizing a key role for FAO early during gestation.

In summary, we have demonstrated the expression and activity of six enzymes involved in the FAO β-oxidation spiral in human placenta, with enzyme expression in a cell-specific manner localized to the syncytiotrophoblast layer, with lesser activity in the cytotrophoblast cells, and with no expression in villous core cells. Fatty acids are used as a major metabolic fuel by human placentas at all gestational ages, and any defect within this energy-producing pathway may hamper the growth, differentiation, and function of the placenta, thereby compromising fetal growth.

Defects of FAO in the fetal-placental unit are associated with accumulation of abnormal metabolic precursors, including hydroxyacylcarnitines and dicarboxylic acids. Such toxic fatty acids are likely transferred to the maternal circulation and may contribute to the pathophysiology of preeclampsia, AFLP, and HELLP syndrome in these families.

We gratefully acknowledge the help of Dr. Beverly Rogers with enzyme kinetics analysis, Karen Hutton with immunohistochemistry, and Prof. James K. Dias from the Medical College of Georgia for statistical data analysis.

This study was supported in part by Grants P30-DK-52574, AM-20407, and DK-02574 from the National Institutes of Health.

REFERENCES

1. **Abumrad N, Harmon C, and Ibrahimi A.** Membrane transport of long-chain fatty acids: evidence for a facilitated process. *J Lipid Res* 39: 2309–2318, 1998.
2. **Bennett MJ, Spotswood SD, Ross KF, Comfort S, Koonce R, Boriack RL, Ijlst L, and Wanders RJ.** Fatal hepatic short-chain L-3-hydroxyacyl-CoA dehydrogenase deficiency: clinical, biochemical, and pathological studies on three subjects with this recently identified disorder of mitochondrial β-oxidation. *Pediatr Dev Pathol* 2: 337–345, 1999.
3. **Bennett MJ, Weinberger MJ, Kobori JA, Rinaldo P, and Burlina AB.** Mitochondrial short-chain L-3-hydroxyacyl-CoA dehydrogenase deficiency: a new defect of fatty acid oxidation. *Pediatr Res* 39: 185–188, 1996.
4. **Campbell FM, Bush PG, Veerkamp JH, and Dutta-Roy AK.** Detection and cellular localization of plasma membrane-associated and cytoplasmic fatty acid-binding proteins in human placenta. *Placenta* 19: 409–415, 1998.
5. **Campbell FM, Taffese S, Gordon MJ, and Dutta-Roy AK.** Plasma membrane fatty-acid-binding protein in human placenta: identification and characterization. *Biochem Biophys Res Commun* 209: 1011–1017, 1995.
6. **Chakrapani A, Olpin S, Cleary M, Walter JH, Wraith JE, and Besley GTN.** Trifunctional protein deficiency: three families with significant maternal hepatic dysfunction in pregnancy not associated with the E474Q mutation. *J Inherit Metab Dis* 23: 826–834, 2000.
7. **Corkey BE, Hale DE, Glennon MC, Kelly RI, Coates PM, Kilpatrick L, and Stanley CA.** Relationship between unusual hepatic acyl-CoA profiles and pathogenesis of Reye syndrome. *J Clin Invest* 82: 782–788, 1988.
8. **Den Boer MEJ, Ijlst L, Wijburg FA, Oostheim W, Van Werkhoven MA, Van Pampus MG, Heymans HSA, and Wanders RJA.** Heterozygosity for the common LCHAD mutation (1528GC) is not a major cause of HELLP syndrome and prevalence of the mutation in the Dutch population is low. *Pediatr Res* 48: 151–154, 2000.
10. **Herrera E.** Metabolic adaptations in pregnancy and their implications for the availability of substrates to the fetus. *Euro J Clin Nutr* 54, *Suppl* 1: S47–S51, 2000.

11. **Ibdah JA, Bennett MJ, Rinaldo P, Zhao Y, Gibson B, Sims HF, and Strauss AW.** A fetal fatty-acid oxidation disorder as a cause of liver disease in pregnant women. *N Engl J Med* 340: 1723–1731, 1999.
12. **Ibdah JA, Paul H, Zhao Y, Binford S, Borgerink H, Salleng K, Cline M, Matern D, Bennett MJ, Rinaldo P, and Strauss AW.** Lack of mitochondrial trifunctional protein in mice causes neonatal hypoglycemia and death. *J Clin Invest* 107: 1403–1409, 2001.
13. **Ibdah JA, Zhao Y, Viola J, Gibson B, Bennett MJ, and Strauss AW.** Molecular prenatal diagnosis in families with fetal mitochondrial trifunctional protein mutations. *J Pediatr* 138: 396–399, 2001.
14. **Illsey NP.** Glucose transporters in the human placenta. *Placenta* 21: 14–22, 2000.
15. **Innes AM, Seargeant LE, Balachandra K, Roe CR, Wanders RJA, Ruiter JPN, Casiro O, Grewar DA, and Greenberg CR.** Hepatic carnitine palmitoyltransferase I deficiency presenting as maternal illness in pregnancy. *Pediatr Res* 47: 43–45, 2000.
16. **Kaufmann P and Scheffen I.** Placental development. In: *Fetal and Neonatal Physiology,* edited by Polin RA and Fox F. New York: Saunders, 1999, p. 47–56.
17. **Kimura RE.** Lipid metabolism in the fetal-placental unit. In: *Principals of Perinatal-Neonatal Metabolism* (2nd ed.), edited by Cowett RM. New York: Springer Verlag, 1998, p. 389–402.
18. **Knoop RH, Warth MR, and Charles D.** Lipoprotein metabolism in pregnancy, fat transport to the fetus and the effects of diabetes. *Biol Neonate* 50: 297–317, 1986.
19. **Kurtz M, Rinaldo P, Rhead WJ, Tian L, Millington DS, Vockley J, Hamm DA, Brix AE, Lindsey JR, Pinkert CA, O'Brien WE, and Wood PA.** Targeted disruption of mouse long-chain acyl-CoA dehydrogenase gene reveals crucial roles for fatty acid oxidation. *Proc Natl Acad Sci USA* 95: 15592–15597, 1998.
20. **Loftus TM, Jaworsky DE, Frehywot GL, Townsend CA, Ronnett GV, Lane MD, and Kuhajda FP.** Reduced food intake and body weight in mice treated with fatty acid synthase inhibitors. *Science* 288: 2379–1381, 2000.
21. **Mak IT, Kramer JH, and Weglicki WB.** Potentiation of free radical-induced lipid peroxidation injury to sarcolemmal membranes by lipid amphiphiles. *J Biol Chem* 261: 1153–1157, 1986.
22. **Manning NJ, Olpin SE, Pollitt RJ, and Webley J.** A comparison of [9,10-^{3} H] palmitic and [9,10-^{3} H]myristic acids for the detection of defects of fatty acid oxidation in intact cultured fibroblasts. *J Inherit Metab Dis* 13: 58–68, 1990.
23. **Matern D, Shehata BM, Shekhawat P, Strauss AW, Bennett MJ, and Rinaldo P.** Placental floor infarction complicating the pregnancy of a fetus with long chain 3-hydroxy-acyl-CoA dehydrogenase deficiency. *Mol Genet Metab* 72: 265–268, 2001.
24. **Moon A and Rhead WJ.** Complementation analysis of fatty acid oxidation disorders. *J Clin Invest* 79: 59–64, 1987.
25. **Nelson DM, Johnson RD, Smith SD, Anteby EY, and Sadovsky Y.** Hypoxia limits differentiation and up-regulates expression and activity of prostaglandin H synthase 2 in cultured trophoblast from term human placenta. *Am J Obstet Gynecol* 180: 896–902, 1999.
26. **Prip Buus C, Pegorier JP, Duee PH, Kohl C, and 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. *Biochem J* 269: 409–415, 1990.
27. **Schoeman MN, Batey RG, and Wilcken B.** Recurrent acute fatty liver of pregnancy associated with a fatty-acid oxidation defect in the offspring. *Gastroenterology* 100: 544–548, 1991.
28. **Tein I.** Metabolic disease in the fetus predisposes to maternal hepatic complications of pregnancy. *Pediatr Res* 47: 6–8, 2000.
29. **Thomas CR, Lowey C, and St. Hillaire RJ.** Studies on the placental hydrolysis and transfer of lipids to the fetal guinea pig. In: *Fetal Nutrition, Metabolism and Immunology: The Role of the Placenta,* edited by Miller RK and Tiede HA. New York: Plenum, 1984, p. 135–146.
30. **Tonsgard JH.** Serum dicarboxylic acids in patients with Reye syndrome. *J Pediatr* 109: 440–445, 1986.
31. **Tonsgard JH and Getz GS.** Effect of Reye's syndrome serum on isolated chinchilla liver mitochondria. *J Clin Invest* 76: 816–825, 1985.
32. **Treem WR, Rinaldo P, Hale DE, Stanley CA, Millington DS, Hyams JS, Jackson S, and Turnbull DM.** Acute fatty liver of pregnancy and long-chain 3-hydroxyacyl-CoA deficiency. *Hepatology* 19: 339–345, 1994.
33. **Tyni T, Ekholm E, and Pihko H.** Pregnancy complications are frequent in long-chain 3-hydroxyacyl-coenzyme A dehydrogenase deficiency. *Am J Obstet Gynecol* 178: 603–608, 1998.
34. **Tyni T, Palotie A, Viinikka L, Valanne L, Salo MK, Dobeln U, Jackson S, Wanders R, Vanizelos N, and Pihko H.** Long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency with the G1528C mutation: clinical presentation of thirteen patients. *J Pediatr* 130: 67–76, 1997.
35. **Venizelos S, Ijlst L, Wanders RJA, and Hagenfeldt L.** β-Oxidation enzymes in fibroblasts from patients with 3-hydroxydicarboxylic aciduria. *Pediatr Res* 36: 111–114, 1994.
36. **Wanders RJA, Ijlst L, Poggi F, Bonnefont JP, Munnich A, Brivet M, Rabier D, and Saudubray JM.** Human trifunctional protein deficiency: a new disorder of mitochondrial fatty acid β-oxidation. *Biochem Biophys Res Commun* 188: 1139–1145, 1992.
37. **Wang Y, Ye J, Ganapathy V, and Longo N.** Mutations in the organic cation/carnitine transporter OCTN2 in primary carnitine deficiency. *Proc Natl Acad Sci USA* 96: 2356–2360, 1999.
38. **Weinberger MJ, Rinaldo P, Strauss AW, and Bennett MJ.** Intact α-subunit is required for membrane-binding of human mitochondrial trifunctional β-oxidation protein, but is not necessary for conferring 3-ketoacyl-CoA thiolase activity to the β-subunit. *Biochem Biophys Res Commun* 209: 47–52, 1995.
39. **Wilcken B, Leung K-C, Hammond J, Kamath R, and Leonard JV.** Pregnancy and fetal long-chain 3-hydroxyacyl-CoA deficiency. *Lancet* 341: 407–408, 1993.
40. **Wittmaack FM, Gafvels ME, Bronner M, Matsuo H, McCrae KR, Tomaszewski JE, Robinson SL, Strickland DK, and Strauss JF.** Localization and regulation of the human very low density lipoprotein/apolipoprotein-E receptor: trophoblast expression predicts a role for the receptor in placental lipid transport. *Endocrinology* 136: 340–348, 1995.