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REVIEW ARTICLE

Acute fatty liver of pregnancy: an update on mechanisms

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Summary: Acute fatty liver of pregnancy (AFLP), characterized by hepatic microvesicular steatosis, is a sudden catastrophic illness occurring almost exclusively in the third trimester of pregnancy. Defective fatty acid oxidation in the fetus has been shown to be associated with this disease. Since the placenta has the same genetic makeup as the fetus and as AFLP patients generally recover following delivery, we hypothesized that the placenta might be involved in pathogenesis of this disease. In an animal model of hepatic microvesicular steatosis (using sodium valproate), we found that microvesicular steatosis results in mitochondrial structural alterations and oxidative stress in subcellular organelles of the liver. In placentas from patients with AFLP, we observed placental mitochondrial dysfunction and oxidative stress in subcellular organelles. In addition, defective placental fatty acid oxidation results in accumulation of toxic mediators such as arachidonic acid. Escape of these mediators into the maternal circulation might affect the maternal liver resulting in microvesicular steatosis.

Keywords: complications, hepatology, maternal–fetal medicine, maternal mortality, metabolism

INTRODUCTION

Five of the unique pregnancy-associated liver diseases are pre-eclampsia with hepatic impairment, haemolysis, elevated liver enzymes and low platelets (HELLP) syndrome, hyperemesis gravidarum, intrahepatic cholestasis of pregnancy and acute fatty liver of pregnancy (AFLP). AFLP is a sudden catastrophic illness which carries significant perinatal and maternal mortality with microvesicular fatty infiltration of hepatocytes causing acute liver failure with coagulopathy and encephalopathy. This requires early diagnosis and intervention to prevent maternal and fetal death.

Management of AFLP patients, their diagnosis, epidemiology, pathophysiology, clinical features and its implications have been extensively reviewed before.[1] The present review highlights the mechanisms of placental damage – mitochondrial dysfunction, oxidative stress in subcellular organelles and accumulation of toxic mediators due to defective placental fatty acid oxidation (FAO) – leading to maternal liver injury in AFLP.

CLUES TO THE MECHANISM OF AFLP FROM THE CLINICAL PRESENTATION

AFLP occurs in the third trimester of pregnancy

It has been well documented that AFLP occurs almost exclusively in the third trimester of pregnancy.[1–3] In the latter stages of pregnancy, the primary source of energy for the mother shifts to fats, while glucose is the primary energy substrate for the fetus.[4–6] If the mother had an inherited defect in fat metabolism, this defect would be expected to become clinically manifest in late pregnancy, when the maternal dependence on fats as the primary source of energy is at its peak. This is further reflected in the liver biopsy in AFLP patients, which is characterized by diffuse/peri-venular microvesicular steatosis, an uncommon histological finding.

Uncommonly, AFLP can present after delivery or marked deterioration of a mother with AFLP can occur after delivery. We speculate that the energy-depleted individual (harbouring a FAO defect which affects mitochondrial functioning leading to decreased ATP production) can be 'compensated' clinically. The added stress/energy requirement of undergoing labour (especially vaginal delivery) will aggravate this energy deficiency and the mother can 'decompensate', leading to worsening liver dysfunction and AFLP manifesting after delivery. In addition, although AFLP occurs mostly in primiparous women, it can also occur after several non-affected pregnancies. AFLP occurs more often in those pregnancies in which the fetus has a homozygous/compound heterozygous FAO mutation. Though all mothers of infants with FAO defects are obligate carriers of the FAO defect, it is not known why only 16% of these pregnancies develop complex maternal liver diseases of pregnancy.[7] Another illness, e.g. an infection, fasting or even emotional stress can trigger metabolic decompensation in a genetically predisposed individual.[8,9]

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Association of fetal FAO defects and AFLP

It has been shown that uncommonly, AFLP can recur in subsequent pregnancies.[2,10] In one report, both infants born in subsequent pregnancies to a woman developed a rapidly progressive illness characterized by widespread fatty infiltration of several vital organs leading to death at six months of age, suggesting a disorder of FAO.[2] These observations led to studies looking for inherited defects of FAO in the fetus, when the mother developed AFLP.

FAO disorders are a category of inborn errors of metabolism that are inherited in an autosomal recessive pattern. Several studies have reported that defects in mitochondrial β-oxidation, especially involving long chain hydroxyacyl CoA dehydrogenase (LCHAD), in the fetus are associated with AFLP,[2,11] and it was recently reported that 79% of pregnant mothers with LCHAD/mitochondrial trifunctional protein (MTP) deficient fetuses develop AFLP.[3] Screening infants of pregnancies complicated by liver disease for FAO disorders has been suggested.[12] However, it is now recognized that AFLP can occur without a mutation in genes that encode LCHAD or MTP,[7,13] and a few cases of maternal liver complications similar to AFLP have also been reported to be associated with fetal deficiency of carnitine palmitoyl transferase I (CPT1) or short or medium chain acyl-CoA dehydrogenase.[1,7] This suggests that the metabolic basis of AFLP is more heterogeneous than previously believed.

Complicating the issue further is the fact that an overlap exists between the various maternal liver diseases in the context of fetal FAO disorders, and studies examining fetal FAO defects and maternal liver disease typically include a variety of conditions such as AFLP, the HELLP syndrome and preeclamspsia evolving into HELLP syndrome. Though recent studies of the incidence of AFLP have used clinical criteria to diagnose AFLP (and in some recent studies these have been referred to as 'Swansea criteria' following the publication of a paper from Wales that described criteria commonly seen in women with AFLP[8,14]), there are scarce data comparing the clinical diagnostic criteria with liver biopsy findings in maternal liver diseases. Clinical criteria (e.g. Swansea criteria) for diagnosing AFLP as a predictor of hepatic microvesicular steatosis had 100% negative predictive value, though specificity was low at 57% when liver biopsy was also used. Hence, though the Swansea criteria are a good bedside screening tool for AFLP, significant overlap in diagnostic criteria is reported for these three maternal liver diseases.[15] Studies into the association of fetal FAO defects with maternal liver diseases characterized on liver biopsy are needed to better understand the association of different maternal liver diseases and fetal FAO.

AFLP dramatically improves after delivery of the baby

The maternal illness improves rapidly after delivery. Three patients who fulfilled Swansea criteria for AFLP underwent liver biopsy at two, five and eight days after delivery; none had hepatic microvesicular steatosis and the biopsies showed hepatocanalicular cholestasis (consistent with resolving phase of AFLP) in two patients and congestion and centrizonal necrosis in one patient.[15]

This recovery of AFLP patients from liver dysfunction immediately following delivery suggests a causative role for the placenta, which is expelled during delivery.

PLACENTAL FATTY ACID TRANSPORT AND OXIDATION

The placenta is an essential organ for the maintenance of pregnancy and development of fetus and it has direct access to the maternal blood for nutrients.[5] The placenta keeps the maternal and fetal blood supplies separate while allowing nutrient access to the fetus.

During pregnancy, it is estimated that the mother deposits approximately 3500 g of fat, which is the same weight of an average new born baby.[5] Maternal body fat increases linearly until around 30 weeks of gestation and slightly decreases after the 30th week with exponential increases in fetal fat accretion.[5] AFLP occurs predominantly in the third trimester of gestation. The decrease in the mother's fat stores is due to increased lipolysis and fatty acid (FA) transfer from the mother to the fetus through the placenta. This might possibly trigger increased lipid metabolism and mitochondrial FAO in the placenta as well as in the fetus.[3,5] FA uptake and efflux from placental syncytiotrophoblasts and the microvillous membrane are carried out by a number of membrane associated FA transport proteins (FATPs). Placental plasma membrane FA -binding protein and FATP 4 are mainly involved in the uptake of long chain FA such as docosahexanoic acid and other FA binding proteins like FABP 1, 3, 4, 5 and 7 have also been detected in the placental syncytiotrophoblast.[16] Two of the most important long chain polyunsaturated FAs such as arachidonic acid (AA) and docosahexanoic acid are markedly increased in the fetal circulation as well as fetal tissues such as brain due to their need for its development.[5]

Activation and acylation of FAs for β-oxidation occurs in the outer mitochondrial membrane by acyl CoA synthase and carnitine acyl transferase I to form acyl-carnitines. Carnitine acyl transferase II then transfers acyl-carnitine into the mitochondria through the mitochondrial inner membrane (Figure 1). Classical β-oxidation involves a four-step pathway, including dehydrogenation, hydration, dehydrogenation and thiolytic cleavage (Figure 1), the initial steps of which are catalysed by the MTP. The first dehydrogenation step is catalysed by acyl-CoA dehydrogenase activity of the enzyme, with formation of a double bond in the FA to form trans-Enoyl CoA. Next, the enoyl CoA hydratase activity hydrates the molecule to form beta hydroxyacyl CoA. This is then oxidized to beta ketoacyl CoA by the beta hydroxyacyl CoA dehydrogenase activity. The final step is catalysed by the ketothiolase activity, which generates an acetyl CoA molecule and an acyl CoA molecule which is two carbons shorter. The MTP has 4α and 4β subunits,[1] with the α-subunit having the long chain 3-enoyl CoA hydratase activity at its amino terminal and the LCHAD activity at the carboxy terminal domain. The β-subunit contains the long-chain 2-ketoacyl-CoA thiolase enzyme activity.[1] The α and β-subunits of the MTP are encoded by the HADHA and HADHB genes, both of which are localized on chromosome 2p23.[1]

Human placental tissue and its chorionic villus contain all eight FA oxidation enzyme activities. The activity of CPT II and very LCHAD are remarkably higher in placenta and chorionic villus compared with the human liver.[17] The other six

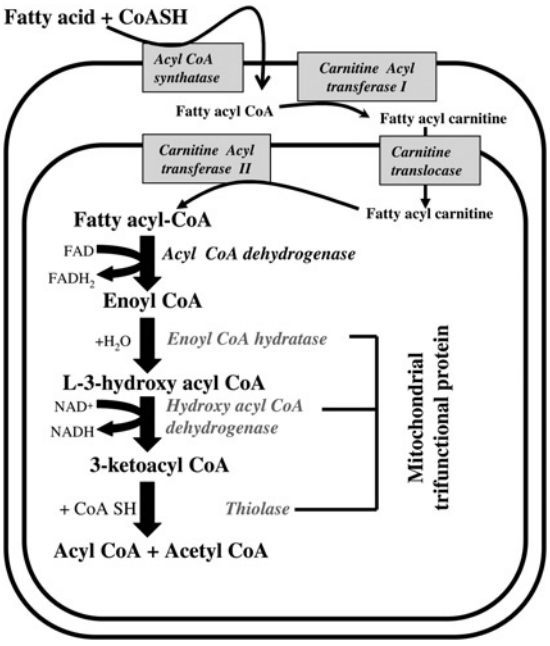

Figure 1 **Classical β-oxidation pathway in the mitochondria. Fatty acids (FAs) are activated to form fatty acyl CoA by the acyl CoA synthatase present in the outer mitochondrial membrane. To import FA into mitochondrial matrix, fatty acyl carnitine is formed by carnitine acyl transferase I and the carnitine translocase helps in transporting it into the mitochondrial matrix. Carnitine acyl transferase II converts fatty acyl carnitine back to fatty acyl CoA. β-Oxidation starts with the action of acyl-CoA dehydrogenase activity. The mitochondrial trifunctional protein, which consists of enoly CoA hydratase, hydroxyl acyl CoA dehydrogenase and thiolase activities leads to the formation of an acyl CoA molecule with two carbons less and acetyl CoA as products**

enzyme activities are two- to four-fold less compared with human liver FAO enzymes levels. It has also been well documented that all these FA oxidation enzymes are active in the placenta during the second and third trimester.[17] The FAO pathway in placenta occurs even in the presence of glucose as an energy source suggesting a metabolic shift from glucose to FA.[6] In addition to the decrease in FAO enzymes in the placenta, it has been reported that liver mitochondrial β-oxidation is also reduced from about 25% to 50% in pregnant mice.[18] The reason for this decrease in FAO in late gestation might be a consequence of female sex hormones.[18,19] It is thus possible that in disorders such as AFLP, the increased maternal lipolysis and defective FAO in the placenta in the third trimester of pregnancy leads to accumulation of toxic metabolites, which escape into maternal circulation and result in maternal liver damage.

PLACENTAL MITOCHONDRIAL DYSFUNCTION IN AFLP

The placenta is essential for fetal development and utilizes FAs as a significant metabolic fuel especially in the third trimester. FAO may involve alpha, beta and omega-oxidation. Children with LCHAD deficiency have been shown to have mitochondrial swelling, increased number and irregular mitochondrial cristae in the skeletal muscle.[20] Data from our laboratory have demonstrated ultrastructural and functional changes in liver mitochondria in an animal model of microvesicular steatosis.[21] We have also demonstrated placental mitochondrial dysfunction in patients with AFLP, accompanied by oxidative stress in the organelles.[22] It has been well demonstrated that 3-hydroxy-palmitoyl-CoA is an inhibitor of mitochondrial oxidative phosphorylation in both the placenta and the maternal liver.[23] Inhibition of oxidative phosphorylation could result in the generation of reactive oxygen species, which would further amplify damage to the placenta and its subcellular organelles. We have also reported increased levels of long chain FAs such as palmitic, arachidonic, oleic and myristic acid in the placenta of AFLP patients.[22]

This increase in long chain FA is suggestive of their defective oxidation and similarly increased levels of these FAs have been reported from LCHAD-deficient children.[3,24] Our data from patients with AFLP also showed an increase in circulating oxidative and nitrosative stress markers along with decreased antioxidants such as retinol and tocopherol (vitamin E),[22] suggesting that oxidative/nitrosative stress may also play a role in liver damage during AFLP. There was a similar increase in oxidative stress parameters both in placental subcellular organelles and in maternal serum. Increased levels of AA and palmitic acid were observed both in placenta as well as in maternal serum of AFLP patients.

HEPATIC MICROVESICULAR STEATOSIS AND OXIDATIVE STRESS

Hepatic microvesicular steatosis is the pathological hallmark of AFLP. Microvesicular steatosis occurs as a result of impaired mitochondrial β-oxidation which leads to accumulation of FAs in the form of triglycerides and formation of small lipid droplets in the cytosol of hepatocytes.[21] In the event of compromised mitochondrial function, oxidation of FAs is channelled to peroxisomal β oxidation, which, unlike mitochondrial FAO, generates hydrogen peroxide.[21] In addition, omega oxidation of FAs in microsomes forms long chain dicarboxylic acids, which are also a substrate for peroxisomal fatty acyl CoA oxidase.[25] The dicarboxylic acids formed can result in increased production of hydrogen peroxide,[21] which can undergo the Fenton's reaction in the presence of heavy metals to form highly reactive hydroxyl radicals and result in oxidative tissue damage. This seems to be occurring in the placenta from AFLP patients and in an animal model of hepatic microvesicular steatosis where oxidative stress was evident in peroxisomes and microsomes.[21,22] We have shown that valproate-induced microvesicular steatosis in rats results in oxidative stress as evidenced by increased lipid and protein oxidation parameters in the liver subcellular organelles. The lipid composition of subcellular organelles was also changed, with an altered cholesterol: phospholipid ratio. The reason for the increased oxidative stress in microvesicular steatosis is due to the generation of hydrogen peroxide and decreased peroxisomal catalase activity.[21] Another reason for the oxidative stress could be the fact that the presence of oxidizable fat in the liver either in acute or in chronic hepatic steatosis can trigger extensive lipid peroxidation. This has been proven in drug-induced steatosis produced by various agents such as valproate, ethanol, tetracycline, amiodarone or pirprofen.[26] In addition, we and others have shown that increased lipid oxidation can result in generation of malonaldehyde, conjugated diene and 4-hydroxynonenal, all of which can trigger hepatic steatosis.[21,26]

The hepatotoxic agent valproic acid can inhibit mitochondrial β-oxidation and produce microvesicular steatosis, which

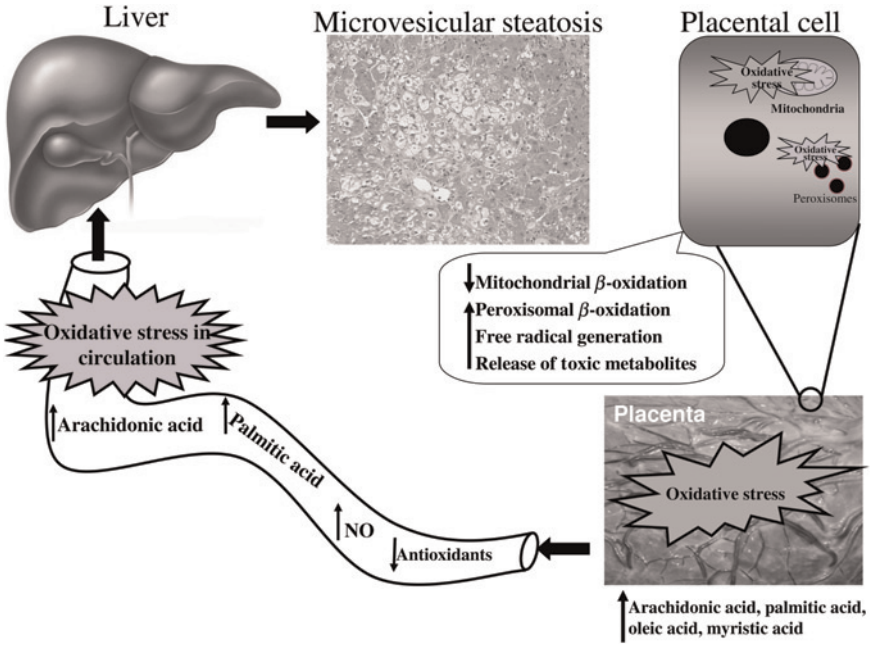

Figure 2 **Role of placental mitochondrial dysfunction and oxidative stress in the maternal liver injury. Defective fatty acid oxidation in the placenta results in accumulation of fatty acid (FA) in the placenta. Increased FA is directed to peroxisomal ω-oxidation resulting increased free radicals production. Release of free radicals, FA and its metabolites into circulation might damage maternal liver leading to microvesicular steatosis**

mimics maternal liver damage during AFLP.[21] Homozygous knockout mice for the MTP which catalyses oxidation of long chain FA develop hepatic steatosis and suffer neonatal death.[27] Mice heterozygous for MTP have a high fasting blood insulin and alanine amino transferase levels along with increased fat stain score and steato-necrosis.[27] These results suggest that mothers heterozygous for MTP might themselves activate hepatic steatosis during pregnancy. This condition is likely to be exacerbated by the generation of toxic metabolites from the placenta in the presence of a fetus homozygous for MTP deficiency in AFLP. From our laboratory, we have shown that a valproate-induced animal model of hepatic microvesicular steatosis develops liver mitochondrial dysfunction due to defective FAO. Increased FAs in the liver can be channelled to peroxisomes and microsomes for oxidation, resulting in the production of hydrogen peroxide that can lead to oxidative stress. In contrast to mitochondria, peroxisomal β-oxidation is not coupled with oxidative phosphorylation systems. Pretreatment with clofibrate (activator of peroxisome proliferators activated receptor-α) to rats prior to valproate administration offers partial reversal of changes occurring in the subcellular organelles, possibly by increasing microsomal and peroxisomal oxidation of FAs which decreases the fatty infiltration in the liver.[21]

IMPACT OF DEFECTIVE PLACENTAL FAO IN AFLP

The placenta consumes a large amount of oxygen and produces ATP for various metabolic process.[23] The placenta is also an important source of free radicals, with lipid and protein oxidation along with decreased antioxidant enzyme function being reported in the placenta of preeclamptic patients. Mitochondrial dysfunction of placentas from preeclamptic patients and its contribution to oxidative stress has been reported.[28] Rakheja *et al.*[24] suggested that in a pregnant mother heterozygous for a defect in FAO with a homozygous fetus, toxic FAO intermediates such as long chain hydroxyacyl metabolites could potentially accumulate. Since the placenta has the same genetic make-up as the fetus, these toxic intermediates can increase lipid peroxidation and decrease antioxidants in the placenta of AFLP patients. In addition, placental macrophages (Hofbauer cells) represent 40% of the placental cell population, and are located close to trophoblasts and fetal capillaries where they can also contribute to free radical generation.[16] However, until now, no study has addressed the significance of these cells in AFLP.

The cytotoxic FAO intermediates are known to inhibit mitochondrial enzymes, including those that catalyse β-oxidation and uncouple oxidative phosphorylation, impairing ATP production leading to damage to mitochondria such as mitochondrial swelling.[29] Elevated levels of metabolites of long chain FAs such as 3-hydroxyacyl carnitines, 3-hydroxyacyl CoAs and 3-hydroxydicarboxylic acids and concomitant decrease in blood carnitines in serum and urine in LCHAD-deficient patients have also been reported.[30] Recently, Eskelin *et al.*[3] reported an increase in long chain 3-hydroxyacyl carnitine molecules of C16 and C18:1 FA intermediates in the mother at the 31st gestational week compared with the 25th week of gestation.

It is now well known that since the placenta has the same genetic makeup as the fetus, it can accumulate toxic metabolites and their escape into maternal circulation can lead to maternal liver complications.[22,24] It has also been suggested that the toxic metabolites can activate the cytokine system and result in multiorgan failure.[23] Our results support the above hypothesis and

we have shown placental mitochondrial damage and increased long chain FAs in the plasma of AFLP patients. Our *in vitro* experiments also showed that increased levels of FAs such as AA (similar to AA levels seen in serum of AFLP patients) can initiate mitochondrial dysfunction, oxidative stress as well as increase the accumulation of fatty droplets in the hepatocyte in culture.[22] Studies of FAO mutations and metabolic changes in maternal serum and placenta in women who develop AFLP are needed to understand the situation better in humans *in vivo*.

In conclusion, in AFLP, defective placental FAO results in accumulation of toxic mediators such as AA and their escape into maternal circulation. In addition, placental mitochondrial dysfunction results in the generation of reactive oxygen and nitrogen species as well as peroxisomal hydrogen peroxide. Escape of free radicals and toxic intermediates of defective FAO into the maternal circulation might result in maternal liver damage seen during AFLP (Figure 2).

DECLARATION

Competing interests: None.

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