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Maternal dietary iron restriction modulates hepatic lipid metabolism in the fetuses

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Maternal dietary iron restriction modulates hepatic lipid metabolism in the fetuses. Am J Physiol Regul Integr Comp Physiol 288: R104–R111, 2005. First published September 23, 2004; doi:10.1152/ajpregu.00343.2004.—Maternal dietary Fe restriction reduced fasting plasma cholesterol and triglyceride (TG) concentrations in the fetuses, as well as decreased plasma TG levels in the adult offspring. To investigate how maternal Fe restriction was affecting fetal lipid metabolism, we investigated whether there were changes in liver lipid metabolism in the full-term fetuses. There was a ~27% (P < 0.05) increase in cholesterol but ~29% reduction (P = 0.01) in TG concentrations in the liver of the Fe-restricted fetuses. Hepatic mRNA levels of cholesterol 7α hydroxylase and liver X receptor-α (LXRα) were reduced by ~50% (P < 0.01) and ~34% (P < 0.01), respectively. As LXRα regulates expression of sterol response element binding protein-1c (SREBP-1c) expression, we measured SREBP-1c expression. There was an ~43% (P < 0.001) reduction in mRNA levels of SREBP-1c and its response genes, including acetyl-CoA carboxylase by ~35% (P = 0.01), fatty acid synthase by ~18% (P = 0.05), and diacylglycerol acyltransferase by ~19% (P = 0.03). Furthermore, protein levels of CD36 were reduced by ~27% (P = 0.02) in Fe-restricted fetuses. In conclusion, changes in liver cholesterol and TG concentrations in Fe-restricted fetuses may be coordinated through reduced expression of heme-containing cholesterol 7α hydroxylase and its regulator LXRα, mainly via down-regulation of expression of genes in bile acid synthesis and fatty acid synthesis pathways.

cholesterol; cholesterol 7 alpha hydroxylase; liver X receptor-alpha; fetus

ABNORMALITIES IN LIPID METABOLISM are associated with the metabolic syndrome, Type 2 diabetes and coronary heart disease (45). Plasma lipid profile may be affected by a change in maternal diet before birth, which has been referred to as the “fetal origins,” “thrifty phenotype,” or “fetal programming” hypothesis (3, 16, 17, 30). This hypothesis is supported by data from epidemiological and animal studies. For example, people who had a small abdominal circumference at birth had raised serum concentrations of total cholesterol and low-density lipoprotein-cholesterol and apolipoprotein B in adulthood (2). People exposed to famine in early gestation had a more atherogenic lipid profile than did those who were not exposed to famine in utero (38). In animal studies, maternal dietary protein restriction during either (both) gestation or (and) lactation reduces total cholesterol and high-density lipoprotein-cholesterol in the rat offspring (31). Increased dietary cholesterol intake in the mother during gestation increases long-term progression of atherosclerosis in the rabbit offspring (33), and treatment of the mother with vitamin E or cholestyramine during gestation markedly reduces the progression of atherosclerotic lesions in the aorta of these offspring (33). In the rat, maternal calorie restriction causes hyperphagia, obesity, and hypertension in the rat offspring (48), and maternal dietary Fe restriction reduces offspring plasma triglyceride (TG) concentrations (29). Thus these data suggest that a change in maternal physiology may have a long-lasting effect on lipid metabolism in the offspring.

Fe deficiency is a common nutritional disorder (51). As many as 4–5 billion people (66–80% of the world’s population) may be Fe deficient, and over 30% of the world’s population (2 billion) are anemic, mainly due to Fe deficiency (51). Pregnant women are among the most susceptible group (12). A number of studies have suggested links between Fe deficiency anemia and altered lipid metabolism; however, these studies do not all find the same effects. For example, children with Fe deficiency anemia have either increased plasma cholesterol concentrations (44) or reduced plasma cholesterol concentrations (8). In animal studies, maternal dietary Fe restriction during gestation either increases plasma lipid concentrations in the offspring (40) or has no effect on plasma cholesterol concentrations in the adult offspring (29). Thus the effect of maternal dietary Fe restriction on fetal lipid metabolism in the offspring requires more detailed investigation.

Changes in adult offspring may originate from changes occurred during fetal development. The liver plays a key role in modulating body lipid homeostasis, and hepatic expression of genes key to lipid metabolism is linked closely with plasma lipids. Thus the aim of this study was to investigate whether hepatic lipid metabolism in the fetuses was sensitive to maternal dietary Fe restriction during gestation. We hypothesized that, in parallel to altered plasma TG and cholesterol concentrations, expression of key genes relevant to TG and choles-

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terol metabolism in the fetuses would be affected by maternal dietary Fe restriction. To test this hypothesis, we have measured hepatic TG and cholesterol concentrations and changes in mRNA and protein levels from key genes regulating TG and cholesterol metabolism in the fetal liver.

MATERIALS AND METHODS

Experimental animals. Maternal and fetal data for this study have been published previously (28). All animal procedures were performed under license in accordance with the Animals (Scientific Procedures) Act (1986). Two groups of rats (n = 6), i.e., control vs. dietary-restricted group, were set up (28), and their fetuses were investigated at 21 days of gestation. The dietary-restricted group were housed individually and maintained at 22°C and fed ad libitum either a Fe-restricted diet (iron = 3 mg/kg diet; Hope Farms) or a control diet (made by adding iron at 150 mg/kg diet to the Fe-restricted diet; Hope Farms). The diets contained 17.5% crude protein, 5.2% crude fat, 66.2% sugar and starch, with a gross energy content of 4.03 kcal/kg diet. Animals were mated between 4 PM and 8 AM. Day 1 of gestation was determined to be the day that a vaginal plug was observed.

On day 21 of gestation, dams were anesthetized with halothane and then killed by decapitation. Fetal trunk blood was collected by using capillary tubes and pooled in tubes containing EDTA. An aliquot of whole blood was taken and centrifuged to prepare plasma. Plasma was stored at −20°C. Fetal livers from each dam were dissected out, pooled, and quickly frozen in liquid nitrogen and stored at −70°C.

Liver lipid extraction and analysis. Livers (∼60 mg) were homogenized in PBS, and protein concentration was determined. Three hundred microliters of homogenate were extracted with 5 ml of chloroform/methanol (2:1) and 0.5 ml of 0.1% sulfuric acid (9). An aliquot of the organic phase was collected, dried under nitrogen, and resuspended in ethanol. Hepatic TG and cholesterol content were determined by using commercially available kits. Data were normalized for differences in protein concentration.

Western blotting. Liver tissues (∼100 mg) were added with homogenized buffer containing 50 mM Tris·HCl (pH 7.6), 0.25% Triton X-100, 0.15 M NaCl, 10 mM CaCl₂, 0.1 mM phenylmethylsulfonyl fluoride, 10 μM leupeptin, 10 μM pepstatin A, 0.1 mM iodoacetamide, and 25 μg/ml aprotinin. The cell homogenates were spun at 15,000 g for 10 min at 4°C. The supernatants were transferred to Eppendorf tubes. Protein concentrations were measured by the Bradford method (28). Sixty micrograms of proteins were mixed with 5× sample buffer (5 ml of sample buffer contains 2.5 ml of glycerol, 1.25 ml of 2-mercaptopropanol, 0.5 g of SDS, 1.04 ml of 1.5 M Tris, pH 6.8, and 1.25 mg of bromphenol blue), boiled for 4 min, and loaded on 7% SDS-polyacrylamide mini-gels that were run at 200 V for 1 h. The gels were transferred on an Immobilon-P transfer membrane (Millipore, Bedford, MA) in 25 mM Tris, 192 mM glycine, and 20% methanol. After transfer, the membranes were blocked in 10 ml of TBST (50 mM Tris, 100 mM NaCl, and 0.1% Tween) and 5% dry milk containing primary antibody (Santa Cruz Biotechnology, Santa Cruz, CA). The membranes were incubated overnight at 4°C, washed, and then incubated for 1–2 h at room temperature with secondary antibody conjugated with horseradish peroxidase. Bands were visualized with an ECL kit (Amersham Pharmacia Biotech, Baie d’Urfe, QC). Distinct bands were quantified by densitometry and normalized for differences in protein concentration.

Table 1. List of PCR primers sequences for target genes

<table>
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<tr>
<th>Target Genes</th>
<th>PCR Primer Sequences (5'-3')</th>
<th>Sizes, bp</th>
<th>Access No.</th>
</tr>
</thead>
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<td>ACCa</td>
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<td>453</td>
<td>J03808</td>
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<tr>
<td></td>
<td>Antisense: GTTGTCACCATGATGTTTC</td>
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<td>AF072411</td>
</tr>
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<td>CD36</td>
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<td>448</td>
<td>J05430</td>
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<td>X62888.1</td>
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<td>450</td>
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<td>LXRα</td>
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<td>450</td>
<td>L16995</td>
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<tr>
<td>HMG-CoA R</td>
<td>Sense: TGTAGATGCTTCTGCTGCTA</td>
<td>450</td>
<td>L16995</td>
</tr>
<tr>
<td>SREBP-1c</td>
<td>Sense: GCACAGAGGAAGACAGGGAC</td>
<td>450</td>
<td>L16995</td>
</tr>
</tbody>
</table>

PCR primer binding sequences were identified for sense and antisense primers to give the amplified DNA products of ∼450 bp. Thus PCR products amplified from cDNA could be differentiated from those amplified from standard DNA competitor (size ∼385 bp) by gel electrophoresis. ACCa, acetyl-CoA carboxylase-α; CYP7α, cholesterol 7α hydroxylase; DGAT, diacylglycerol acyltransferase; FAS, fatty acid synthase; LXRα, liver X receptor-α; HMG-CoA R, 3-hydroxy-3-methylglutaryl-CoA reductase; PPARα, peroxisomal proliferator-activated receptor-α; SREBP, sterol response element binding protein.
nology). At the end of the incubation periods, the membranes were washed in TBST for 30 min at room temperature and then incubated for 1 h in TBST and 5% dry milk containing secondary antibodies conjugated to horseradish peroxidase (Santa Cruz, 1:4,000). The membranes were washed in TBST for 30 min at room temperature and then exposed to the Amersham enhanced chemiluminescent detection system, according to the manufacturer’s instructions.

To quantify intensities of protein bands from developed film, a picture was taken with a digital camera (UVP Led, Cambridge, UK) by placing the film on a white screen. The picture was saved on a floppy disk, and the intensity of the bands was quantified by using Phoretix 1 D advanced version 4.01 software (Newcastle upon Tyne, UK). Units of protein band intensities were only comparable within the same blot, but not comparable between different blots, as the film was exposed for different periods.

Preparation of total RNA. Total RNA was extracted from fetal liver tissues by using a Quick Prep total RNA extraction kit (Pharmacia Biotek), according to the manufacturer’s instructions. Total RNA was quantified by spectrophotometry. The integrity of the RNA was assessed by examination of 28S and 18S bands following gel electrophoresis. Only total RNA of good quality was used in quantitative analysis of mRNA expression.

Preparation of PCR primers. The PCR primers were designed so that the sizes of PCR products were ~445 bp (see Table 1 for primer sequences). The percentage of A/T was similar to that of C/G in primer sequences, and their melting temperatures were ~60°C. Sense and antisense primers spanned at least one intron, if possible. All primer sequences were searched against existing sequences submitted to the Genebank using blastn software at the National Center for Biological Information to ensure that each of the primer sequences did not form a good match with sequences of other genes. Two hundred micromolar stock solutions of synthesized primers were prepared in 1× Tris·HCl-EDTA buffer (10 mM Tris·HCl, 1 mM EDTA, pH 8.0).

Preparation of standard DNA and standard DNA dilution series. A fragment containing PCR primer binding sequences for 16 genes (Fig. 1) was constructed by using a technique of oligonucleotide overlap extension followed by PCR amplification (21). The construct (~800 bp; Fig. 1) was cloned into a pGEM-T easy vector (Promega, Southampton, UK) in Escherichia coli JM 109 strain, according to the manufacturer’s protocol. Colonies harboring the fragment were identified (the plasmid was designated as pR01). Plasmids (pR01) were prepared from bacteria grown from a single colony and digested with EcoR I enzyme to release the fragment containing PCR primer binding sequences. The construct fragment was gel purified, dissolved in H2O, and quantified by spectrophotometry.

A dilution series of standard DNA (sDNA) was prepared for quantification of mRNA levels (55). The pR01 sDNA dilution series was prepared by a sequential twofold dilution (55). For example, the concentrations for pR01 sDNA dilution series were 10.8, 5.4, 2.7, 1.35, 0.67, 0.33, 0.17 and 0.084 pM, and were amplified by PCR for 21–28 cycles. Thirteen target genes were analyzed by using pR01 dilution series. For the rest of the genes, sDNA for each single gene was prepared individually, and isomolar concentrations from each of these target genes were mixed (55). A second sDNA dilution series (MR02) with concentrations similar to pR01 sDNA dilution series were prepared. Thus the two sDNA dilution series were ready for analysis of mRNA levels by using medium-throughput quantitative competitive PCR (qcPCR).

cDNA synthesis. Five micrograms of total RNA in 50 μl of RNase-free H2O were denatured at 70°C for 5 min and then chilled to 4°C. Denatured total RNA was added with 0.5 mM dNTP mix, 5 μM oligo(dT)15 primer, 10 U/μl of Moloney murine leukemia virus reverse transcriptase (Promega, Southampton, UK), 1.0 U/μl RNasin (Promega) in 1× Moloney murine leukemia virus reverse transcriptase buffer [Promega; containing 50 mM Tris·HCl (pH 8.3 at 25°C), 75 mM KCl, 3 mM MgCl2, 10 mM DTT], and H2O to make a final volume of 100 μl. The cDNA reaction was incubated for 1 h at 42°C and stopped by heating at 95°C for 5 min.

mRNA quantification from multiple genes. mRNA levels were quantified by using a qcPCR (medium-throughput qcPCR) developed

Fig. 2. Plasma and liver lipid concentrations in the fetuses. A: see Ref. 28. Fasting plasma triglyceride (TG), cholesterol [total cholesterol (TC)], and free fatty acid (FFA) concentrations in the fetuses (n = 6 for both groups). Liver cholesterol (B) and TG (C) (n = 6 for both groups) concentrations in the fetuses were measured as described in MATERIALS AND METHODS. CN, controls; Fe-res, Fe restricted. Values are means ± SD. *P ≤ 0.05, **P ≤ 0.01.
in our laboratory which can detect a ~15% difference in mRNA levels (53–56). Briefly, cDNA (containing ~25 ng of total RNA), multiplex sDNA (concentrations depending on the cycle number to be amplified as indicated above), 0.4 μM gene-specific PCR primers for one gene, and 1× PCR Ready-Mix (containing 0.3 unit Taq polymerase, 10 mM Tris·Cl, 50 mM KCl, 1.5 mM MgCl2, 0.001% gelatin, 0.2 mM dNTP, and stabilizers) in a total of 10-μl reaction volume were added to each well in a 96-well plate. Reactions in the 96-well plate were amplified by a given PCR cycle under the following conditions: 4 min of preliminary heating at 94°C, followed by 50-s denaturation at 94°C, 50-s annealing at 57°C, and 1-min extension at 72°C, and a final 5-min extension at 72°C. For each gene analyzed, data were obtained from PCR reactions with four different cycle numbers, i.e., for simultaneous analysis of mRNA levels from six genes × 16 samples, four different PCR plates were set up, amplified by four different, increasing cycle numbers. The PCR reaction conditions between these plates were identical except that the concentrations of sDNA were reduced in relation to increasing PCR cycle numbers. The assay was repeated to verify the reproducibility of data.

**Data analysis.** After the PCR amplification was completed, 2.5 μl (6×) of DNA loading buffer was mixed with each PCR reaction, and 5 μl of each mixed PCR products were loaded on a prestained (in 1 μg/ml ethidium bromide in 1× Tris-borate-EDTA buffer for 20 min) microplate diagonal gel electrophoresis gel containing 5% polyacrylamide. After 70 min of running at 150 V, the gel was destained in H2O for 30 min. The picture was taken with a digital camera (UVP, Cambridge, UK), with the gel facing toward the ultraviolet transilluminator (the glass support facing up). The image of the gel was saved onto a floppy disk, and the fluorescence of DNA bands was analyzed by using Phoretix 1 D advanced version 4.01 (Newcastle upon Tyne, UK) software. The detailed procedure for data analysis has been described before (55). mRNA levels were calculated and presented as arbitrary units, assuming that the efficiency of cDNA synthesis is 100%.

**Statistical analysis.** All statistical calculations were performed by using SPSS (10.1) software. Differences in mean mRNA levels between different target genes were examined by unpaired Student’s t-test. Results are presented as means ± SD.

**RESULTS**

Phenotypic data for this study have been reported previously (28). A Fe-restricted diet fed to the mother 1 wk before and during the gestation period reduced fetal weight by ~19.1% (P < 0.005) (28). Plasma cholesterol and TG concentrations were decreased by ~32% and ~30%, respectively, in the restricted fetuses (P < 0.01 for cholesterol and P < 0.05 for TG, respectively, Fig. 2A) (28). However, plasma free fatty acid (FFA) concentrations were not significantly different between the two groups (P = 0.49, Fig. 2A) (28). In contrast, hepatic cholesterol (total cholesterol) concentrations were increased by ~27% (P = 0.028, Fig. 2B), whereas hepatic TG concentrations were reduced by ~29% in the Fe-restricted fetuses compared with the controls (P = 0.012, Fig. 2C).

Fig. 3. Expression of key genes in cholesterol biosynthesis. A: Western blotting analysis of sterol response element binding protein (SREBP)-2. Sixty micrograms of proteins were loaded per lane (n = 6 for both CN and Fe-res fetal liver), separated on a 10% SDS-PAGE, blotted onto polyvinylidene difluoride membrane, and probed with a polyclonal antibody against SREBP-2, as described in MATERIALS AND METHODS. Two bands were detected, which corresponded to the membrane-bound form (band I) and the processed form (band II). The intensity of these bands [arbitrary units (AU)] was quantified as described in MATERIALS AND METHODS. mRNA levels of HMG-CoA R (B) and lanosterol 14α-demethylase (CYP 51) (C) were quantified by quantitative competitive PCR. Equal amounts of cDNA from each sample (n = 6 for both CN and Fe-res fetal liver) were amplified with decreasing amount of standard DNA by increasing consecutive PCR cycle numbers (n = 4) as described in MATERIALS AND METHODS. For each sample to be analyzed, the mean value was calculated from 3–4 measurements to represent the mRNA levels of that sample. The values (units: amol/μg total RNA) presented either in the CN or Fe-res group were taken from mRNA levels of 6 samples. Values are means ± SD.
We then determined whether hepatic expression of key genes regulating cholesterol and TG metabolism was altered, in parallel to altered liver lipid concentrations using both a qcPCR technique developed in our laboratory (53–56) and Western blotting.

Sterol response element binding protein (SREBP)-2 is a key transcription factor regulating cholesterol metabolism. Both forms of protein levels of SREBP-2 were similar between the Fe-restricted fetuses and the controls (Fig. 3A). We did not measure SREBP-2 mRNA levels, as we could not locate mRNA sequences for SREBP-2 at the time of the study. mRNA levels from the rate-limiting enzyme in cholesterol biosynthesis, 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase (HMG-CoA R), were similar between the two groups (Fig 3B). mRNA levels of SREBP-2 at the time of the study.

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We have presented novel data showing that maternal dietary Fe restriction during gestation increased hepatic cholesterol, but reduced hepatic TG concentrations, in association with reduced plasma cholesterol and TG concentrations. Increased hepatic cholesterol concentration was linked with reduced gene expression in the bile acid biosynthesis pathway, as mRNA levels of CYP7α and LXRα were reduced in the restricted fetuses. In contrast, reduced hepatic TG concentrations were

**DISCUSSION**

We have presented novel data showing that maternal dietary Fe restriction during gestation increased hepatic cholesterol, but reduced hepatic TG concentrations, in association with reduced plasma cholesterol and TG concentrations. Increased hepatic cholesterol concentration was linked with reduced gene expression in the bile acid biosynthesis pathway, as mRNA levels of CYP7α and LXRα were reduced in the restricted fetuses. In contrast, reduced hepatic TG concentrations were
linked with reduced expression of genes in hepatic fatty acid and TG biosynthesis, as mRNA levels of SREBP-1c, ACC, FAS, and DGAT were all reduced. Hepatic expression of CD36, a fatty acid transporter, was also decreased in the Fe restricted fetuses. Thus maternal dietary Fe restriction has pleiotropic effects on hepatic lipid metabolism in the fetuses.

Increased hepatic cholesterol concentrations may be caused by increased cholesterol biosynthesis, or reduced conversion of cholesterol to bile acids, or reduced hepatic cholesterol output. Our data show that hepatic mRNA levels of HMG-CoA R, the rate-limiting enzyme of cholesterol biosynthesis (5), and that of CYP 51, which catalyzes the first step in the conversion of lanosterol into cholesterol (13), are not affected in Fe-restricted fetuses. Furthermore, protein levels of SREBP-2, a key transcription factor regulating HMG-CoA R expression (23), are similar between Fe-restricted fetuses and the control. Thus these consistent data suggest that expression of key genes in the cholesterol biosynthesis pathway is not affected in the Fe-restricted fetuses.

CYP7α is a key enzyme converting cholesterol into bile acid, which reduces the amount of cholesterol in the liver, and expression of CYP7α is regulated by LXRα (26, 49). Bile acid pool size and synthesis rates in the fetuses increase during the final third of gestation and the perinatal period. Bile acid secretion is a developing function during the final third of gestation and is incompletely developed at birth (27). The exact role of bile acid formation (and hence the function of CYP7α) in the fetus requires further investigation, as the fetus does not absorb fat from the diet. In adults, however, a mutation in the CYP7α gene, and hence a marked decrease in CYP7A1 activity, is associated with 70% increased hepatic cholesterol concentrations in humans (36). In LXRα-deficient mice, transcription of CYP7α is suppressed, and liver cholesterol concentrations are markedly increased (34). If similar mechanisms exist in the fetuses (i.e., reduced CYP7α mRNA producing reduced CYP7α activity and less cholesterol being converted to bile acid), reduced CYP7α mRNA may be relevant to increased hepatic cholesterol through downregulation of the bile acid synthesis pathway.

CYP7α is also linked to the assembly of very-low-density lipoprotein (VLDL) (25). In CYP7α-deficient mice, VLDL production is decreased (35), whereas overexpressing CYP7α in hepatoma cells increases the assembly and secretion of VLDL (25). As fetal plasma cholesterol is mainly derived from fetal hepatic cholesterol biosynthesis rather than from the maternal supply crossing the placenta (4, 24, 50), it is plausible to hypothesize that CYP7α regulation of VLDL secretion plays an important role in the fetus in regulating fetal circulation cholesterol concentrations. Thus reduced CYP7α mRNA will tend to increase hepatic cholesterol concentration in the fetus by decreasing VLDL (and cholesterol) secretion to the circu-
lation and reducing fetal circulation cholesterol concentration (which is indeed reduced in the Fe-restricted fetuses).

Unlike cholesterol, hepatic TG concentrations in the fetuses were reduced in Fe-restricted fetuses. Reduced hepatic TG concentrations may be due to increased hepatic fatty acid oxidation, reduced reesterification of FFA uptake from the circulation, and reduced fatty acid biosynthesis.

Liver TG concentrations in adults can be affected by altered gene expression regulating lipid oxidation such as PPARα and CPT-1 (19, 20). However, our data showing that hepatic CPT-1 and PPARα protein do not change in the Fe-restricted fetuses suggest that hepatic fatty acid oxidation in the fetuses is not affected, which is consistent with our data showing that plasma β-hydroxybutyrate levels are similar between Fe-restricted fetuses and the controls (28). Therefore, hepatic fatty acid oxidation in the fetuses may have little relevance to the reduced hepatic TG concentrations in the Fe-restricted fetuses.

Hepatic TG concentrations may be affected by reesterification of FFA uptake from the circulation (14). In adult liver, plasma FFA contributes to the bulk of hepatic TG concentration (14). Data from studies with perfused liver in rats show that liver has an extremely high capacity for TG synthesis from circulating FFA (39), and the accumulation of liver TG is proportional to the FFA concentrations (47). If we assume that a similar mechanism occurs in the fetal liver, hepatic TG concentrations may also be affected by circulating FFA concentrations. CD36 is a high-affinity fatty acid transporter and lipoprotein receptor (1, 6). Our data show that protein levels of CD36 are reduced in the Fe-restricted fetuses, which is consistent with our laboratory’s previous data (28) showing a nonsignificant reduction of ~12.5% in fasting plasma FFA concentrations in the Fe-restricted fetuses (which is consistent with reduced body weights, suggesting less body fat in these fetuses). Thus these data suggest a role of reduced CD36 protein levels linking a lower plasma FFA with reduced hepatic TG concentrations in the Fe-restricted fetuses.

SREBP-1c is a key transcription factor regulating hepatic fatty acid and TG biosynthesis via upregulating expression of key genes in fatty acid and TG biosynthesis, such as ACC, FAS, and DGAT (22, 42). Current evidence suggests that altered expression of SREBP-1c affects liver TG concentrations. For example, liver-specific knock out of SREBP-1c reduces liver and plasma TG concentrations (52), whereas overexpression of nuclear SREBP-1c in the liver in mice produces a TG-enriched fatty liver (42). Our data showing reduced expression of SREBP-1c and key genes in TG biosynthesis including ACC, FAS, and DGAT suggest that SREBP-1c may play a role in reduced TG biosynthesis in the Fe-restricted fetuses, which may be relevant to reduced hepatic TG concentrations in these fetuses.

Expression of SREBP-1c is regulated by LXRα (37). Administration of synthetic LXRα agonists upregulates expression of SREBP-1c and is associated with massive hepatic steatosis (15). CYP7α also plays a role in regulating hepatic expression of SREBP-1c. For example, in CYP7α-deficient mice, expression of SREBP-1c is reduced (35). In contrast, hepatoma cells overexpressing CYP7α increase expression of the mature form of SREBP-1c with a coordinate induction of lipogenesis (25). Thus reduced SREBP-1c mRNA levels in the Fe-restricted fetuses may be relevant to reduced LXRα and CYP7α. As we have discussed above, CYP7α and LXRα play an important role in regulating hepatic cholesterol metabolism. Taken together, these data suggest that reduced CYP7α and LXRα may play a role in coordinating changes in expression of genes in both hepatic TG and cholesterol pathways in Fe-restricted fetuses.

CYP7α is a heme-containing enzyme (43, 46). Here we show that reduced mRNA levels of CYP7α in the fetal liver are associated with maternal dietary Fe restriction. The exact mechanism underlying reduced CYP7α mRNA in the fetuses due to maternal dietary Fe restriction require further investigation. However, previous studies show that the reduction of hepatic Fe levels in the fetuses is almost in proportion to maternal dietary Fe restriction (11). As our Fe-restricted fetuses are anemic (28), it is likely that the Fe level is reduced in the Fe-restricted fetuses (although we have not measured liver Fe content), and reduced hepatic Fe content may be relevant to reduced CYP7α mRNA levels in the Fe-restricted fetuses.

In conclusion, maternal dietary Fe restriction during pregnancy increased hepatic cholesterol and reduced TG concentrations in the fetuses. Changes in hepatic cholesterol and TG concentrations may be coordinated through reduced expression of heme-containing CYP7α and its regulator LXRα, mainly via downregulation of expression of genes in bile acid synthesis and fatty acid synthesis pathways.

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REFERENCES

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MATERNAL DIET MODULATES FETAL HEPATIC LIPID METABOLISM


