Fetal liver X receptor activation acutely induces lipogenesis but does not affect plasma lipid response to a high-fat diet in adult mice

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van Straten EM, van Meer H, Huijkman NC, van Dijk TH, Baller JF, Verkade HJ, Kuipers F, Plösch T. Fetal liver X receptor activation acutely induces lipogenesis but does not affect plasma lipid response to a high-fat diet in adult mice. Am J Physiol Endocrinol Metab 297: E1171–E1178, 2009. First published September 1, 2009; doi:10.1152/ajpendo.00021.2009.—There is increasing evidence that the metabolic state of the mother during pregnancy affects long-term glucose and lipid metabolism of the offspring. The liver X receptors (LXR)α and -β are key regulators of cholesterol, fatty acid, and glucose metabolism. LXRα are activated by oxysterols and expressed in fetal mouse liver from day 10 of gestation onward. In the present study, we aimed to elucidate whether in utero pharmacological activation of LXR would influence fetal fatty acid and glucose metabolism and whether this would affect lipid homeostasis at adult age. Exposure of pregnant mice to the synthetic LXR agonist T0901317 increased hepatic mRNA expression levels of LXR target genes and hepatic and plasma triglyceride levels in fetuses and dams. T0901317 treatment increased absolute de novo synthesis and chain elongation of hepatic oleic acid in dams and fetuses. T0901317 exposure in utero influenced lipid metabolism in adulthood in a sex-specific manner; hepatic triglyceride content was increased (+45%) in male offspring and decreased in female offspring (~42%) when they were fed a regular chow diet compared with untreated sex controls. Plasma and hepatic lipid contents and hepatic gene expression patterns in adult male or female mice fed a high-fat diet were not affected by T0901317 pretreatment. We conclude that LXR treatment of pregnant mice induces immediate effects on lipid metabolism in dams and fetuses. Despite the profound changes during fetal life, long-term effects appeared to be rather mild and sex selective without modulating the lipid response to a high-fat diet.

fetus; lipid metabolism; gestation; long-term effects

IN HUMANS, THE NUTRITIONAL CONDITION during pregnancy has been shown to have a persistent effect on aspects of lipid and carbohydrate metabolism of the developing fetus. Maternal overnutrition leads to a higher incidence of insulin resistance, obesity, hypertension, and cardiovascular diseases in adult offspring (6). Effects of a high-fat maternal diet during pregnancy on long-term health status of the offspring are relatively well studied in animal models (3). However, relatively little is known about long-term consequences of targeted alterations in fetal lipid metabolism in utero.

The liver X receptors (LXR) have been identified as key players in the regulation of cholesterol, fatty acid, and carbohydrate metabolism in adult mammals, LXRα (NR1H3) and LXRβ (NR1H2) are members of the nuclear receptor superfamily of ligand-activated receptors. Both LXR isoforms are activated by oxidized cholesterol metabolites (oxysterols), with no LXRα/β specificity documented (19). Expression of LXRα is restricted mainly to liver, intestine, adipose tissue, and macrophages, whereas LXRβ is broadly expressed (22). Activated LXRs heterodimerize with ligand-activated retinoid X receptor (RXR) at LXR response elements present in the promoters of target genes to induce their transcription. Activation of LXRs induces expression of the sterol regulatory element-binding protein-1c (SREBP1c) that subsequently activates the lipogenic genes acetyl-CoA carboxylase (ACC), fatty acid synthase (FASN), and stearoyl-CoA desaturase 1 (SCD1) (28). It has been postulated that activation of LXR could induce carbohydrate response element-binding protein (ChREBP) and in this way indirectly influence glycolysis via influencing expression of liver-specific pyruvate kinase (PKLR) (7).

Expression of Lxra and Lxrb has been demonstrated in mouse fetuses from day 11.5 postcoitum onward (2); however, the functional role of LXRs during fetal development and in the newborn has not been defined. LXR does not appear to be essential for normal fetal development, because mice with targeted inactivation of Lxra, Lxrb, or Lxra/b (1, 17, 20) show normal fetal development.

Mechanisms by which fetal and early postnatal environment influence lipid and glucose homeostasis in adult life are poorly defined. We previously showed that treatment of pregnant mice with the widely used LXR agonist T0901317 affects cholesterol metabolism in the fetus. T0901317 treatment of pregnant mice activated LXR in the fetuses, resulting in induction of genes involved in fetal cholesterol metabolism. Interestingly, the treatment did not profoundly influence cholesterol metabolism at adult age (26). To delineate the functional importance of LXRs in control of fetal fatty acid and carbohydrate metabolism, we supplemented the diet of pregnant mice with T0901317. In the present study, we aimed to 1) determine whether pharmacological activation of Lxr in fetuses through maternal treatment with T0901317 activated Lxra or Lxrb in the fetal liver and influenced fetal fatty acid and glucose metabolism and 2) assess whether Lxr activation during fetal development changed lipid homeostasis into adulthood when mice were weaned onto standard chow or a high-fat diet. Our results indicate that maternal T0901317 treatment activates LXRs and strongly affects lipid metabolism in the fetus. The prenatal treatment had only relatively minor, sex-selective effects on lipid homeostasis in (young) adulthood and did not influence the response to a short-term high-fat diet.
MATERIALS AND METHODS

Animals. Animals were housed in temperature-controlled rooms (23°C) with 12-h light cycling and received standard RMH-B mouse chow (Arie Blok, Woerden, The Netherlands) and water ad libitum. Pregnant C57BL/6J mice were obtained from Harlan (Horst, The Netherlands) at 2 days postcoitum (dpc). Lxra+/− female mice (24) on a C57BL/6J background were crossed with Lxra+/− male mice on the same background in our laboratory to obtain offspring with Lxra+/+, Lxra−/−, and Lxra+/− genotypes, as described previously (26). All experimental procedures were approved by the Ethics Committee for Animal Experiments of the University of Groningen.

Experimental procedures. From day 10 postcoitum until day 1 after delivery, C57BL/6J wild-type females received standard chow only or chow supplemented with 0.015% wt/wt T0901317 (Cayman Chemicals, Ann Arbor, Michigan), as described in Ref. 26. On days 13.5, 15.5, 17.5, and 19.5, postcoitum pregnant C57BL/6J mice were anesthetized with isoflurane and terminated by heart puncture. Livers and intestines of fetuses and pups were collected, immediately snap-frozen in liquid nitrogen, and stored at −80°C. Blood was collected in EDTA-containing tubes. Liver samples of the dams were snap-frozen in liquid nitrogen. Fetuses were re-anesthetized with isoflurane and terminated by heart puncture. Lxra+/− females received the T0901317 diet from day 10 postcoitum until day 19.5 postcoitum and were terminated on day 19.5 postcoitum. Blood was collected in EDTA-containing tubes. Liver samples of the dams were snap-frozen in liquid nitrogen. Fetuses were terminated by decapitation and dissected. Pups were euthanized on day 1 postpartum. Blood samples were taken by exsanguination. Livers and intestines of fetuses and pups were collected, immediately snap-frozen in liquid nitrogen, and stored at −80°C until mRNA isolation or biochemical analysis. Samples for microscopic evaluation were snap-frozen in liquid nitrogen for Oil Red O staining. Pups were euthanized by 10.220.32.247 on June 3, 2017 http://ajpendo.physiology.org/ Downloaded from
thickness) capillary column (Phenomenex, Torrance, CA). The oven temperature was started at 100°C for 1 min, increased to 200°C at a rate of 50°C/min, then increased to 270°C at a rate of 5°C/min, and finally increased to 300°C at a rate of 50°C/min, and it remained at 300°C for 4 min. MS analysis was performed by electron capture negative ionization, with methane as a moderating gas. The ion monitored were \[m/z\] 255–259 corresponding to the \(m_0–m_4\) mass isotopomers for C16:0, \[m/z\] 269–273 for C17:0, \[m/z\] 283–287 for C18:0, and \[m/z\] 281–285 for C18:1.

Calculations on the newly synthesized polymers, the isotope enrichments of their monomer precursor (acetyl-CoA), and synthesis and chain elongation of stearic acid and oleic acid are described elsewhere (Ref. 21 and Oosterveer MH, unpublished observation).

RNA isolation and PCR procedures. Total RNA was extracted from frozen tissues with TriReagent (Sigma, St. Louis, MO) and quantified using a NanoDrop ND1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE). cDNA synthesis and real-time quantitative PCR were performed as described by Ploesch et al. (20). Primer and probe sequences for 18S, Srebp1c, Acc1, Fasn, glucokinase (Gck), glucose-6-phosphatase (G6pc), phosphoenolpyruvate carboxykinase (Pck1), liver-specific pyruvate kinase (Pklr) (12), Scd1 (24), Fxr, peroxisome proliferator-activated receptor-\( \gamma \) (Ppara) (15), Lxra, and Lxrb (26) have been published, with the exception of Rxra: sense 5'-GGCAAACATGGGGCTGAAC-3', antisense 5'-GCTTGTGCTGCTTTGACAGAT-3', and probe 5'-CCAGCTACACCAATGACCTGTGTTACCACAC-3' (accession no. NM_011305); and insulin receptor (Insr): sense 5'-TGGATCTAGGCATGTCCTCAGAA-3', antisense 5'-ACTACGGACATGTCGCTT-3', and probe 5'-CCATCGTGGTCGCCGCTT-3' (accession no. NM_010568).

Expression levels were normalized to those of 18S ribosomal RNA. 18S rRNA was analyzed in separate runs and not found to be significantly different between the experimental groups.

Histology. Liver histology was examined on frozen liver sections after Oil Red O staining for neutral lipids using standard procedures.

Statistics. Statistical analyses were performed using SPSS 14.0 for Windows (SPSS, Chicago, IL). Differences between the groups were analyzed by Kruskal-Wallis test followed by Mann-Whitney U-test. Data presented are means ± SD. A \( P \) value <0.05 was considered to be statistically significant.

RESULTS

T0901317 treatment to dams strongly induces genes involved in lipogenesis in dams and fetuses and acts mainly via Lxra. As described previously, dams receiving T0901317 from 10.5 dpc to 19.5 dpc had slightly lower body weights on day 19.5 of gestation compared to control dams (Fig. 2). Hepatic gene expression analysis revealed that T0901317 treatment to dams strongly induces genes involved in lipogenesis in dams and fetuses and acts mainly via Lxra (Fig. 3).
levels of lipogenic LXR target genes (Srebp-1c, HMG-CoA reductase) in heterozygous dams resulted in significantly lower expression of pooled plasma triglyceride levels (B) in the fetus compared with control dams (26). LXR activation in pregnant dams and fetuses on day 19 of gestation revealed that the reduction in triglycerides in the LXR-induced dams was mainly in VLDL-sized fractions (Fig. 3C).

Maternal T0901317 treatment increased hepatic triglyceride concentrations in the fetuses (Fig. 4A). However, on day 1 after delivery, triglyceride concentrations in livers of T0901317-exposed pups remained at (induced) fetal level, whereas concentrations in control pups surged. Fetal plasma triglyceride concentrations, measurable at 19.5 dpc and on day 1 after delivery, were significantly elevated upon LXR activation (Fig. 4B). Both in the control and in the T0901317-exposed pups, plasma lipid levels strongly increased after delivery. FPLC analysis showed a profile typical for fetal murine plasma and revealed that the increase in plasma triglyceride reflects elevated intermediate-density lipoprotein/LDL levels (Fig. 4C). Consistent with gene expression data, in Lxra knockout fetuses, T0901317 administration increased hepatic triglyceride concentrations to a lower extent compared with wild-type fetuses (~70% vs. ~150%, respectively, each P < 0.01; Fig. 4D). Oil Red O staining for neutral lipids confirmed lipid accumulation in treated wild-type pups on days 13.5, 15.5, 17.5, and 19.5 of gestation and on day 1 after delivery. Livers of control and T0901317-treated wild-type fetuses on day 17.5 and day 1 after delivery are shown in Fig. 5, A–D.

T0901317 treatment increases absolute synthesis and total pool of oleate in pregnant dams and fetuses on day 19.5. Using a [14C]oleate method, we quantified to what extent T0901317 treatment affected the de novo hepatic synthesis of palmitate, stearate, and oleate and the hepatic pool size of these fatty acids in dams and fetuses. T0901317 treatment increased total

![Fig. 4. Hepatic (A) and plasma and triglyceride levels (B) on several days of the gestation of fetuses. C] triglyceride levels in FPLC samples of pooled plasma (n = 20/group) of 19 days postcoitum (dpc) fetuses. D: hepatic triglyceride concentrations in wild-type and Lxra knockout fetuses on day 19 of gestation upon treatment of the heterozygote mother with T0901317. Black bars or solid line, control mice; open bars or dotted line, mice receiving 0.015% T0901317 in the diet. Values represent means ± SD; n = 6 dams/group. *P < 0.05 for treated vs. control; #P < 0.05 for knockout vs. wild type.
de novo synthesis and chain elongation of oleate (C18:1) in dams and fetuses on day 19.5 of gestation (Fig. 6, A and B) but did not significantly alter palmitate (C16:0) or stearate (C18:0) synthesis. Chain elongation of stearate was reduced in treated fetuses. In dams, T0901317 administration increased the total hepatic pool of oleate, and it increased both the oleate and the palmitate pool in fetuses (Fig. 6, C and D).

In utero Lxr activation does not influence basal insulin signaling. To test the hypothesis that Lxr activation influences carbohydrate metabolism in the wild-type mouse via induction of gene expression of Srebp1c and Chrebp and their subsequent target genes, we measured hepatic expression levels of several genes involved in carbohydrate metabolism in fetuses from untreated and treated wild-type dams. Treatment of dams with T0901317 had no influence on hepatic gene expression levels of G6pc, Pck1, Gck, Chreb, Pklr, or Insr in the fetuses (Supplemental Fig. S3, A–F). Since gene expression levels do not necessarily reflect physiological changes, we examined whether T0901317 treatment influenced insulin signaling in offspring. We performed an adapted insulin tolerance test in 1-day-old pups of treated and untreated dams. Intraperitoneal injection of 1-day-old pups with 0.75 U/kg insulin lowered
blood glucose levels by 50% compared with pups injected with saline. Prenatal T0901317 treatment of the dams did not influence this insulin response compared with controls.

Long-term effects of prenatal Lxr activation in offspring into adulthood. We determined whether T0901317 treatment of the dam had long-lasting effects in the fetuses, i.e., into adulthood. Previous studies have indicated that a perinatal programming phenotype may only become apparent in adulthood upon exposure to a metabolic challenge. We administered T0901317 from day 10 of gestation until day 1 after delivery to C57BL/6J females via the diet (0.015% wt/wt in chow). Nest sizes were comparable in the two treatment groups, with an average of six pups per nest. After weaning, offspring received chow until 6 wk of age; subsequently, offspring received either chow or a semisynthetic Western-type diet (high fat/high cholesterol) containing 15% (wt/wt) cacao butter and 0.25% (wt/wt) cholesterol for 2 wk.

Body weight, liver weight, and liver weight to body weight were described previously (26) and were similar in all male and all female offspring. In chow-fed offspring, gene expression levels of Srebp1c, Fasn, and Scd1 were not different between pretreated and untreated male offspring on control diet (Fig. 7A). However, control-fed females from T0901317-treated dams showed lower Fasn expression levels (~30%) compared with untreated females (Fig. 7B). Male offspring on control diet from treated dams had ~42% higher hepatic triglyceride levels than male offspring on control diet from control dams, whereas female offspring on control diet from treated dams had ~42% lower hepatic triglyceride levels than female offspring on control diet from untreated dams (Table 1). Plasma triglycerides in males and females were not influenced by T0901317 pretreatment (Table 1), although FPLC analysis showed that control male offspring receiving control diet had slightly higher VLDL levels than other male offspring groups (Fig. 7C). No change in triglyceride FPLC profiles was seen between the different female offspring groups (Fig. 7D).

The high-fat diet increased hepatic gene expression levels of the lipogenic LXR target genes Srebp1c and Scd1 in all male and female offspring regardless of exposure to T0901317 during gestation (Fig. 7, A and B). Interestingly, a sex specificity was observed concerning Fasn expression. Fasn expression levels were significantly higher in high-fat male offspring from untreated dams compared with control-fed male offspring from untreated dams (+200%, P = 0.017), whereas Fasn expression levels were not upregulated by the high-fat diet in pretreated male offspring compared with pretreated control males (P = 0.247). Similarly, females pretreated with T0901317 showed no upregulation of Fasn upon receiving the high-fat diet. In all male offspring, hepatic triglyceride levels were increased in animals that received the high-fat diet independently from pretreatment with T0901317 (Table 1). The high-fat diet did not lead to higher hepatic triglyceride levels in female offspring. Feeding the high-fat diet did not significantly change

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Table 1. Parameters of adult offspring

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<tr>
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<th>Hepatic TG, umol/g</th>
<th>Plasma TG, mM</th>
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<tr>
<td></td>
<td>CON T0901317 CON T0901317</td>
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<tr>
<td>Male CON</td>
<td>2.4 ± 0.6</td>
<td>0.43 ± 0.08</td>
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<tr>
<td>Male HFHC</td>
<td>6.6 ± 1.2</td>
<td>6.7 ± 2.1</td>
</tr>
<tr>
<td>Female CON</td>
<td>11.1 ± 2.3</td>
<td>6.4 ± 2.9</td>
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<tr>
<td>Female HFHC</td>
<td>11.6 ± 4.5</td>
<td>9.5 ± 2.0</td>
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Data are means ± SE. TG, triglyceride; CON, control; HFHC, high fat/high cholesterol. Parameters of C57BL/6J/OlaHsd offspring of mothers that received either chow or high-fat/high cholesterol. Pretreatment levels were significantly higher in high-fat male offspring from untreated dams compared with control-fed male offspring from untreated dams (+200%, P = 0.017), whereas Fasn expression levels were not upregulated by the high-fat diet in pretreated male offspring compared with untreated control males (P = 0.247). Similarly, females pretreated with T0901317 showed no upregulation of Fasn upon receiving the high-fat diet. In all male offspring, hepatic triglyceride levels were increased in animals that received the high-fat diet independently from pretreatment with T0901317 (Table 1). The high-fat diet did not lead to higher hepatic triglyceride levels in female offspring. Feeding the high-fat diet did not significantly change

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Fig. 7. Changes in relative hepatic gene expression in offspring of mice fed chow containing 0.015% T0901317 or control chow during gestation and FPLC profiles of this offspring. The offspring received either chow or a high fat/high cholesterol diet from 6 until 8 wk of age. Data are means ± SD; n = 6. *P < 0.05 HFHC vs. CON; †P < 0.05 T0901317 vs. CON.
plasma triglyceride levels or FPLC profiles in either males or females from chow-fed or T0901317-fed dams (Table 1 and Fig. 7, B and C).

**DISCUSSION**

We previously showed that targeted treatment of pregnant mice with the synthetic LXR agonist T0901317 activates Lxr in the fetuses. In the present paper, we investigated whether in utero Lxr activation by T0901317 changes fatty acid and glucose metabolism in the fetus and in adult offspring. Our data show that this treatment results in acute effects on lipid homeostasis in dams and fetuses, but not on glucose homeostasis in fetuses, and in relatively minor but persistent consequences on fatty acid metabolism during adulthood.

Supplementation of the diet of the dam with T0901317 induced LXR target genes Srebp1c, Acc1, Fasn, and Scd1 in the fetal liver, suggesting that T0901317 transported across the placenta activates Lxr. We previously showed that maternal T0901317 administration did not influence fetal hepatic gene expression levels of Lxra or -b (26). Our present results in Lxra-knockout fetuses show that hepatic Lxr effects are caused mainly by Lxra and that Lxb compensates about 50% of Lxr action in the absence of Lxra.

Lxr-specific effects on lipid metabolism in the mother and in the fetus are comparable with the effects seen in nonpregnant adult mice (20, 23). T0901317 administration increased hepatic triglyceride levels in dams, which was similar to studies in adult male mice (12, 14). Hepatic triglyceride accumulation decreased during gestation in treated dams, possibly by increased transport from the liver, either to other tissues or to the rapidly growing fetus. The hepatic lipid concentrations in 1-day-old pups varied considerably within one group. Possibly, this is caused by the variation in feeding time of the pups. Prolonged fasting leads to hepatic lipid accumulation (25). Because we could not control the breastfeeding times by the mother, some pups could have been fasted for a longer time than other pups, leading to higher liver triglyceride concentrations compared with pups that were fed prior to termination.

Grefhorst et al. (11) found no difference in plasma triglycerides in wild-type male mice treated with T0901317 compared with controls. T0901317-treated dams showed a lowering of plasma triglycerides compared with controls, representing a decrease in VLDL levels. Since plasma triglycerides in fetuses and pups from treated dams were increased, this seems to imply that treated dams transfer more lipid to the fetus compared with controls. Further studies have to elucidate how LXR activation influences lipid transport to the fetus.

Treated dams and fetuses showed an increase in absolute hepatic synthesis of oleic acid. Based on the [13C]acetate method used and the absence of increased palmitate and stearate synthesis, we interpreted that this observation is due to increased palmitate elongation and subsequent desaturation by Scd1. Scd1 catalyzes the synthesis of monounsaturated fatty acids from saturated fatty acids (10). Scd1 is a target gene of Lxr, and its expression was increased in maternal and fetal liver upon T0901317 exposure.

Apart from the well-characterized role of Lxr in cholesterol metabolism, a potential role controlling glucose homeostasis via activation of Srebp1c and Chrebp has been suggested (9, 16), although this role remains controversial (8, 18). Using an adapted insulin tolerance test in 1-day-old pups, no effect of the T0901317 pretreatment was found on glucose levels. Our results show that Lxr activation did not quantitatively influence the hepatic expression of any of the tested genes related to glucose metabolism in the fetus.

We next investigated whether Lxr activation by T0901317, which induced profound changes in lipid metabolism in perinatal life, also had long-lasting effects in lipid homeostasis, i.e., into adulthood. Female chow-fed offspring from T0901317-treated dams showed lower Fasn expression levels and lower hepatic triglyceride levels compared with untreated chow-fed female offspring. Strikingly, although no changes were seen on gene expression levels, in the male chow-fed offspring hepatic triglyceride levels were higher than in untreated chow-fed male offspring. Apparently, treatment with T0901317 in utero reduces hepatic storage of triglycerides in female offspring, whereas it induces hepatic storage of triglycerides in male offspring.

The subtle changes in adult lipid metabolism caused by in utero Lxr activation could be enlarged by receiving a high-fat diet at adult age. As expected (5), short-term (2 wk) high-fat feeding led to higher expression levels of lipogenic genes in all offspring and higher hepatic triglyceride levels in male offspring. However, these effects were present regardless of in utero treatment with T0901317. Apparently, receiving a high-fat diet for 2 wk only eliminates rather than increases the sex-specific effects caused by in utero Lxr activation. On the basis of this observation, it is tempting to speculate that long-term high-fat feeding would possibly override the relatively small effects of the prenatal treatment.

We conclude that Lxr activation by T0901317 treatment of pregnant mice induces immediate effects on lipid metabolism in dams and fetuses, including increases in fatty acid synthesis and triglyceride levels. Lxr activation did not significantly influence expression of carbohydrate metabolism-related genes or the glucose response to insulin administration. Despite profound changes in lipid metabolism during fetal life, long-term effects on lipid metabolism appear to be rather mild and not related to the lipid response to a high-fat diet.

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**GRANTS**

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