Liver X receptors regulate de novo lipogenesis in a tissue-specific manner in C57BL/6 female mice

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Korach-Andrè M, Archer A, Gabbi C, Barros RP, Pedrelli M, Steffensen KR, Pettersson AT, Laurencikiene J, Parini P, Gustafsson JA. Liver X receptors regulate de novo lipogenesis in a tissue-specific manner in C57BL/6 female mice. Am J Physiol Endocrinol Metab 301: E210–E222, 2011.—The liver X receptors (LXRs) play a key role in cholesterol and bile acid metabolism but are also important regulators of glucose metabolism. Recently, LXRs have been proposed as a glucose sensor affecting LXR-dependent gene expression. We challenged wild-type (WT) and LXRoβ−/− mice with a normal diet (ND) or a high-carbohydrate diet (HCD). Magnetic resonance imaging showed different fat distribution between WT and LXRoβ−/− mice. Surprisingly, gonadal (GL) adipocyte volume decreased on HCD compared with ND in WT mice, whereas it slightly increased in LXRoβ−/− mice. Interestingly, insulin-stimulated lipogenesis of isolated GL fat cells was reduced on HCD compared with ND in LXRoβ−/− mice, whereas no changes were observed in WT mice. Net de novo lipogenesis (DNL) calculated from VO2 and VCO2 not in LXR groups, but insulin sensitivity was decreased by the HCD in WT but increased in LXR−/− mice. Glucose tolerance was not different between groups, but insulin sensitivity was decreased by the HCD in WT but not in LXRoβ−/− mice. Glucose tolerance was not different between groups, but insulin sensitivity was decreased by the HCD in WT but not in LXRoβ−/− mice. Finally, gene expression analysis of adipose tissue showed induced expression of genes involved in DNL in LXRoβ−/− mice compared with WT animals as opposed to the liver, where expression of DNL genes was repressed in LXRoβ−/− mice. We thus conclude that absence of LXRs stimulates DNL in adipose tissue, but suppresses DNL in the liver, demonstrating opposite roles of LXR in DNL regulation in these two tissues. These results show tissue-specific regulation of LXR activity, a crucial finding for drug development.

high carbohydrate; energy regulation; metabolism; insulin resistance

Obesity and associated complications such as cardiovascular diseases and diabetes represent a major health problem in Western countries. The molecular mechanisms behind metabolic disorders are poorly understood but typically involve deregulation of cholesterol, lipid, and carbohydrate metabolism as well as impaired insulin signaling. The principal function of insulin is to maintain blood glucose and nonesterified fatty acid (NEFA) concentrations nearly constant despite the fluctuations during dietary intake of carbohydrates and lipids. De novo lipogenesis (DNL), i.e., the synthesis of fatty acids from nonlipid substrates, mainly carbohydrates, is nutritionally regulated. Both glucose and insulin signaling pathways are engaged in response to dietary carbohydrates to synergistically induce expression of glycolytic and lipogenic genes in the liver (12).

Liver and adipose tissue are the two main sites of DNL in mice (32, 46). In humans, DNL capacity of adipose tissue remains controversial. Diraion et al. (14) found that a high-carbohydrate diet (HCD) in humans does not upregulate DNL or expression of lipogenic genes in adipocytes, whereas hepatic DNL is increased two- to threefold. Letexier et al. (32) concluded that DNL is reduced in human adipose tissue compared with rodents due to a lower abundance of sterol-responsive element-binding protein-1c (SREBP-1c) protein (32). This is in conflict with Swierczynski et al. (46), who showed that the difference between DNL capacity of human and rat adipose tissue was negligible when humans and rats were studied while receiving comparable diets.

The nuclear receptors liver X receptor (LXR)α and β play an important role in metabolic homeostasis and are involved in many physiological events. Data obtained in different experimental models show that LXRs are involved in metabolic disorders (7). Several studies have shown that LXRoβ−/− mice are resistant to diet-induced obesity (27, 29). However, despite the wealth of data on the metabolic impact of LXRs, the physiological role played by LXRs in regulation of glucose metabolism and fat storage remains unclear. Mitro et al. (36) proposed glucose as a direct LXR agonist at physiological concentrations, and Anthonisen et al. (2) recently suggested that O-GlcNAcylation of LXRs is a potential mechanism by which LXRs act as a glucose sensor. However, Denechaud et al. (11) demonstrated that LXRs are not required for the induction of glucose-regulated genes in mouse liver. Several studies have shown that LXRs stimulate DNL in mouse liver (8, 42, 54) and fetus (51). Recent evidence suggests that the regulation of hepatic lipogenic gene expression by insulin is mediated by SREBP-1c and in part by LXRs, whereas the effects of glucose and glucagon are suggested to be mediated by the carbohydrate response element-binding protein (ChREBP) (22, 49). Less information is available concerning the regulation of DNL in adipocytes, although it has been shown that the insulin-responsive glucose transporter GLUT4 is directly regulated by LXRs (10, 31).

Available data demonstrate the complexity of regulation of glucose and lipid metabolism and that the role of LXR signaling in this context is not yet fully understood. In addition,
LXRs have been shown to play an important role in insulin secretion/action (17, 29). We thus hypothesize that LXRs may be significant in the regulation of glucose homeostasis by modulating insulin sensitivity. Therefore, in this study we have compared the effects of a 3-wk normal diet (ND) with those of an HCD on metabolic homeostasis in wild-type (WT) and LXR-deficient (LXReβ−/−) 10-wk-old female mice. We carried out a comprehensive in vivo analysis of metabolic responses combined with assessment of modulation of expression of genes as well as protein quantification involved in glucose and lipid metabolism in liver, muscle tissues, and gonadal adipose tissue. Our results indicate that LXRs elicit distinct or even opposite metabolic responses in different tissues.

**MATERIALS AND METHODS**

**Animals and experimental design.** Ten-week-old female C57Bl/6 WT and LXRα and β knockout mice [as previously described (1)] were housed on a regular 12:12-h light-dark cycle with free access to water and food unless specified. Mice were fed for 3 wk either an ND (TD 7001, Harlan Teklad) containing 4% (wt/wt) of total lipids (<12% of its calories as animal fat) and ≤0.04% (wt/wt) of cholesterol or an HCD (TD 98090, Harlan Teklad) containing 70.4% (wt/wt) of total carbohydrate (64.5% from sucrose) and 11.8% (wt/wt) of fat with ≤0.04% (wt/wt) of cholesterol. Sucrose is the organic compound known as saccharose. The molecule is a disaccharide derived from glucose and fructose with the molecular formula C12H22O11. Glucose and fructose are rapidly absorbed into the bloodstream in the small intestine. Saccharose is very rapidly absorbed by the small intestine and in the bloodstream. Mice fed a high glycerol index carbohydrate diet show after 4 wk liver steatosis compared with low glycerol index carbohydrate diet (Feldstein, 2003 no. 70; Scriber, 2007 no. 69). In our study, we wanted to study the role of LXR under physiologically “stable” conditions and not during a pathophysiological state. We thus ran the whole study with a short-period diet (3–4 wk before euthanasia) to investigate LXR regulation of carbohydrate metabolism under physiological conditions. Body weight was recorded at day 0 and every 3 days after the beginning of the diet period. Food consumption was measured for 8 consecutive days in the middle of the diet period. Three days after the last in-vivo experiment, mice were euthanized under 4% isoflurane after 4 h of fasting, and blood was collected by heart puncture. Tissues (liver, gonadal fat pad, and tibialis anterior and soleus muscle tissues) were quickly collected and immediately frozen in liquid nitrogen for further analysis unless specified. All experiments were approved by the local Ethics Committee on Animal Research in Stockholm.

**In vivo MRI.** In vivo MRI was made under 1.5–2% isoflurane in O2 anesthesia. Fat distribution was measured in every mouse within 1 wk after the 3-wk diet period. All measurements were performed as previously described (29). Briefly, the mouse was laid on a support and positioned in the center of the coil. A total of ~36 contiguous 1.5-mm-thick transversal slices covering the mouse body were recorded. Image analysis was carried out with Paravision 3.1 image analysis software (Bruker). As a result, regional changes in fat were assessed on the basis of total, abdominal, visceral, and subcutaneous fat within the abdominal region. MRI-visible visceral fat comprises omental, retroperitoneal, and mesenteric fat depots. Two-dimensional image series were then imported into Biomap platform (Boulder, CO) for pixel counting-based determination of fat volumes. A density factor of 0.9 g/ml was used to convert fat volumes (in ml) into fat mass (in g).

**Isolation of adipocytes from adipose tissue and measurement of fat cell volume.** Fat cells were isolated from the gonadal adipose tissue (GLAT) according to the procedure described by Rodbell (39). In brief, tissue was cut into ~20-µg pieces and incubated (1 g tissue/ml medium) in Krebs-Ringer phosphate (KRP) buffer (pH 7.4) containing 4% bovine serum albumin (BSA) and 0.5 mg/ml collagenase Type I for 60 min at 37°C in a shaking water bath. The isolated fat cells were collected on a nylon mesh filter and were washed four or five times with 0.1% KRP-BSA buffer. Mean adipocyte volume was determined as described previously (13, 39).

**Measurement of lipogenesis in isolated fat cells.** Lipogenesis was isolated from fat cells. Lipogenesis was performed as described in detail previously (26). In brief, isolated gonadal fat cells from one animal group were pooled and incubated for 2 h at 37°C in a buffer containing [3H]glucose without (basal) or with different concentrations of insulin. After incubation, lipids were extracted, and radioactive glucose incorporation into total lipid was used as an index of lipogenesis. The ability of insulin to stimulate basal lipogenesis was calculated.

**Lipolysis.** Isolated gonadal fat cells were prepared by collagenase treatment of adipose tissue, fat cell size was determined, and lipolysis experiments were done on isolated fat cells as described (34). In brief, diluted fat cell suspensions (2%, vol/vol) were incubated in an albumin buffer (pH 7.4) supplemented with glucose and ascorbic acid. The cells were incubated at 37°C for 2 h without (basal) or with increasing concentrations (10−16 to 10−5 mol/l) of norepinephrine. Thereafter, glycerol release into the incubation medium (lipolysis index) was determined by bioluminescence (21).

**Energy consumption.** Mice were individually housed in calorimeter cages and acclimatized to the respiratory chambers for 2 days prior to gas exchange measurements. Indirect calorimetry was performed using a computer-controlled SOMEDIC metabolic system (INCA; SOMEDIC Sales, Stockholm, Sweden). Measurements were performed in both fed and fasting states. Animals were fed during the first 12 h light-dark cycle and then fasted for 6 h during the next 12-h light cycle. VO2 and VCO2 were measured for each mouse at 20-min intervals over a 48-h period. The respiratory quotient (RQ) was calculated as the ratio of VCO2 over VO2. Total protein oxidation and the associated amount of energy provided were assumed to be equivalent of protein energy intake as previously described (33) and VO2 and VCO2 were corrected for protein oxidation (2.9 g protein oxidized/g urea, and 1.01 liter O2 and 0.843 liter CO2/g protein oxidized) (33). Glucose and fat oxidation and the amount of energy provided were computed when the nonprotein respiratory quotient (NPoRQ) was <1.0 (33), whereas the amount of glucose converted into fat and the amount of fat synthesized were computed when the NPoRQ was >1.0 (15). After the second overnight feeding at 23°C, cage temperature was increased to 30°C for 3 h to measure the basal activity (nonmotion activity).

**Chemical analysis of serum and tissue.** At the end of the experiment, animals were euthanized, blood was collected for serum preparation, and tissues were quickly collected and frozen in liquid nitrogen for further analysis. Small intestine was harvested and immediately washed with PBS and divided into three segments: duodenum, jejunum, and ileum, before frozen in liquid nitrogen. Glucose was determined by a colorimetric enzymatic kit (Roche Diagnostics, Roche, Stockholm, Sweden). NEFA were determined by a commercially available kit (Wako Chemicals, Richmond, VA). C-peptide was determined by RIA following the manufacturer’s instructions (LINCO Research, St. Charles, MO). Serum insulin was determined by ELISA according to the instruction manual (LINCO Research, St. Charles, MO). Serum levels of α-amylase were determined using a QuantiChrom kit (DAMY-100, BioAssay Systems) according to manufacturer’s instructions. Tissue glycogen content was quantified enzymatically in liver pool samples from each group (n = 6/group) after tissue digestion in HClO4. Briefly, liver pool samples were digested overnight in HClO4 (4%) and then centrifuged. The supernatants were neutralized by K2CO3 (1 M); thereafter, the digested free glycogen was converted into free glucose and fructose molecules. This reaction was carried out in triplicate, incubating samples with amyloglucosidase (1 mg/ml at 55°C for 1 h). D-Glucose/
d-fructose was quantified using an enzymatic kit (R-Biopharm/Roche Diagnostics, Mannheim, Germany) according to the manufacturer’s instructions.

Liver lipids were extracted according to Folch et al. (16). Triglyceride (TG) and total (TC) and free (FC) cholesterol masses were measured by enzymatic assay using commercially available kits (Roche Diagnostics; mti Diagnostic, Idstein, Germany). Liver cholesterol ester was calculated as the difference between TC and FC content. Data were corrected for the hepatic protein content, measured according to the Lowry method in the tissues digested with NaOH (1 M).

**Histology of liver tissue.** The median lobe of the liver was removed and fixed overnight in 4% paraformaldehyde at 4°C and embedded in paraffin. Sections (4 μm thickness) were stained with hematoxylin-eosin according to standard histological procedures.

**Oral glucose and intraperitoneal insulin and pyruvate tolerance tests.** Mice were fasted ~6 h before each test. An oral glucose tolerance test (OGTT) was performed on day 15 (±2 days) when mice were administered 1.0 g/kg b-glucose by gavage. Insulin (0.8 U/kg Actrapid; Penfill, Novo Nordisk) and pyruvate (2 g/kg) were injected intraperitoneally. Blood samples were obtained via tail nick at 0, 15, 30, 60, and 120 min in each test, and glucose was measured with the OneTouch Ultra glucometer (Accu-Chek Sensor, Roche Diagnostics). The tolerance tests were conducted at least 5 days apart. For the insulin tolerance test (ITT), we repeated the ITT as percentage of starting glucose prior to recalculating the area under the curve (AUC). AUC was calculated as follows: Glc(T1) + Glc(T2) × (T2 – T1)/2.

**Quantitative PCR.** RNA was prepared using the RNeasy minikit (Qiagen, Valencia, CA) according to the manufacturer’s instructions. Total RNA (0.5 μg) was reverse transcribed into cDNA using SuperScript II and random hexamer primers (Invitrogen). mRNA expression levels were quantified using the ABI 7500 instrument and the SYBR Green technology (Applied Biosystems, Foster City, CA). All primers were designed with the Primer Express software version 2.0, a program specifically provided for primer design using ABI qPCR instruments. Primers (100–300 nM) were used, and for each primer pair a dissociation curve analysis was carried out to ensure the specificity of the qPCR amplification. We calculated relative changes employing the comparative Ct method using 18S as the internal reference gene for inter- and intra-tissue variability for all other tissues.

**Western blotting.** Proteins were extracted from GLAT and liver samples with a lysis buffer complemented with anti-protease and anti-phosphatase (Roche). Briefly, ~20 mg of tissues were homogenized in 1 ml of lysis buffer and incubated on ice for 35 min and centrifuged (16,000 rpm/10 min) at 4°C. Supernatant containing total cellular protein extract was collected. Fifty micrograms of proteins was separated on a 7.5% SDS-PAGE, and electrophoresed to a nitrocellulose membrane (Hybond-C, Amersham). The following primary antibodies were used: anti-p47-3Akt (M-17, sc 6529; Cell Signaling), anti-Akt(pan) (C67E7; Cell Signaling), anti-HSL (hormone-sensitive lipase) total (4107; Cell Signaling), anti-p563-HSL (4139; Cell Signaling), anti-p565-HSL (4137; Cell Signaling), anti-adipose triglyceride lipase (ATGL, 2138; Cell Signaling), anti-perilipin A (PLIN, ab3526; abcam), anti-β-actin, and anti-α-tubulin. Secondary antibodies were horseradish peroxidase-conjugated anti-rabbit (Cell Signaling) or anti-mouse (Amersham). p47–3Akt and p563-HSL and p565-HSL levels were normalized to total Akt (Akt) and total HSL (HSL) expression, respectively, and total level of protein was normalized to β-actin in the liver and α-tubulin in the GLAT. Visualization was carried out using ECL plus (Amersham), and protein quantification was performed by Image J software.

**Immunohistochemical staining.** Representative blocks of paraffin-embedded tissues were cut at 4-μm thickness, dehydrated, and rehydrated. Sections were incubated in 0.5% Triton X-100 in PBS for 30 min. To block nonspecific binding, sections were incubated in 3% BSA for 1 h at 4°C and then incubated with anti-GLUT4 antibodies at a dilution of 1:200 in 1% BSA and 0.1% NP-40 in PBS overnight at 4°C. BSA replaced primary antibodies in negative controls. After washing, sections were incubated with the corresponding secondary antibodies (1:200 dilution) for 2 h at room temperature. Sections were counterstained with DAPI and directly mounted in Vectashield antifading medium (Vector Laboratories). GLUT4-stained sections were examined under a light microscope or a Zeiss fluorescence microscope with suitable filter for selectively detecting the fluorescence of FITC (green). GLUT4 pixel intensity was quantified at the cell perimeter with Image J software and expressed as the ratio to intracellular staining. This quantification was done on 10 different cells in each section, both in cytoplasm and membrane, in a total of 4 animals per group.

**Statistical analysis.** All values are expressed as means ± SE. Differences between groups or diet were determined by either two-tailed Student’s t-test or one-way analysis of variance (ANOVA), as indicated for specific experiments in figure legends. Tukey’s post hoc test was used to identify the location of significant differences between the two diets when appropriate, and Dunnett’s post hoc test was used to identify the location of significant differences between the knockout animals compared with the WT group when appropriate. The level of significance was set at P < 0.05.

**RESULTS**

**Different fat distribution in WT vs. LXRαβ−/− mice on HCD.** To further understand the role of LXRs in the metabolic changes following an HCD, we measured body weight (BW) as well as whole body fat content and distribution by magnetic resonance imaging (MRI) in all animals on ND or HCD. BW was similar between genotypes on both ND and HCD (Fig. 1A). After HCD, a significant increase of BW was observed in both WT and LXRαβ−/− mice compared with ND. No effect of the diet was observed on food intake (FI), although on HCD LXRαβ−/− mice showed a slight but significantly lower FI than WT mice (Fig. 1B). Total fat content measured by MRI was significantly higher in WT mice than in LXRαβ−/− mice on ND but became similar on HCD (Fig. 1C). Interestingly, fat distribution was different between the two genotypes on HCD (Fig. 1, D–F). While HCD led to a significant increase of total body fat in both genotypes (Fig. 1C), WT mice showed a higher fat accumulation in the visceral (VS) region (Fig. 1D) as opposed to LXRαβ−/− mice that accumulated fat in the subcutaneous (SC) region. The ratio between visceral fat and subcutaneous fat was significantly lower in LXRαβ−/− mice compared with WT animals on HCD but not those on ND (Fig. 1E).

**LXRs reduce DNL and induce lipolysis in GLAT on ND.** HCD has been shown to promote whole body DNL. Liver and adipose tissue are the two main tissues where DNL occurs. At least four different adipose tissues are present in the visceral region i.e., gonadal (GL), peritoneal, retroperitoneal, and omental, with different functions in the regulation of lipid metabolism (4, 50, 52). We harvested GL fat and measured DNL in these cells. Metabolism (4, 50, 52). We harvested GL fat and measured DNL in these cells.
stimulated lipogenesis in all four groups in a concentration-dependent manner (Fig. 2B). On ND, basal lipogenesis in GLAT was 50% higher in LXRαβ−/− mice than in WT mice (Table 1). Interestingly, when fed the HCD, basal lipogenesis was not changed in WT mice but was reduced by 50% in LXRαβ−/− mice (Table 1). LXRαβ−/− mice fed ND showed a much higher insulin-induced lipogenesis response in GLAT compared with all other groups. The HCD, reduced insulin response in LXRαβ−/− mice by 70%, whereas HCD had no effect on insulin-induced lipogenesis in WT mice. The higher insulin responsiveness observed in GL adipocytes in LXRαβ−/− mice on HCD compared with WT mice under low insulin concentration suggested a better insulin sensitivity of GL adipocytes in LXRαβ−/− mice compared with WT mice. Under high insulin concentration this difference disappeared between groups; one possible explanation could be that, when insulin concentration was too high, glucose transporters were saturated and this was the limiting step for an increased lipogenesis (5). Nevertheless, overall data on lipogenesis showed higher insulin responsiveness in GL adipocytes of LXRαβ−/− mice compared with WT mice on ND and HCD (Fig. 2B and Table 1). Since fat content depends on the difference between lipid storage and lipid utilization, a catecholamine-induced lipolysis in GLAT of WT and LXRαβ−/− mice was significantly lower in WT mice but was reduced by 50% in LXRαβ−/− animals compared with WT animals (Fig. 2D). 

Gene expression analysis was performed in GLAT samples (Fig. 2D). Expression of genes involved in lipogenesis [Srebp1c, Mel (malic enzyme-1)] and lipolysis (Atgl) was upregulated in LXRαβ−/− mice compared with WT on both diets respectively (Fig. 2D). Interestingly, expression of Plin1, a well-known regulator of lipolysis in adipocytes (47), was significantly higher after the HCD in the LXRαβ−/− animals compared with WT animals (Fig. 2D). Atgl expression in GLAT was 70% higher in LXRαβ−/− mice than in WT mice on both diets. No significant differences were observed between diets in LXRαβ−/− mice, whereas a slight but significant increase was observed in WT animals on HCD compared with ND. Moreover, significantly higher expression levels of the insulin receptor substrate-1 and -2 (Irs1 and Irs2) as well as glucose transporter (Glut4 and Glut5) genes would suggest a higher sensitivity to insulin-stimulated glucose and fat storage of GLAT in LXRαβ−/− animals compared with WT animals (Fig. 2D), further supporting the finding of enhanced insulin sensitivity in the GLAT of LXRαβ−/− animals. To examine potential HCD-mediated tissue-specific shifts in metabolic capacity, we determined the expression of Pgc-1α and Pgc-1β, two transcriptional regulators of mitochondriogenesis and oxidative metabolism. As shown in Fig. 2D, both Pgc-1α and Pgc-1β expression in GLAT was significantly induced in LXRαβ−/− mice compared with WT mice on ND and HCD. Finally, quantification of ATGL, PLIN and total and phosphorylated HSL (tHSL and pHSL, respectively) proteins involved in the regulation of lipid metabolism in adipocytes was performed on GLAT collected after 4 h of fasting (Fig. 2E). ATGL expression in GLAT was significantly higher in LXRαβ−/− mice than in WT mice on ND diet but similar on HCD due to an 80% increased expression in WT mice only [Fig. 2E and Supplementary Fig. S1 (supplementary materials are found online at the Journal}
PLIN expression was higher in LXRαβ−/− mice than in WT mice on ND, but no differences were observed between genotypes on HCD. In addition, tHSL expression was 96% higher in LXRαβ−/− mice than in WT mice on ND but similar on HCD due to a significantly decreased expression in LXRαβ−/− mice on HCD, whereas no diet effect was observed in WT mice (Fig. 2E and Supplementary Fig. S1). Protein kinase A (PKA) is known to phosphorylate HSL at Ser563 (p563-HSL), which stimulates HSL activity and thus lipolysis (3); amount of p563-HSL/tHSL was similar between genotypes on both diets, with a significant increase on HCD compared with ND in both genotypes. Interestingly, the amount of p563-HSL was similar on ND in both genotype but significantly increased in LXRαβ−/− mice with the HCD but not in WT mice (Fig. 2E and Supplementary Fig. S1).

Table 1. Adipocyte volume, lipogenesis, and lipolysis related to adipocyte TG in female mice

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<th>Wild Type</th>
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<td>HCD</td>
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<tr>
<td>Adipocyte volume, μm³</td>
<td>112 ± 8</td>
<td>58 ± 12†</td>
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<tr>
<td>Lipogenesis</td>
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<tr>
<td>Basal, μmol/g TG</td>
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<tr>
<td>Insulin, μmol/g TG</td>
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<td>3.6</td>
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<tr>
<td>Lipolysis</td>
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<tr>
<td>Basal, μmol/g TG</td>
<td>2.5 ± 0.4</td>
<td>4.6 ± 0.9†</td>
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<tr>
<td>NA, μmol/g TG</td>
<td>38.0 ± 6.4</td>
<td>116.9 ± 18.8†</td>
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Data are means ± SE; n = 8 animals pooled samples. KO, LXRαβ−/−; ND, normal diet; HCD, high-carbohydrate diet; TG, triglyceride; NA, norepinephrine-induced, 10−6 M glycerol release minus basal glycerol values. *P < 0.05 KO vs. WT mice; †P < 0.05 ND vs. HCD.
HCD induces liver TG accumulation in liver of WT mice but not in LXRαβ−/− mice. Increased visceral fat storage as well as reduced whole body insulin sensitivity are two main factors that eventually lead to hepatic steatosis and fatty liver. MRI showed a robust increase of fat content in liver with the HCD diet in both genotypes (Fig. 3A). Histological examination of liver sections from WT and LXRαβ−/− mice fed ND showed no difference, whereas there was a marked difference between the genotypes after HCD; micro/macrosteatosis, in particular in the periporal region, was detected in the liver of WT but not in the liver of LXRαβ−/− mice (Fig. 3B). Quantification of TG in liver showed significantly lower TG accumulation in LXRαβ−/− mice compared with WT mice on ND, whereas HCD tended to induce TG liver content in WT mice, although this induction did not reach significance (Fig. 3C). FC as well as cholesterol ester content was higher in LXRαβ−/− mice than in WT mice on both diets, with no effect of the HCD (Fig. 3, D and E). Expression of well-known genes involved in lipogenesis and lipolysis in liver tissue of WT and LXRαβ−/− mice was analyzed (Fig. 3F). As already shown by our group and others (29, 42), genes involved in the regulation of lipogenesis (Srebp1c, Me1) were all repressed in liver of LXRαβ−/− mice on ND, and we show here that this was the case on HCD as well. Also, a completely opposite response to that in liver was observed in GLAT, where expression of lipogenesis genes was upregulated in LXRαβ−/− mice. Moreover, Hsl, a gene well known to be involved in lipolysis, was significantly upregulated in LXRαβ−/− mice compared with WT mice on HCD. Furthermore, expression of Elov6 (elongase of very-long-chain fatty acids), a new marker of insulin resistance in the liver (35), was significantly higher in WT liver compared with LXRαβ−/− liver on both diets. These data support the finding of liver steatosis in HCD-fed WT liver but not in HCD-fed LXRαβ−/− liver. Total HSL expression was quantified in liver extracts after 4 h of fasting (Fig. 3G and Supplementary Fig. S1). Total HSL expression was significantly decreased by the HCD in WT mice but not in LXRαβ−/− mice (25%) compared with ND, with no differences between genotypes. ATGL was not detected in the liver. Glucose and fatty acid absorption in small intestine is not modified in LXRαβ−/− mice. To exclude a pancreatic insufficiency in LXRαβ−/− mice and to better characterize LXR KO mice, expression levels of genes involved in DNL, lipolysis, and insulin sensitivity in liver analyzed by qPCR. Relative fold changes in expression levels observed in LXR KO mice were compared with WT mice in the ND group, where expression was set to 1.0, and to mice in HCD group. Data are means ± SE; n = 5–7 animals/group. *P < 0.05 LXR KO vs. WT mice; #P < 0.05 ND vs. HCD.
function in substrate absorption in the small intestine, we measured 1) serum α-amylase concentration in mice fed HCD (Table 2) and 2) expression of several genes involved in lipid and glucose metabolism in the small intestine in ND or HCD fed WT and LXRαβ−/− female mice (Table 3). Recently, Gabbi et al. (17) showed that LXRβ regulates pancreatic exocrine secretion and that the absence of LXRβ leads to pancreatic exocrine insufficiency in 11-mo-old mice, one of the main causes of malabsorption syndrome (37). In the present study, no differences in serum amylase concentration were observed between genotypes on HCD (Table 2). We then investigated whether LXRαβ−/− mice showed differences in expression of glucose and fatty acid transporter genes that could modify absorption of glucose or fatty acids. On ND, no differences were observed between WT and LXRαβ−/− mice in the expression of the glucose transporters (Glut2, Glut5, and Slc5a1) and in apolipoproteins (ApoA4, ApoB) involved in the synthesis of TG-rich chylomicrons in the intestine (Table 3). After 3 wk on HCD, ApoA4, ApoB, and sucrase isomaltase (Slc5a1) were significantly upregulated compared with ND in both WT and LXRαβ−/− mice. Interestingly, expression of genes involved in glucose and lipid metabolism in the intestine, such as Ppara and Srebp1c, was significantly increased by the HCD in WT but not in LXRαβ−/− mice (Table 3). Expression of both LXRα and LXRβ was unchanged on HCD compared with ND in WT animals. On the basis of these results, it may be suggested that glucose and fatty acid absorption in the small intestine was likely not modified in LXRαβ−/− mice.

**Increased energy expenditure and net DNL in LXRαβ−/− mice.** Indirect calorimetry has long since been successfully used for the calculation of heat production in animals and in humans (24, 38, 48). For the past decade, measurements of gas exchange have been used to calculate the oxidation of protein, carbohydrate, and fat in the intact body of different species (9, 24, 38). Oxygen consumption and CO2 release were registered in WT and LXRαβ−/− mice before and after 3 wk of an HCD for 48 h in temperature-controlled (23°C) individual chambers (Fig. 4, A and B). Cage temperature was raised to 30°C for 3 h to measure basal metabolism (nonmotion activity) at the end of the 48-h registration period (Fig. 4, A and B). Energy expenditure (EE) was significantly higher in LXRαβ−/− mice than in WT animals during all metabolic conditions except at 30°C on HCD (Fig. 4C) but not at baseline (data not shown). After HCD, glucose oxidation during the light 23°C period increased five- and fourfold in WT (9.2 ± 2.9 and 47.2 ± 11.7 mg/min) and LXRαβ−/− mice (15.1 ± 3.8 and 57.7 ± 14.8 mg/min), respectively, compared with baseline. On HCD, fat oxidation was higher in LXRαβ−/− mice than in WT during both the dark 23°C and light 23°C period animals but remained unchanged between genotypes at 30°C (data not shown). Interestingly, on HCD, net DNL was higher in LXRαβ−/− mice than in WT mice under all metabolic conditions except during the 30°C period (Fig. 4D). Consequently, net DNL over the 48-h registered period was eight times higher in LXRαβ−/− mice than in WT mice (Fig. 4E).

The above data prompted us to analyze the expression of genes involved in the regulation of EE and substrate oxidation in muscle, the most important tissue for the regulation of energy metabolism under tempering (23°C) conditions. We thus harvested two different muscle tissues, tibialis anterior (TA) and muscle (glycolytic) and soleus muscle (lipolytic) and analyzed the expression of well-known genes involved in the regulation of EE (Ucp2 and Pgc1α) as well as lipid (Srebp1c, Me1, Hsl, and Atgl) and glucose (Hk and Glut4) homeostasis. In TA muscle, no differences were observed in expression of analyzed genes involved in energy metabolism between genotypes on either diet (data not shown). Expression of Srebp1c gene was highly downregulated in LXRαβ−/− mice compared with WT on both diets (Fig. 4F). In contrast, expression of Me1 was similar between genotypes on ND and slightly downregulated in LXRαβ−/− mice compared with WT on HCD. In addition, expression of Hk1 and Hk2 was not different between genotypes on ND, but Hk1 expression was significantly higher in LXRαβ−/− mice than in WT mice on HCD. Finally, Glut4 expression was slightly upregulated in LXRαβ−/− mice compared with WT on both diets. To further investigate the effect of the HCD as well as the absence of LXRαs on TA muscle GLUT4 regulation, we stained TA muscle for GLUT4 in WT and LXRαβ−/− mice on ND and HCD (Fig. 4F and Supplementary Fig. S2). A more intense GLUT4-positive reaction

**Table 2. Effects of HCD on plasma metabolites and liver glycogen content in female mice**

<table>
<thead>
<tr>
<th>Diet</th>
<th>Wild Type</th>
<th>LXRαβ−/−</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ND</td>
<td>HCD</td>
</tr>
<tr>
<td>Liver glycogen, mg/g</td>
<td>22.1</td>
<td>26.6</td>
</tr>
<tr>
<td>Glucose, mM</td>
<td>9.4 ± 0.7</td>
<td>9.4 ± 0.3</td>
</tr>
<tr>
<td>Insulin, ng/ml</td>
<td>0.43 ± 0.03</td>
<td>0.50 ± 0.06</td>
</tr>
<tr>
<td>C-peptide, mM</td>
<td>0.37 ± 0.02</td>
<td>0.48 ± 0.06</td>
</tr>
<tr>
<td>FFA, mM</td>
<td>1.06 ± 0.03</td>
<td>1.11 ± 0.03</td>
</tr>
<tr>
<td>C-peptide/glucose</td>
<td>0.040 ± 0.00</td>
<td>0.051 ± 0.01</td>
</tr>
<tr>
<td>Amylase, U/l</td>
<td>125 ± 12</td>
<td>122 ± 10</td>
</tr>
</tbody>
</table>

Data are means ± SE; n = 6–8 animals per group. All animals were fasted for 4 h prior to euthanasia. Blank cells, not measured. *P < 0.05 LXR KO vs. WT mice.

**Table 3. Intestine, jejuneum gene expression in WT and LXRαβ−/− female mice fed ND or HCD**

<table>
<thead>
<tr>
<th>Genes/Diet</th>
<th>Wild Type</th>
<th>LXRαβ−/−</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ND</td>
<td>HCD</td>
</tr>
<tr>
<td>ApoA4</td>
<td>1.0 ± 0.1</td>
<td>2.3 ± 0.3†</td>
</tr>
<tr>
<td>ApoB</td>
<td>1.0 ± 0.1</td>
<td>1.5 ± 0.2†</td>
</tr>
<tr>
<td>Chrebp</td>
<td>1.0 ± 0.1</td>
<td>1.3 ± 0.2</td>
</tr>
<tr>
<td>Gapdh</td>
<td>1.0 ± 0.1</td>
<td>1.4 ± 0.2</td>
</tr>
<tr>
<td>Glut5</td>
<td>1.0 ± 0.1</td>
<td>2.7 ± 0.5†</td>
</tr>
<tr>
<td>Ifabp</td>
<td>1.0 ± 0.1</td>
<td>0.8 ± 0.2</td>
</tr>
<tr>
<td>LDH</td>
<td>1.0 ± 0.1</td>
<td>1.6 ± 0.3</td>
</tr>
<tr>
<td>MPT</td>
<td>1.0 ± 0.1</td>
<td>1.3 ± 0.2</td>
</tr>
<tr>
<td>Peck</td>
<td>1.0 ± 0.1</td>
<td>1.7 ± 0.3</td>
</tr>
<tr>
<td>PPARα</td>
<td>1.0 ± 0.1</td>
<td>3.0 ± 0.3†</td>
</tr>
<tr>
<td>Slc5a1</td>
<td>1.0 ± 0.1</td>
<td>2.3 ± 0.6‡</td>
</tr>
<tr>
<td>Slc5a2</td>
<td>1.0 ± 0.1</td>
<td>2.7 ± 0.5‡</td>
</tr>
<tr>
<td>Srebp1c</td>
<td>1.0 ± 0.2</td>
<td>1.9 ± 0.2‡</td>
</tr>
<tr>
<td>LXRα</td>
<td>1.0 ± 0.1</td>
<td>1.7 ± 0.2</td>
</tr>
<tr>
<td>LXRβ</td>
<td>1.0 ± 0.1</td>
<td>1.4 ± 0.4</td>
</tr>
</tbody>
</table>

Data are means ± SE; n = 6–8 animals per group. Relative mRNA expression levels analyzed by quantitative PCR in WT and KO mice on ND and HCD. *P < 0.05 KO vs. WT mice; †P < 0.05 ND vs. HCD.
was observed in the cytoplasm of TA cells of LXRαβ−/− mice compared with WT mice on ND. On HCD, GLUT4-positive reaction migrated to the membrane in LXRαβ−/− mice with no clear differences in GLUT4-positive reaction intensity between genotypes (Supplementary Fig. S2). In addition, the pixel intensity of GLUT4 at the cellular perimeter was quantified and expressed as a ratio of intracellular staining (Supplementary Fig. S2). No significant differences were found between groups and diets. However, on HCD, a significant pixel GLUT4 intensity difference was observed between cytoplasm and membrane in both WT and LXRαβ−/− mice compared with ND. Moreover, relative pixel intensity was significantly increased in the membrane of LXRαβ−/− mice on HCD compared with WT mice. As a result, GLUT4 expression was upregulated in LXRαβ−/− mice compared with WT mice on ND. On HCD, GLUT4 expression was decreased in both genotypes but remained significantly higher in LXRαβ−/− mice compared with WT mice.

Insulin sensitivity is reduced by HCD in WT mice but not in LXRαβ−/− mice. Since VS fat accumulation is associated with insulin resistance (28), we performed OGTT and ITT in vivo. No difference in glucose tolerance was observed between groups and diets (Fig. 5, A–C). Whole body insulin sensitivity was decreased by the HCD in WT mice but remained unchanged in LXRαβ−/− mice (Fig. 5, D–F). The observed VS fat accumulation (Fig. 1E) is in line with the decreased insulin sensitivity in WT but not in LXRαβ−/− mice. A PTT was performed to induce liver gluconeogenesis and showed a slight
but significantly higher glucose output from the liver of LXRαβ−/− mice compared with WT mice on ND. Surprisingly, after HCD, gluconeogenesis was significantly decreased in LXRαβ−/− mice but not in WT mice (Fig. 5, G–I). These results may suggest a reduced insulin response from the liver of WT mice on HCD compared with ND. As insulin resistance is derived from defects in insulin signaling in peripheral tissues, and insulin/Akt signaling regulates many of the metabolic actions of insulin (6), we investigated phosphorylation of Akt in liver and GLAT in our mice on ND and HCD. In liver, no differences were observed in the pAkt/Akt ratio between genotypes on ND. Interestingly, on HCD, a much higher pAkt/Akt ratio was observed in LXRαβ−/− mice than in WT mice (Fig. 5J and Supplementary Fig. S1). In GLAT, a significantly higher pAkt/Akt ratio was observed in LXRαβ−/− mice compared with WT mice however, pAkt/Akt was significantly increased by the HCD in WT mice, whereas no diet effect was observed in LXRαβ−/− mice (Fig. 5J and Supplementary Fig. S1). In addition, although no differences in liver glycogen content were observed between genotypes on ND, HCD resulted in a 20% increase in glycogen concentration in the liver of WT mice compared with ND, whereas no changes were observed in LXRαβ−/− mice (Table 2). Accordingly, on HCD, liver glycogen concentration was ~70% higher in WT mice compared with LXRαβ−/− mice (Table 2). Plasma glucose, insulin, C-peptide, and NEFA levels were measured after 4 h of fasting. Analysis of these circulating metabolic factors in serum showed no significant changes in response to HCD or difference due to genotype in any of the measured parameters except for C-peptide on HCD (Table 2). As a consequence, the C-peptide/glucose ratio was significantly higher in WT mice than in LXRαβ−/− mice on HCD, further suggesting a lower response to insulin in the regulation of serum glucose concentration in WT mice. Moreover, NEFA serum analysis after 10 h of fasting showed that NEFA concentration was increased by 30% with the HCD in WT mice (0.37 ± 0.05 vs. 0.49 ± 0.02 mM, respectively), whereas it was not changed in LXRαβ−/− mice (0.40 ± 0.06 vs. 0.38 ± 0.04 mM, respectively), in line with the increased lipolysis in GLAT in WT compared with LXRαβ−/− mice.

**DISCUSSION**

A wealth of data has shown that LXRs are key stimulators of the DNL pathway in the liver mainly through the insulin-mediated activation of Srebp1c and downstream genes such as Fas, Acc1, and Scd1 (8, 42, 54). The present study reveals an unexpected metabolic pathway involving LXRs. LXRs act as repressors of DNL in GL adipocytes and, to a smaller extent, in myocytes, a completely opposite action to the one observed in liver. In vivo and ex vivo experiments converge to demonstrate that, when both LXR isoforms are absent, LXRs act as regulators of DNL (49). While expression of Srebp1c and Chrebp...
genes was low in the liver of LXRαβ−/− mice on ND, only Chrebp was significantly increased upon HCD (Fig. 3F). These findings support recent data from Denechaud et al. (11), demonstrating that glucose induces lipogenesis through activation of the ChREBP pathway. Most interestingly, in GLAT, a completely opposite response to that observed in liver was observed. Expression of lipogenesis genes (Srebp1c, Mel, and Plin) was upregulated in LXRαβ−/− mice compared with WT animals on both ND and HCD (Fig. 2D). Thus, we show for the first time that transcriptional regulation of DNL is regulated by LXRs in a tissue specific manner.

In human fat cells, LXR agonist has been shown to suppress PLIN expression and upregulate basal lipolysis (45). In the current study, in line with Stenson et al. (45), PLIN expression (Fig. 2E and Supplementary Fig. S1) as well as basal lipolysis (Table 1) were higher in LXRαβ−/− mice than in WT mice on ND, indicating that in murine adipocytes LXR could be a possible repressor of PLIN expression and thus upregulate lipolysis (Fig. 2E). In addition, higher expression of ATGL protein in GLAT in LXRαβ−/− mice compared with WT mice might indicate that LXR could be a negative regulator of ATGL in murine WAT, which again would stimulate lipolysis. Finally, expression of HSL under basal conditions was higher in LXRαβ−/− mice than in WT mice on ND, but this difference disappeared on HCD. These results might indicate that stimulation of LXRs by the HCD would increase basal lipolysis, as already described by Stenson et al. (45). The mobilization of metabolic energy from adipocytes depends on a tightly regulated balance between hydrolysis and lipogenesis. Hydrolysis is stimulated by β-adrenergic signaling to PKA that mediates phosphorylation of HSL on Ser563 (p563-HSL). Surprisingly, the p563-HSL/tHSL ratio was not different between genotypes and significantly increased in WT and LXRαβ−/− mice on HCD compared with ND. However, the significant increase of p563-HSL/tHSL with the HCD in LXRαβ−/− mice is due to a significant decrease of total HSL expression but not due to a significant increase of p563-HSL, as opposed to WT animals that showed a significant increase of both ATGL and p563-HSL with the HCD, in line with the increased lipolysis observed in isolated adipocytes of GLAT (Fig. 2C). These results indicate that under basal conditions the PKA pathway was significantly affected by the HCD and that this regulation might be LXR dependent. Furthermore, a single lipogenesis/ lipolysis experiment performed on isolated fat cells from GLAT extracted from our ND- and HCD-treated WT/LXRαβ−/− mice supported the finding of enhanced lipogenesis on ND and HCD and reduced lipogenesis on HCD in LXRαβ−/− mice compared with WT mice (Fig. 2, B and C, and Table 1). These results are in line with the higher net DNL observed in vivo with indirect calorimetry in LXRαβ−/− mice than in WT mice on HCD (Fig. 4E). A recent study by Stenson et al. (44) showed that LXRs play an important role in the regulation of substrate oxidation and the switch between carbohydrates and lipids as cellular fuel in both human and murine white adipocytes. Our results support their main findings that LXRs stimulate fatty acid oxidation in murine fat cells. In our study, HCD induced lipolysis in GLAT of WT mice compared with LXRαβ−/− mice (Fig. 2C and Table 1).

The induced lipolysis observed in WT mice could be the consequence of reduced insulin sensitivity. The release of C-peptide into the bloodstream at an equimolar concentration of insulin makes it a useful marker of insulin release because, unlike insulin, C-peptide is subjected to negligible first-pass metabolism by the liver. As a consequence, increased C-peptide serum concentration on HCD would be correlated with increased active insulin in WT compared with LXRαβ−/− mice that could activate the Akt pathway, but this needs to be further investigated in future studies. Moreover, whole body insulin sensitivity was reduced by the HCD in WT but not in LXRαβ−/− mice (Fig. 5F). These data support an overall defect of insulin sensitivity in WT animals on HCD, but this would need to be further investigated in different tissues.

Insulin is the most important hormone that inhibits gluconeogenesis. It acts predominantly by suppressing the expression of the genes for the key gluconeogenic enzymes PEPCK and G-6-Pase. The initial stages of type 2 diabetes (T2D) are characterized by insulin resistance (41). This leads to the inability of insulin to control the activity of gluconeogenic enzymes. Gene knockout studies have demonstrated that insulin decreases hepatic glucose production mainly via IRS-2, which suggests that this isoform has a predominant role in the signaling pathway that controls gluconeogenesis (30, 53). In our study, while the presence of LXR reduced Iris2 expression in liver (Fig. 3F) with the HCD, the expression did not change in LXRαβ−/− mice. In addition, in WT mice, insulin seems to fail to inhibit liver Pepck expression on HCD and therefore glucose output from the liver (Figs. 3F and 5F). These results would be in favor of reduced insulin sensitivity in the liver of WT mice but not of LXRαβ−/− mice on the HCD. In addition, relative GLUT4 pixel intensity in the membrane of LXRαβ−/− mice TA muscle was increased on HCD compared with ND, whereas no changes were observed in WT mice. This observation correlates with the improvement of insulin sensitivity of HCD-fed LXRαβ−/− mice compared with WT mice on the same diet.

It is largely accepted that LXRs are key lipogenic factors in hepatocytes (1, 43). In the present study, liver TG content was found to be significantly lower in LXRαβ−/− mice than in WT mice on ND and tended to be lower (not significantly) on HCD (Fig. 3C). Furthermore, staining of liver showed hepatic steatosis in WT mice fed HCD but not in LXRαβ−/− mice. These observations could be the consequence of LXR activation by dietary HCD (mainly sucrose). Indeed, glucose has been shown to be a potential LXR activator (2, 36), even though the mechanism behind this regulation remains controversial (2, 11, 36, 40). Interestingly, Anthonisen et al. (2) recently suggested that O-GlcNAcylation of LXRs could be a mechanism by which LXR acts as a glucose sensor and activates transcription of SREBP-1c. Our data would support such hypothesis of action through LXRs.

The amount as well as the distribution of adipose tissue is associated with many of the obesity-related diseases, such as cardiovascular disease and T2D; the distribution of body fat appears to be even more important than the total fat content (20). VS adiposity has been strongly linked to insulin resistance, T2D, hypertension, and dyslipidemia, leading to increased risk of cardiovascular disease, whereas such associations seem to be much less consistent with respect to SC fat mass (20). The increased VSAT observed in central obesity would be associated with greater mobilization of NEFA into the portal circulation, impairing liver metabolism and leading to
to glucose intolerance, insulin resistance, and dyslipidemia (19, 20). Conversely, SCAT seems to be much less related to insulin sensitivity (25). In the current study, the differences in fat distribution (Fig. 1, D and E) between WT (viscerally) and LXRαβ−/− (subcutaneously) mice are associated with the lower insulin sensitivity and liver steatosis observed in WT mice as opposed to LXRαβ−/− mice on HCD, in line with the concept that adipose tissue distribution affects hepatic glucose and lipid metabolism. This is further supported by the higher expression of Elovl6 and Srebp1c (Fig. 3F), key players in hepatic insulin resistance (14, 35), in WT mice compared with LXRαβ−/− mice. Moreover, a study by Ishikawa et al. (23) showed that SC-lipectomized mice showed a decrease in systemic insulin sensitivity. More importantly, transplantation of fat into the SC region improved the insulin resistance of SC-lipectomized mice. Those authors concluded that the ratio between SCAT and VSAT should be considered a better marker of insulin sensitivity than the amount of VSAT. These results support the finding of higher insulin sensitivity in LXRαβ−/− mice compared with WT mice. Basal pAkt/tAkt ratio (after 4-h fasting) was increased in LXRαβ−/− mice on ND and HCD in GLAT and liver, respectively. This result would support an overall insulin sensitivity in LXRαβ−/− mice compared with WT mice, but insulin-stimulated pAkt in different fat tissues would be necessary in future studies to completely address this issue.

Finally, our results reveal an important, previously unknown regulation of fatty acid metabolism by LXR. LXR has previously been shown to be an important regulator of fat metabolism, since LXRβ−/− and LXRαβ−/− mice are resistant to diet-induced obesity (27, 29). In the present study, we show another mechanism that regulates fat storage in LXRαβ−/− mice. We show that, on HCD, LXRαβ−/− mice are not protected against fat storage since they accumulate the same amount of fat as WT animals (Fig. 1). In addition, we show that, on HCD, the presence of LXR causes reduced insulin sensitivity and increased lipolysis, two phenomena that would lead to insulin resistance. Finally, our data support the idea of an indirect or direct activation of LXRs by glucose, as proposed recently by Anthonisen et al. (2) and previously by Mitro et al. (36). A novel mechanism by which LXRs regulate fat and glucose homeostasis in mice is summarized in Fig. 6, although this hypothesis still has to be further investigated. Briefly, excess glucose coming from the HCD directly or indirectly stimulates LXRs. In the liver, LXR stimulation induces DNL and TG accumulation (Fig. 3), a completely opposite effect to that observed in the GLAT (Fig. 2) and, to a lesser extent, in muscle tissue (Fig. 4). In these tissues, the presence of LXRs reduces DNL and induces lipolysis (Fig. 6).

In summary, the role of LXR in dual sensing of lipids and carbohydrates, in conjunction with its functional networking with insulin signaling, establishes LXR as a critical regulator of adipocyte metabolism. Our findings have important potential implications for the development of drugs for treatment of diseases characterized by metabolism deregulation, involving several different target tissues and meta-

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**Fig. 6.** Schematic metabolic response as well as changes in gene expression due to HCD in WT and LXRαβ−/− mice. Loss of insulin sensitivity appeared after HCD in WT mice but not in LXRαβ−/− mice. Moreover, liver steatosis is observed in WT but not in LXRαβ−/− mice. GLAT volume increased with HCD in LXRαβ−/− mice, but decreased in WT mice. Whole body lipogenesis as well as lipogenesis in isolated GLAT was increased in LXRαβ−/− mice vs. WT mice. Interestingly, analysis of GLAT showed a large induction of lipolysis in adipocytes in WT mice after HCD, whereas a significant decrease was observed in LXRαβ−/− mice. EE was induced in LXRαβ−/− mice vs. WT mice, and GLUT4 proteins were translocated to the membrane with the HCD in both WT and LXRαβ−/− mice.
bolic pathways. Results from this study emphasize the
essence of tissue-specific targeting in the development of
antidiabetic LXR-based drugs.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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