Liver X receptors (LXR) α and β are nuclear oxysterol receptors with established roles in cholesterol, lipid, and carbohydrate metabolism. Although LXRs have been extensively studied in liver and macrophages, the importance for development and metabolism of other tissues and cell types is not as well characterized. We demonstrate here that although LXRα and LXRβ are not required for adipocyte development per se, LXRβ is required for the increase in adipocyte size that normally occurs with aging and diet-induced obesity. Similar food intake and oxygen consumption in LXRβ−/− mice suggest that reduced storage of lipid in adipose tissue is not due to altered energy balance. Despite reduced amounts of adipose tissue, LXRβ−/− mice on a chow diet have insulin sensitivity and levels of adipocyte hormones similar to wild type mice. However, these mice are glucose-intolerant due to impaired glucose-induced insulin secretion. Lipid droplets in pancreatic islets may result from accumulation of cholesterol esters as analysis of islet gene expression reveals that LXRα is required for expression of the cholesterol transporters, ABCA1 and ABCG1. Our data establish novel roles for LXRβ in adipocyte growth, glucose homeostasis, and β cell function.

LXRβ is Required for Adipocyte Growth, Glucose Homeostasis, and β Cell Function*§

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*mRNA levels in liver, macrophages, and white adipose tissues (5, 6), LXRβ is expressed more ubiquitously (5–7). These nuclear hor-
enhancer-binding protein \(\alpha\), and SREBP-1c, raising the possibility that LXRs regulate adipogenesis or lipid metabolism (24–26). However, conflicting results obtained from various laboratories in vitro underscore the need for further experimentation in vivo (24–28). In this study, we report that although LXR\(\alpha\) and LXR\(\beta\) are not required for adipocyte development in mice, LXR\(\beta\) is required for the increase in adipocyte size that occurs with age or obesity. Despite reduced amounts of adipose tissue, LXR\(\beta\)\(-/-\) mice do not have substantially altered circulating levels of adipocyte hormones, nor are they resistant to insulin. However, LXR\(\beta\)\(-/-\) mice are intolerant to glucose due to impaired glucose-stimulated insulin secretion. Analyses of islets suggest that reduced expression of cholesterol transporters results in accumulation of cholesterol esters and perhaps other neutral lipids. Our data establish previously unrecognized roles for LXR\(\beta\) in adipocyte hypertrophy, glucose homeostasis, and \(\beta\) cell function.

**MATERIALS AND METHODS**

**Animal Experiments**—LXR\(\alpha\)\(-/-\), LXR\(\beta\)\(-/-\), and LXR\(\alpha\)/LXR\(\beta\)\(-/-\) mice were generated by gene targeting as described previously (29, 30). Mice used in this study were backcrossed to C57BL/6 mice for at least 10 generations. Animals were housed with a regular 12-h light/12-h dark cycle and ad libitum access to standard rodent chow diet (Laboratory Rodent Diet 5001, LabDiet, St. Louis, MO). All experiments were approved by the University Committee on Use and Care of Animals and were overseen by the Unit for Laboratory Animal Medicine (University of Michigan).

**Diets**—Where indicated, 4-week-old mice were assigned to receive ad libitum access to a low fat diet (10% fat, D12450B, Research Diets, New Brunswick, NJ) or a high fat diet (45% fat, D12451, Research Diets) for 6 months.

**Energy Balance and Body Composition**—Measurement of oxygen consumption (VO\(_2\)) of individually housed wild type \((n = 5)\) and LXR\(\beta\)\(-/-\) \((n = 5)\) mice with indirect calorimetry was performed on 7-month-old mice over 2 days with the Oxymax\(^{\text{TM}}\) system (Columbus Instruments, Columbus, OH). Animals were fed standard laboratory chow and nectar fluid and maintained on 12-h light and dark cycles beginning at 6 a.m. and 6 p.m., respectively. Animals were acclimated in measuring chambers for 1 week prior to recording. Measurements of VO\(_2\) were made every 24 min for each animal over a period of 2 days. Body composition was estimated with dual energy x-ray absorptiometry with pDEXA Sabre software (Norland Medical Systems, Fort Atkinson, WI). Differences between genotypes were evaluated with Student's t test.

**Glucose, Insulin, and Pyruvate Tolerance Tests**—For the glucose tolerance test, mice were fasted for 16 h and then intraperitoneally injected with glucose \((1.5 \text{ g/kg of body weight})\). Blood glucose was measured with the OneTouch Ultra\(^{\text{TM}}\) glucometer (LifeScan, Burnaby, British Columbia, Canada). Serum insulin was determined using an enzyme-linked immunosorbent assay kit (Crystal Chem, Downers Grove, IL) for wild type and LXR\(\beta\)\(-/-\) mice. For the insulin tolerance test, mice were deprived of food for 6 h and then injected intraperitoneally with insulin \((0.75 \text{ units/kg of body weight})\), and blood glucose levels were measured. For the pyruvate challenge, animals were fasted for 16 h prior to an intraperitoneal injection of pyruvate dissolved in saline \((2 \text{ g/kg})\) and measurement of blood glucose.

**Chemistry**—Serum adiponectin, resistin, and leptin were determined with the Lincoplex system on a Luminex 100 machine (Linco, St. Charles, MO). Triacylglycerol determinations were performed with an Infinity triglyceride reagent kit (Sigma) with glycerol as the standard. Non-esterified fatty acids were measured using the half-micro test (Roche Diagnostics GmbH).

**Real-time PCR**—Total RNA from adipose tissue or pancreatic islets was extracted using RNA Stat60 (Tel-Test, Friendswood, TX) and then was purified using RNeasy mini-kits (Qiagen, Valencia, CA). cDNA was synthesized using the TaqMan system (Applied Biosystems, Foster, CA) and random hexamer primers. Quantitative PCR was performed according to the manufacturer's protocol. SYBR Green I was used to monitor amplification of DNA on the iCycler and IQ real-time PCR detection system (Bio-Rad Laboratories). Gene expression was normalized to 18 S RNA levels. Primer sequences are available upon request.

**Histochemistry, Staining, and Immunostaining**—Gonadal adipose tissue and pancreata were dissected, fixed in 10% neutral buffered formalin, embedded in paraffin, and stained with hematoxylin and eosin. For immunostaining, sections were deparaffinized and rehydrated in xylene and ethanol. Sections were incubated in blocking buffer \((10% \text{ normal goat serum, } 0.3\% \text{ Triton X-100 in phosphate-buffered saline})\) and then with antibodies against insulin and glucagon (Linco). After washes, sections were incubated with Alexa Fluor 488 goat anti-guinea pig and goat anti-rabbit secondary antibody, respectively (Molecular Probes, Eugene, OR). After final washes, sections were counterstained with 4',6'-diamidino-2-phenylindole-blue and visualized using fluorescence microscopy. For Oil Red-O staining, pancreata were snap-frozen in Tissue-Tek (Sakura Finetek, Torrance, CA). Neutral lipid was visualized by staining 10-\(\mu\)m cryosections with Oil Red-O.

**Islet Isolation**—Islets of Langerhans were obtained by a previously described method (31). Briefly, mice were sacrificed by cervical dislocation followed by ductal injection of collagenase type XI (Sigma). The pancreas was dissected and incubated in 5 ml of collagenase solution at 37 °C for 7 min. Islets were hand-picked under stereomicroscope and selected for an oblong to spherical shape, a smooth surface (indicative of an intact islet membrane), and a diameter of 100–200 \(\mu\)m. The islets were placed in RPMI 1640 cell culture medium supplemented with 10% fetal bovine serum, 100 units/ml penicillin, and 100 \(\mu\)g/ml streptomycin and incubated at 37 °C, 5% CO\(_2\). Islets were used 1–6 days following isolation.

**Insulin Immunoassay**—Single islets were assayed for insulin secretion using a microfluidic device described previously (32). Briefly, single islets were loaded onto the device and perfused at 1 \(\mu\)l/min with balanced salt solution containing varying glucose concentrations. Perfusion was sampled and reacted on-chip with 50 nm fluorescein isothiocyanate-labeled insulin (Molecular Probes) and 25 nm monoclonal anti-insulin antibody (Biodiagnostic International, Saco, ME). The reaction mixture was injected onto a capillary electrophoresis channel every 5.5 s for 0.5 s where the antibody-insulin complex and free labeled-insulin were separated at 600 V/cm to quantify the amount of secreted insulin.

**RESULTS**

**Reduced Weight of Adipose Tissue Depots in LXR\(\alpha\)/LXR\(\beta\)\(-/-\) Mice Primarily Due to Decreased Size of Adipocytes**—Nebb and co-workers (25) reported that LXR\(\alpha\)/LXR\(\beta\)\(-/-\) mice at 18 months of age have significantly less adipose tissue than wild type mice. To determine whether reduced fat mass is due to a loss of LXR\(\alpha\)/LXR\(\beta\)\(-/-\) or, both, we analyzed wild type, LXR\(\alpha\)/LXR\(\beta\)\(-/-\), and LXR\(\beta\)\(-/-\) mice at 1 year of age. In male LXR\(\alpha\)/LXR\(\beta\)\(-/-\) and LXR\(\beta\)\(-/-\) animals, the gonadal fat pad as a percentage of body weight decreased by 80 and 65%, respectively (Fig. 1A). Body weights were not different between genotypes under these conditions (data not shown). Although gonadal fat pads from LXR\(\alpha\)/LXR\(\beta\)\(-/-\) mice were not significantly different from wild type mice, there was a trend toward less adipose tissue (Fig. 1A). The weight of other tissues examined, including heart, lung, liver, spleen, kidney, and pancreas, was not changed (data not shown). Histological analyses revealed that the relative size of adipocytes from LXR\(\alpha\)/LXR\(\beta\)\(-/-\) and LXR\(\beta\)\(-/-\) mice was decreased by 60–70% when compared with wild type mice (Fig. 1, B and C). The trend for LXR\(\alpha\)/LXR\(\beta\)\(-/-\) mice to have slightly less adipose tissue may be the result of a 30% decrease in adipocyte size (Fig. 1B). Decreased triacylglycerol levels in liver may suggest that adipocyte growth is impaired due to reduced delivery of hepatic triacylglycerol to adipocytes (Table 1). The reduced weight of gonadal white adipose tissue in LXR\(\alpha\)/LXR\(\beta\)\(-/-\), LXR\(\alpha\)/LXR\(\beta\)\(-/-\), and LXR\(\beta\)\(-/-\) is proportional to the decrease in adipocyte size, suggesting that the total number of adipocytes per depot is approximately the same between the different genotypes. Similar results are also observed in female mice (data not shown).

**LXR\(\beta\)\(-/-\) Mice Are Resistant to Diet-induced Obesity**—To investigate whether LXR\(\beta\) is required for diet-induced obesity, female wild type and LXR\(\beta\)\(-/-\) mice were fed a low fat or high fat diet for 6 months. Wild type mice gained ~12 g more body weight on a high fat diet when compared with a low fat diet; however, LXR\(\beta\)\(-/-\) mice were resistant to this weight gain (Fig. 1D). In wild type mice on a high fat diet, the gonadal fat...
FIG. 1. Reduced weight of adipose tissue depots in aged LXRA−/−β−/− and LXRB−/− mice is largely due to decreased size of adipocytes. A, weight of the gonadal white adipose tissue (GWAT) as a percentage of body weight (%BW) of wild type (WT), LXRA−/−β−/−, LXRA−/−, and LXRB−/− mice (n = 4) at 1 year of age. B, the relative size of fat cells within a microscopic field was determined by quantitation of cells in at least three different randomly chosen fields per mouse. Data are presented as mean ± S.D. Statistical differences between LXRA−/−β−/−, LXRA−/−, and LXRB−/− versus wild type mice were evaluated with Student's t test; *, p < 0.01; **, < 0.001. C, photomicrograph of gonadal white adipose tissue after staining with hematoxylin and eosin. D-F, body weight (D), gonadal fat pad weight (E), and relative adipocyte size (F) in female wild type and LXRB−/− mice fed a low fat (LF) or high fat (HF) diet for 6 months (n = 4–6). Open columns indicate wild type, filled columns indicate LXRB−/−. G, gene expression determined by quantitative real-time PCR in gonadal white adipose tissue from mice with the indicated dietary treatments and genotypes. FAS, fatty acid synthase. H, expression of UCP-1 in gonadal white adipose tissue of female mice fed a high fat diet, determined by quantitative real-time PCR. UCP-1 was not detected (ND) in white adipose tissue from mice fed a low fat diet. Data are presented as mean ± S.D. Statistical differences between treatments are indicated: #, p < 0.05; *, < 0.01; **, < 0.001.

To investigate mechanisms whereby adipocyte growth is impaired in LXRB−/− mice, we quantified expression of a number of adipocyte genes involved in lipid metabolism in mice fed a low fat or a high fat diet (Fig. 1) and resisted the increase in adipocyte size normally observed with diet-induced obesity (Fig. 1F).

To investigate mechanisms whereby adipocyte growth is impaired in LXRB−/− mice, we quantified expression of a number of adipocyte genes involved in lipid metabolism in mice fed a low fat or a high fat diet (Fig. 1, G and H). No consistent difference was observed in expression of hormone-sensitive lipase, perilipin, CD36 (fatty acid translocase), GLUT4, lipoprotein lipase, or cytochrome c mRNAs in gonadal adipose tissue (Fig. 1G; data not shown). Reduced lipid accumulation cannot be explained by expression of lipogenic genes as expres-
FIG. 2. LXRβ is required for the hypertrophy of adipocytes that occurs with aging. A, gonadal (GWAT) adipose tissue as a percentage of body weight (%BW) (A) and the relative size of adipocytes in gonadal white adipose tissue (B) for male wild type and LXRβ−/− mice at 2, 6, and 12 months of age. C and D, weight of perirenal (PWAT) and mesenteric (MWAT) adipose tissues for wild type and LXRβ−/− mice at 2 and 6 months of age. Male wild type (n = 4) and LXRβ−/− (n = 4) mice were fed a normal chow diet. Data are presented as mean ± S.D. Open columns indicate wild type, filled columns indicate LXRβ−/−. Statistical differences between LXRβ−/− mice and wild type were determined with Student’s t test. ∗, p < 0.01; #, p < 0.05.

FIG. 3. Energy balance and body composition of LXRβ−/− mice. Daily food intake (A) and oxygen consumption (B) were determined for male wild type and LXRβ−/− mice at 6 months of age. Lean body mass (LBM) (C) and total body lipid (D) were estimated by dual energy x-ray absorptiometry. Open bars, wild type; closed bars, LXRβ−/−. Data are presented graphically as mean ± S.D. (n = 4–5). No statistical differences were observed in two independent experiments.

sion of SREBP-1c and fatty acid synthase in the gonadal white adipose tissue of LXRβ−/− mice was not suppressed by a high fat diet. Expression of leptin mRNA was less under both dietary conditions (Fig. 1G). As expected (33), mice on a high fat diet had increased expression of UCP-1 in gonadal adipose tissue due to adaptive thermogenesis (Fig. 1H). Unexpectedly, we discovered that expression of UCP-1 was increased by a further −15-fold in LXRβ−/− mice (Fig. 1H), suggesting that LXRβ normally suppresses expression of UCP-1 expression in white adipose tissue. Thus, uncoupling of mitochondria in adipocytes of LXRβ−/− mice may help confer resistance to the adipocyte growth and weight gain observed in wild type mice fed a high fat diet.

**LXRβ Is Required for the Increase in Adipocyte Size That Occurs with Aging**—To investigate further the adipose phenotype of LXRβ−/− mice, tissues at 2 and 6 months of age were examined. At 2 months of age, LXRβ−/− mice did not show differences in the weight of gonadal fat pads nor in the size of adipocytes (Fig. 2A and B), again indicating that LXRβ is not required for adipocyte development. Between 2 and 6 months of age, gonadal fat mass of the wild type mice more than doubled (Fig. 2A), due in large part to adipocyte hypertrophy (Fig. 2B). In contrast, the weight of gonadal fat pads and the size of adipocytes in LXRβ−/− mice did not change during this period. Diminished adipocyte hypertrophy in LXRβ−/− mice is not specific to the gonadal fat pad as other visceral depots, such as the perirenal and mesenteric fat depots, do not expand between 2 and 6 months of age (Fig. 2, C and D). Taken together, these data indicate that LXRα and LXRβ are not required for adipogenesis per se, but instead, LXRβ appears to play a specific role in growth of adipocytes.

**Energy Balance and Body Composition**—Impaired growth of adipocytes in LXRβ−/− mice could arise from slight alterations in energy balance or from differences in lipid metabolism within adipocytes and/or hepatocytes. To investigate effects on energy balance, we analyzed male wild type and LXRβ−/− mice at 6 months of age maintained on a standard chow diet. Under these conditions, LXRβ does not appear to play a role in energy balance as differences in daily food intake or oxygen consumption were not detected (Fig. 3, A and B). Although total oxygen consumption did not differ, further analysis revealed a slight statistical decrease in oxygen consumption in LXRβ−/− mice during the light cycle (data not shown). Consistent with LXRβ not having dramatic effects on energy balance, body weights of wild type and LXRβ−/− mice were not different when compared at 2 months, 6 months, or 1 year (Table I; data not shown). Furthermore, analysis of body composition by dual energy x-ray absorptiometry did not reveal differences in either
lean body mass or total body lipid at these ages (Fig. 3, C and D; data not shown). Thus, despite a decrease in lipid stored within adipose tissue depots and within liver (Table I), there appears to be a compensatory increase in lipids elsewhere in the body. Therefore, under standard laboratory conditions, LXRβ does not appear to play a major role in regulation of energy balance or body composition. Although impaired hypertrophy could be due to regulation by LXRβ of a lipogenic gene not examined here or through small effects on many adipocyte genes, reduced adipocyte size could also be an indirect effect through altered metabolism elsewhere within the body.

LXRβ−/− Mice on Chow Diet Are Glucose-intolerant Due to Impaired Insulin Secretion—Since total body lipid is similar despite reduced adipose tissue mass, we speculated that the storage of lipid in non-adipose tissues might cause insulin resistance and impair glucose homeostasis. To assess potential differences in glucose homeostasis, we performed glucose tolerance tests with female mice at 6 and 9 months of age. Although LXRα−/−β−/− mice tended toward glucose intolerance, statistical differences in glucose tolerance were not observed in either LXRα−/−β−/− or LXRα−/− mice (Fig. 4, A and B). In contrast, both female and male LXRβ−/− mice showed impaired glucose tolerance (Fig. 4, C and D). Contrary to our hypothesis, insulin tolerance tests did not reveal differences in sensitivity between wild type, LXRα−/−, and LXRβ−/− animals (Fig. 4E). Therefore, LXRβ−/− mice are glucose-intolerant and yet do not exhibit insulin resistance. Paradoxically, LXRβ−/− mice on low and high fat diets both show improved glucose tolerance (data not shown), suggesting that this phenotype is strongly influenced by dietary factors.

Consistent with reduced fat mass not causing insulin resistance in mice devoid of LXRβ and on a chow diet, the serum concentrations for secreted adipocyte hormones such as resistin and leptin are similar (Table I). Serum adiponectin is slightly reduced in mice devoid of both LXRα and LXRβ, contrary to other mouse models in which adiponectin is secreted at higher levels from small adipocytes (Table I). These data suggest that the smaller adipocytes in LXRβ−/− mice produce comparable levels of adipocyte hormones as the larger wild type adipocytes. To investigate the mechanism for the decreased ability of LXRβ−/− animals to clear glucose from the circulation, serum insulin was measured. Although the insulin levels of mice fasted overnight were not different, the insulin levels of random fed LXRβ−/− mice were ~50% lower than the wild type mice (Table I). Furthermore, serum insulin levels measured 30 min after injection of glucose were ~65% lower than those for wild type mice despite hyperglycemia in the LXRβ−/− mice at this time point (Fig. 4F). In addition, LXRβ−/− mice have increased glucose production in pyruvate tolerance tests at 45 and 60 min (Supplemental Fig. 1), consistent with impaired insulin secretion causing elevated hepatic glucose production and decreased peripheral glucose disposal. These data suggest that glucose intolerance in LXRβ−/− mice is due to a pancreatic defect, with impaired glucose-induced insulin secretion.

Lipid Accumulation in β Cells of LXRβ−/− Mice—To assess whether LXRβ is required to maintain proper β cell function, we examined the pancreata of mice at 6 months of age. Histological analysis revealed the presence of vacuoles in β cells of islets from LXRα−/−β−/− and LXRβ−/− mice (Fig. 5A) but not in β cells of wild type and LXRα−/− animals. To characterize these vacuoles further, cryosections of pancreata were prepared, and neutral lipids were stained with Oil Red-O. Islets from LXRα−/− mice showed massive accumulation of neutral lipids (Fig. 5B), whereas lipid stores were not observed in islets from either wild type mice (Fig. 5B) or LXRα−/− mice (data not shown). Mice devoid of LXRβ did not have altered islet morphology or insulin and glucagon content as assessed by immunohistochemistry (Fig. 5C). Furthermore, histomorphometric analyses of pancreatic sections did not reveal any change in the numbers or size of islets (data not shown).

To investigate the role of LXRβ in regulating pancreatic cholesterol and lipid homeostasis, we isolated islets and determined the relative mRNA expression of selected genes by...
and 80%, respectively, but differences in scavenger receptor
cholesterol transporters ABCA1 and ABCG1 was reduced by 60

LXR

mRNAs was determined by quantitative PCR. In LXR

mice at 5 months of age (Fig. 5A). Reduced efflux of cholesterol
is expected to result in intracellular accumulation of free
cholesterol and cholesterol esters. SREBP-1c is known to
activate genes required for fatty acid synthesis, whereas
SREBP-2 preferentially activates genes involved in choles-
terol biosynthesis (34). Although SREBP-1c and SREBP-2
mRNA levels were significantly reduced in LXR

mice, changes in expression of these genes were not observed in
islets isolated from LXR

mice (Fig. 5D). Despite reduced expression of SREBPs, differences in fatty acid synthase, lipoprotein lipase, acetyl CoA carboxylase 1, and 3-hydroxy-3-methylglutaryl CoA reductase were not detected (Fig. 5D). Furthermore, expression of peroxisome proliferator-activated receptor γ, UCP-2, GLUT2, and glucokinase were also similar, suggesting that differences in energy expenditure or glucose uptake are not regulated by LXRβ in islets. LXRα and LXRβ mRNAs are expressed in islets from wild type mice, consistent with a cell-autonomous mechanism (data not shown). Taken together, these data suggest that LXRβ−/− mice accumulate cholesterol esters due to impaired choles-
terol efflux. Whether accumulation of neutral lipids in islets
impairs insulin secretion warrants further investigation.

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Fig. 5. Lipid accumulation in β cells of LXRβ−/− mice. A, photo-
micrographs of pancreas from male wild type (WT), LXRα−/−β−/−, LXRα−/−, and LXRβ−/− mice at 1 year of age stained with hematoxy-
lin and eosin. B, Oil Red-O staining of islets after cryosectioning of
pancreata from wild type and LXRβ−/− mice. C, immunohistochem-
istry of fixed cryosections of mouse pancreas for insulin (red) and gluca-
gon (green). D, gene expression in pancreatic islets isolated from wild
type (open bars), LXRα−/− (shaded bars), and LXRβ−/− (closed bars)
mice at 5 months of age (n = 4). Relative expression of the indicated
mRNAs was determined by quantitative PCR. Bars indicate means ±
S.D. #, p < 0.05; *, < 0.01; **, < 0.001 versus wild type. All data are
representative of at least two independent experiments. FAS, fatty acid
synthase; LPL, lipoprotein lipase; GK, glucokinase.

quantitative PCR. In LXRβ−/− mice, expression of the
cholesterol transporters ABCA1 and ABCG1 was reduced by 60
and 80%, respectively, but differences in scavenger receptor
B1 were not observed (Fig. 5D). Reduced efflux of cholesterol
is expected to result in intracellular accumulation of free
cholesterol and cholesterol esters. SREBP-1c is known to
activate genes required for fatty acid synthesis, whereas
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terol biosynthesis (34). Although SREBP-1c and SREBP-2
mRNA levels were significantly reduced in LXRβ−/− mice, changes in expression of these genes were not observed in
islets isolated from LXRα−/− mice (Fig. 5D). Despite reduced expression of SREBPs, differences in fatty acid synthase, lipoprotein lipase, acetyl CoA carboxylase 1, and 3-hydroxy-3-methylglutaryl CoA reductase were not detected (Fig. 5D). Furthermore, expression of peroxisome proliferator-activated receptor γ, UCP-2, GLUT2, and glucokinase were also similar, suggesting that differences in energy expenditure or glucose uptake are not regulated by LXRβ in islets. LXRα and LXRβ mRNAs are expressed in islets from wild type mice, consistent with a cell-autonomous mechanism (data not shown). Taken together, these data suggest that LXRβ−/− mice accumulate cholesterol esters due to impaired choles-
terol efflux. Whether accumulation of neutral lipids in islets
impairs insulin secretion warrants further investigation.

Fig. 6. Impaired glucose-stimulated insulin secretion in single
islets from LXRβ−/− mice. A, insulin was assayed every 6 s from wild
type (black) and LXRβ−/− (gray) islets upon step increases in glucose
concentration from 3 to 8 to 15 mM (arrows). Traces are the average of
eight islets from two mice of each genotype; ±S.E. Error bars are shown
at every 10th point for clarity. B, basal insulin levels at 3 mM glucose
and the maximum first phase of insulin secretion upon increases to 8
and 15 mM glucose are compared (n = 8 for each genotype). Error bars
are ±S.E. The differences between genotypes were evaluated with
Student’s t test; *, p < 0.05; **, < 0.005.

Impaired Glucose-stimulated Insulin Secretion in Islets from
LXRβ−/− Mice—To determine whether decreased insulin secretion
after injection of glucose is due to a defect in islet function, we isolated islets and investigated their ability to
secrete insulin in response to glucose. After isolation and cul-
ture for 1–2 days, single islets were exposed to increasing
concentrations of glucose, and secretion of insulin was moni-
tored (Fig. 6A). Although islets from LXRβ−/− mice had de-
creased secretion of insulin at basal levels of glucose (3 mM), the
impaired glucose-stimulated insulin secretion was far more
striking at 15 mM glucose (Fig. 6A), with islets from LXRβ−/− mice secreting 50–80% less insulin than control islets (Fig.
6B). Insulin content of cultured islets from wild type and
LXRβ−/− islets was similar (data not shown). Examination of
dividual insulin secretion traces revealed that, in addition to
suppressed secretion, LXRβ−/− islets tended to have damp-
ened and irregular oscillations when compared with controls, suggesting that LXRβ is required for maintenance of normal pulsatility (data not shown). Taken together, these data indicate that LXRβ−/− mice have a defect in secretion of insulin from islets.

**DISCUSSION**

A potential role for LXRs in adipogenesis has been appealing for a number of reasons. First, expression of LXRα increases during adipogenesis, and LXRβ is expressed throughout adipocyte conversion (24, 25). Second, LXRs regulate lipogenesis in hepatocytes (17). Third, adipocytes have a very high level of cellular cholesterol, suggesting a ready supply of LXR ligands (35). Finally, both LXRα and LXRβ are highly expressed in adipose tissue (36). However, the testing of this hypothesis has led to considerable controversy within the literature. Although expression and activation of LXRα in 3T3-L1 preadipocytes was reported to inhibit adipocyte conversion (24), a similar experiment in NIH-3T3 cells did not affect the ability of ectopic peroxisome proliferator-activated receptor γ to stimulate adipogenesis (27). Furthermore, activation of endogenous LXRs in 3T3-L1 preadipocytes has been reported to have no effect on adipogenesis or accumulation of lipid (24, 27), whereas other studies find that LXR agonists stimulate adipogenesis (28) and/or lipid accumulation (25, 28). Although knock-down of LXRα by siRNA in 3T3-L1 cells suggests that this transcription factor is required for adipogenesis (28), mice deficient for LXRα and LXRβ have adipose tissue (25). Although differences in LXR agonists, cell culture conditions, and 3T3-L1 subclones are possible causes of such discrepancies, further experimentation is clearly warranted to unify the field on the roles of LXRs in adipocyte biology.

A prior report indicated that LXRα−/−β−/− mice at 18 months of age have reduced levels of white adipose tissue; however, these data were confounded by dramatic differences in body weight not observed in our studies (Table 1) (Ref. 25). To explore roles of LXR transcription factors in adipocyte biology, we used mice deficient for LXRα and/or LXRβ and confirmed that LXRα−/−β−/− mice at 1 year of age have less adipose tissue. The basis for reduced fat mass appears to be due to a lack of LXRβ rather than LXRα, consistent with these transcription factors regulating distinct sets of adipocyte genes (36). The decrease in fat depot weights is age-dependent and largely due to decreased adipocyte size rather than reduced adipocyte number. Although adipose tissue weights and adipocyte size are similar at 2 months of age, impaired adipocyte growth in LXRβ−/− mice over subsequent months results in decreased amounts of adipose tissue. Taken together, these data indicate that LXRs are not required for adipocyte development, but LXRβ is required for the increase in adipocyte size that occurs with age.

The impaired adipocyte hypertrophy observed in LXRβ−/− mice as they age could arise through differences in energy balance. Although we did not observe differences in daily food intake or oxygen consumption, insensible differences in either of these variables over a period of months could result in less triacylglycerol stored in fat cells. However, total body lipid is similar when assessed by dual energy x-ray absorptiometry, suggesting that altered energy balance is unlikely to be the cause. The non-adipose lipid is not stored in liver (Table 1) (Ref. 30) but appears to be distributed through other non-adipose tissues, including vasculature, macrophages, motor neurons, spinal chord, and pancreatic islets (Fig. 5) (Refs. 30, 37, and 38).

The lack of triglyceride in adipocytes of LXRβ−/− mice could also arise due to reduced fatty acid synthesis in adipose tissue or liver or decreased uptake and storage of dietary or hepatic lipid. Alternatively, reduced stores could be due to increased lipolysis or mitochondrial uncoupling. Reduced de novo fatty acid synthesis in adipocytes is unlikely to be the cause because SREBP-1c and fatty acid synthase are expressed at similar or increased levels in adipose tissue of mice (Fig. 1G) (Ref. 39). It should be noted that interpreting changes in adipocyte gene expression in LXRβ−/− mice is confounded by increased expression of LXRα (data not shown and Ref. 39). In addition, genes regulated by LXRβ may increase or decrease in LXRβ−/− mice depending upon whether they are actively repressed or transactivated under the conditions examined (2). Decreased hepatic triacylglycerol and reduced fasting triacylglycerol levels are consistent with the decreased size of adipocytes resulting from impaired delivery of lipids to adipose tissue from liver (Table 1).

Increased adipose expression of UCP-1 may contribute to reduced lipid storage under certain circumstances and is consistent with suppression of UCP-1 by LXR agonists (40, 41). Although induction of UCP-1 in adipose tissue of LXRα−/−β−/− and LXRβ−/− mice fed a chow diet was observed in some experiments (Fig. 1H), the lack of response in others indicates sensitivity to environment, age, or other conditions not controlled for in these studies. However, UCP-1 was greatly increased in white adipose tissue of mice fed a high fat diet (Fig. 1H), suggesting that at least part of the decrease in adipocyte size may be accounted for by mitochondrial uncoupling. Finally, it may be that reduced adipocyte hypertrophy in LXRβ−/− mice is secondary to alterations in insulin secretion from pancreas (Fig. 6) or due to metabolic alterations in other tissues.

Although our observation that LXRβ−/− mice are glucose-intolerant appears on the surface to coincide with prior work indicating that the LXR agonists improve glucose tolerance (21, 22), the mechanisms are undoubtedly different. LXR agonists act to suppress gluconeogenesis (i.e. phosphoenol-pyruvate carboxykinase and glucose 6-phosphatase) and increase glucose flux into the liver by metabolic trapping (i.e. glucokinase) and into adipose tissue by increasing expression of the insulin-sensitive glucose transporter (i.e. GLUT4). Although our hypothesis was that reduced adipose tissue mass in LXRβ−/− mice led to lipodystrophy, we observe that insulin sensitivity, as assessed by insulin tolerance tests, is not altered by the absence of LXRα or LXRβ and that adipokines do not differ substantially. Instead, glucose intolerance in LXRβ−/− mice is more likely caused by impaired glucose-induced insulin secretion (Figs. 4 and 6). LXRβ−/− islets have reduced secretion of insulin in response to basal levels of glucose as well as stimulatory concentrations. Consistent with these observations, incubation of rat islets with the LXR agonist T091317 increases insulin secretion in response to glucose (23). Although a link to islet dysfunction remains speculative, lipid deposits are observed in islets from LXRβ−/− mice. Reduced expression of cholesterol transporters, ABCA1 and ABCG1, likely leads to reduced efflux of cholesterol and to the accumulation of cholesterol esters and perhaps other neutral lipids (Fig. 5D). These studies demonstrate that LXRβ plays an important role in coupling of glucose metabolism to insulin secretion.

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