Hepatic Insulin Resistance Is Sufficient to Produce Dyslipidemia and Susceptibility to Atherosclerosis

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SUMMARY

Insulin resistance plays a central role in the development of the metabolic syndrome, but how it relates to cardiovascular disease remains controversial. Liver insulin receptor knockout (LIRKO) mice have pure hepatic insulin resistance. On a standard chow diet, LIRKO mice have a proatherogenic lipoprotein profile with reduced high-density lipoprotein (HDL) cholesterol and very low-density lipoprotein (VLDL) particles that are markedly enriched in cholesterol. This is due to increased secretion and decreased clearance of apolipoprotein B-containing lipoproteins, coupled with decreased triglyceride secretion secondary to increased expression of Pgc-1α (Ppargc1a), which promotes VLDL secretion, but decreased expression of Srebpb-1c (Srebfp1), Srebpb-2 (Srebfp2), and their targets, the lipogenic enzymes and the LDL receptor. Within 12 weeks on an atherogenic diet, LIRKO mice show marked hypercholesterolemia, and 100% of LIRKO mice, but 0% of controls, develop severe atherosclerosis. Thus, insulin resistance at the level of the liver is sufficient to produce the dyslipidemia and increased risk of atherosclerosis associated with the metabolic syndrome.

INTRODUCTION

Atherosclerotic cardiovascular disease is a major cause of morbidity and mortality in Western society, and much effort has been directed toward understanding its pathogenesis and identifying the risk factors associated with it. One of the most important predisposing factors for atherosclerosis is the metabolic syndrome. The metabolic syndrome is a constellation of abnormalities including central obesity; glucose intolerance and type 2 diabetes; hypertension; and a dyslipidemia characterized by increased serum triglycerides, decreased high-density lipoprotein (HDL) cholesterol, and increased small dense low-density lipoprotein (LDL) particles (Haffner et al., 1998; Grundy et al., 2004; Ford, 2005). The metabolic syndrome affects more than 27% of adults in the United States (Ford et al., 2004; Hedley et al., 2004) and increases the risk of cardiovascular disease 2- to 3-fold (Framingham Heart Study Cooperative Research Group, 1995; Isomaa et al., 2001). Despite the alarming prevalence of the metabolic syndrome and the magnitude of risk it confers, however, defining the pathogenic links between the metabolic syndrome and cardiovascular disease has been difficult, and even the question of whether they are united by some common underlying pathophysiology remains a matter of intense debate (Kahn et al., 2005; Zimmet et al., 2005).

Insulin resistance has long been considered to be central to the pathophysiology of the metabolic syndrome (Reaven, 1988; Biddinger and Kahn, 2006). In the liver, insulin resistance is manifested by the blunted ability of insulin to activate its receptor kinase and its downstream targets (Saad et al., 1992; Kerouz et al., 1997), resulting in incomplete suppression of hepatic glucose production (Lewis et al., 1996). It is not clear, however, whether all metabolic pathways become resistant to insulin (Shimomura et al., 2000; Saad et al., 1993). For example, lipogenesis, which is positively regulated by insulin, is increased in the metabolic syndrome. This could be because lipogenesis remains sensitive to insulin and is driven excessively by the compensatory hyperinsulinemia that occurs in insulin-resistant states (Reaven, 1988, 2004; Biddinger et al., 2005; Biddinger and Kahn, 2006). Alternatively, lipogenesis could also become resistant to insulin but be driven by other factors in the metabolic syndrome, such as excessive carbohydrate intake (Schwarz et al., 2003).

The coexistence of hyperinsulinemia and other hormonal and metabolic changes has made it difficult to dissect the role of insulin resistance in the pathogenesis of the metabolic syndrome. This is particularly true of very low-density lipoprotein (VLDL) secretion, which is increased in the metabolic syndrome and is thought to drive the other aspects of the dyslipidemia associated with this disorder (Sparks and Sparks, 1994; Zammit, 2002; Ginsberg, 1996; Ginsberg and Huang, 2000). Under normal conditions, insulin targets apolipoprotein B (apoB), the principal protein component of VLDL, for intracellular degradation and thereby decreases VLDL secretion acutely (Sparks and Sparks, 1994; Ginsberg et al., 2006). However, insulin also stimulates...
lipogenesis, which can promote VLDL secretion (Horton et al., 2002). Therefore, the increase in VLDL secretion associated with the metabolic syndrome could be due either to insulin resistance driving VLDL secretion by failing to degrade apoB or to hyperinsulinemia driving VLDL secretion through excessive lipogenesis (Sparks and Sparks, 1994; Lewis et al., 1995; Malmstrom et al., 1997b; Reaven, 1997; Reaven and Laws, 1994). Determining the relative roles played by insulin resistance versus hyperinsulinemia or other factors in the development of the dyslipidemia associated with the metabolic syndrome remains a fundamental but unanswered question with important therapeutic implications.

In the present study, we used the liver insulin receptor knockout (LIRKO) mouse as a model of pure hepatic insulin resistance. These mice develop hyperinsulinemia, but their livers are unable to respond to it. Therefore, the LIRKO model is a unique tool with which to dissect the effects of hepatic insulin resistance on the development of dyslipidemia (Sparks and Sparks, 1994; Lewis et al., 1995; Malmstrom et al., 1997b; Reaven, 1997; Reaven and Laws, 1994). Determining the relative roles played by insulin resistance versus hyperinsulinemia or other factors in the development of the dyslipidemia associated with the metabolic syndrome remains a fundamental but unanswered question with important therapeutic implications.

### RESULTS

LIRKO mice were created by breeding mice carrying insulin receptor alleles modified with flox sites with mice carrying the Cre recombinase driven by the albumin promoter, as described previously (Michael et al., 2000). On normal chow (9% fat with negligible amounts of cholesterol), LIRKO mice were nonobese, with normal serum free fatty acid levels and mildly to moderately elevated serum glucose levels (Table 1) relative to their littermate controls (Lox mice). LIRKO mice also developed marked hyperinsulinemia due to the compensatory secretion of insulin from the β cells of the pancreas coupled with a defect in insulin clearance due to the lack of insulin receptors in the liver (Michael et al., 2000). We have previously shown that the LIRKO liver fails to respond to this hyperinsulinemia, as insulin is unable to stimulate phosphorylation of its downstream targets, the insulin receptor substrates 1 and 2 (IRS-1 and -2), or normally suppress expression of phosphoenolpyruvate carboxykinase (PEPCK) and glucose 6-phosphatase (Michael et al., 2000). As observed previously, liver weights were decreased by approximately 30%, due at least in part to decreased glycogen stores (Michael et al., 2000). Hepatic triglyceride content was unchanged, but there was a 2-fold increase in hepatic cholesterol, a 4-fold increase in hepatic cholesterol esters, and a 40% increase in free hepatic cholesterol (Table 1 and data not shown).

### Dyslipidemia in LIRKO Mice

Despite the fact that serum cholesterol levels were normal (Figure 1A) and serum triglyceride levels were reduced by 50% (Figure 1B) on a normal chow diet, hepatic insulin resistance alone was sufficient to produce atherogenic changes in lipoprotein metabolism. Thus, fast protein liquid chromatography (FPLC) analysis of serum from LIRKO and Lox control mice revealed that HDL cholesterol, the major form of circulating cholesterol in the mouse, was decreased by 50%, whereas VLDL cholesterol was increased 3-fold (Figure 1C). Consistent with the decreased total serum triglyceride levels, FPLC analysis also showed that VLDL and LDL/intermediate-density lipoprotein (IDL) particles in LIRKO mice contained reduced levels of triglycerides (Figure 1D). Concomitant with these differences, the levels of apoB100 and apoB48, the major lipoproteins of VLDL, LDL, and chylomicrons, were increased in LIRKO mice, whereas the levels of apoA-I and apoE were not (Figure 1E). Interestingly, apoE was present in the more buoyant lipoprotein fractions of LIRKO, but not control, serum. Therefore, even though insulin resistance did not alter total serum cholesterol levels, it produced a distinctly proatherogenic distribution of cholesterol with a shift toward apoB-containing lipoproteins.

Lipid analysis of VLDL particles isolated from these mice by ultracentrifugation confirmed their abnormal lipid composition (Table 2). In control mice, VLDL particles were composed largely of triglycerides, with an 8:1 weight ratio of triglycerides to total cholesterol. In contrast, triglycerides accounted for only 43% of the lipids in VLDL isolated from LIRKO serum, producing a 1:1 ratio of triglycerides to total cholesterol. Therefore, isolated hepatic insulin resistance is sufficient to produce VLDL particles enriched in cholesterol.

### Dysregulation of PGC-1α, SREBP-1c, and SREBP-2

Several transcription factors, including the sterol regulatory element-binding proteins (SREBPs) and their coactivators, especially peroxisome proliferator-activated receptor γ coactivator (PGC)-1α (Yoon et al., 2001), have been shown to regulate the synthesis and secretion of VLDL (Lin et al., 2005; Kalaany and Mangelsdorf, 2006; Wolfrum and Stoffel, 2006; Horton et al., 2002) In nonfasted mice on a standard chow diet, Pgc-1α (Ppargc-1a) and Pgc-1β (Ppargc-1b), coactivators involved in regulation of gluconeogenic and mitochondrial gene expression (Yoon et al., 2001), were increased 2- to 3-fold at the mRNA and protein levels (Figure 2A). In contrast, mRNA for SREBP-1c (Sreb1α), which regulates lipogenic gene expression, and SREBP-2 (Sreb2), which regulates expression of cholesterologenic enzymes and the LDL receptor, were decreased by 40%–80% in LIRKO livers. After fasting and refeeding, which normally leads to a dramatic
induction of SREBP-1c (Matsuzaka et al., 2004), nuclear SREBP-1c, which represents the active form of SREBP-1c, was reduced >95% in LIRKO livers relative to controls (Figure 2A).

Many of the targets of SREBP-2, including the cholesterogenic enzymes HMG-CoA reductase (Hmgcr), squalene synthase (SS), and farnesyl diphosphate synthetase (Fdps), as well as the LDL receptor (Ldlr), were decreased by 60%–90% in LIRKO livers. However, the targets of SREBP-1c, the genes of fatty acid and triglyceride metabolism, showed a mixed pattern of change in LIRKO livers. Some enzymes, like glycerol 3-phosphate acyltransferase (Gpat) and diacylglycerol acyltransferase (Dgat), which are involved in the packaging of fatty acids into triglycerides, and phospholipid transfer protein (Ptlp), which also traffics lipids, were significantly increased in LIRKO livers. In contrast, stearoyl-CoA desaturase 1 (Scd1) and fatty acid synthase (Fas) were decreased by 40%–90%, and acetyl-CoA carboxylase (Acc) was unchanged (Figure 2B).

Srebp-1c is regulated in part by insulin and in part by liver X receptor (LXR) (Repa et al., 2000; Tobin et al., 2002; Chen et al., 2004; Liang et al., 2002), but how these two factors interact in the control of Srebp-1c remains a subject of debate (Hegarty et al., 2005; Dif et al., 2006; Cagen et al., 2005). Lxr mRNA (Figure 2A) and protein levels (data not shown) were essentially unchanged in LIRKO mice. To determine whether activation of LXR could rescue lipogenic gene expression in the absence of insulin signaling, we treated LIRKO mice and controls with an LXR agonist, T090137, for 2 days (Figure 2C). The LXR agonist markedly increased expression of SREBP-1c and its lipogenic targets in both Lox and LIRKO livers. However, expression of these genes remained significantly lower in LIRKO livers even after pharmacological stimulation of LXR. Therefore, insulin action, in addition to LXR activation, is necessary for maximal induction of Srebp-1c and lipogenesis.

Increased Secretion and Decreased Clearance of apoB Lipoproteins

Given the changes in gene expression and the presence of abnormal, cholesterol-enriched VLDL particles in LIRKO serum, we measured VLDL secretion in chow-fed Lox and LIRKO mice by injecting them with Triton WR1339, which inhibits lipoprotein lipase and therefore VLDL clearance. As shown in Figure 3A, the rate of triglyceride secretion was decreased by 66% in LIRKO mice. Three hours after injection of Triton WR1339, VLDL was isolated by ultracentrifugation, and consistent with the decreased rate of triglyceride secretion, VLDL from LIRKO mice contained 50% less triglyceride (Figure 3B). However, VLDL cholesterol levels were similar in control and LIRKO mice (Figure 3C). Thus, insulin resistance in the liver leads to an uncoupling of cholesterol and triglyceride secretion such that only triglyceride secretion is impaired, resulting in cholesterol-rich VLDL particles.
but it also contains cholic acid, which increases intestinal cholesterol absorption and produces an even greater degree of hypercholesterolemia. Interestingly, on this diet, although LDLR protein was markedly decreased, Ldlr mRNA levels were similar (see Figure S1 available online). After 2 months on the atherogenic diet, serum cholesterol levels rose to 310 ± 24 mg/dl in control mice but to 781 ± 98 mg/dl in LIRKO mice (Figure 4A). FPLC fractionation revealed that the excess cholesterol was found exclusively in the apob-containing lipoproteins: VLDL, LDL, and IDL (Figure 4B). Remarkably, despite this dramatic increase in total serum cholesterol, HDL cholesterol levels were still significantly lower in LIRKO mice than in Lox mice. Similar changes were observed upon FPLC analysis of serum from mice placed on the Western diet (data not shown). In contrast, the amount and distribution of serum triglycerides were not different between Lox and LIRKO mice on either the atherogenic Paigen diet (Figures 4C and 4D) or the Western diet (data not shown).

The effects of hepatic insulin resistance and the atherogenic diet on atherosclerosis were striking. After 12 weeks on this diet, all of the LIRKO mice of both sexes, but not a single Lox mouse, developed overt atherosclerosis (n = 10–15 mice of each sex per genotype). Atherosclerotic plaques were easily visible on transillumination of the unstained aortic arch of LIRKO mice in situ (Figure 4F, left). Oil red O staining of sections through the aortic sinus revealed intimal fat accumulation in LIRKO mice (Figure 4F, center). Staining of the entire aorta with Sudan IV demonstrated disease throughout the aorta of LIRKO mice, but no visible atheroma in control mice subjected to the same dietary stress (Figure 4F, right). By quantitation, 1.7% of the surface of LIRKO aortas was stained by Sudan IV, whereas no staining was detectable in Lox aortas (Figure 4E). Thus, by multiple criteria, LIRKO mice developed severe atherosclerosis whereas controls did not.

**DISCUSSION**

Although the association between the metabolic syndrome, dyslipidemia, and increased cardiovascular disease risk is generally accepted, how each of these relates to insulin resistance is controversial (Kahn et al., 2005). Our studies in LIRKO mice show that hepatic insulin resistance causes a proatherogenic distribution of serum cholesterol, with a 50% decrease in HDL cholesterol and an increase in non-HDL cholesterol. Furthermore, on both the Western diet and the atherogenic Paigen diet, HDL cholesterol levels remained low, yet LIRKO mice developed severe hypercholesterolemia, with the excess cholesterol associated with apob-containing lipoproteins, i.e., VLDL, LDL, and IDL. As a consequence of these proatherogenic changes in lipid and lipoprotein metabolism, LIRKO mice, but not control mice, developed atherosclerosis within 12 weeks on an atherogenic diet. Therefore, hepatic insulin resistance alone is sufficient for the development of dyslipidemia and, when coupled with a permissive diet, the development of atherosclerosis.

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Insulin resistance acts at several levels to produce dyslipidemia in the LIRKO mouse. First, it increases apoB secretion by decreasing intracellular degradation of apoB (Sparks and Sparks, 1994; Fisher et al., 2001). Second, it alters the expression of Srebp-1c, Srebp-2, Pgc-1μ, and Pgc-1α. SREBP-1c, which mediates the lipogenic response to insulin (Shimomura et al., 1999b), is decreased in LIRKO mice, even in the presence of...
of an LXR agonist, indicating that insulin resistance suppresses SREBP-1c through mechanisms independent of LXR ligand generation. This decrease in SREBP-1c expression results in reduced VLDL triglyceride secretion and hypotriglyceridemia on a chow diet. Insulin resistance also promotes the accumulation of cholesterol, which inhibits SREBP-2 by preventing its maturation (Brown and Goldstein, 1997), and thereby decreases expression of its target, LDLR, and apoB clearance. Insulin may also play a role in stabilizing LDLR protein since the differences in LDLR expression were greater at the protein level than at the mRNA level. In addition, PGC-1 expression is upregulated in LIRKO mice, and this, along with other factors such as Foxa2, could further increase apoB secretion (Lin et al., 2005; Kalaany and Mangelsdorf, 2006; Wolfrum and Stoffel, 2006; Horton et al., 2002). Finally, hepatic insulin resistance could alter lipoprotein metabolism through secondary changes in the hormonal and metabolic milieu, such as hyperglycemia and hyperinsulinemia.

Dietary cholesterol and fat intake clearly interact with hepatic insulin resistance in the production of the dyslipidemia of the metabolic syndrome. On a chow diet, serum triglyceride levels are low and total serum cholesterol is normal. Nonetheless, the distribution of this cholesterol is distinctly proatherogenic, with reduced levels of HDL and increased levels of non-HDL cholesterol. When LIRKO mice are challenged with a high-fat, high-cholesterol diet, serum triglycerides normalize to the level of Lox controls, but cholesterol levels increase progressively to almost 800 mg/dl while still retaining their atherogenic profile with low HDL cholesterol. Therefore, hepatic insulin resistance is able to produce a significant derangement of cholesterol metabolism that progresses in the presence of dietary stress to marked hypercholesterolemia and atherosclerosis.

The distribution of serum cholesterol in LIRKO mice recapitulates the dyslipidemia of the human metabolic syndrome, with increased VLDL cholesterol and decreased HDL cholesterol. In contrast, most commonly used mouse models of the metabolic syndrome, such as leptin deficient ob/ob mice and mice with diet-induced obesity, show increased HDL cholesterol (Table S1). Yet LIRKO mice do not develop the hypertriglyceridemia that is characteristic of human metabolic disease. Serum triglyceride levels are under complex regulation (reviewed in Ginsberg, 1996), but one important factor driving hypertriglyceridemia is insulin-stimulated hepatic de novo lipogenesis, and this is mediated largely by SREBP-1c (Shimomura et al., 1998, 1999b).

Figure S2 and Table S2 show that SREBP-1c and its targets are reduced in mice deficient in hepatic insulin signaling, such as LIRKO mice and mice made insulinopenic by streptozotocin.

Figure 2. Insulin Resistance Alters Gene Expression

(A) Left: expression of the transcriptional regulators Pgc-1α, Pgc-1β, Lxr, Srebpb-1c, and Srebpb-2 was measured in the livers of 2-month-old nonfasted mice on a chow diet by real-time PCR (n = 4–8, *p < 0.05). Right: PGC-1 protein was measured in liver extracts from 2-month-old mice sacrificed in the nonfasted state, and SREBP-1c was measured in liver nuclear extracts prepared from 2-month-old mice after fasting and refeeding. See text for gene names.

(B) Real-time PCR analysis of cDNA prepared from livers of 2- to 4-month-old nonfasted chow-fed mice. See text for gene names.

(C) Two-month-old Lox and LIRKO mice were gavaged with 40 mg/kg LXR agonist (T090137) or vehicle every 24 hr for 2 days and sacrificed 4 hr after the second dose in the nonfasted state. Real-time PCR was performed on cDNA prepared from these animals’ livers (n = 5–6, *p < 0.05, **p < 0.005).

(D) Hepatocytes were isolated from 2- to 3-month-old Lox and LIRKO mice and cultured overnight in the presence of 100 nM insulin and either 5 μM LXR agonist or vehicle. RNA was extracted and subjected to real-time PCR analysis (n = 4–6, *p < 0.05).

Error bars represent SEM.
increasing HDL and decreasing non-HDL cholesterol (Figure S3). Serum glucose and the distribution of serum cholesterol by in-
genic diet using a constitutively active form of Akt normalizes hypothesis, as restoring Akt activity in LIRKO mice on the athero-
 genesis continues to be activated by hyperinsulinemia or some alternative pathway (Siri et al., 2001; Biddinger et al., 2005; Elam et al., 2001). In particular, it appears that the phosphatidylinositol 3-kinase (PI3K)/Akt arm of the insulin signaling pathway, which is thought to mediate many of insulin’s metabolic effects (Garg, 1996), becomes resistant to insulin whereas the MAPK alternative pathway (Siri et al., 2001; Biddinger et al., 2005; Elam et al., 2001). Finally, several rodent models of obesity have demonstrated, preliminary studies show that HDL clearance is normal in LIRKO mice (S.B.B., E.F.S., D.E.C., and C.R.K., unpublished data). Therefore, hepatic insulin resistance reduces HDL cholesterol by either decreasing HDL production or promoting a shift of HDL cholesterol to non-HDL lipoproteins.

In relating the LIRKO model to the metabolic syndrome, it is important to remember that insulin resistance in human meta-
 bolic disease differs in several respects from the LIRKO model. First, the insulin resistance associated with the metabolic syn-
 drome and type 2 diabetes involves many tissues of the body in addition to the liver, including muscle, fat, β cells, the vascula-
 ture, and other cells. Recent studies have shown that these other tissues may also participate in the dyslipidemia and accelerated atherosclerosis associated with these disorders. For example, Baumgartl et al. (2006) have shown that insulin resistance in cells of the myeloid lineage may protect against atherosclerosis in apoE-deficient mice, whereas Han et al. (2006) found that insulin receptor deficiency in macrophages may increase ER stress-induced apoptosis and necrosis in advanced atherosclerotic lesions. Therefore, the development of dyslipidemia and atherosclerosis in the metabolic syndrome is ultimately dependent upon the net effects of insulin resistance in both hepatic and extrahepatic tissues. Moreover, as noted above, in human disease, the effects of dietary and other genetic factors are superimposed on the effects of insulin resistance in the production of the metabolic syndrome. Despite these differences, however, many of the changes in cholesterol metabolism observed in the LIRKO model are also observed in mice and humans with the metabolic syndrome. First, obese, insulin-resistant mice and humans show increased secretion of apoB and VLDL (Lewis et al., 1993; Malmstrom et al., 1997a; Bartels et al., 2002; Cohen et al., 2002; Siri et al., 2001). Second, several rodent models of obesity have also been shown to have decreased expression of LDLR (Roberts et al., 2004; Lundasen et al., 2003; Liao et al., 1997). Finally, humans with metabolic disease show a defect in the catabolism of HDL through cholesteryl ester transfer protein (CETP), an enzyme present in humans but not mice (Nagashima et al., 1988; de Grooth et al., 2004), which removes cholesteryl esters on HDL by exchanging them for triglycerides on VLDL (Garg, 1996; Ginsberg, 1996; Rashid et al., 2002). The fact that LIRKO mice show a major reduction in HDL cholesterol levels even in the absence of hypertriglyceridemia and CETP indicates that insulin resistance alone can decrease HDL cholesterol independently of hypertriglyceridemia. Although the exact mechanism by which insulin resistance produces this effect remains to be determined, preliminary studies show that HDL clearance is normal in LIRKO mice (S.B.B., E.F.S., D.E.C., and C.R.K., unpublished data). Therefore, hepatic insulin resistance reduces HDL cholesterol by either decreasing HDL production or promoting a shift of HDL cholesterol to non-HDL lipoproteins.
of apoB-containing lipoproteins (Ouguerram et al., 2003; Duvilleard et al., 2000; Howard et al., 1987; Chan et al., 2003). Therefore, insulin resistance in the context of the metabolic syndrome may drive dyslipidemia and atherosclerosis by the same mechanisms as it does in the LIRKO model.

In summary, we have shown that insulin resistance plays a critical and central role in the development of dyslipidemia and atherosclerosis. This finding is of clinical importance because it suggests that the metabolic syndrome is not merely a collection of abnormalities that should be considered and treated independently, as some experts have advocated (Kahn et al., 2005). Rather, it appears that the metabolic syndrome is truly a syndrome, in which disturbances in glucose and cholesterol metabolism both stem from a defect in insulin signaling. These data further suggest that finding and reversing the molecular lesions that produce the insulin resistance associated with the metabolic syndrome will result in more effective treatment of this disorder.

**EXPERIMENTAL PROCEDURES**

**Mice, Diets, and Treatments**

Generation and genotyping of LIRKO (Cre$^{+/-}$,IR$^{flox/flox}$) mice and their littermate Lox controls (Cre$^{+/-}$,IR$^{flox/flox}$) were performed as described previously (Michael et al., 2000). LIRKO mice were generated on a mixed genetic background.
background, including 129Sv, C57BL/6, FVB, and DBA, and were inbred for more than ten generations.

In most studies, mice were male, chow-fed (Purina Lab Diet 9F Mouse Chow 5020, Pharmaserv, containing 9.0% fat and 0.221 ppm cholesterol), and sacrifi"""cated in the nonfasted state. Alternatively, mice were given ad libitum access to either an atherogenic Paigen diet (The Jackson Laboratory) or a Western diet (21% milk fat, 34.1% sucrose, 0.15% cholesterol). For refeeding studies, mice were fasted for 24 hr and then refed for 8 hr with a high-carbohydrate diet. LXR agonist (Sigma) was prepared in 1% carboxymethylcellulose and given at a dose of 40 mg/kg by gavage every 24 hr for 2 days to 3-month-old mice, which were sacrificed 4 hr after the second dose in the nonfasted state. Ade


nukrines encoding myr-Akt or LacZ (Ono et al., 2003) were prepared as pre


-teristic plaques. Quantitation was performed with ImageJ software (http://rsb.info.nih.gov/ij/).


LDL Turnover Studies

LDL (d: 1.025–1.060) was isolated from the plasma of C57BL/6J male mice by sequential ultracentrifugation, labeled with [125I]iodide monochloride (Bilheim et al., 1972), dialyzed, and filter sterilized. 0.15 μCi of [125I]LDL was injected via the femoral vein (n = 3 for each group), and radioactivity was measured in plasma samples collected at multiple time points thereafter. Mice had free access to food and water throughout the experiment. The radioactivity present in plasma at 30 s accounted for more than 98% of the injected LDL and was used to calculate the percent of injected LDL remaining in the circulation at the later time points.

Real-Time PCR Analysis

RNA (RNaseasy, QIAGEN) was isolated from mouse liver and used as a template for cDNA synthesis (High Capacity cDNA Synthesis Kit, Applied Biosystems) (Biddinger et al., 2005). Quantitative real-time PCR was performed using a fluoro


LDL apoB Secretion

After a 4 hr fast, apoB secretion rates were determined as described previously (Siri et al., 2001). Mice were injected with 200 μCi [35S]methionine (PerkinElmer Life Sciences) and 500 mg/kg Triton WR1339 in normal saline. Plasma samples were collected after 60 min and subjected to SDS-PAGE and autoradiography. The apoB100 and apoB48 bands were excised and counted in scintillation fluid (Ecocent H LS-275). The rate of apoB secretion was calculated as the amount of radioabeled apoB present in the serum after 1 hr.

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Statistical Analyses

Statistical significance was calculated by unpaired Student’s t test, and p < 0.05 was considered significant. All data are expressed as the mean ± SEM.

Supplemental Data

Supplemental Data include Supplemental Results and Discussion, Supplemental References, four figures, and four tables and can be found with this ar


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