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Supplemental Data

Article

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

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Supplemental Results and Discussion

LDL Receptor mRNA and Protein on the Paigen Diet

The atherogenic Paigen diet decreases LDL receptor mRNA in control mice but not LIRKO mice; consequently, the differences in LDL receptor mRNA do not significantly differ on the atherogenic diet (Figure S1A). Nonetheless, the LDL receptor protein is still markedly decreased in LIRKO mice, compared to controls, on the atherogenic diet (Figure S1B). Therefore, post-transcriptional regulation of the LDL receptor also contributes to the reduced LDL receptor protein levels in LIRKO mice.

Dyslipidemia in Other Mouse Models of the Metabolic Syndrome

To understand how the dyslipidemic phenotype of LIRKO mice compares to other models of the metabolic syndrome, we examined serum lipids in several mouse models with perturbed insulin signaling.

In particular, we chose two commonly used models of the metabolic syndrome, mice with diet induced obesity—in this case, mice fed a high fat diet (HFD, 55% calories from fat) for 18 weeks, and leptin deficient *ob/ob* mice (OB). These mice have partial insulin resistance due to defects in insulin receptor, IRS-1, IRS-2 and PI3K (Kerouz et al., 1997) (Shimomura et al., 2000).

Our own data are summarized together with published data in Table S1. First, it is important to note that of all these models of perturbed insulin signaling, only LIRKO mice show the low HDL and increase in non-HDL that is observed in humans with the metabolic syndrome. Second, hypertriglyceridemia does not always develop in mice with perturbed insulin signaling. OB mice have a modest 33% increase in serum triglycerides (32 ± 2 versus 24 ± 1 mg/dL, $n = 4\text{-}8$; $p < 0.05$), and HFD mice tend to have reduced serum triglycerides. This is due to the fact that many factors regulate serum triglyceride production and clearance (Ginsberg, 1996).

One factor driving hepatic triglyceride secretion is the supply of free fatty acids from the serum. STZ mice, the model with the most impressive hypertriglyceridemia (378 $\pm$ 108 versus 70 $\pm$ 4 mg/dL, n = 6; $p < 0.05$), have serum free fatty acids that are increased three-fold (3.76 $\pm$ 0.70 versus 1.31 $\pm$ 0.08, n = 6; p = 0.01).

Another important factor driving hypertriglyceridemia is insulin stimulated de novo lipogenesis, which is mediated largely by SREBP-1c. Figure S2 shows nuclear SREBP-1 protein and Table S2 shows lipogenic gene expression as measured by microarray analysis. Despite the fact that STZ mice are hypertriglyceridemic, they show dramatically decreased levels of nuclear SREBP-1c and lipogenic gene expression, with several genes, including fatty acid synthase, reduced more than 3-fold. On the other hand, HFD and OB mice have higher levels of SREBP-1c than their controls, and markedly increased lipogenic gene expression (Figure S2B). For example, OB livers show two- to three-fold elevations in fatty acid synthase, stearoyl CoA desaturase (SCD1), and fatty acid elongase. Therefore, mice with decreased hepatic insulin signaling (LIRKO and STZ mice) fail to activate SREBP-1c, but mouse models of the metabolic syndrome (HFD and OB mice) show activation of SREBP-1c. These data are consistent with prior reports in the literature and support the hypothesis that, in the metabolic syndrome, SREBP-1c remains activated even while other pathways, presumably those regulated by PI3K/Akt, have become resistant to insulin (Shimomura et al., 2000).

Dyslipidemia in LIRKO Mice Is Due to Decreased Signaling through PI3K and Akt

To further dissect the molecular mechanisms by which insulin resistance leads to dyslipidemia and atherosclerosis, we manipulated the downstream targets of the insulin receptor, PI 3-kinase (PI3K) and Akt. In particular, we studied mice with whole-body knockout of the p85β regulatory subunit of PI3K (βKO), mice with a liver specific knockout of p85α (αLKO), and mice harboring both of these deletions (p85α/β–DKO). These mice have been previously described by our lab (Taniguchi et al., 2006). p85α/β–DKO mice entirely lack PI3K activity in the liver and fail to activate Akt in response to insulin; they are therefore hyperinsulinemic and hyperglycemic (Taniguchi et al., 2006).

Consistent with prior reports (Taniguchi et al., 2006), p85α/β–DKO mice on a chow diet show decreased levels of SREBP-1c mRNA (Figure S3A). They also show a 50% decrease in SREBP-2, and its targets, HMG CoA reductase, and farnesyl diphosphate synthetase, and a 25% reduction in LDL receptor (Figure S3A) similar to LIRKO mice. Furthermore, when p85α/β–DKO mice were placed on an atherogenic diet (15% dairy fat, 1% cholesterol, 0.5% cholic acid) for four weeks, they developed severe hypercholesterolemia relative to their βKO controls. FPLC analysis of the serum of these mice (Figure S3C) showed that the excess cholesterol was associated exclusively with non-HDL particles, thereby recapitulating the LIRKO lipoprotein profile. Despite this marked increase in serum cholesterol, however, serum triglyceride profiles (Figure S3E) were similar to controls. These changes paralleled those observed in LIRKO mice, and indicate that hepatic insulin resistance at the level of either PI3K or the insulin receptor is capable of producing hyperglycemia, hypercholesterolemia, and decreased levels of SREBP-2, but not hypertriglyceridemia, or activation of SREBP-1c.

To determine the specific role of Akt in the dyslipidemia phenotype, we restored Akt signaling in LIRKO mice. LIRKO mice were fed a lithogenic diet for four weeks and then injected with adenovirus encoding either a constitutively active form of Akt (myr-Akt) or LacZ, as a control. Myr-akt produced an almost 50% decrease in serum glucose (Figure S4A), consistent with the known association between Akt and glucose homeostasis (Ono et al., 2003).

In addition, myr-Akt led to a redistribution of serum cholesterol such that HDL was increased more than 50%, whereas VLDL and LDL/IDL were decreased (Figure S4D). Total cholesterol and triglyceride levels were unchanged in the both the serum (Figures S4B and 4C) and liver (data not shown). Table S3 shows that myr-Akt had mild effects on gene expression, as it tended to decrease expression of the gluconeogenic genes and increase expression of the lipogenic and cholesterologenic genes; however, none of these changes was greater than 55%, and few reached significance. Therefore, Akt has important effects on both cholesterol and glucose metabolism, but less of an effect on triglyceride metabolism.

In summary, we show that either ablation of the insulin receptor or a complete loss of PI3K signaling in the liver produces hyperglycemia and an atherogenic distribution of serum cholesterol, which are normalized by restoration of Akt signaling, and hypotriglyceridemia, which is not normalized by Akt. These data indicate that partial insulin resistance due to defects in the PI3K/Akt signaling pathway result in impaired regulation of glucose and cholesterol metabolism. However, such defects do not activate SREBP-1c, which must be induced through other regulatory pathways in the metabolic syndrome.

Supplemental References

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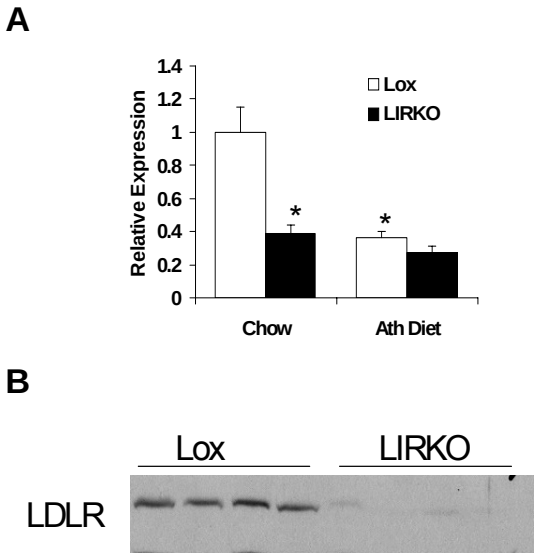

Figure S1. LDL Receptor Expression

4 week old male mice were either placed on the atherogenic Paigen diet (Ath Diet), or continued on a chow diet, and sacrificed 6 weeks later in the non fasted state.

(A) mRNA expression of LDL receptor was measured using real-time PCR (n = 4-6, *p < 0.01 versus Lox Chow).

(B) Liver extracts from Lox and LIRKO mice on the lithogenic diet were subjected to immunoblotting with antibodies against the LDL receptor.

Figure S2. SREBP-1c Is Activated in Mice with Partial Insulin Resistance

LIRKO mice were compared to their littermate controls (LOX), mice treated with STZ were compared to their untreated controls (Con), 129Sv mice were fed either a high fat (HFD) or low fat (LFD, taken from Biddinger, 2005) and *ob/ob* mice were compared to their lean littermates (Lean). SREBP-1c protein was measured by immunoblotting hepatic nuclear extracts prepared from at least three mice per group as described previously (Biddinger, 2005).

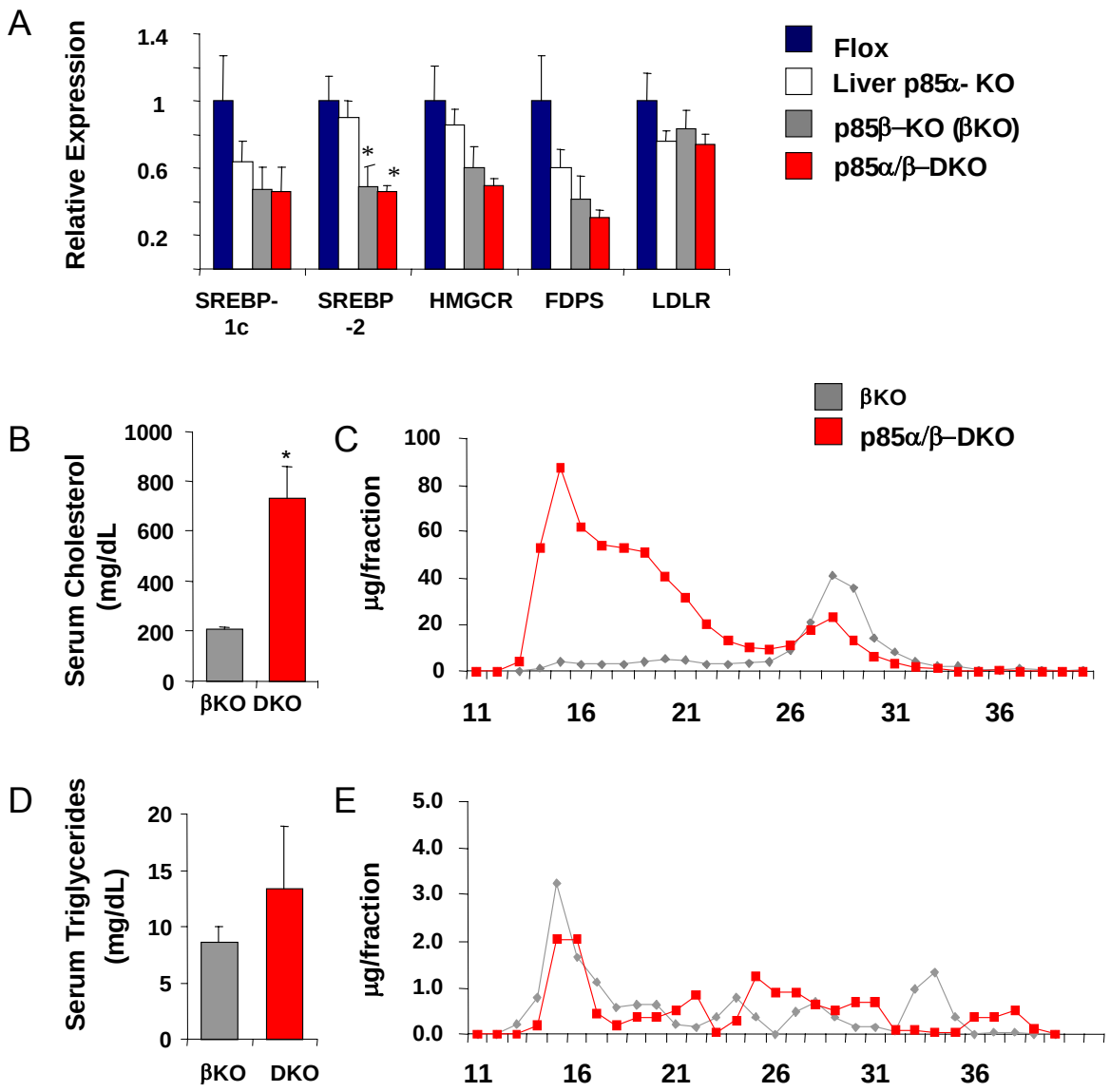

Figure S3. PI3K-Deficient Mice Show Changes in Lipid Metabolism Similar to LIRKO Mice

(A) Gene expression was measured in the livers of 8-12 week old male mice on a chow diet using real-time PCR.

(B–E) Two-month-old male β-DKO (con) and p85α/β-DKO (DKO) mice were placed on the atherogenic diet for one month, and serum was obtained after a four-hour fast.

(B and D) Total cholesterol (B) and triglycerides (D) were measured in the serum (n = 5, *p < 0.05).

(C and E) Equal amounts of serum were pooled from five mice of each genotype and subjected to FPLC fractionation. Cholesterol (C) and triglycerides (E) were measured in each fraction.

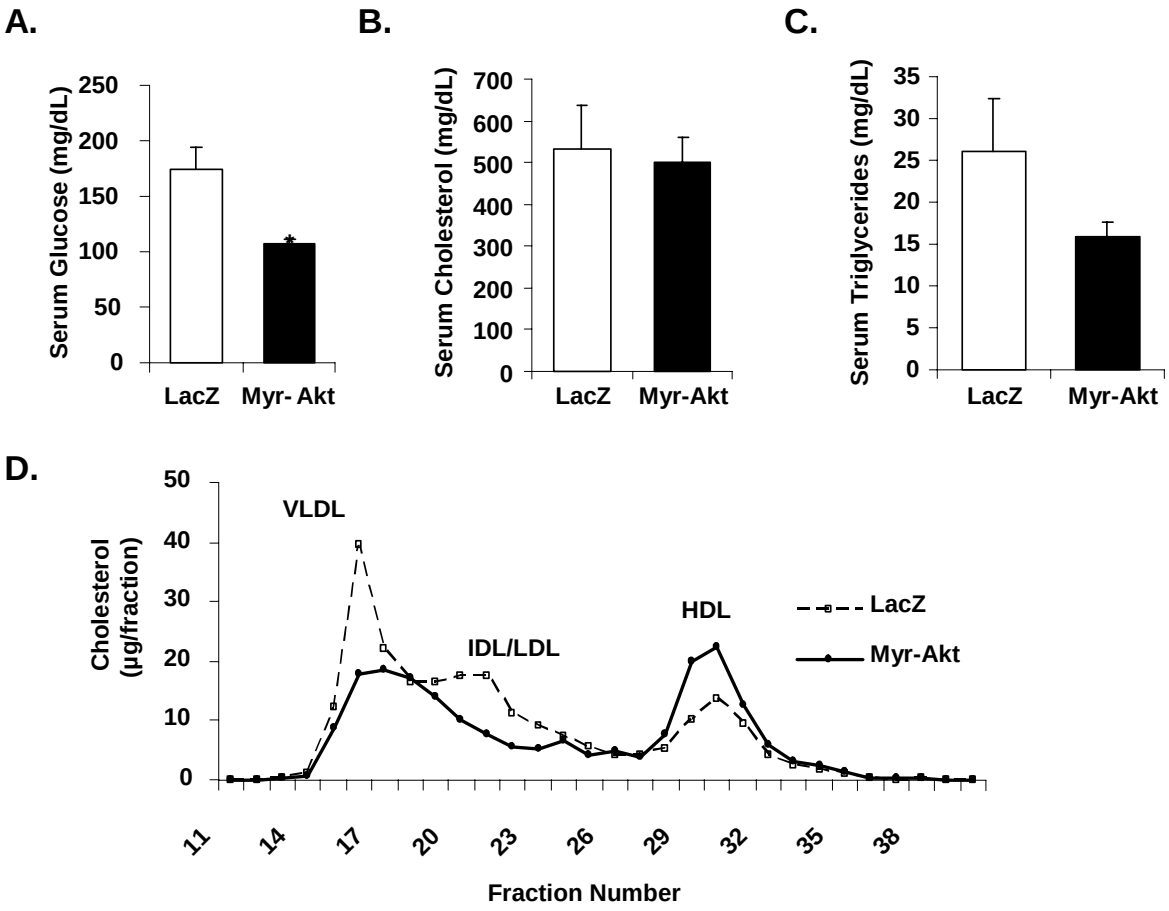

Figure S4. Restoration of Akt Activity in LIRKO Mice Improves Dyslipidemia

Two-month-old LIRKO mice fed the atherogenic diet for four weeks were injected with adenovirus encoding myr-Akt or lacZ, and sacrificed four days later.

(A) Serum glucose was measured in the non-fasted state ($n = 5$, $*p < 0.05$).

(B and C) Serum was obtained after a four hour fast, and total cholesterol (B) or triglycerides (C) were measured.

(D) Equal volumes of serum from 5 mice were pooled and subjected to FPLC analysis, and cholesterol was measured in each fraction.

Table S1. Serum Lipids in Mouse Models of Insulin Deficiency and Metabolic Syndrome

	LIRKO	STZ	HFD	*ob/ob*
Total Cholesterol	↑	↑	↑	↑
HDL Cholesterol	↓	↔	↑	↑
Non-HDL Cholesterol	↑	↑	↔	↔
Total Triglycerides	↔	↑	↔	↑
FFA	↔	↑	↔	↑

(References: Bar-on et al., 1976; Biddinger et al., 2005; Salmon and Hems, 1973.)

Table S2. SREBP-1c Is Activated in Mice with Partial Insulin Resistance

	FOLD CHANGE			
	LIRKO/LOX	**STZ/Con**	**HFD/LFD**	**OB/Lean**
Malic Enzyme	0.59	**0.23**	0.83	**2.98**
Fatty Acid Synthase	**0.59**	**0.24**	**1.86**	**3.09**
Fatty Acid Elongase 1	0.77	0.59	**1.97**	**2.54**
Glycerol-3-Phospate Acyltransferase	**0.64**	0.69	**1.41**	2.19
ATP Citrate Lyase	0.84	0.71	1.19	1.90
Δ5-Fatty Acid Desaturase	**0.56**	0.80	**1.51**	1.21
Glycerol Kinase	**0.62**	1.15	0.94	1.18
Pyruvate Carboxylase	0.86	1.51	1.02	**1.50**
Stearoyl-Coenzyme A desaturase 1	0.60	**0.02**	**1.85**	**2.24**

LIRKO mice were compared to their littermate controls (LOX), mice treated with STZ were compared to their untreated controls (Con), 129Sv mice were fed either a high-fat (HFD) or low-fat (LFD, taken from Biddinger et al., 2005), and *ob/ob* mice were compared to their lean littermates (Lean). Lipogenic gene expression was measured using microarray analysis. Data are presented as the ratio of the mean expressions in each group (n = 3-4 chips per group, with RNA from 2-3 mice pooled for each chip). Blue and red indicate a decrease or increase, respectively, of gene expression by more than 15%. Boldface indicates significant change ($p \leq 0.05$).

Table S3. Restoration of Akt Activity in LIRKO Mice Improves Dyslipidemia

	Fold Change Induced by myr-AKT
Gluconeogenesis	
G6P	0.70
FBP	0.81
PEPCK	0.82
Lipogenesis	
LXR	1.19*
SREBP-1c	1.53*
SCD1	1.48*
FAS	1.13
Cholesterol Metabolism	
SREBP-2	1.09
HMGCoAR	1.08
SS	0.90
FDPS	1.30
LDLR	1.19

Two-month-old LIRKO mice fed the atherogenic diet for four weeks were injected with adenovirus encoding myr-Akt or lacZ, and sacrificed four days later. Gene expression was measured using real-time PCR ($n = 5$, $*p \leq 0.05$).

Table S4. Primer Sequences Used for Real-Time PCR

Gene Name	Primers
ACC-R	TGGAGAGCCCCACACACA
ACC-F	TGACAGACTGATCGCAGAGAAAG
FAS-R	AGAGACGTGTCACTCCTGGACTT
FAS-F	GCTGCGGAAACTTCAGAAAAT
SCD1-R	CATCATTCTCATGGTCCTGCT
SCD1-F	CCCAGTCGTACACGTCATTTT
HMGR-F	CTTGTGGAATGCCTTGTGATTG
HMGR-R	AGCCGAAGCAGCACATGAT
SREBP2-F	GCGTTCTGGAGACCATGGA
SREBP2-R	ACAAAGTTGCTCTGAAAACAAATCA
SREBP1C-F	GGAGCCATGGATTGCACATT
SREBP1C-R	GGCCCGGGAAGTCACTGT
FDPS-F	ATGGAGATGGGCGAGTTCTTC
FDPS-R	CCGACCTTTCCCGTCACA
SS-F	CCAACTCAATGGGTCTGTTCCT
SS-R	TGGCTTAGCAAAGTCTTCCAACT
PTLP-F	GGCCGTCTCAGTGCTAAGTT
PTLP-R	CGAAGTTGATACCCTCAGGAA
DGAT-F	GTGCCATCGTCTGCAAGATT
DGAT-R	CTGGATAGGATCCACCAGGA
GPAT-F	CATCCTCTTTTGCCACAACAT
GPAT-R	ACAGAATGTCTTTGCGTCCA

See main text for abbreviations.