SUPPLEMENTAL METHODS

Mice. *Albcre tg*$^{+}$ mice were mated with *Lepr*$^{flox/flox}$ (129 X FVB N6) mice to generate *Lepr*$^{flox/wt}$ mice either with or without the *Albcre* transgene. These offspring (~50% C57BL6/J, 50% FVB, and less than 1% 129) were bred, with one mouse of each pair harboring the *Albcre tg*$^{+}$. The offspring that were *Lepr*$^{flox/flox}$ *Albcre tg*$^{+}$ and *Lepr*$^{flox/flox}$ *Albcre tg*$^{-}$ were mated to produce *Lepr*$^{flox/flox}$ *Albcre tg*$^{+}$ mice and *Lepr*$^{flox/flox}$ *Albcre tg*$^{-}$ littermate controls for experiments. For all experiments, *Lepr*$^{flox/flox}$ *Albcre tg*$^{-}$ littermate controls were compared with *Lepr*$^{flox/flox}$ *Albcre tg*$^{+}$ mice in order to minimize differences in genetic background, which can affect the phenotype of leptin deficiency (1). Mice were housed with a 12 hour light-12 hour dark cycle and had *ad libitum* access to food and water. Unless otherwise specified, mice were fed a chow diet (5015 Lab Diet, St Louis, MO). For studies on high fat diet fed mice, mice were switched from a chow diet to a high fat diet (#D12330, Research Diets, New Brunswick, USA) at 4 weeks of age. All procedures were approved by the University of British Columbia Animal Care Committee.

PCR and RT-PCR Analysis. For *Lepr*^{flox} PCR reactions from genomic DNA, the forward primer was primer mLepr101; ATG CTA TCG ACA AGC AGC AGA ATG ACG and the reverse primer was primer mLepr102; CAG GCT TGA GAA CAT GAA CAC AAC AAC (Integrated DNA Technologies Inc., Coralville, USA). RT-PCR from liver cDNA was performed with primer G; TAT TCC CAT CGA GAA ATA TCA, and primer 60; AGG CTC CAA AAG AAG AGG ACC (Integrated DNA Technologies Inc., Coralville, USA).

Measurement of Lean to Lipid Mass. The NMR signal from the body was acquired with a quadrature volume RF coil tuned to 300 MHz. The "free" water component corresponding to body fluids, e.g. urine and CSF, was typically less than 5% of the total signal.

Measurement of Hepatic Lipid Content. Liver was homogenized in 3 mL of chloroform:methanol (2:1) and extracted twice with water. 500 µL of the organic layer was dried down under $N_2(g)$ and 10 µL of Thesit (Sigma-Aldrich, St Louis, USA) was added and mixed under $N_2(g)$. Water (100 µL) was added and incubated at 37°C for 1 hour with intermittent vortexing. Triglycerides were quantified using the Serum Triglyceride Determination kit (Sigma-Aldrich, St Louis, USA) and cholesterol was assayed using the Cholesterol E kit (Wako Chemicals USA, Richmond, USA).

Hyperinsulinemic-Euglycemic Clamps. Mice were fasted overnight for 16 hours and anaesthetized with acepromazine (5 mg/kg), midazolam (5 mg/kg), and fentanyl (0.25 mg/kg). The tail vein was then cannulated and a 1 hour basal infusion of ^{3}H-D-glucose (1.2 µCi/h) was initiated. Duplicate blood samples were obtained from the tail vein at the end of the basal period to measure fasting blood glucose levels and for basal insulin measurements. Hyperinsulinemia was induced with a constant infusion of insulin (6.8 mU/h). Euglycemia (~5.0 mM) was maintained by variable infusion of 12.5% D-glucose for 45 minutes. Once glucose clamping was achieved, triplicate blood samples (50 µL) were obtained from the tail vein at 10 min intervals. Animals were sacrificed by cervical dislocation and tissues were dissected and frozen in liquid N_2. Plasma samples were counted using a Beckman LS6000IC scintillation counter after extraction by TCA precipitation. Whole body glucose utilization (µmol/kg·min) was determined as the ratio of the specific activity of glucose to the rate of ^{3}H-D-glucose appearance. Endogenous glucose production (µmol/kg·min) was calculated as the difference between whole body glucose utilization and exogenous glucose infusion.

Measurements of β-Cell Mass. Sections were incubated with 3% hydrogen peroxide (Fisher Scientific, Fair Lawn, USA), blocked in serum free protein block (DakoCytomation, Inc., Carpinteria, USA), and stained with guinea pig anti-insulin antibody (LINCO Research Inc., St.

Charles, USA) overnight at 4°C. The sections were then incubated for 1 hour at room temperature in biotinylated goat anti-guinea pig IgG and then 30 minutes in Vectastain R.T.U. Elite ABC reagent. They were then stained with Peroxidase Substrate Kit DAB (all from Vector Laboratories, Burlingame, USA) and mounted. Whole sections were scanned with a ScanScope CS System, and insulin positive area was measured by V9 Positive Pixel Count Algorithm on ImageScope software (all from Aperio, Vista, USA).

Western Blot Analysis. Liver lysates were prepared by homogenization in ice cold lysis buffer containing 50 mM Tris-HCl at pH 8, 120 mM NaCl, 30 mM NaF, 5 mM EDTA, 1% (w/v) NP-40 (Sigma-Aldrich, St Louis, USA), protease inhibitor cocktail (Sigma-Aldrich, St Louis, USA), and phosphatase inhibitor cocktail (Sigma-Aldrich, St Louis, USA). SDS-PAGE was performed on an 8% acrylamide gel and proteins transferred onto a PVDF membrane using the Bio-Rad Trans-Blot Semi-Dry Transfer Cell (Mississauga, Canada). Membranes were blocked with 5% skim milk in Tris-buffered saline and probed with antibodies against phosphorylated Akt (Cell Signaling Technologies, #4060, Danvers, USA) and total Akt (Cell Signaling Technologies, #9272, Danvers, USA).

Measurement of Plasma Leptin Binding Proteins. Levels of plasma leptin binding proteins were assessed based on the method of Guo *et al.* (2). Briefly, plasma was incubated overnight with ^{125}I-leptin (NEX340, PerkinElmer, Wellesley, MA) and then subjected to size-exclusion chromatography on a G-75 Sephadex column with a buffer consisting of 0.05 M $NaPO_4$ and 0.15 M NaCl at pH 7.4. The incubation and chromatography were performed at 4°C. Fractions were collected and analyzed by scintillation counting on a Beckman LS6000IC scintillation counter.

Measurement of PEPCK and G6Pase transcript levels. Livers were extracted following a 4 hour fast and immediately placed in RNA*later* (Qiagen, Mississauga, Canada) overnight at 4°C after which the RNA*later* was removed and tissue was stored at -80°C. RNA was prepared from the tissue using TRI Reagent (Applied Biosciences, Carlsbad, USA). Genomic DNA was removed from the RNA samples by treatment with DNase I (RNase-free) (New England Biolabs, Pickering, Canada) according to the manufacturer instructions. Then 1 µg of RNA was used for cDNA synthesis using a polyT primer with the iScript Select cDNA synthesis kit (BioRad, Mississauga, Canada). For each sample 2 µL of 100-fold diluted cDNA was used with the desired primers and SsoFast EvaGreen Supermix with Low ROX master mix (BioRad, Mississauga, Canada) as specified by the manufacturer instructions. Reactions were monitored in a StepOne Plus thermocycler (Applied Biosystems, Carlsbad, USA). The Hprt1 reference gene was pre-selected from other potential reference genes by assessing transcript stability using geNorm software. Transcript abundance was calculated by the Pfaffl method using a single control sample as the calibrator point. Primer sequences were as follows; PEPCK GCAGAACACAAGGGCAAGATCATC and GATGTAGCCGATGGGCGTG, G6Pase CGTCACAGTTTTCTCCTCCTCAGC and CTCTGCAAATCAGCCGAGGCAG.

Pyruvate Tolerance Test. Mice were fasted overnight for 16 hours and then given an intraperitoneal injection of 2 g/kg body weight pyruvate (Sigma-Aldrich, St Louis, MO) in saline. Blood glucose levels were monitored for the next 2 hours with a One Touch Ultra Glucometer (Life Scan Inc., Burnaby, Canada) from the saphenous vein.

Table S1. Fasting Plasma Lipids in Mice with and without Hepatic Leptin Signaling.

			Fasting Lipids [a]		
			Cholesterol (mg/dL)	**Triglycerides (mM)**	**Free Fatty Acids (mM)**
6 wks	♂	*Lepr*$^{flox/flox}$ *Albcre tg*$^{-}$	156±10 (7)	0.57±0.05 (18)	0.70±0.08 (8)
		Lepr$^{flox/flox}$ *Albcre tg*$^{+}$	166±6 (12)	0.65±0.05 (23)	0.88±0.08 (8)
		C57BL/6	77±1 (8)	0.52±0.03 (8)	0.73±0.06 (8)
		db/db	119±4 (8)*	0.54±0.02 (8)	1.03±0.16 (8)*
	♀	*Lepr*$^{flox/flox}$ *Albcre tg*$^{-}$	137±15 (7)	0.57±0.10 (16)	0.59±0.11 (4)
		Lepr$^{flox/flox}$ *Albcre tg*$^{+}$	134±10 (10)	0.48±0.06 (23)	0.78±0.08 (4)
		C57BL/6	80±7 (4)	0.40±0.04 (4)	0.96±0.09 (4)
		db/db	185±9 (8)*	0.56±0.06 (8)	1.66±0.06 (8)*
12 wks	♂	*Lepr*$^{flox/flox}$ *Albcre tg*$^{-}$	185±10 (10)	0.42±0.03 (15)	0.93±0.07 (5)
		Lepr$^{flox/flox}$ *Albcre tg*$^{+}$	199±9 (16)	0.34±0.04 (21)	0.70±0.06 (5)*
		C57BL/6	100±4.5 (8)	0.46±0.02 (8)	1.14±0.08 (8)
		db/db	125±7.1 (8)*	0.48±0.07 (8)	1.26±0.14 (8)
	♀	*Lepr*$^{flox/flox}$ *Albcre tg*$^{-}$	154±9 (8)	0.22±0.04 (14)	0.84±0.11 (6)
		Lepr$^{flox/flox}$ *Albcre tg*$^{+}$	142±8 (12)	0.19±0.03 (14)	0.70±0.15 (2)
		C57BL/6	108±4 (8)	0.30±0.03 (8)	0.90±0.07 (8)
		db/db	225±15 (8)*	0.42±0.07 (8)	1.98±0.12 (8)*
16 wks	♂	*Lepr*$^{flox/flox}$ *Albcre tg*$^{-}$	176±20 (6)	0.56±0.05 (6)	0.88±0.09 (6)
		Lepr$^{flox/flox}$ *Albcre tg*$^{+}$	183±11 (7)	0.50±0.05 (7)	0.96±0.07 (7)
		C57BL/6	129±4.6 (8)	0.37±0.04 (8)	1.31±0.10 (8)
		db/db	161±5.2 (7)*	0.47±0.09 (8)	1.33±0.10 (8)
	♀	*Lepr*$^{flox/flox}$ *Albcre tg*$^{-}$	167±14 (6)	0.45±0.06 (5)	1.01±0.09 (5)
		Lepr$^{flox/flox}$ *Albcre tg*$^{+}$	141±13 (6)	0.36±0.07 (5)	0.95±0.15 (5)
		C57BL/6	96±3 (7)	0.21±0.03 (7)	0.97±0.04 (5)
		db/db	237±10 (8)*	0.38±0.08 (8)*	1.68±0.12 (8)*

[a] Mice were fasted for 4 hours during the light cycle
* $P \leq 0.05$ versus control (*Lepr*$^{flox/flox}$ *Albcre tg*$^{-}$ or C57BL/6)

FIG. S1. Levels of plasma leptin binding proteins are not altered in male mice lacking hepatic leptin signaling. Twenty-one week old male mice were fasted for four hours and then a cardiac puncture was performed to collect plasma. Plasma was incubated overnight with ^{125}I-leptin at 4°C and then subjected to size-exclusion chromatography. Fractions were collected and analyzed by scintillation counting, n=3 in each group. Representative traces for one *Lepr$^{flox/flox}$ Albcre tg^{-}* male (A) and one *Lepr$^{flox/flox}$ Albcre tg^{+}* male (B) are shown.

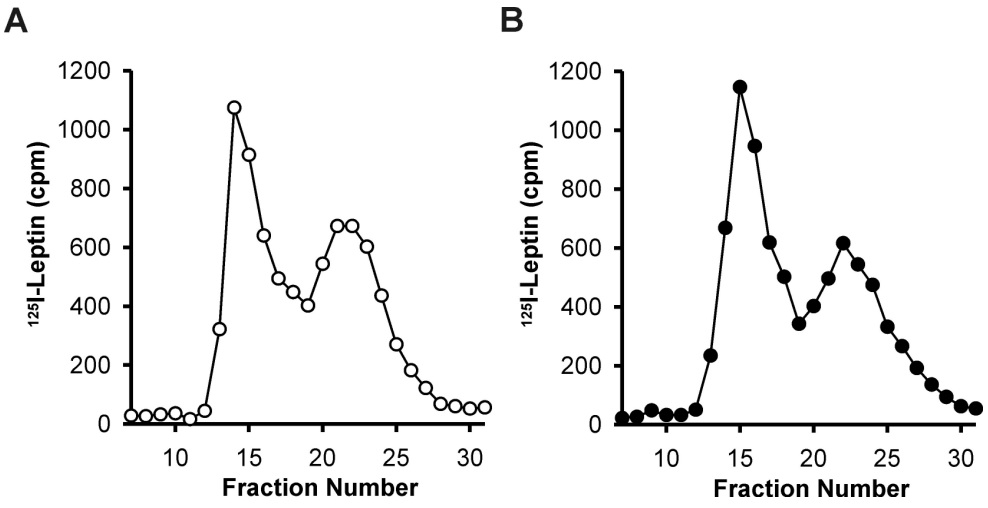


FIG. S2. Loss of hepatic leptin signaling does not affect glucose metabolism in the fasted state. (A) Levels of PEPCK and G6Pase transcripts were measured in the livers of 22 week old male mice following a 4 hour fast, n≥8. (B) Thirty week old male *Lepr$^{flox/flox}$ Albcre tg^{-}* and *tg^{+}* mice were fasted overnight and then given an injection of 2 g/kg body weight pyruvate. Blood glucose measurements were then monitored for 2 hours, n=8 in each group. Data are expressed as mean ± SEM.

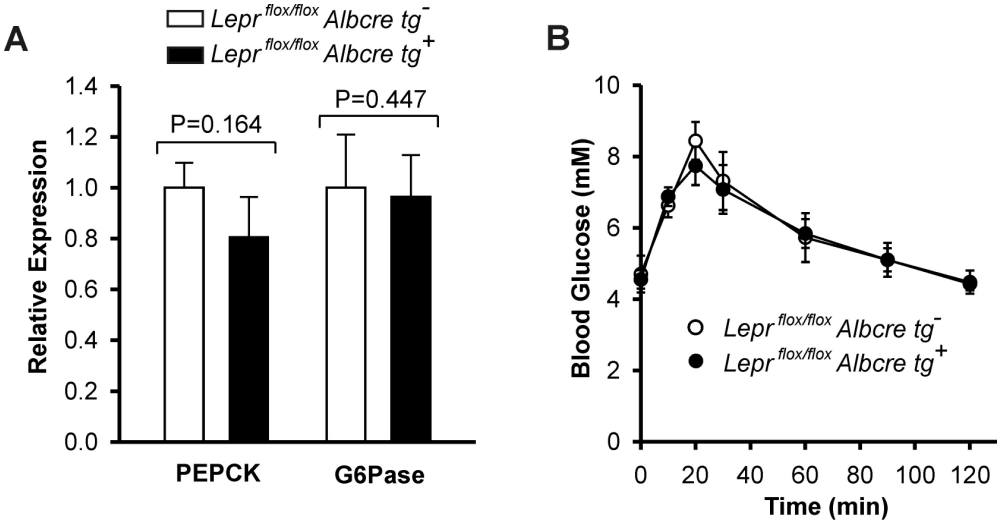


FIG. S3. Attenuation of hepatic leptin signaling does not alter glucose tolerance in female mice. Oral glucose tolerance tests were performed on female *Lepr*$^{flox/flox}$ *Albcre tg*$^{+}$ mice and littermate controls at 6 (A), 12 (B), and 16 (C) weeks of age. Mice were fasted for 4 hours and gavaged with 1.5 mg/g glucose. For *Lepr*$^{flox/flox}$ *Albcre tg*$^{+}$, n=3 and for *Lepr*$^{flox/flox}$ *Albcre tg*$^{-}$, n≥7. Data are expressed as mean ± SEM.

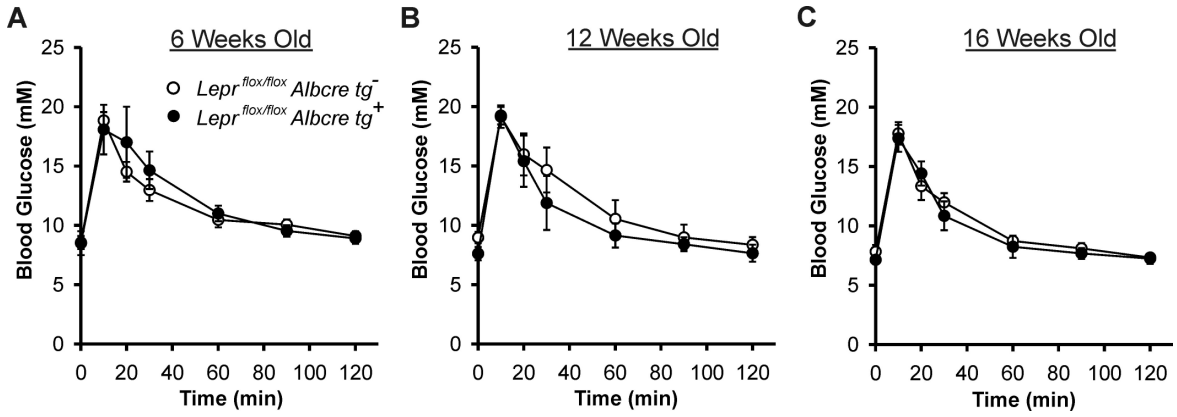
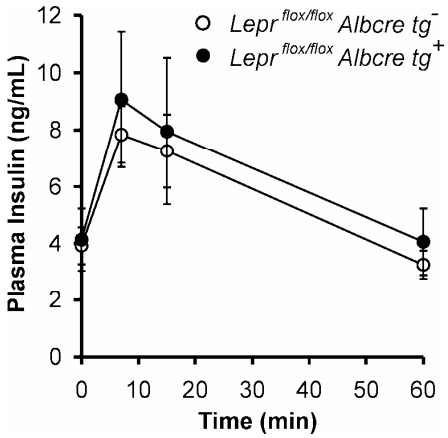


FIG. S4. Effect of attenuated hepatic leptin signaling on glucose-stimulated insulin levels during high fat feeding. Plasma insulin levels were monitored following a gavage of 1.22 mg/g body weight glucose to assess steady state levels of glucose-stimulated insulin secretion in 18 week old *Lepr*$^{flox/flox}$ *Albcre tg*$^{+}$ and *tg*$^{-}$ mice fed a high fat diet for 14 weeks, n≥5 in each group. Data are expressed as mean ± SEM.



SUPPLEMENTAL REFERENCES

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2. Guo K, McMinn JE, Ludwig T, Yu YH, Yang G, Chen L, Loh D, Li C, Chua S, Jr., Zhang Y. Disruption of peripheral leptin signaling in mice results in hyperleptinemia without associated metabolic abnormalities. Endocrinology 2007;148:3987-3997