Supplemental data

Critical role of STAT3 in leptin's metabolic actions

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Table S1. General characteristics

Treatment	**Vehicle**	**Leptin**	**Leptin plus STAT3 PI**
N:	7	6	7
Basal:			
Daily Food Intake (kcal/rat)	135.9 ± 3	131.2 ± 4	136.3 ± 4
Body Weight (g)	295 ± 7	299 ± 4	315 ± 3
Glucose (mmol/l)	7.8 ± 0.7	7.4 ± 0.2	8.0 ± 0.3
Insulin (ng/ml)	2.7 ± 0.2	2.6 ± 0.3	2.1 ± 0.1
FFA (mM)	0.8 ± 0.0	1.1 ± 0.1	0.9 ± 0.2
Clamp:			
Glucose (mM)	7.6±0.3	7.8±0.2	7.2±0.4
Insulin (ng/ml)	1.±0.28	1.6±0.18	1.3±0.15
Leptin (ng/ml)	1.3±0.2	1.3±0.1	1.5±0.2
FFA (mM)	0.8±0.1	0.7±0.1	1.0±0.2

Effect of central leptin and STAT3 inhibition on the circulating fasting levels of glucose, insulin, free fatty acids in rats that were fed a high fat diet for 3 days at basal levels and during clamp studies.. Biochemical parameters represent the average ± S.E. of at least five basal measurements in each rat. Food intake represents the average ± S.E. of the last three days preceding the study.

Table S2. Effect of central leptin and STAT3 inhibition on the "direct" and "indirect" pathway of hepatic UDP glucose formation

Group	[3H]Glc	[3H]UDPGlc	% Direct	[14C]PEP	[14C]UDPGlc	% Indirect
	dpm/nmol	dpm/nmol		dpm/nmol	dpm/nmol	
Vehicle	49.5 ± 3.9	8.8 ± 1.6	18.2±3.9	8.9 ± 2.0	4.4 ± 0.7	28.3 ±4.6
Leptin	30.7±2.2*	4.1 ± 0.3*	14.9±1.9	5.4 ± 0.5	2.3 ± 0.2*	24.0±2.8
Leptin + STAT3PI	58.1 ± 2.9	8.5 ± 0.5	15.3±2.0	7.9 ± 1.1	1.9±0.4	24.1±3.6

Specific activities of plasma glucose, hepatic UDP-glucose (UDP-Glc) and phosphoenolpyruvate (PEP) were used to calculate the contribution of plasma glucose and PEP-gluconeogenesis to the hepatic UDP-glucose pool following [^{3}H-3]-glucose and [U-^{14}C]-lactate infusions in rats at the completion of pancreatic/insulin clamp studies. Data are means ± SE. *$p < 0.05$ vs. vehicle or controls.

Table S3. Metabolic characteristics of *s/s* and *db/db* mice under fasting and clamped conditions

	s/s	***db/db***	**ttest**
N:	7	6	
Food Intake (kcal/d)	11.4 ± 0.2	11.4 ± 0.2	
Body Weight (g)	27.9 ± 0.94	26.6 ± 0.4	0.148
Glucose (mmol/l)	8.3 ± 0.4	15.8 ± 1.6	<0.001
Insulin (ng/ml)	1.6±0.25	3.7±0.7	<0.001
Leptin (ng/ml)	66±15	260±23	<0.001
Glucagon (pg/ml)	216±23	319±60	0.04
FFA (mM)	0.94 ± 0.2	1.0 ± 0.1	0.34

Biochemical parameters represent the average ± S.E. *$p < 0.01$ vs. vehicle or regular chow.

Figure S1. Effect of leptin on gucose metabolism in rats with MBH expression of STAT3 dominant negative

A) Time line for the implantation of catheters and the MBH infusion of the adenovirus expressing a dominant negative mutant of STAT3 (STAT3 DN) in rats. All rats received a lard-enriched (high fat) diet for three days to induce hepatic insulin resistance.

B) Western blots of MBH extracts confirms the over-expression of the STAT3 DN leading to impaired leptin-induced STAT3 Y705 phosphorylation one week after MBH adenoviral injection. For leptin signaling studies 2.5 ug of leptin was injected ICV and the animals were sacrificed 30 min. later.

C) Central administration of leptin markedly increases the rate of glucose infusion in rats receiving LacZ but not STAT3DN adenovirus.

D) This effect was entirely accounted for by increased rate of glucose production in the STAT3DN compared with the LacZ group. *p <0.05 vs. LacZ infused group.