Supplemental Information

Role of the hypothalamic-pituitary-thyroid axis in metabolic regulation by JNK1

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Figure S1. Comparison of floxed Jnk1 deletion in pancreatic islets of Nes-Cre and Rip-CreESR mice.

Genomic DNA prepared from pancreatic islets of Jnk1+/+ Cre+ and Jnk1LoxP/LoxP Cre+ mice was examined by PCR analysis to detect the Jnk1+, Jnk1LoxP, and Jnk1Δ alleles. The results obtained with nestin promoter (Nes)-Cre mice (NWT and NKO mice) are presented. No deletion of Jnk1LoxP was detected in the pancreatic islets of NKO mice. Control studies were performed using rat insulin promoter (Rip)-CreESR mice (RWT and RKO mice) that were treated with 4-hydroxytamoxifen. Efficient deletion of Jnk1LoxP was detected in RKO mice.
Figure S2. Comparison of organ weight in $N^{WT}$ and $N^{KO}$ mice.

$N^{WT}$ and $N^{KO}$ mice were fed a chow diet (ND) or a HFD (16 wks.). The weight of the heart, white epididymal fat, brown intrascapular fat, quadriceps muscle, and liver were measured (mean ± SD, n = 10). Statistically significant differences between $N^{WT}$ and $N^{KO}$ mice are indicated (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$).
Figure S3. Blood concentration of cytokines in $N^{WT}$ and $N^{KO}$ mice.

$N^{WT}$ and $N^{KO}$ mice were fed a chow diet (ND) or a HFD (16 wks.). The concentration of IL2, IL5, and IL6 in the blood was measured by ELISA (Millipore) (mean ± SD, n = 9 – 15). Statistically significant differences between $N^{WT}$ and $N^{KO}$ mice are indicated (*, P < 0.05).
NWT and NKO mice were fed a chow diet (ND) or a HFD (16 wks.) and then fasted overnight. The concentration of adrenocorticotropic hormone (ACTH) and growth hormone (GH) in the blood was measured by ELISA (Millipore Inc.) (mean ± SD, n = 15). Statistically significant differences between NWT and NKO mice are indicated (*, P < 0.05).

The blood concentration of ACTH was greatly increased in HFD-fed NWT mice compared with chow-fed NWT mice, as expected (Edwardson and Hough, 1975). ACTH production by the anterior pituitary gland acts on the adrenal cortex to increase the secretion of corticosteroids. Increased corticosteroid production by HFD-fed NWT mice may contribute to insulin resistance (Walker, 2006). Moreover, ACTH can inhibit TSH production by the pituitary gland (Natori et al., 1994). Consequently, the HFD-induced expression of ACTH in NWT mice may contribute to feed-back regulation of TSH production. Loss of HFD-induced expression of ACTH may therefore contribute to the increased amount of TSH and thyroid hormone (T4 and T3) in the blood of NKO mice.

Feeding NWT mice a HFD caused a decrease in the blood concentration of GH. In contrast, HFD-fed NKO mice did not cause a significant reduction in blood GH concentration. These data demonstrate that blood GH is dysregulated in NKO mice. Studies of chow-fed NKO mice indicated that blood GH was decreased compared with NWT mice (although not statistically significant). Decreased blood GH correlates with increased hypothalamic expression of somatostatin (Fig. 5), an inhibitor of pituitary gland GH production. Decreased GH could also be mediated by thyroid hormone (de Picoli Souza et al., 2006). Reduced GH could contribute to the reduced lean mass of NKO mice compared with NWT mice.
Figure S5. Expression of thyroid hormone-responsive genes in $N^{WT}$ and $N^{KO}$ mice.

Intrascapular brown fat was isolated from $N^{WT}$ and $N^{KO}$ mice that were treated without (A) or with PTU (B) in the drinking water for 12 wks. Thyroid hormone-responsive gene expression (Obregon, 2008) was examined by quantitative RT-PCR analysis of the amount of uncoupling protein 1 ($Ucp1$), glucose transport 4 ($Glut4$), phosphoenol pyruvate carboxykinase ($Pck1$), Acetyl-CoA carboxylase β ($Accβ$), lactate dehydrogenase β ($Ldhβ$), and Spot 14 mRNA. The data were normalized to the expression of Gapdh mRNA in each sample. The data are presented as the mean ± SD (n = 6). Statistically significant differences between $N^{WT}$ and $N^{KO}$ mice are indicated (*, P < 0.05; **, P < 0.01).
PTU-treated N<sup>WT</sup> and N<sup>KO</sup> mice were fed a chow diet (ND) or a HFD (12 wks.) and then fasted overnight. The weight of the heart, white epididymal fat, brown intrascapular fat, quadriceps muscle, and liver were measured (Mean ± SD, n = 10). No statistically significant differences between N<sup>WT</sup> and N<sup>KO</sup> mice were detected (P > 0.05).
Figure S7. Effect of PTU-treatment of wild-type and Jnk1−/− mice on body weight and the blood concentrations of glucose and insulin.

Wild-type (WT) and Jnk1−/− mice were treated without (left panels) or with PTU (right panels). The effect of feeding a chow diet (ND) or a HFD was examined (12 wks.). (A) Body weight. (B) Blood glucose concentration in fed mice. (C) Blood insulin concentration in mice fasted overnight. The data presented are the mean ± SD (n = 10). Statistically significant differences between WT and Jnk1−/− mice are indicated (*, P < 0.05; **, P < 0.01; ***, P < 0.001).
Figure S8. Effect of PTU-treatment of wild-type and Jnk1−/− mice on insulin tolerance, glucose tolerance, and glucose-induced insulin release.

Wild-type (WT) and Jnk1−/− mice were treated without (left panels) or with (right panels) PTU in the drinking water. The effect of feeding a chow diet (ND) or a HFD was examined (12 wks.). (A) Insulin tolerance test. (B) Glucose tolerance test. (C) Glucose-induced insulin release. The data presented are the mean ± SD (n = 10). Statistically significant differences between WT and Jnk1−/− mice are indicated (*, P < 0.05; **, P < 0.01; ***, P < 0.001).