Supplemental Data

Supplemental Methods

Leptin Resistance and pSTAT3 Immunohistochemistry

Group housed (n=5/cage) animals were injected i.p. with mouse recombinant leptin (n=3/group; 1mg/kg; Dr. Parlow, National Hormone and Peptide Program, Torrance, CA) in phosphate buffered saline (PBS), or PBS alone (n=2/group), and returned to their homecage. Thirty minutes following injection, animals were deeply anesthetized (pentobarbital: 200 mg/kg i.p.), and perfused intracardially with 5ml saline followed by 25ml of 4% paraformaldehyde in 0.1 M phosphate buffer (PB), pH 7.3. Brains were post-fixed for 4hr at 4°C, and cryoprotected in 20% sucrose in 0.1M PB overnight. Brains were sectioned at 35µm on a cryostat. Briefly, free-floating sections were incubated in a 1% hydrogen peroxide/1% NaOH buffer for 20min. Sections were then incubated in 3% glycine for 10min, followed by incubation in 1% SDS for 10min. Then, sections were washed 3 X 10min in PBS, incubated in normal goat serum for 1hr, and then placed in anti-pSTAT3 made in rabbit primary antibody (Cell Signaling, Beverly, MA) for 24 hrs. Sections were then washed 5 X 10 min in PB with 1% Triton-XP (PBT), incubated in biotinylated goat anti-rabbit secondary (Vector Labs, Burlingame, CA; 1:250) for 1 hr, and washed. Sections were then treated with avidin-biotin complex (ABC, Vector Labs) for 1 hr, and washed. Staining was visualized with nickel chloride enhanced diaminobenzidine (Sigma Aldrich, St.Louis, MO). Sections were then mounted on gelatin-coated glass slides and coverslipped with Permount (Fisher Scientific, Hampton, NH). Slides were analyzed on a Nikon 90i microscope at 20X magnification. An optical grid covering 10,000µm^2 was overlayed on the arcuate nucleus, and cells counted throughout the depth of focus.

Supplemental Results

The phosphorylation of STAT3 can be induced by leptin acting through the leptin receptor, and is a standard assay of leptin resistance (19). To probe if high-CORT treatment, which results in very high plasma leptin levels, also results in leptin insensitivity, we examined leptin induced phospho-STAT3

(pSTAT3) immunoreactivity in the arcuate nucleus (ARC) of the hypothalamus in high-CORT and vehicle treated animals. We found a statistically significant interaction (One-way ANOVA; $F_{1,6}$=11.74, p=0.04), with leptin inducing an increase in ARC pSTAT3 staining in vehicle animals (p<0.01) while there was no statistically significant induction in high-CORT animals (p>0.05). These results suggest that high-CORT treatment induces leptin resistance, at least as measured by leptin-induced pSTAT3 staining in the ARC.

Supplemental Figure 1 Caption

High-CORT animals show blunted pSTAT3 staining in the arcuate nucleus following leptin challenge

Bar graphs depicting the number of pSTAT3 immunoreactive cell profiles in the ARC of vehicle and high-CORT animals following leptin injection. While robust pSTAT3 staining is induced following leptin challenge in vehicle animals, this response is significantly blunted in high-CORT animals. Asterisk indicates significant difference between the PBS and leptin treated groups (p<0.05).