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Rapid onset and reversal of peripheral and central leptin resistance in rats offered chow, sucrose solution, and lard[☆]

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Abstract

We previously reported that rats offered choice diet (chow, 30% sucrose solution, lard) increase body fat by 130% within 3 weeks. We tested the effects of choice diet on the development of leptin resistance in rats. Intraperitoneal injection of 2 mg/kg leptin inhibited 14 h food intake and weight gain of all rats after 2 days and 4 days of diet. On day 8, choice rats were leptin insensitive and by day 16 they were resistant. Chow rats remained leptin responsive. A second study showed that on day 16 choice, but not chow rats, were centrally leptin resistant (1.5 µg leptin, 3rd ventricle). In both studies, rats were switched back to chow only after approximately 3 weeks on choice diet and were leptin responsive after 4 days. A third study showed that carcass fat was reduced by 30% 4 days after switching back to chow. A final experiment showed that leptin responsive chow rats, but not leptin resistant choice rats, increased energy expenditure by 12% during the 2.6 h after a central leptin injection. Thus, choice diet rapidly induces leptin resistance, but leptin responsiveness is quickly restored when choice is replaced with chow. This rapid onset and reversal of leptin resistance may be associated with changes in either substrate metabolism or adiposity.

Keywords

Glucose; Insulin; Energy expenditure; Obesity; 3rd Ventricle; Macronutrient self-selection; Energy intake; Body fat; Body weight; Choice diet

Introduction

Leptin, a hormone secreted from adipocytes, is hypothesized to play a role in long-term energy homeostasis functioning as a negative feedback signal informing the brain of the size of body fat stores (Harris, 2000; Woods & D'Alessio, 2008). Signal transducer and activator

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of transcription (STAT) 3 is critical for leptin signaling (Bates & Myers, 2004) and others have suggested that leptin lowers energy intake and body weight through this pathway (Halaas et al., 1997; Myers, Cowley, & Munzberg, 2008). However, this effect is attenuated in humans that are obese (Considine et al., 1996; Heymsfield et al., 1999) and in animals which are habituated to a high fat diet (El-Haschimi, Pierroz, Hileman, Bjorbaek, & Flier, 2000; Harris, Bowen, & Mitchell, 2003; Lin, Thomas, Storlien, & Huang, 2000). This lack of a response is referred to as leptin resistance.

The 'choice' model of diet induced obesity offers rats free ad libitum access to chow, 30% sucrose solution, and lard. In a previous study, rats consuming the choice diet increased energy intake by about 50% and body fat by 133% in 3 weeks (Apolzan & Harris, 2011). Serum leptin was doubled in choice compared to chow rats, but the rats were not tested for leptin resistance (Apolzan & Harris, 2011). Thus in studies described here animals fed the choice diet were tested for the time of onset of leptin resistance. Leptin resistance is defined here as a condition in which leptin fails to inhibit body weight gain and energy intake (Munzberg, 2010). The primary objective of the studies was to determine the time of onset of peripheral and central leptin resistance in rats consuming the choice diet. Subsequently we tested whether the resistance was reversible if the rats were switched from choice to chow and whether the reversal of leptin resistance was associated with a decrease in body fat.

Leptin is known to affect both energy intake and expenditure. Energy expenditure has been suggested to be controlled by the same sites (hypothalamus and brainstem) and mechanisms (long form receptor-STAT3 signaling) as energy intake (Bates et al., 2004; Elmquist, Coppari, Balthasar, Ichinose, & Lowell, 2005; Hayes et al., 2010). Previously others have reported that peripheral leptin increases energy expenditure (Scarpace, Matheny, Pollock, & Tumer, 1997) by activating sympathetic nervous system stimulation of brown fat (Ziylan, Baltaci, & Mogulkoc, 2009). It has been reported that leptin resistance includes the arcuate nucleus (ARC) (Munzberg, Flier, & Bjorbaek, 2004) and ventral tegmental area (VTA) (Matheny, Shapiro, Tumer, & Scarpace, 2011) and that leptin can increase sympathetic outflow even in a state of leptin resistance (Enriori, Sinnayah, Simonds, Garcia Rudaz, & Cowley, 2011). However, currently, it is unknown if leptin resistance identified by a failure of leptin to inhibit energy intake or change body weight also results in an attenuation of the increase in energy expenditure. Therefore, after confirming that the choice diet caused leptin resistance based on changes in body weight and energy intake, we determined whether choice diet made rats resistant to the effects of leptin on energy expenditure.

Methods

Animals

All animal procedures were approved by the Georgia Health Sciences University Institutional Care and Use Committee and followed the recommendations of the NIH Intramural Animal Care and Use program. All rats were housed in a climate controlled room at ~20 °C with lights on for 12 h/day starting at 7 am.

Experiment 1: Effects of choice diet on peripheral leptin responsiveness

The purpose of this experiment was to determine the time course of onset of peripheral leptin responsiveness in rats offered free access to chow (Harlan Teklad Rodent Diet 8604) or chow, 30% sucrose solution (w/v; Kroger Sugar, Hood Packing Corporation, Hamlet, NC), and lard (Armour, ConAgra Foods, Omaha, NE). Thirty-six male rats (Sprague Dawley, Harlan, Indianapolis, IN) weighing ~260 g were individually housed in hanging wire mesh cages. During the baseline period, all rats had free access to chow (Harlan Teklad Rodent Diet 8604) and water. After a week, the animals were divided into two weight matched groups. One group was offered ad libitum chow ($n = 18$) and the second group was offered chow, liquid sucrose (LS), and lard (choice; $n = 18$). The bottle containing LS was placed next to the water bottle and the lard was given in a dish inside the cage with the chow. Body weight and energy intake corrected for spillage were measured daily throughout the experiment. Peripheral leptin responsiveness was tested in rats fed choice or chow on days 2, 4, 8, and 16. Rats were fasted for 10 h and half the rats in each treatment group were injected i.p. with 2 mg/kg leptin and the other half with phosphate buffered saline (PBS) on the test days. After initial randomization on day 2, rats alternated treatments on each day of testing. The chow and choice diets were provided to each group, respectively, at 6:00 pm. Energy intakes were measured at 14 h. Body weight was measured at 14 h and 24 h.

On day 20, the choice rats were switched back to chow and the control group continued to be fed chow. On day 4 of reversal back to chow (chow only), rats were again tested for leptin responsiveness using the protocol described above.

Blood glucose was measured after a 5 h fast on day 17, day 2 of reversal, and day 9 of reversal. Tail blood samples were used for measurement of whole blood glucose (EasyGluco Blood Glucose Monitoring System, US Diagnostics, Inc., New York, NY).

Experiment 2: Effects of choice diet on central leptin responsiveness

The previous study showed that choice rats were unresponsive to peripheral leptin after 16 days on diet, and this experiment determined the time course of changes in central leptin responsiveness in rats offered free access to chow or choice. The rats were housed and fed as described in experiment 1. Approximately 1 week after arrival, thirty-two rats were fitted with a 3rd ventricle 22-gauge guide cannula (Plastics One, Inc., Roanoke, VA) using stereotaxic techniques and coordinates (anteroposterior -2.8 mm, lateral 0.0 mm, ventral -8.3 mm relative to the bregma) based on the Paxinos and Watson rat brain atlas (Paxinos & Watson, 1998). The cannula was attached to the skull using super glue, two screws, and dental cement. One week after surgery, cannula placement was confirmed using angiotensin II (Sigma-Aldrich, St. Louis, MO). Angiotensin (20 ng/ 2 μ L) was infused into the 3rd ventricle over 1 min using a Harvard Apparatus infusion pump (PhD 2000, Holliston, MA). If the rats drank voraciously within 1 min, the cannula placement was deemed accurate. Rats were given 3 days to recover from cannula testing before the baseline period of the experiment started.

Baseline food intakes were measured in 31 rats for 5 days as described in experiment 1. After baseline the rats were weight matched into two groups, chow and choice. Since

peripheral leptin resistance was seen on day 16 in experiment 1, we started testing for central leptin responsiveness on that day in this study. At 5:00 pm, 1.5 μ g leptin in 2 μ L of solution was infused over 1 min. Following a 10 h fast, the chow and choice diets were provided to each group, respectively, at 6:00 pm. Energy intakes were measured 14, 24, and 38 h after infusion. Body weight was measured at 14, 24, and 38 h. On day 22, the choice rats were switched back to chow and the control group continued to be fed chow. On day 4 of reversal back to chow (chow only), rats were again tested for central leptin responsiveness using the same procedures as described above.

Tail blood samples were collected after a 5 h fast on day 18 of choice and day 7 of reversal for measurement of whole blood glucose, serum insulin (Rat Insulin RIA kit, Millipore, St. Charles, Missouri), and serum leptin concentrations (Multi-species Leptin RIA, Millipore, Billerica, MA).

Experiment 3: Effects of the choice diet and central leptin responsiveness on energy expenditure

Experiments 1 and 2 identified a time course for development of leptin resistance in the choice model. This experiment tested the effects of centrally administered leptin on energy expenditure of choice rats compared with chow rats at a time when they were unresponsive to the effects of leptin on weight change. Fourteen rats arrived weighing ~270 g and were housed and fed as described above. Body weights were recorded daily. The rats were fitted with 3rd ventricle cannulas and cannula placement was tested with angiotensin II. Rats were divided into two weight matched groups, chow and choice. Rats were on diet for 25 days and then tested to confirm peripheral leptin responsiveness in chow rats and peripheral leptin resistance in choice rats. The rats were divided into 2 sets of 7 animals. One set of rats at a time were then placed in a 12-cage indirect calorimeter (TSE LabMaster – Metabolic Research Platform, TSE Systems International, Chesterfield, MO) and adapted to the cages for 1 week. Oxygen consumption, carbon dioxide production, and activity were recorded from every cage for 1 min every 39 min. Energy expenditure was calculated on a per metabolic body weight basis (kcal/h/body weight^{0.75}) and a per rat basis (kcal/h/rat). Respiratory exchange ratio (RER; CO₂/O₂) was also calculated. Activity was determined through the Infra-Mot program which uses infrared radiation and spatial displacement over time. Chow and LS intake were also recorded every 39 min. In the choice rats, lard intake was recorded daily.

On test days, rats were fasted for 10 h starting at 8:00 am. At 5:00 pm, half of the rats in the choice and chow groups were infused over 1 min with 1.5 μ g leptin in a volume of 2 μ L. The other rats were infused with an equivalent volume of PBS. At 6:00 pm, food was returned to the cages. Measurements were taken from 6:00 pm until 7:00 am the following morning (13 h; night 1). Then rats were weighed and food hoppers and water refilled. The calorimeter was restarted at about 8:00 am and ran until 7:30 am the following morning (day 1; 8:00 am–7:00 pm). The rats were given 1 week to recover before the opposite treatment was given.

Experiment 4: Effects of choice reversal on obesity

Previous experiments showed that the choice rats had a reduced energy intake when choice rats switch to chow only and were leptin responsive within 4 days. This experiment tested whether the 4 days of diet reversal of the choice rats to chow only reduced total carcass fat in male rats. Twenty male Sprague Dawley rats were housed and fed as described in experiment 1. Rats arrived weighing ~275 g. Approximately 4 days after arrival the rats were placed on the choice diet (chow, LS and lard). All rats remained on the choice diet for 38 days. Then on day 39, they were weight matched and half the rats were switched from the choice diet to a chow only diet while the remaining rats remained on the choice diet. Body weights were recorded daily. The final 29 days food intakes were performed as described in experiment 1. These include the final 25 days of the choice diet for both groups and the 4 days of reversal for the choice reversal (now chow only group) and the rats that continued the choice diet. On day 28, serum leptin was measured on tail blood collected at 1:00 pm following a 5 h fast (Rat Leptin RIA, Millipore, Billerica, MA, USA). On day 42 (day 4 of reversal), food was taken away at 7 am and the rats were decapitated starting at 9:45 am. The liver and epididymal, retroperitoneal, and mesenteric fat pads were dissected, weighed and returned to the carcass. Trunk blood was collected to measure serum leptin. Carcass composition, less the gastrointestinal tract, was analyzed as described previously (Harris, 1991).

Statistical analysis

Values are mean $\pm$ SEM. Power calculations from previous data on male Sprague–Dawley rat suggested that 28 rats (14 rats per group) would permit detection of test day food intake effects equal to a standardized difference of 1.1 at the 5% probability level with 80% power. Statistically significant differences in experiments 1, 2, and 3 were determined by two-way ANOVA using Statistica (ver. 9, Statsoft, Inc., Tulsa, OK, USA). Post hoc tests were performed using *t*-tests in Microsoft Excel. In experiment 3, the one choice and 2 chow rats with RER average values (below 0.70) were excluded from all analyses. Statistically significant differences for experiment 4 were determined using one-way ANOVA.

Results

Experiment 1

The purpose of this experiment was to determine the time course of onset of peripheral leptin responsiveness in rats offered free access to chow or choice diet. At baseline, body weights of chow and choice rats were similar (Table 1). However, by day 3 chow rats were heavier than choice rats ($F(1,33) = 2.44, p = 0.02$) and this continued for the remainder of the study. Choice rats consumed 33% chow, 25% LS, and 42% lard and consumed 21% more energy ($F(1,33) = 9.31, p < 0.0001$) than chow rats (Table 1). During the first 4 days of reversal, choice rats (now chow only) consumed 40% less energy than the chow fed controls ($F(1,33) = 20.22, p < 0.001$).

Choice rats remained leptin responsive on days 2 (data not shown) and 4 (14 h weight Δ , PBS 5.9 ± 0.2 g leptin -0.1 ± 0.7 g; $F(1,16) = 3.91, p = 0.001$). By Day 8, however, the choice rats were leptin insensitive according to 14 h and 24 h weight change (W) and 14 h

energy intake (EI; 24 h data not shown; Fig. 1A and B). By day 16, chow rats remained leptin responsive, but the choice rats were leptin resistant since 14 h food intake and 14 h and 24 h body weight did not change following leptin injection (24 h data not shown; Fig. 1C and D). The choice rats were then switched to chow. On day 4 of reversal (1st testing day), the choice rats were again leptin responsive according to 14 h weight change and 14 h energy intake (Fig. 1E and F).

During this study, fasting glucose was higher on day 18 in the choice rats (6.1 ± 0.1 mmol/L) compared with the controls (5.4 ± 0.1 mmol/L; ($F(1,33) = 4.42, p = 0.0001$). This increase continued on day 2 of the reversal (choice 5.9 ± 0.1 , chow 5.5 ± 0.1 mmol/L; $F(1,33) = 2.72, p = 0.01$). By day 9 of the reversal, however, there was no difference in fasting glucose values between the choice reversal (5.8 ± 0.1 mmol/L) and control chow (5.7 ± 0.1 mmol/L) animals.

Experiment 2

This experiment determined the time course of changes in central leptin responsiveness in rats offered free access to chow or choice. At baseline, body weights of chow and choice rats were similar (Table 2). Choice and chow rats body weight and change from baseline body weight remained similar for the duration of the study. Choice rats consumed 39% more energy than chow rats (choice 2001 ± 76 kcal/22 d, Chow 1437 ± 35 kcal/22 d; $F(1,26) = 6.97, p < 0.0001$). Choice rats consumed 24% of energy as chow, 23% as LS, and 53% as lard. During reversal, choice rats (consuming chow only) consumed 34% less energy than the chow fed controls (Choice reversal 187 ± 11 kcal/4 d, Chow 285 ± 8 kcal/4 d; $F(1,26) = 7.80, p < 0.0001$).

On day 16, chow rats were leptin sensitive, however choice rats were leptin unresponsive with no changes in 24 h and 38 h body weight or 14 h, 24 h, and 38 h food intake in response to a 3rd ventricle leptin injection (14 h and 24 h data not shown; Fig. 2A and B). Again the choice rats were then switched to chow. On day 4 of reversal, the choice rats were leptin responsive based on 24 h and 38 h body weight change and 38 h energy intake (14 h and 24 h data not shown; Fig. 2C and D).

On day 18 of the experimental diet there were no differences in fasting insulin (Table 3), but fasting glucose was higher in the choice rats compared to the chow controls ($F(1,26) = 2.59, p = 0.015$). By day 7 of reversal, there was no difference in either insulin or glucose between the choice rats and the control rats. On day 18 of diet, leptin was higher in choice vs. chow fed rats ($F(1,25) = 4.26, p = 0.0002$), but leptin concentrations were not different when the choice rats were fed chow only for 7 days.

Experiment 3

This experiment tested the effects of centrally administered leptin on energy expenditure in choice rats compared with chow rats at a time when they were unresponsive to the effects of leptin on weight change. After 25 days on diet, the chow rats were leptin responsive (24 h weight ; Leptin 4 ± 1 g, PBS 7 ± 2 g; $F(1,6) = 2.51, p = 0.045$) whereas the choice rats were leptin unresponsive (24 h weight , Leptin 5 ± 3 g PBS 6 ± 3 g). The rats had similar

body weights at the start of acclimation to the calorimeter (choice 420 ± 11 , chow 429 ± 17 g). During the first 156 min of the test night (2.6 h), chow leptin rats had a higher energy expenditure per unit metabolic size than chow saline rats (Fig. 3A) and energy expenditure expressed per rat tended to be higher ($p = 0.07$; Fig. 3B). Energy expenditure of choice rats was higher than that of chow rats ($F(1,8) = 6.45$, $p = 0.039$), but leptin had no effect on energy expenditure expressed per metabolic body size or per rat. There was no effect of diet or leptin on RER or activity (Fig. 3C) during the 156 min after the leptin injection.

During the entire test night and day 1, choice rats had higher average energy expenditure than controls whether expressed per unit metabolic size (night, $F(1,8) = 6.43$, $p = 0.035$; day, $F(1,8) = 14.925$, $p = 0.005$) or per rat (night, $F(1,8) = 5.19$, $p = 0.05$; day, $F(1,8) = 10.228$, $p = 0.013$) but there were no leptin treatment effects (data not shown). During the entire test night and day 1 no group or treatment differences were seen with average RER. During night 1, choice had higher average activity than saline rats ($F(1,8) = 5.09$, $p = 0.05$). During night 1, the average activity of choice leptin and choice saline rats was higher from the chow saline rats. The chow leptin group was not different than any other group. During day 1, there were no group or treatment differences with average activity. There was no effect of diet or leptin on body weight change or food intake (Fig. 3D).

Experiment 4

This experiment tested whether the 4 days of diet reversal of the choice rats to chow only reduced total carcass fat in male rats. The choice and choice reversal groups had similar body weight and body weight gain from baseline throughout the study (Table 4). Also, choice and choice reversal rats consumed a similar amount of chow (choice 33%; choice reversal 33%), LS (choice 25%; choice reversal 21%), lard (choice 41%; choice reversal 46%), and total energy before the diet switch. During reversal, choice rats consuming chow only consumed 30% less energy ($F(1,17) = 2.96$, $p = 0.008$) than the choice rats. On day 28, fasting leptin levels were similar between choice groups but at the end of the experiment (day 42, day 4 of reversal) choice reversal rats had lower serum leptin ($F(1,17) = 7.95$, $p < 0.0001$) than the choice rats. Epididymal ($F(1,17) = 2.48$, $p = 0.024$), retroperitoneal ($F(1,17) = 2.44$, $p = 0.025$), and mesenteric ($F(1,17) = 3.80$, $p = 0.001$) fat pad weights were smaller in the choice reversal rats compared to the rats that continued the choice diet, and carcass fat ($F(1,17) = 3.74$, $p = 0.002$) was higher in the choice compared to the choice reversal rats (Table 4).

Discussion

Previously many studies have reported that rats or mice fed high fat diets take 8 weeks (56 days) or longer to develop leptin resistance (de Lartigue, Barbier de la Serre, Espero, Lee, & Raybould, 2011; Harris et al., 2003; Matheny et al., 2011; White et al., 2009) but not all studies (Lin, Martin, Schaffhauser, & York, 2001; Zhang, Matheny, Tumer, Mitchell, & Scarpace, 2007) and 17 weeks after reverse to become leptin responsive following 20 weeks of high fat feeding (Enriori et al., 2007). However not all these studies examined for leptin resistance at time points earlier than 8 weeks. At least one study found Osborne–Mendel rats fed a low fat diet (10% fat) were peripherally leptin responsive whereas the rats fed a high

fat diet (56%) were insensitive to a 0.5 mg/kg i.p. leptin following dietary treatment for a minimum of 2 weeks (Lin et al., 2001). When rats consuming the high fat diet were switched to the low fat diet, they remained leptin insensitive on day 1 but were leptin responsive to food intake on day 5. The rats adapted to the low fat diet but tested on the high fat diet were leptin responsive on day 1 but unresponsive on days 5 and 15 (Lin et al., 2001). These results from the high fat diet switch are similar to those presented in the current study but do suggest a previous precedent for shorter-term dietary induced leptin resistance and thus some aspects of leptin resistance can occur rapidly with high fat feeding. In our time course for the development of leptin resistance in choice fed rats, we found that peripheral and central leptin resistance was present as early as 16 days, which represents a 71% reduction in time for the development of leptin resistance compared to most studies where rats were fed a traditional high fat diet. When choice rats have liquid sucrose and lard taken away, peripheral and central leptin responsiveness is restored within 4 days. During those first 4 days of reversal, rats lose about 10 g (~30%) of body fat without a significant drop in body weight. This confirms a previous report that female rats that had been fed 160% of their energy need by stomach tube for 21 days demonstrated a reduction in body fat and carcass weight following 4 days of ad libitum food intake (Harris, Kasser, & Martin, 1986). Also, male and female rats have shown the ability to reverse obesity (Bartness, Polk, McGriff, Youngstrom, & DiGirolamo, 1992; Hill, Dorton, Sykes, & DiGirolamo, 1989) over 5 weeks (Bartness et al., 1992) if on high fat diet for less than 30 weeks (Hill et al., 1989).

Besides high-fat diets, rats fed a high-sugar, high-fat diet (Shapiro, Tumer, Gao, Cheng, & Scarpace, 2011) and a high-sugar, low-fat diet (Haring & Harris, 2011) have developed peripheral leptin resistance. Shapiro et al. tested peripheral leptin resistance in Sprague–Dawley rats consuming a sugar-free, high-fat diet (0% sugar, 50% carbohydrate; 30% fat) or a high-sugar, high-fat diet (40% fructose, 50% carbohydrate; 30% fat) (Shapiro et al., 2011). Rats consuming high-sugar, high-fat diet were unresponsive to a peripheral leptin injection (0.6 mg/kg) on day 65 whereas the sugar-free, high-fat rats had reduced food intake suggesting leptin responsiveness (Shapiro et al., 2011). The choice diet demonstrated peripheral leptin insensitivity on day 8 and peripheral and central leptin resistance based on body weight and food intake on day 16. Shapiro et al. did not test for central leptin resistance, but performed leptin injections when rats were switched from the high-sugar, high fat diet to the sugar-free, high-fat diet (Shapiro et al., 2011). Eighteen days after the switch they decreased food intake in response to a peripheral leptin injection of leptin, indicating a recovery of leptin sensitivity, they decreased food intake. Day 18 was the first day after the switch that these rats were tested. In choice rats, peripheral and central leptin unresponsive rats became leptin responsive on day 4 of reversal. Also during reversal, Shapiro et al. performed a tail bleed on day 3 after the diet switch. We performed a tail bleed on day 7 of reversal. Both studies found decreased serum leptin levels (Shapiro et al., 2011). Thus, the results of the two studies were similar, but our study demonstrated the rapid onset and reversal of peripheral and central leptin resistance with the choice diet. It is possible that leptin resistance and reversal also occurred more rapidly than reported by Shapiro et al. (2011) but earlier time points were not tested.

The experiments described here suggested that leptin resistance may be an effect of obesity or energy overconsumption. In the current study, there was a 34–40% reduction in energy

intake when the rats no longer had access to liquid sucrose and lard, and Shapiro et al. (2011) demonstrated a ~10% reduction in energy intake during their diet switch. Also, with the choice diet the rats were exposed to and continued to consume chow pre and post reversal. All rats consumed the same or more chow (0–190%) during reversal suggesting it was not a chow specific response. It was first hypothesized by Havel that leptin responds to changes in cellular energy status (Havel, 2004). However this hypothesis was further refined by Morrison (Morrison, 2008). Morrison stated that if leptin primarily signaled for negative energy balance, then studies enhancing Suppressor of cytokine signaling (SOCS) 3 and Protein Tyrosine Phosphatase (PTB) 1B signaling should not reduce food intake and body weight (Cheng et al., 2002; Howard et al., 2004; Zabolotny et al., 2002). SOCS3 and PTB1B inhibit STAT3 using feedback inhibition (Munzberg, 2010; Myers et al., 2008). Thus, extended energy overconsumption may be needed for leptin resistance and a decrease in energy consumption (feeding an ad libitum low fat diet) may be needed to regain leptin responsiveness following a period of energy overconsumption in non-genetically modified animals. These changes in energy intake were shown with the current studies, but we also found a reduction in body fat.

In a previous study by Morrison, food intake was measured in rats following the end of overfeeding using a gastric cannula (White et al., 2009). Rats were overfed with a low fat liquid diet or water using a protocol that gradually increased the infusion volume during the 17 day study. Subgroups were killed on days 17 or day 2 (day 19) or day 4 (day 21) of the cessation of liquid overfeeding. On day 17, serum leptin concentrations in overfed rats were 8-fold those of water infused control rats, but by day 2 of stopping overfeeding leptin concentrations were similar to those of water infused controls. Food intakes for the overfed rats were 52% and 21% lower than controls on days 2 and 4 respectively. Thus leptin concentrations normalized far faster than food intake. It was theorized that leptin is not tied to adiposity, instead to nutrient status (White et al., 2009), but body fat was not measured in the study. However, our experiment 4 suggests that we cannot eliminate body fat as a regulator of leptin levels and leptin sensitivity as body fat was significantly reduced when peripheral and central leptin responsiveness was demonstrated in choice rats that had been switched back to chow.

As described in the previous paragraph it is important to examine both body weight and body fat. In experiment 1, choice rats had a lower body weight than chow rats. In experiments 2 & 3 the body weights were not different between the groups. Also, in the original paper examining the choice model, there were no difference in body weight or body weight gain between the choice and chow groups over 21 days (Apolzan & Harris, 2012). However, in Harris and Apolzan (Harris & Apolzan, 2012) choice were heavier than chow rats. The authors feel the lack of a body weight response is a reflection that body weight is not an adequate measure of body composition. Body composition was consistent among studies. The difference in body weight is why controls groups are necessary for every experiment. It should be noted that the previous study (Apolzan & Harris, 2012) and in the present study no differences in lean body mass were shown. The lack of a body weight response suggests it is critical to measure body composition to determine if body fat differences are present.

It has been speculated that leptin production is regulated by insulin secretion caused by alterations in energy intake (Havel, 2000). This hypothesis was based on observations that circulating leptin increased following insulin infusions. However, in the studies described here fasting values for leptin values did not correlate with serum insulin concentrations. This may be due to glucose metabolism being affected by the composition of the choice diet (la Fleur, Luijendijk, van Rozen, Kalsbeek, & Adan, 2011). Thus energy intake and/or obesity over an intermediate period remains a likely regulator of leptin production, and the hypothesis of other hormonal and nutrient signals regulating leptin production remains a possibility for acute change in circulating concentrations of leptin (Lee & Fried, 2009).

It has previously been reported that high energy diets increase heat production (Rothwell & Stock, 1982). This was confirmed in the current study and is related to increased diet induced thermogenesis (Hill, Fried, & DiGirolamo, 1983) associated with higher energy intake in the choice compared to chow fed rats. Leptin has also been shown to increase energy expenditure. Energy expenditure was higher in male F-344 X BN rats infused with a high dose of leptin (1 mg/day) from Alzet osmotic pumps than in their controls (Scarpace et al., 1997). In male Sprague Dawley rats, 10 µg of leptin or vehicle was administered daily into the lateral ventricle for 4 days (Gullicksen, Flatt, Dean, Hartzell, & Baile, 2002) and on day 5 (1 day post injection) indirect calorimetry was performed. Energy expenditure relative to lean body mass was increased in the leptin treated group compared to controls (Gullicksen et al., 2002). Also, injections of leptin into the lateral ventricle were found to increase energy expenditure per metabolic body size when doses were 2.5 µg and 10 µg but not 0.156 µg or 0.625 µg (Wang et al., 1999). Thus, both peripheral and central leptin administration have previously been shown to increase energy expenditure which supports our observation of a 12% increase in energy expenditure per metabolic size in chow fed rats. Also following a central leptin injection, RER has been shown to decrease suggesting greater fat oxidation (Wang et al., 1999) or increase suggesting greater carbohydrate oxidation (Gullicksen et al., 2002). Overall, the prevailing view is that leptin increases fat oxidation lowering RER (Chen & Heiman, 2000; Hwa et al., 1997; Wein, Ukropec, Gasperikova, Klimes, & Sebkova, 2007). However in the current study we did not see changes in RER following central leptin injections suggesting no change in macronutrient oxidation rates in choice or chow rats.

Leptin regulates the sympathetic nervous system through the long form leptin receptor (ObRb)-STAT3 pathway (Bates et al., 2004). This is the same signaling pathway through which leptin affects energy expenditure. In a previous study by Scarpace et al. (2002) 18 month old mildly obese F-344 X Brown Norway rats were given recombinant adeno-associated virus encoding rat leptin cDNA (rAAV-leptin group) or a control vector into the 3rd ventricle. The rAAV-leptin group's food intake reached its nadir on day 11 and gradually rose back to vector control feeding levels on day 25. Energy expenditure was significantly elevated in the rAAV-leptin group compared to vector controls on days 7, 21 and 57 but not on day 120 suggesting that the decrease in energy expenditure at the early time point may be a dietary effect. Enriori et al. suggested that leptin stimulates sympathetic tone and increases brown adipose tissue temperature even in a state of leptin resistance (Enriori et al., 2011). C57BL/6J mice were fed a high fat or low fat control diet for 20 weeks (Enriori et al., 2011). Peripheral leptin administration (6 mg/kg) significantly increased

IBAT temperature for at least 1 h in the high fat mice and the low fat fed control mice. Central leptin administration (1 µg) also resulted in increased IBAT temperature in both the high fat and low fat fed mice. The increased IBAT temperature following leptin administration corresponds with a previous study that reported increased UCP-1 mRNA expression following a 3-day i.p. infusion of 0.9 mg/d of leptin using Alzet mini pumps (Scarpace & Matheny, 1998). Although increased IBAT temperature (Enriori et al., 2011) and UCP-1 expression (Scarpace & Matheny, 1998) was previously shown following a leptin injection, leptin did not increase energy expenditure in leptin resistant choice fed rats described here in experiment 3. Thus, the definition of leptin resistance by Munzberg (Munzberg, 2010) may be expanded to include inhibition of energy expenditure.

A possible hypothesis for the mechanism of leptin resistance may include obesity itself or high triacylglycerol (TG) concentrations inhibiting leptin transport into the brain (Banks, 2008; Banks et al., 2004). The latter is an unlikely culprit since TG concentrations are not different between choice and control animals when leptin resistance is established. Previous research from the Harris laboratory has shown that a high fat diet (Research Diets, Inc., 60% fat, D12492) does not result in peripheral leptin resistance after 21 days whereas chow + 30% sucrose solution and choice rats were leptin resistant. At the time of kill (day 23), chow plus 30% sucrose solution, choice and the high fat diet rats had similar carcass fat suggesting it is not an obesity or body fat effect. Others have shown that increased O-GlcNAc modification of insulin signaling proteins leads to insulin resistance (Teo, Wollaston-Hayden, & Wells, 2010). Leptin and insulin share insulin receptor substrates (IRSs) and phosphatidylinositol-3 kinase (PI3K) as a common signaling pathway (Morrison, Morton, Niswender, Gelling, & Schwartz, 2005). Therefore, while speculative we hypothesize that high levels of O-GlcNAc glycosylation on leptin's intracellular signaling proteins are causing leptin resistance. Previously unpublished glucose tolerance test data, which were used as a measure of insulin sensitivity, suggest that choice rats have lower insulin sensitivity than chow rats. While speculative, the rapid onset and reversal of leptin resistance and the glucose tolerance test lend support to the glycosylation hypothesis.

In conclusion, the choice diet rapidly induces peripheral and central leptin resistance. However, when liquid sucrose and lard are no longer available, peripheral and central leptin responsiveness are quickly restored. As was previously found, the choice rats have an increased energy expenditure in addition to increased energy intake. However, following a central leptin injection chow rats, but not choice rats, increase energy expenditure per metabolic size. Thus, choice rats were unresponsive to the effect of leptin on energy expenditure in addition to the effects on food intake and weight gain. The rapid onset and reversal of leptin responsiveness of rats fed choice diet suggests a direct effect of metabolism on signaling pathways. However, obesity cannot yet be eliminated as a regulator of leptin resistance. The rapid onset and reversal of leptin resistance in choice rats provides a new model in which to explore mechanisms of leptin resistance.

References

- Apolzan JW, Harris RB. Differential effects of chow and purified diet on the consumption of sucrose solution and lard and the development of obesity. *Physiology & Behavior*. 2011; 105(2):325–331. [PubMed: 21893075]

- Apolzan JW, Harris RB. Differential effects of chow and purified diet on the consumption of sucrose solution and lard and the development of obesity. *Physiology & Behavior*. 2012; 105(2):325–331. [PubMed: 21893075]
- Banks WA. The blood–brain barrier as a cause of disease. *Current Pharmaceutical Design*. 2008; 14(16):1553–1554. [PubMed: 18673196]
- Banks WA, Coon AB, Robinson SM, Moinuddin A, Shultz JM, Nakaoka R, et al. Triglycerides induce leptin resistance at the blood–brain barrier. *Diabetes*. 2004; 53(5):1253–1260. [PubMed: 15111494]
- Bartness TJ, Polk DR, McGriff WR, Youngstrom TG, DiGirolamo M. Reversal of high-fat diet-induced obesity in female rats. *American Journal of Physiology*. 1992; 263(4 Pt 2):R790–797. [PubMed: 1415790]
- Bates SH, Dundon TA, Seifert M, Carlson M, Maratos-Flier E, Myers MG Jr. LRb-STAT3 signaling is required for the neuroendocrine regulation of energy expenditure by leptin. *Diabetes*. 2004; 53(12):3067–3073. [PubMed: 15561935]
- Bates SH, Myers MG. The role of leptin → STAT3 signaling in neuroendocrine function. An integrative perspective. *Journal of Molecular Medicine (Berlin)*. 2004; 82(1):12–20.
- Chen Y, Heiman ML. Chronic leptin administration promotes lipid utilization until fat mass is greatly reduced and preserves lean mass of normal female rats. *Regulatory Peptides*. 2000; 92(1–3):113–119. [PubMed: 11024573]
- Cheng A, Uetani N, Simoncic PD, Chaubey VP, Lee-Loy A, McGlade CJ, et al. Attenuation of leptin action and regulation of obesity by protein tyrosine phosphatase 1B. *Developmental Cell*. 2002; 2(4):497–503. [PubMed: 11970899]
- Considine RV, Sinha MK, Heiman ML, Kriauciunas A, Stephens TW, Nyce MR, et al. Serum immunoreactive-leptin concentrations in normal-weight and obese humans. *New England Journal of Medicine*. 1996; 334(5):292–295. [PubMed: 8532024]
- de Lartigue G, Barbier de la Serre C, Espero E, Lee J, Raybould HE. Diet-induced obesity leads to the development of leptin resistance in vagal afferent neurons. *American Journal of Physiology – Endocrinology and Metabolism*. 2011; 301(1):E187–195. [PubMed: 21521717]
- El-Haschimi K, Pierroz DD, Hileman SM, Bjorbaek C, Flier JS. Two defects contribute to hypothalamic leptin resistance in mice with diet-induced obesity. *The Journal of Clinical Investigation*. 2000; 105(12):1827–1832. [PubMed: 10862798]
- Elmqvist JK, Coppari R, Balthasar N, Ichinose M, Lowell BB. Identifying hypothalamic pathways controlling food intake, body weight, and glucose homeostasis. *The Journal of Comparative Neurology*. 2005; 493(1):63–71. [PubMed: 16254991]
- Enriori PJ, Evans AE, Sinnayah P, Jobst EE, Tonelli-Lemos L, Billes SK, et al. Diet-induced obesity causes severe but reversible leptin resistance in arcuate melanocortin neurons. *Cell Metabolism*. 2007; 5(3):181–194. [PubMed: 17339026]
- Enriori PJ, Sinnayah P, Simonds SE, Garcia Rudaz C, Cowley MA. Leptin action in the dorsomedial hypothalamus increases sympathetic tone to brown adipose tissue in spite of systemic leptin resistance. *Journal of Neuroscience*. 2011; 31(34):12189–12197. [PubMed: 21865462]
- Gullicksen PS, Flatt WP, Dean RG, Hartzell DL, Baile CA. Energy metabolism and expression of uncoupling proteins 1, 2, and 3 after 21 days of recovery from intracerebroventricular mouse leptin in rats. *Physiology & Behavior*. 2002; 75(4):473–482. [PubMed: 12062312]
- Halaas JL, Boozer C, Blair-West J, Fidathusein N, Denton DA, Friedman JM. Physiological response to long-term peripheral and central leptin infusion in lean and obese mice. *Proceedings of the National Academy of Sciences of the United States of America*. 1997; 94(16):8878–8883. [PubMed: 9238071]
- Haring SJ, Harris RB. The relation between dietary fructose, dietary fat and leptin responsiveness in rats. *Physiology & Behavior*. 2011; 104(5):914–922. [PubMed: 21684298]
- Harris RB. Growth measurements in Sprague–Dawley rats fed diets of very low fat concentration. *Journal of Nutrition*. 1991; 121(7):1075–1080. [PubMed: 2051227]
- Harris RB. Leptin – much more than a satiety signal. *Annual Review of Nutrition*. 2000; 20:45–75.
- Harris RB, Apolzan JW. Changes in glucose tolerance and leptin responsiveness of rats offered a choice of lard, sucrose, and chow. *American Journal of Physiology Regulatory, Integrative and Comparative Physiology*. 2012; 302(11):R1327–1339.

- Harris RB, Bowen HM, Mitchell TD. Leptin resistance in mice is determined by gender and duration of exposure to high-fat diet. *Physiology & Behavior*. 2003; 78(4–5):543–555. [PubMed: 12782207]
- Harris RB, Kasser TR, Martin RJ. Dynamics of recovery of body composition after overfeeding, food restriction or starvation of mature female rats. *Journal of Nutrition*. 1986; 116(12):2536–2546. [PubMed: 3543262]
- Havel PJ. Role of adipose tissue in body-weight regulation. Mechanisms regulating leptin production and energy balance. *Proceedings of the Nutrition Society*. 2000; 59(3):359–371. [PubMed: 10997652]
- Havel PJ. Update on adipocyte hormones. Regulation of energy balance and carbohydrate/lipid metabolism. *Diabetes*. 2004; 53(Suppl 1):S143–151. [Review]. [PubMed: 14749280]
- Hayes MR, Skibicka KP, Lechner TM, Guarnieri DJ, DiLeone RJ, Bence KK, et al. Endogenous leptin signaling in the caudal nucleus tractus solitarius and area postrema is required for energy balance regulation. *Cell Metabolism*. 2010; 11(1):77–83. [PubMed: 20074530]
- Heymsfield SB, Greenberg AS, Fujioka K, Dixon RM, Kushner R, Hunt T, et al. Recombinant leptin for weight loss in obese and lean adults. A randomized, controlled, dose-escalation trial. *JAMA*. 1999; 282(16):1568–1575. [PubMed: 10546697]
- Hill JO, Dorton J, Sykes MN, DiGirolamo M. Reversal of dietary obesity is influenced by its duration and severity. *International Journal of Obesity*. 1989; 13(5):711–722. [PubMed: 2583925]
- Hill JO, Fried SK, DiGirolamo M. Effects of a high-fat diet on energy intake and expenditure in rats. *Life Sciences*. 1983; 33(2):141–149. [PubMed: 6865651]
- Howard JK, Cave BJ, Oksanen LJ, Tzamelis I, Bjorbaek C, Flier JS. Enhanced leptin sensitivity and attenuation of diet-induced obesity in mice with haploinsufficiency of *Socs3*. *Nature Medicine*. 2004; 10(7):734–738.
- Hwa JJ, Fawzi AB, Graziano MP, Ghibaudi L, Williams P, Van Heek M, et al. Leptin increases energy expenditure and selectively promotes fat metabolism in *ob/ob* mice. *American Journal of Physiology*. 1997; 272(4 Pt 2):R1204–1209. [PubMed: 9140021]
- la Fleur SE, Luijckendijk MC, van Rozen AJ, Kalsbeek A, Adan RA. A free-choice high-fat high-sugar diet induces glucose intolerance and insulin unresponsiveness to a glucose load not explained by obesity. *International Journal of Obesity (London)*. 2011; 35(4):595–604.
- Lee MJ, Fried SK. Integration of hormonal and nutrient signals that regulate leptin synthesis and secretion. *American Journal of Physiology – Endocrinology and Metabolism*. 2009; 296(6):E1230–1238. [PubMed: 19318513]
- Lin L, Martin R, Schaffhauser AO, York DA. Acute changes in the response to peripheral leptin with alteration in the diet composition. *American Journal of Physiology Regulatory, Integrative and Comparative Physiology*. 2001; 280(2):R504–509.
- Lin S, Thomas TC, Storlien LH, Huang XF. Development of high fat diet-induced obesity and leptin resistance in C57Bl/6J mice. *International Journal of Obesity and Related Metabolic Disorders*. 2000; 24(5):639–646. [PubMed: 10849588]
- Matheny M, Shapiro A, Tumer N, Scarpace PJ. Region-specific diet-induced and leptin-induced cellular leptin resistance includes the ventral tegmental area in rats. *Neuropharmacology*. 2011; 60(2–3):480–487. [PubMed: 21059361]
- Morrison CD. Leptin resistance and the response to positive energy balance. *Physiology & Behavior*. 2008; 94(5):660–663. [PubMed: 18508097]
- Morrison CD, Morton GJ, Niswender KD, Gelling RW, Schwartz MW. Leptin inhibits hypothalamic *Npy* and *Agrp* gene expression via a mechanism that requires phosphatidylinositol 3-OH-kinase signaling. *American Journal of Physiology – Endocrinology and Metabolism*. 2005; 289(6):E1051–1057. [PubMed: 16046456]
- Munzberg H. Leptin-signaling pathways and leptin resistance. *Forum of Nutrition*. 2010; 63:123–132. [PubMed: 19955780]
- Munzberg H, Flier JS, Bjorbaek C. Region-specific leptin resistance within the hypothalamus of diet-induced obese mice. *Endocrinology*. 2004; 145(11):4880–4889. [PubMed: 15271881]
- Myers MG, Cowley MA, Munzberg H. Mechanisms of leptin action and leptin resistance. *Annual Review of Physiology*. 2008; 70:537–556.

- Paxinos, G.; Watson, C. The rat brain in stereotaxic coordinates. 4. Academic Press; 1998.
- Rothwell NJ, Stock MJ. Energy expenditure of 'cafeteria'-fed rats determined from measurements of energy balance and indirect calorimetry. *Journal of Physiology*. 1982; 328:371–377. [PubMed: 7131317]
- Scarpance PJ, Matheny M. Leptin induction of UCP1 gene expression is dependent on sympathetic innervation. *American Journal of Physiology*. 1998; 275(2 Pt 1):E259–264. [PubMed: 9688627]
- Scarpance PJ, Matheny M, Pollock BH, Tumer N. Leptin increases uncoupling protein expression and energy expenditure. *American Journal of Physiology*. 1997; 273(1 Pt 1):E226–230. [PubMed: 9252501]
- Scarpance PJ, Matheny M, Zhang Y, Shek EW, Prima V, Zolotukhin S, et al. Leptin-induced leptin resistance reveals separate roles for the anorexic and thermogenic responses in weight maintenance. *Endocrinology*. 2002; 143(8):3026–3035. [PubMed: 12130569]
- Shapiro A, Tumer N, Gao Y, Cheng KY, Scarpance PJ. Prevention and reversal of diet-induced leptin resistance with a sugar-free diet despite high fat content. *British Journal of Nutrition*. 2011; 106(3):390–397. [PubMed: 21418711]
- Teo CF, Wollaston-Hayden EE, Wells L. Hexosamine flux, the O-GlcNAc modification, and the development of insulin resistance in adipocytes. *Molecular and Cellular Endocrinology*. 2010; 318(1–2):44–53. [PubMed: 19799964]
- Wang T, Hartzell DL, Rose BS, Flatt WP, Hulsey MG, Menon NK, et al. Metabolic responses to intracerebroventricular leptin and restricted feeding. *Physiology & Behavior*. 1999; 65(4–5):839–848. [PubMed: 10073490]
- Wein S, Ukropec J, Gasperikova D, Klimes I, Sebkova E. Concerted action of leptin in regulation of fatty acid oxidation in skeletal muscle and liver. *Experimental and Clinical Endocrinology & Diabetes*. 2007; 115(4):244–251. [PubMed: 17479441]
- White CL, Whittington A, Barnes MJ, Wang Z, Bray GA, Morrison CD. HF diets increase hypothalamic PTP1B and induce leptin resistance through both leptin-dependent and -independent mechanisms. *American Journal of Physiology – Endocrinology and Metabolism*. 2009; 296(2):E291–299. [PubMed: 19017730]
- Woods SC, D'Alessio DA. Central control of body weight and appetite. *Journal of Clinical Endocrinology and Metabolism*. 2008; 93(11 Suppl 1):S37–50. [PubMed: 18987269]
- Zabolotny JM, Bence-Hanulec KK, Stricker-Krongrad A, Haj F, Wang Y, Minokoshi Y, et al. PTP1B regulates leptin signal transduction in vivo. *Developmental Cell*. 2002; 2(4):489–495. [PubMed: 11970898]
- Zhang J, Matheny MK, Tumer N, Mitchell MK, Scarpance PJ. Leptin antagonist reveals that the normalization of caloric intake and the thermic effect of food after high-fat feeding are leptin dependent. *American Journal of Physiology Regulatory, Integrative and Comparative Physiology*. 2007; 292(2):R868–874.
- Ziylan YZ, Baltaci AK, Mogulkoc R. Leptin transport in the central nervous system. *Cell Biochemistry and Function*. 2009; 27(2):63–70. [PubMed: 19205004]

Fig. 1.

Chow and choice rats tested for peripheral leptin responsiveness in experiment 1. (A and B) Peripheral leptin responsiveness of rats tested on day 8 of diet. Data are mean $\pm$ SEM for groups of 9 rats. Values with a different superscript are different at $p < 0.05$. (C and D) Peripheral leptin responsiveness of rats tested on day 16 of diet. Data are mean $\pm$ SEM for groups of 9 rats. Values with a different superscript are different at $p < 0.05$. (E and F) Peripheral leptin responsiveness of rats tested on day 4 of choice diet reversal. Data are mean $\pm$ SEM for groups of nine rats. Values with a different superscript are different at $p < 0.05$.

Fig. 2.

Chow and choice rats tested for central leptin responsiveness in experiment 2. (A and B) Central leptin responsiveness of rats tested on day 16 of diet. Data are mean $\pm$ SEM for groups of 7 and 8 rats. Values with a different superscript are different at $p < 0.05$. (C and D) Central leptin responsiveness of rats tested on day 4 of choice diet reversal. Data are mean $\pm$ SEM for groups of 7 and 8 rats. Values with a different superscript are different at $p < 0.05$.

Fig. 3.

Chow and choice rats tested for effects of central leptin responsiveness on energy expenditure in experiment 3. (A) Energy expenditure per metabolic body size of rats in experiment 3 during the first 156 min of the test. Data are mean $\pm$ SEM for groups of 5 or 6 rats. Values with a different superscript are different at $p < 0.05$. (B) Energy expenditure per rat of rats in experiment 3 during the first 156 min of the test. Data are mean $\pm$ SEM for groups of 5 or 6 rats. Values with a different superscript are different at $p < 0.05$. (C) Activity of rats in experiment 3 during the first 156 min of the test. Data are mean $\pm$ SEM for groups of 5 or 6 rats. (D) Energy intake of rats in experiment 3 during the test night. Data are mean $\pm$ SEM for groups of 5 or 6 rats.

Table 1

Body weights and energy intake of rats in experiment 1.

	Chow	Choice
<i>Body weight</i>		
Baseline (day 0) (g)	282 ± 3	282 ± 2
Day 20 (g)	352 ± 4	342 ± 4 *
(from baseline, g)	70 ± 3	60 ± 3 *
Day 4 reversal (g)	362 ± 4	349 ± 3 *
(from baseline, g)	80 ± 3	66 ± 3 *
<i>Energy intake (kcal/20 day)</i>		
	1614 ± 19	1959 ± 32 *
Chow	1614 ± 19	654 ± 21 *
Liquid sucrose		480 ± 66
Lard		824 ± 85
<i>Energy intake reversal (kcal/4 day)</i>		
Chow	325 ± 4	194 ± 5 *

Mean ± SEM.

Day 20 was the final day of the choice diet.

*
 $p < 0.05$ between groups.

Table 2

Body weights of rats in experiment 2.

	Chow	Choice
<i>Body weight</i>		
Baseline (day 0) (g)	288 ± 2	293 ± 3
Day 20 (g)	320 ± 5	331 ± 8
(from baseline, g)	32 ± 5	38 ± 6
Day 4 reversal (g)	317 ± 5	328 ± 8
(from baseline, g)	28 ± 6	34 ± 7

Table 3

Fasting glucose, insulin, and leptin concentrations of rats tested for central leptin resistance in experiment 2.

	<u>Choice</u>		<u>Chow</u>	
	Choice	Day 7 (reverse) chow	Chow	Day 7 (reverse) chow
Glucose (mmol/L)	5.66 ± 0.11 ^a	5.22 ± 0.17 ^b	5.33 ± 0.11 ^b	5.33 ± 0.11 ^b
Insulin (ng/mL)	0.19 ± 0.02	0.16 ± 0.03	0.18 ± 0.02	0.14 ± 0.01
Leptin (ng/mL)	5.7 ± 0.8 ^a	2.1 ± 0.3 ^b	1.3 ± 0.2 ^b	2.0 ± 0.2 ^b

Mean ± SEM; values with a different superscript differ $p < 0.05$.

Table 4

Characteristics of male rats in experiment 4.

	Choice	Choice reversal
<i>N</i>	9	10
<i>Body weight (g)</i>		
Day 0	296 ± 2	293 ± 2
Day 38	384 ± 7	382 ± 7
Day 42, day 4 reversal	394 ± 7	388 ± 6
<i>Energy intake (kcal/25 day)</i>		
Total	2190 ± 54	2257 ± 51
Chow	747 ± 30	733 ± 47
Liquid sucrose	555 ± 91	472 ± 98
Lard	902 ± 102	1038 ± 115
<i>Energy intake reversal (kcal/4 day)</i>		
Total	427 ± 65	252 ± 6*
Chow	115 ± 11	252 ± 6*
Liquid sucrose	121 ± 17	
Lard	191 ± 66	
<i>Serum (ng/mL)</i>		
Leptin (day 28)	4.6 ± 0.6	5.1 ± 0.6
Leptin (day 42, Day 4 reversal)	5.2 ± 0.5	1.8 ± 0.2*
<i>Fat pad weights (g)</i>		
Epididymal	5.6 ± 0.2	4.7 ± 0.3*
Retroperitoneal	4.3 ± 0.2	3.3 ± 0.3*
Mesenteric	4.2 ± 0.3	2.9 ± 0.2*
Carcass weight (g)	350.5 ± 6.3	341.3 ± 6.0
Fat (g)	29.2 ± 2.0	19.0 ± 2.0*
Water (g)	211 ± 4	215 ± 3
Ash (g)	13.5 ± 0.6	13.6 ± 0.6
Protein (g)	96.8 ± 2.1	95.4 ± 1.8
LBM (g)	308 ± 6	312 ± 5

LBM, lean body mass (water + protein). Data are expressed as mean ± SEM.

An asterisk represents a difference at $p < 0.05$.