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Hexosamine biosynthetic pathway activity in leptin resistant sucrose-drinking rats

Ruth B.S. Harris and John W. Apolzan*

Department of Physiology, Medical College of Georgia, Georgia Regents University, Augusta, GA 30912

Abstract

Rats offered 30% sucrose solution in addition to chow and water become leptin resistant therefore we investigated the effect of sucrose solution consumption on leptin signaling. In Experiment 1 rats were resistant to 3rd ventricle injections of 1 μ g leptin after 36 days of sucrose and western blot indicated that resistance was associated with increased basal levels of signal transducer and activator of transcription 3 phosphorylation (pSTAT3). In Experiment 2 rats were resistant to a peripheral injection of 2 mg leptin/kg after 26 days of sucrose. Immunohistochemistry indicated that increased basal pSTAT3 was limited to the medial and lateral arcuate nucleus of the hypothalamus. Increased availability of glucose and fructose can stimulate the hexosamine biosynthetic pathway (HBP) which *O*-GlcNAc-modifies proteins. This has the potential to change protein bioactivity. We tested whether this pathway could account for the leptin resistance. There was no increase in the expression of HBP enzymes in tissues from sucrose rats in Experiment 1, however, direct activation of the HBP with a 3 hour intravenous infusion of 30 μ mol/kg/min glucosamine significantly increased hypothalamic pSTAT3. Although sucrose consumption and activation of the HBP both increase hypothalamic pSTAT3 experiments described here did not provide evidence of a direct link between sucrose consumption, HBP activity and leptin resistance. Unexpectedly, we found that the HBP enzyme glutamine fructose-6-phosphate amidotransferase (GFAT) in liver and *O*-GlcNAcase in hypothalamus were increased 30 minutes after leptin injection in leptin responsive animals, implying a complex interaction between activity of the HBP and leptin responsiveness.

Keywords

Hypothalamic pSTAT3; hexosamine biosynthetic pathway; glucosamine; GFAT; OGT

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Contact Information: Ruth B.S. Harris, Department of Physiology, CA 1020, Georgia Regents University, 1120 15th Street, Augusta, GA 30912, Telephone: 706 721 4479, FAX: 706 721 7299, ruharris@gru.edu.

*Present address for JWA: Pennington Biomedical Research Center, 6400 Perkins Road, Baton Rouge, LA 70808

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INTRODUCTION

The importance of leptin, an adipose-derived cytokine, in the regulation of energy balance is well established. Animals and humans that have a deficit in leptin signaling or leptin production are hyperphagic, diabetic, infertile and obese [1–4]. In experimental conditions, peripheral or central administration of leptin inhibits food intake and weight gain of lean, chow-fed, wild type rats and mice [5–7]. By contrast, experimental animals that become obese and hyperleptinemic due either to aging [8] or consumption of a high-fat diet [9] are unresponsive to the effect of leptin administration on food intake. This lack of response to leptin is referred to as “leptin resistance” and has been attributed to both a failure of leptin to cross the blood brain barrier [10], a decrease in the number of central leptin receptors [11–12] and increased expression of inhibitors of leptin receptor signaling [13]. Therefore, although the individual has high circulating concentrations of leptin, central receptors involved in the control of food intake are not fully activated.

There are multiple reports that feeding rats or mice a composite high-fat diet induces leptin resistance [9, 14]. Some investigators have reported that rats offered a palatable high-fat diet become leptin resistant within only a few days [15–16] other have found that it can take months for resistance to develop [9, 17]. We found that rats offered a choice diet in which they had free access to chow, 30% sucrose solution and lard increased their caloric intake and became resistant to both peripheral and central leptin administration within 18 days [18]. Subsequently we determined that access to 30% sucrose solution, chow and water is sufficient for the induction of leptin resistance [19]. In the experiments described here we have examined the impact of sucrose consumption on the hexosamine biosynthetic pathway (HBP) which has the potential to influence leptin production [20] and leptin responsiveness [21].

When glucose availability is increased activity of the “nutrient sensing” HBP is stimulated [22]. The end product of the pathway, uridine diphosphate N-acetylglucosamine (UDP-GlcNAc), is used for *O*-linked N-acetylglucosamine modification (*O*-GlcNAcylation) of threonine and serine residues in hundreds of bioactive proteins and transcription factors [23] (see Figure 1). There is a significant literature showing a relation between increased activity of the HBP and development of insulin resistance [24–26] and it has been shown that this resistance is associated with *O*-GlcNAcylation of the insulin receptor and some of the proteins involved in the insulin signaling cascade [27–28]. In adipocytes increased activity of the HBP not only leads to insulin resistance [29], but also increases leptin expression and release [20]. Consistent with these observations, mice that over express glutamine:fructose-6-phosphate amidotransferase (GFAT), the enzyme that regulates entry of glucose into the HBP, are hyperleptinemic, but maintain a normal body fat mass [30], which implies that they are leptin resistant. We have previously reported a preliminary study that found that rats infused with glucosamine, which enters the HBP independent of GFAT, were resistant to the inhibitory effects of peripheral injection of leptin on food intake and weight gain [21]. The studies described here examine the changes in leptin signaling associated with sucrose-induced leptin resistance and further investigate the potential involvement of the HBP in this resistance.

METHODS

Male Sprague Dawley rats weighing 275–300 g at the start of an experiment (Harlan Sprague Dawley, Indianapolis, IN) were used for these studies. The animals were housed in temperature controlled rooms at 70°F with lights on for 12 hours a day from 7.00 a.m. All animal procedures were approved by the Institutional Animal Care and Use Committee of Georgia Regents University.

Experiment 1: The response to central leptin injections in sucrose rats

The objective of this study was to examine the effect of leptin administration on activation of leptin signaling proteins in the hypothalamus and hindbrain of sucrose-consuming rats that had become leptin resistant and to determine whether there were changes in activity of the HBP in rats that consumed 30% sucrose solution. Thirty six rats were individually housed in hanging wire mesh cages. They were divided into two weight matched groups, one had free access to chow (Harlan Teklad Rodent Diet 8604) and water the other had access to chow, water and 30% sucrose solution (Kroger Sugar, Hood Packing Corporation, Hamlet, NC). The rats were weighed daily and chow and sucrose intake were recorded from Days 29 to 35. On Day 15 all of the rats were fitted with a 3rd ventricle guide cannula as described previously [31] and on Day 23 cannula placement was confirmed, as described previously [32]. On Days 26 and 36 central leptin responsiveness was tested. Food and sucrose were removed from the cages at 7.00 a.m. At 5.00 p.m. each rat received a 2 µl 3rd ventricle injection of either saline or 1.5 µg leptin (R&D Systems rat recombinant leptin, Minneapolis, MN). Food and sucrose were returned to the cages at 6.00 p.m. and energy intakes and body weights were recorded 14, 24, 38, 48 and 62 hours after injection. Seven days later (Day 44) food and sucrose were removed from the cages at 7.00 a.m. and starting at 11.00 a.m. each rat received an i.p. injection of either saline or 1.0 mg leptin/kg body weight. Exactly 30 minutes later the rat was euthanized and trunk blood was collected for measurement of serum leptin (Rat leptin RIA EMD Millipore Corporation, Billerica, MA), glucose (Glucose assay kit GAGO20; Sigma-Aldrich, St. Louis, MO) glycerol (Free glycerol reagent F6428; Sigma-Aldrich), and triglycerides (TG: L-Type TG H kit; Wako Chemicals). Inguinal, epididymal, retroperitoneal and mesenteric fat depots and the liver were dissected and weighed. The inguinal fat and the liver were snap frozen for subsequent Western blot analysis of GFAT, O-linked N-acetylglucosamine transferase (OGT), which catalyzes the addition of a single N-acetyl-D-glucosamine to serine or threonine residues of intracellular proteins, O-GlcNAcase which catalyzes the reversal of protein O-GlcNAcylation and N-acetyl-D-glucosamine (GlcNAc), as a measure of protein O-linked glycosylation. All western blot analyses followed the same general procedure as has been described previously [19]. The antibodies are listed below. One lobe of the liver was used to measure liver lipid content by chloroform methanol extraction. Tissue blocks of hypothalamic and hindbrain tissue were dissected and snap frozen, as described previously [33–34], and used for western blot analysis of pSTAT3(Tyr705), pSTAT3(Ser747), STAT3 suppressor of cytokine signaling 3 (SOCS3), GFAT, OGT, O-GlcNAcase and GlcNAc.

Experiment 2: Hypothalamic pSTAT3 in rats given access to 30% sucrose solution

We previously reported that rats given access to a 30% sucrose solution were resistant to the effects of peripheral injection of leptin on food intake and weight gain after three weeks on diet [19] and Experiment 1 showed an increase in basal pSTAT3(Tyr705) in sucrose rats. The objective of this experiment was to confirm those observations, to test the insulin responsiveness of the animals in glucose tolerance (GTT) and insulin tolerance (ITT) tests and at the end of the study to examine hypothalamic pSTAT3(Tyr705) in non-stimulated conditions. Fourteen rats were housed in individual wire-mesh cages with free access to water and chow. They were divided into two weight-matched groups and one group was offered free access to 30% sucrose solution in addition to chow and water. Body weight, chow intake and sucrose consumption were recorded daily except on Days 17 and 18 of the experiment. A GTT was performed on Day 12 of the experiment. Food and water were removed from the cages at 7.00 a.m. Starting at 1.00 p.m. a small sample of tail blood was collected for measurement of insulin (Rat insulin RIA kit, Millipore Corporation, Massachusetts) and glucose (EasyGlucose blood glucose test strips, US Diagnostics, New York). The rat then received an intraperitoneal (i.p.) injection of 1 mg/kg glucose (0.1 ml/100 g body weight). This dose of glucose is lower than is often used by others [35–36], but allows for detection of subtle changes in glucose clearance. Blood glucose was measured 15, 30, 45, 60, 90 and 120 after injection. Insulin was measured 15, 30, 60 and 120 minutes after injection. Leptin responsiveness of the rats was tested on Days 14, 19, 26 and 31 of the experiment. On the test day food and sucrose were removed from the cages at 7.00 a.m. At 5.00 p.m. each rat received an i.p. injection of PBS or 2 mg/kg leptin. Food and sucrose were returned to the cages at 6.00 p.m. Energy intake and body weight were recorded 14, 24 and 36 hours after the injection. Treatments were alternated so that half of the rats received PBS injections on Days 14 and 26 and received leptin injections on Days 19 and 31. The other half of the rats received leptin injections on Days 14 and 26 and received PBS injections on Days 19 and 31. There was no significant effect of day of injection on leptin response when Days 14 and 19 were compared or when Days 26 and 31 were compared. The insulin responsiveness of the rats was measured on Day 34. Food was removed from the cage at 7.00 a.m. Starting at 12.00 p.m. a small sample of tail blood was collected for measurement of baseline insulin and glucose. Each rat then received an i.p. injection of 0.75 unit/kg of Humulin (Eli Lilly, Indianapolis, IN). A blood sample was collected 10 minutes after injection for measurement of insulin and blood glucose was measured 5, 10, 15, 20, 30, 40 and 50 minutes after injection.

On Day 36 of the experiment food and sucrose were removed from the cages at 7.00 a.m. Starting at 10.00 a.m. a tail blood sample was collected for measurement of serum total TG, insulin and leptin. The rats were anesthetized with ketamine/xylazine (90 mg/kg, 10 mg/kg) the epididymal, inguinal and retroperitoneal fat were dissected and weighed. The rats were then transcardially perfused with ice-cold heparinized saline and 4% paraformaldehyde as described previously [33]. The brain was collected and stored in 4% paraformaldehyde at 4°C overnight and was then transferred to 25% sucrose, 1% sodium azide and stored at 4°C until phosphorylated STAT3 (pSTAT3(Tyr 705)) was detected by immunohistochemistry on 30 µm sections made through the hypothalamus and hindbrain, as described previously [33]. Images were captured with an Olympus BX51 microscope. The number of pSTAT3 positive

nuclei was counted manually in the lateral and medial arcuate nucleus (ArcL and ArcM), the dorsomedial nucleus, compact (DMC) and the ventromedial nucleus, dorsomedial (VMHDM) at -3.14 and 3.30 to the bregma (plates 32 and 33 Paxinos Watson Rat Brain Atlas [37]). pSTAT3 positive cells were also counted in the medial posterior arcuate nucleus (ArcMP) at -4.16 and -4.30 mm to the bregma (Plates 36 and 37) and in the medial nucleus of the solitary tract (SolM) at -13.80 and -14.08 mm to the bregma in the hindbrain (Plates 75 and 76). These sites were selected because they were the only ones that consistently showed significant levels of STAT3 activation. There was no evidence of pSTAT3(Tyr705) in the ventral tegmental area of the midbrain, an area of the brain that expresses leptin receptors and directs reward-seeking behavior [38].

Experiment 3: Effect of acute intravenous glucosamine infusion on leptin signaling protein activation

The objective of this experiment was to further examine the relation between leptin resistance and the HBP by testing whether acute activation of the HBP by glucosamine (GlcN) infusion increased hypothalamic pSTAT3. Blood glucose was monitored during the infusion because activation of the HBP inhibits glucose clearance [39].

Thirty two rats were housed individually with free access to chow and water. Each rat was fitted with a jugular vein catheter (Tygon tubing 0.02 inch ID, 0.06 inch OD). Groups of 4 to 6 rats were catheterized under isoflurane anesthesia on a daily basis and two days after surgery the rats were moved to a testing room in which each rat was housed in a shoe-box cage without access to food or water. Their catheter was attached to an infusion pump and the rat was allowed to settle for 30 minutes. Tail blood glucose was measured at 10 minute intervals (EasyGluco blood glucose test strips) until a stable glucose value was obtained. The rats were then infused with either GlcN at a rate of $30 \mu\text{mol/kg/min}$ in a volume of $3.2 \mu\text{l}/100 \text{ g body weight/min}$ or an equivalent volume of saline. Blood glucose was sampled at 15 minute intervals for 150 minutes. At this time each rat received an i.p. injection of either saline or $1 \text{ mg leptin/kg body weight}$. Thus there were 4 treatment groups: Saline/Saline, Saline/Leptin, GlcN/Saline, GlcN/Leptin. Blood glucose was measured at 10 minute intervals for the next 30 minutes and then the rats were euthanized by decapitation. Hypothalamic tissue blocks were collected as described for experiment 2, the inguinal fat pads and the liver were snap frozen and trunk blood was collected for measurement of serum insulin and leptin. Hypothalamus pSTAT3 (Tyr705), SOCS3, PI3K and pERK 1/2, and liver and inguinal *O*-GlcNAc, GFAT, OGT and *O*-GlcNAcase were measured by western blot.

Antibodies— β -actin (A3853 Sigma Chemical Co, St Louis, MO), OGlcNAcase (14711-1-AP Proteintech Group, Chicago, IL), OGT (sc32921 Santa Cruz Biotechnology, Dallas, TX), OGlcNAc (sc59623 Santa Cruz Biotechnology), GFAT (5322 Cell Signaling Technology, Danvers, MA) STAT3 (4904 Cell Signaling Technology). PSTAT3 (Tyr 705) (9145 Cell Signaling Technology), phospho-STAT3 (ser727) (9134 Cell Signaling Technology), SOCS3 (NBP2-20451 Novus Biologicals, Littleton, CO), phospho-ERK1/2 (4370 Cell Signaling Technology), ERK 1/2 (4695 Cell Signaling Technology), phospho-PI3-kinase p85 (4228 Cell Signaling Technology), PI3K p85 (4292 Cell Signaling Technology). Goat horseradish peroxidase-anti-rabbit antibody (111-035-144 Jackson

ImmunoResearch Laboratories, West Grove, PA), Goat horseradish peroxides – anti-mouse antibody (115-035-003 Jackson ImmunoResearch Laboratories).

Data Analysis—Statistically significant differences between treatment groups were determined using Statistica software version 9.0 (StatSoft, Tulsa, OK). Differences were considered significant at $P < 0.05$. Repeated measures of food intake, body weight, glucose or insulin were compared by repeated measures analysis of variance. Single end point measures for rats in Experiment 1 were compared using a Student's T-test assuming equal variance. Single end point measures and measures at a specific time point in Experiments 2 and 3 were compared by two-way ANOVA. Post-hoc differences were determined using Tukey's test.

RESULTS

Experiment 1: The response to central leptin injection in sucrose rats

Energy intakes of sucrose rats were 10% greater than those of chow rats and sucrose represented 60% of their daily intake, but there was no effect of sucrose on weight gain (Table 1). At the end of the experiment white fat depots were significantly larger in sucrose than chow rats. The total weight of dissected fat was increased by 46% in sucrose rats (Table 1), but the effect was greatest in retroperitoneal (RP) fat which doubled in size. Third ventricle injection of leptin on Days 26 and 36 of the experiment significantly inhibited food intake for at least 62 hours in chow fed rats. In sucrose-fed rats food intake was inhibited on Day 26, but the rats were resistant to the feeding effects of leptin on Day 36 (Figure 2A). There was no significant effect of leptin on weight gain of either group of rats at any of the time points tested (data not shown).

Western blot analysis of tissue samples collected at the end of the study 30 minutes after an i.p. injection of 1 mg leptin/kg body weight showed an increase in hypothalamic pSTAT3(Tyr705) for leptin-injected chow-fed rats, but not in sucrose rats. Hypothalamic pSTAT3(Tyr705) in sucrose rats was not different from leptin-stimulated levels in chow-fed rats (Figure 2B). By contrast, there was no significant effect of peripheral leptin injection on hindbrain pSTAT3(Tyr705), but phosphorylation was substantially increased in sucrose rats compared with controls (Figure 2C). There was no effect of diet or leptin of pSTAT3(Ser727) in either hypothalamic or hindbrain tissue (data not shown) and there was no effect of diet or leptin on total STAT3 expression (data not shown). Hypothalamic SOCS3 was inhibited by both diet and leptin (Figure 2D, Diet: $P < 0.03$, leptin: $P < 0.04$, Interaction: NS), but was not reliably detected in the hindbrain tissue.

Measures of different aspects of HBP activity in the hypothalamus revealed no difference in GFAT, OGT or *O*-GlcNAcylation (data not shown) of cellular proteins. Leptin stimulated expression of *O*-GlcNAcase (Diet: NS, Leptin: NS, Interaction $P < 0.01$) in chow-fed, but not sucrose rats (Figure 3). In the hindbrain there was no effect of either diet or leptin on *O*-GlcNAcylation of proteins, expression of OGT or of *O*-GlcNAcase. There was an overall interaction between diet and leptin on hindbrain GFAT (Diet: NS, Leptin: NS, Interaction: $P < 0.05$), but differences between specific treatment groups did not reach significance. In the liver there were no differences in *O*-GlcNAcase or *O*-GlcNAcylation of cellular proteins,

but leptin stimulated GFAT in chow-fed, but not sucrose rats (Diet: NS, Leptin: $P<0.05$, Interaction: NS). OGT was higher in leptin-injected sucrose rats compared with those fed chow (Diet $P<0.05$, leptin: NS, Interaction: NS). In inguinal fat (Figure 3) there was no effect of diet or leptin on *O*-GlcNAcylation of proteins or on OGT protein expression. Leptin stimulated GFAT in sucrose rats, but not chow-fed controls (Diet: NS, leptin: $P<0.04$, Interaction: $P<0.03$). Leptin also stimulated *O*-GlcNAcase in sucrose, but not chow-fed rats. However, the level of *O*-GlcNAcase in inguinal fat from chow-fed rats was not different from that in sucrose rats.

Experiment 2: Hypothalamic pSTAT3 in rats given access to 30% sucrose solution

Rats offered sucrose solution in addition to chow consumed approximately 17% more energy than the chow-fed controls and sucrose represented approximately 60% of their intake (Figure 4A). There were no differences in body weights of the two groups of rats (Figure 4B), but at the end of the experiment the size of all fat pads was increased by about 20% in the sucrose rats (Figure 4C). Both chow and sucrose rats were leptin responsive on Days 14 and 19 of the experiment (data not shown), but the sucrose rats were leptin resistant on Days 26 and 31. Energy intake and weight gain were inhibited in chow-fed rats 12 hours after leptin injection, but there was no effect of leptin in the sucrose rats (Figure 4D, E). Food intake was inhibited by 19% in chow rats, which is a relatively small effect, but consistent with the degree of inhibition reported by others for rats receiving peripheral injections of leptin [17] and similar to the 25% suppression reported for rats receiving leptin injections directly in the arcuate nucleus [40]. Weight gained during the 14 hours following the leptin or PBS injection was greater than the normal daily weight gain of the rats because it included a change in gut fill. We compared the weight of the rats at the end of the dark period during which they had been eating with their weight at the end of the light period during which they had been food deprived and gut fill was likely to have been substantially reduced. Serum glucose was significantly higher in sucrose than chow rats during the GTT (Figure 5A: Sucrose: $P<0.002$, Time: $P<0.0001$, Interaction: NS). Repeated measures analysis of insulin during the GTT indicated a significant effect of sucrose ($P<0.05$) and time ($P<0.006$), but there was no difference between groups at any of the individual time points (Figure 5B). Serum insulin was significantly higher in the sucrose rats during the ITT (Figure 5D: Sucrose $P<0.001$, Time $P<0.0001$, Interaction NS), but there was no difference in glucose clearance (Figure 5C). At the end of the experiment there was a 250% increase in serum leptin and a doubling of serum triglycerides in sucrose rats compared with controls (Figure 5E, F).

Immunohistochemistry indicated that pSTAT3(Tyr705) was significantly increased in the medial and lateral arcuate nucleus of the hypothalamus of sucrose rats compared with their controls. There were no significant differences in the other brain sites evaluated (Figure 6).

Experiment 3: Effect of acute intravenous glucosamine infusion on leptin signaling protein activation

GlcN infusion caused a significant rise in blood glucose concentration which stabilized after 60 minutes (Figure 7). Leptin injection had no effect on blood glucose in either group of rats and there was no effect of leptin or GlcN on serum insulin concentration (Table 2). At the

end of infusion there was an overall effect of GlcN to increase hypothalamic pSTAT3 ($P<0.03$), but there were no significant differences between individual groups. There also was an overall effect of leptin to suppress hypothalamic SOCS3 ($P<0.01$), but the differences between individual groups did not reach significance. There was no effect of GlcN or leptin on the phosphorylation of hypothalamic PI3K, ERK1 or ERK2 (data not shown). As expected, GlcN infusion had no effect on GFAT expression in the liver (Figure 8), but it did significantly increase OGT (Glucosamine: $P<0.01$, Leptin: NS, Interaction: NS). Leptin significantly increased protein *O*-GlcNAcylation (Glucosamine: NS, Leptin: $P<0.03$, Interaction: NS), but there were no significant effects of GlcN or leptin on *O*-GlcNAcase.

DISCUSSION

The objectives of these studies were to confirm that rats given free access to a 30% sucrose solution in addition to chow and water develop a reliable and early onset of leptin resistance and to investigate the changes in leptin signaling associated with this resistance. The results confirm that sucrose rats are resistant to peripheral injections of leptin within 26 days and are resistant to central injections of leptin within 36 days. This is a much shorter period of time for leptin resistance to develop than the 8 to 20 weeks that has been reported in some studies for rats or mice offered a high fat diet [9, 14, 41–42] or the 6 months for rats fed a 60% fructose diet [43]. Leptin resistance in mice fed a high-fat diet is associated with a failure of leptin to fully activate STAT3 [42, 44], whereas the leptin resistance in sucrose rats appears to be associated with an increase in basal levels of pSTAT3(Tyr705) which precludes further stimulation by leptin. Scarpace et al [44] previously reported that rats made leptin resistant by central adeno-associated virus over-expression of leptin or by feeding a high-fat diet had a 2 to 3-fold increase in basal levels of hypothalamic pSTAT3(Tyr705). In these rats an icv injection of leptin increased pSTAT3(Tyr705) another 2 to 3-fold, but this represented a smaller maximal signaling capacity than was found in leptin-treated control, leptin-responsive rats. In the sucrose-fed rats we also found that hypothalamic and hindbrain pSTAT3(Tyr705) was elevated in basal conditions, a peripheral leptin injection had no further stimulatory effect and there was no difference between hypothalamic pSTAT3(Tyr705) in sucrose rats compared with leptin-injected controls. Immunohistochemistry indicated that the increase in hypothalamic pSTAT3(Tyr705) in sucrose rats was limited to the medial and lateral arcuate nucleus. This is an area of the brain that mediates leptin's inhibition of food intake [45] and is one of the first to develop leptin resistance in high-fat fed mice [46]. We did not find any effect of sucrose on the other proteins involved in leptin signaling in hypothalamic tissue, which is consistent with STAT3 activation being essential for leptin's effects on food intake and energy balance [47].

Although the sucrose rats were leptin resistant they were only 50% fatter than controls. This is a much smaller change in body fat than the 150–500 % increase in leptin resistant high fat fed rats [41, 48]. These data confirm our results from a previous study in which we compared leptin responsiveness in rats fed chow, chow plus lard, chow plus sucrose solution, chow plus lard and sucrose solution or formulated high or low fat diets [19]. The only rats that were leptin resistant after 17 days on the different diets were those offered either chow and sucrose solution, or chow, lard and sucrose solution. The leptin resistance

was not related to total sucrose intake, serum leptin levels or body fat mass [19]. The results described here confirm these results in that sucrose rats in both studies were leptin resistant even though serum leptin levels were elevated only in the sucrose rats from Experiment 2. Similarly, serum TG were increased only in sucrose rats from Experiment 2, implying that the resistance was also independent of circulating TG levels which have been reported to inhibit leptin transport across the blood brain barrier [49]. The total weight gain and daily energy intake of both chow and sucrose rats were reduced in Experiment 1 compared with Experiment 2 and this may explain the differences in serum leptin and TG between the two experiments, but the increase in TG is consistent with stimulation of liver TG synthesis by diets containing very high concentrations of either glucose or fructose [50]. The relatively mild obesity in sucrose rats was associated with insulin insensitivity and glucose clearance was impaired as early as Day 12, but the insulin insensitivity was not severe because glucose clearance was normal during the ITT.

As noted above, we previously reported that sucrose-induced leptin resistance was specific to consumption of sucrose in solution because rats that consumed a similar quantity of sucrose as part of a dry composite diet remained leptin responsive [19]. We have not investigated the differential response to sucrose in a liquid versus a dry diet, but it may be because consumption of sucrose solution results in delivery of significant quantities of glucose and fructose independent of any other dietary component. This bolus delivery of glucose and fructose would be expected to increase activity of pathways that metabolize carbohydrate, including the HBP. Normally the HBP accounts for 1–3% of glucose metabolism [24], but this is increased when glucose availability increases and for this reason it is referred to as a “nutrient sensing” pathway [20]. There has been intense interest in the role that this pathway plays in the development of insulin insensitivity that can result from *O*-GlcNAcylation of the insulin receptor and insulin signaling proteins [29, 51]. It has also been shown that increased activity of the HBP stimulates adipocyte leptin expression and release [52] and it is possible that this mediates the diurnal rhythm of leptin release that is entrained by meals [53].

Because leptin signaling involves some of the same proteins as insulin signaling, including IRS1 and PI3K which can be *O*-GlcNAcylated and because of the literature on increased HBP activity and development of insulin resistance [51], we have started to explore the potential involvement of protein *O*-GlcNAcylation in the development of leptin resistance in rats that drink sucrose solution. In Experiment 1 described here western blot did not show an overall increase in *O*-GlcNAcylation of protein in any of the tissues tested. We used total cell lysates for the blots and it is possible that if nuclear proteins had been isolated that we would have found an increase in protein *O*-GlcNAcylation in tissues from sucrose rats. Similarly there were no consistent effects of sucrose consumption on the expression of any of the enzymes that catalyze critical steps of the HBP. Because immunohistochemistry subsequently indicated that the increase in pSTAT3(Tyr705) was limited to the arcuate nucleus, it also is possible that site-specific changes in HBP activity and *O*-GlcNAc protein were masked in Experiment 1 by analyzing a block of tissue that contained multiple brain areas. Although the dietary studies clearly demonstrated that consumption of sucrose increased hypothalamic pSTAT3(Tyr705) specifically in an area of the brain that has a

fenestrated blood brain barrier and could therefore respond to circulating concentrations of nutrients, the measurements of HBP activity in these animals did not provide any clear evidence that increased hypothalamic pSTAT3(Tyr705) was related to protein *O*-GlcNAcylation.

Previously we reported that rats infused peripherally for 2 days with GlcN from Alzet pumps were resistant to the inhibitory effect of leptin on food intake [21]. We have since found that this inhibition is transient and that rats are leptin responsive after a 7 day infusion (unpublished observations). Experiment 3 described here showed that an acute infusion of GlcN inhibited glucose clearance, indicative of insulin insensitivity. The increase in serum glucose did not stimulate insulin release which was consistent with in vitro studies that suggest that glucosamine inhibits glucose-induced insulin release from pancreatic islets [54]. GlcN infusion also increased hypothalamic pSTAT3(Tyr705) and because GlcN entry into the HBP as glucosamine-6-phosphate is concentration dependent, these results show that increased activity of the HBP increases phosphorylation of pSTAT3. Further studies are needed to test for a direct association between consumption of sucrose solution, protein *O*-GlcNAcylation and development of leptin resistance.

An unexpected observation in Experiment 1 was that expression of GFAT in the liver and *O*-GlcNAcase in the hypothalamus of chow-fed rats were increased following leptin treatment. These effects were lost in sucrose rats, either because the enzymes were already stimulated by sucrose consumption or because the rats were leptin resistant and the resistance extended to the transcriptional or translational control of GFAT expression by leptin. By contrast, leptin treatment increased GFAT and *O*-GlcNAcase in adipose tissue from sucrose, but not chow fed rats. Leptin inhibits adipocyte lipogenesis [55] whereas protein *O*-GlcNAcylation increases the activity of lipogenic enzymes in adipocytes [56]. *O*-GlcNAcase reverses protein *O*-GlcNAcylation, therefore, these data imply that although sucrose rats were resistant to the effects of leptin on food intake, other aspects of leptin activity remained intact. This is one of the first reports of HBP activity responding to leptin treatment as as most studies focus on substrate-driven regulation of the pathway [24, 57]. The metabolic significance of this effect has not been investigated, but because the stimulation occurred within 30 minutes of leptin administration it may represent a pathway by which leptin amplifies phosphorylation of STAT3. These observations add to the complexity of the relation between leptin and HBP activity as it would appear that leptin increases activity of the HBP and stimulation of the HBP in adipose tissue increases leptin secretion [20], but chronic activation also increases hypothalamic pSTAT3(Tyr705) and is associated with leptin resistance.

In summary, rats offered a 30% sucrose solution in addition to water and chow develop both peripheral and central leptin resistance much faster than rats offered a high-fat diet [9, 17] and the resistance is associated with only a moderate increase in body fat. Basal hypothalamic pSTAT3 (Tyr705) in sucrose rats is not different from that in leptin treated, leptin responsive rats. This form of leptin resistance is unlike that in rats made leptin resistant on a high-fat diet or by leptin over-expression in which leptin is unable to stimulate pSTAT3 to the same levels as in control leptin-responsive animals [44]. Activation of the HBP by intravenous infusion of GlcN also stimulates hypothalamic pSTAT3(Tyr705), but

we did not demonstrate activation of the HBP in tissue from leptin resistant sucrose-drinking rats. An unexpected observation was that leptin stimulated GFAT in the liver and *O*-GlcNAcase in the hypothalamus of chow-fed, but not leptin resistant sucrose rats. This is one of the first reports of leptin control of the pathway and suggests a complex interaction between leptin, activity of the HBP pathway and leptin responsiveness in different tissues.

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References

1. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. *Nature*. 1994; 372:425–432. [PubMed: 7984236]
2. Lee G, Li C, Montez J, Halaas J, Darvishzadeh J, Friedman JM. Leptin receptor mutations in 129 db3J/db3J mice and NIH fapc/fapc rats. *Mammalian Genome*. 1997; 8:445–447. [PubMed: 9166593]
3. Lee GH, Proenca R, Montez JM, Carroll KM, Darvishzadeh JG, Lee JI, Friedman JM. Abnormal splicing of the leptin receptor in diabetic mice. *Nature*. 1996; 379:632–635. [PubMed: 8628397]
4. Farooqi S, Rau H, Whitehead J, O'Rahilly S. *ob* gene mutations and human obesity. *Proc Nutr Soc*. 1998; 57:471–475. [PubMed: 9794006]
5. Harris RB, Zhou J, Redmann SM Jr, Smagin GN, Smith SR, Rodgers E, Zachwieja JJ. A leptin dose-response study in obese (*ob/ob*) and lean (+/?) mice. *Endocrinology*. 1998; 139:8–19. [PubMed: 9421392]
6. Penn DM, Jordan LC, Kelso EW, Davenport JE, Harris RB. Effects of central or peripheral leptin administration on norepinephrine turnover in defined fat depots. *Am J Physiol Regul Integr Comp Physiol*. 2006; 291:R1613–1621. [PubMed: 16840651]
7. 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. *Proc Natl Acad Sci U S A*. 1997; 94:8878–8883. [PubMed: 9238071]
8. Zhang Y, Matheny M, Tumer N, Scarpance PJ. Aged-obese rats exhibit robust responses to a melanocortin agonist and antagonist despite leptin resistance. *Neurobiol Aging*. 2004; 25:1349–1360. [PubMed: 15465633]
9. El-Haschimi K, Pierroz DD, Hileman SM, Bjorbaek C, Flier JS. Two defects contribute to hypothalamic leptin resistance in mice with diet-induced obesity. *J Clin Invest*. 2000; 105:1827–1832. [PubMed: 10862798]
10. Banks WA, Kastin AJ, Huang W, Jaspan JB, Maness LM. Leptin enters the brain by a saturable system independent of insulin. *Peptides*. 1996; 17:305–311. [PubMed: 8801538]
11. Wilsey J, Scarpance PJ. Caloric restriction reverses the deficits in leptin receptor protein and leptin signaling capacity associated with diet-induced obesity: role of leptin in the regulation of hypothalamic long-form leptin receptor expression. *J Endocrinol*. 2004; 181:297–306. [PubMed: 15128278]
12. Madiehe AM, Schaffhauser AO, Braymer DH, Bray GA, York DA. Differential expression of leptin receptor in high- and low-fat-fed Osborne-Mendel and S5B/Pl rats. *Obes Res*. 2000; 8:467–474. [PubMed: 11011914]
13. Bjorbaek C, El-Haschimi K, Frantz JD, Flier JS. The role of SOCS-3 in leptin signaling and leptin resistance. *J Biol Chem*. 1999; 274:30059–30065. [PubMed: 10514492]
14. Widdowson PS, Upton R, Buckingham R, Arch J, Williams G. Inhibition of food response to intracerebroventricular injection of leptin is attenuated in rats with diet-induced obesity. *Diabetes*. 1997; 46:1782–1785. [PubMed: 9356026]
15. Wang J, Obici S, Morgan K, Barzilai N, Feng Z, Rossetti L. Overfeeding rapidly induces leptin and insulin resistance. *Diabetes*. 2001; 50:2786–2791. [PubMed: 11723062]

16. Lin L, Martin R, Schaffhauser AO, York DA. Acute changes in the response to peripheral leptin with alteration in the diet composition. *Am J Physiol Regul Integr Comp Physiol*. 2001; 280:R504–509. [PubMed: 11208581]
17. Lin S, Thomas TC, Storlien LH, Huang XF. Development of high fat diet-induced obesity and leptin resistance in C57Bl/6J mice. *Int J Obes Relat Metab Disord*. 2000; 24:639–646. [PubMed: 10849588]
18. Apolzan JW, Harris RB. Rapid onset and reversal of peripheral and central leptin resistance in rats offered chow, sucrose solution, and lard. *Appetite*. 2012; 60:65–73. [PubMed: 23022555]
19. Harris RB, Apolzan JW. Changes in glucose tolerance and leptin responsiveness of rats offered a choice of lard, sucrose, and chow. *Am J Physiol Regul Integr Comp Physiol*. 2012; 302:R1327–1339. [PubMed: 22496363]
20. Wang J, Liu R, Hawkins M, Barzilai N, Rossetti L. A nutrient-sensing pathway regulates leptin gene expression in muscle and fat. *Nature*. 1998; 393:684–688. [PubMed: 9641678]
21. Vasselli JR, Scarpace PJ, Harris RB, Banks WA. Dietary components in the development of leptin resistance. *Adv Nutr*. 2013; 4:164–175. [PubMed: 23493533]
22. Rossetti L. Perspective: Hexosamines and nutrient sensing. *Endocrinology*. 2000; 141:1922–1925. [PubMed: 10830271]
23. Zeidan Q, Hart GW. The intersections between O-GlcNAcylation and phosphorylation: implications for multiple signaling pathways. *J Cell Sci*. 2010; 123:13–22. [PubMed: 20016062]
24. Marshall S, Bacote V, Traxinger RR. Discovery of a metabolic pathway mediating glucose-induced desensitization of the glucose transport system. Role of hexosamine biosynthesis in the induction of insulin resistance. *J Biol Chem*. 1991; 266:4706–4712. [PubMed: 2002019]
25. Wells L, Vosseller K, Hart GW. A role for N-acetylglucosamine as a nutrient sensor and mediator of insulin resistance. *Cell Mol Life Sci*. 2003; 60:222–228. [PubMed: 12678487]
26. McClain DA. Hexosamines as mediators of nutrient sensing and regulation in diabetes. *J Diabetes Complications*. 2002; 16:72–80. [PubMed: 11872372]
27. Ball LE, Berkaw MN, Buse MG. Identification of the major site of O-linked beta-N-acetylglucosamine modification in the C terminus of insulin receptor substrate-1. *Mol Cell Proteomics*. 2006; 5:313–323. [PubMed: 16244361]
28. Klein AL, Berkaw MN, Buse MG, Ball LE. O-linked N-acetylglucosamine modification of insulin receptor substrate-1 occurs in close proximity to multiple SH2 domain binding motifs. *Mol Cell Proteomics*. 2009; 8:2733–2745. [PubMed: 19671924]
29. Teo CF, Wollaston-Hayden EE, Wells L. Hexosamine flux, the O-GlcNAc modification, and the development of insulin resistance in adipocytes. *Mol Cell Endocrinol*. 2010; 318:44–53. [PubMed: 19799964]
30. McClain DA, Alexander T, Cooksey RC, Considine RV. Hexosamines stimulate leptin production in transgenic mice. *Endocrinology*. 2000; 141:1999–2002. [PubMed: 10830282]
31. Chotiawat C, Harris RB. Antagonism of specific corticotropin-releasing factor receptor subtypes selectively modifies weight loss in restrained rats. *Am J Physiol Regul Integr Comp Physiol*. 2008; 295:R1762–1773. [PubMed: 18922964]
32. Chotiawat C, Kelso EW, Harris RB. The effects of repeated restraint stress on energy balance and behavior of mice with selective deletion of CRF receptors. *Stress*. 2010; 13:203–213. [PubMed: 20392192]
33. Haring SJ, Harris RB. The relation between dietary fructose, dietary fat and leptin responsiveness in rats. *Physiol & Behav*. 2011; 104:914–922. [PubMed: 21684298]
34. Kasser TR, Harris RB, Martin RJ. Level of satiety: fatty acid and glucose metabolism in three brain sites associated with feeding. *Am J Physiol*. 1985; 248:R447–452. [PubMed: 3985186]
35. Hayes MR, Kanoski SE, De Jonghe BC, Lechner TM, Alhadeff AL, Fortin SM, Arnold M, Langhans W, Grill HJ. The common hepatic branch of the vagus is not required to mediate the glycemic and food intake suppressive effects of glucagon-like-peptide-1. *Am J Physiol Regul Integr Comp Physiol*. 2011; 301:R1479–1485. [PubMed: 21849636]
36. Chambers AP, Jessen L, Ryan KK, Sisley S, Wilson-Perez HE, Stefater MA, Gaitonde SG, Sorrell JE, Toure M, Berger J, D'Alessio DA, Woods SC, Seeley RJ, Sandoval DA. Weight-independent

- changes in blood glucose homeostasis after gastric bypass or vertical sleeve gastrectomy in rats. *Gastroenterology*. 2011; 141:950–958. [PubMed: 21699789]
37. Paxinos, G.; Watson, C. The rat brain. New York: Academic Press; 1998.
 38. Figlewicz DP, Evans SB, Murphy J, Hoen M, Baskin DG. Expression of receptors for insulin and leptin in the ventral tegmental area/substantia nigra (VTA/SN) of the rat. *Brain Res*. 2003; 964:107–115. [PubMed: 12573518]
 39. Einstein FH, Fishman S, Bauman J, Thompson RF, Huffman DM, Atzmon G, Barzilai N, Muzumdar RH. Enhanced activation of a “nutrient-sensing” pathway with age contributes to insulin resistance. *Faseb J*. 2008; 22:3450–3457. [PubMed: 18566293]
 40. Zheng H, Patterson LM, Rhodes CJ, Louis GW, Skibicka KP, Grill HJ, Myers MG Jr, Berthoud HR. A potential role for hypothalamomedullary POMC projections in leptin-induced suppression of food intake. *Am J Physiol Regul Integr Comp Physiol*. 2010; 298:R720–728. [PubMed: 20071607]
 41. Enriori PJ, Evans AE, Sinnayah P, Jobst EE, Tonelli-Lemos L, Billes SK, Glavas MM, Grayson BE, Perello M, Nilni EA, Grove KL, Cowley MA. Diet-induced obesity causes severe but reversible leptin resistance in arcuate melanocortin neurons. *Cell Metab*. 2007; 5:181–194. [PubMed: 17339026]
 42. Knight ZA, Hannan KS, Greenberg ML, Friedman JM. Hyperleptinemia is required for the development of leptin resistance. *PLoS One*. 2010; 5:e11376. [PubMed: 20613882]
 43. Shapiro A, Mu W, Roncal C, Cheng KY, Johnson RJ, Scarpance PJ. Fructose-induced leptin resistance exacerbates weight gain in response to subsequent high-fat feeding. *Am J Physiol Regul Integr Comp Physiol*. 2008; 295:R1370–1375. [PubMed: 18703413]
 44. Scarpance PJ, Matheny M, Tumer N, Cheng KY, Zhang Y. Leptin resistance exacerbates diet-induced obesity and is associated with diminished maximal leptin signalling capacity in rats. *Diabetologia*. 2005; 48:1075–1083. [PubMed: 15864530]
 45. Morton GJ, Niswender KD, Rhodes CJ, Myers MG Jr, Blevins JE, Baskin DG, Schwartz MW. Arcuate nucleus-specific leptin receptor gene therapy attenuates the obesity phenotype of Koletsky (fa(k)/fa(k)) rats. *Endocrinology*. 2003; 144:2016–2024. [PubMed: 12697710]
 46. Olofsson LE, Unger EK, Cheung CC, Xu AW. Modulation of AgRP-neuronal function by SOCS3 as an initiating event in diet-induced hypothalamic leptin resistance. *Proc Natl Acad Sci U S A*. 2013; 110:E697–706. [PubMed: 23386726]
 47. Bates SH, Stearns WH, Dundon TA, Schubert M, Tso AW, Wang Y, Banks AS, Lavery HJ, Haq AK, Maratos-Flier E, Neel BG, Schwartz MW, Myers MG Jr. STAT3 signalling is required for leptin regulation of energy balance but not reproduction. *Nature*. 2003; 421:856–859. [PubMed: 12594516]
 48. Wilsey J, Zolotukhin S, Prima V, Scarpance PJ. Central leptin gene therapy fails to overcome leptin resistance associated with diet-induced obesity. *Am J Physiol Regul Integr Comp Physiol*. 2003; 285:R1011–1020. [PubMed: 14557234]
 49. Banks WA, Coon AB, Robinson SM, Moinuddin A, Shultz JM, Nakaoke R, Morley JE. Triglycerides induce leptin resistance at the blood-brain barrier. *Diabetes*. 2004; 53:1253–1260. [PubMed: 15111494]
 50. Waddell M, Fallon HJ. The effect of high-carbohydrate diets on liver triglyceride formation in the rat. *J Clin Invest*. 1973; 52:2725–2731. [PubMed: 4748509]
 51. Slawson C, Copeland RJ, Hart GW. O-GlcNAc signaling: a metabolic link between diabetes and cancer? *Trends Biochem Sci*. 2010; 35:547–555. [PubMed: 20466550]
 52. Wang J, Liu R, Liu L, Chowdhury R, Barzilai N, Tan J, Rossetti L. The effect of leptin on Lep expression is tissue-specific and nutritionally regulated. *Nat Med*. 1999; 5:895–899. [PubMed: 10426312]
 53. Schoeller DA, Cella LK, Sinha MK, Caro JF. Entrainment of the diurnal rhythm of plasma leptin to meal timing. *J Clin Invest*. 1997; 100:1882–1887. [PubMed: 9312190]
 54. Yoshikawa H, Tajiri Y, Sako Y, Hashimoto T, Umeda F, Nawata H. Glucosamine-induced beta-cell dysfunction: a possible involvement of glucokinase or glucose-transporter type 2. *Pancreas*. 2002; 24:228–234. [PubMed: 11893929]

55. Harris RB. Direct and indirect effects of leptin on adipocyte metabolism. *Biochim Biophys Acta*. 2013; 1842:414–423. [PubMed: 23685313]
56. McClain DA, Hazel M, Parker G, Cooksey RC. Adipocytes with increased hexosamine flux exhibit insulin resistance, increased glucose uptake, and increased synthesis and storage of lipid. *Am J Physiol Endocrinol Metab*. 2005; 288:E973–979. [PubMed: 15613679]
57. Love DC, Hanover JA. The hexosamine signaling pathway: deciphering the “O-GlcNAc code”. *Sci STKE*. 2005:re13. [PubMed: 16317114]

Highlights

Rats with access to sucrose solution are peripherally and centrally leptin resistant

Basal phosphorylated STAT3 is increased in the arcuate nucleus of the hypothalamus

Leptin does not further stimulate STAT3 phosphorylation in sucrose drinking rats

Glucosamine stimulates hypothalamic STAT3 phosphorylation

Figure 1.

A simplified schematic of the hexosamine biosynthetic pathway (HBP) identifying the point of entry of glucose, fructose and glucosamine into the pathway. GFAT= glutamine:fructose-6-phosphate aminotransferase, the enzyme which controls flux of glucose into the HBP. OGT= *O*-linked N-acetylglucosamine transferase, the enzyme which transfers a single N-acetylglucosamine by *O*-glycosidic (*O*-GlcNAc) linkage to serine or threonine residues of proteins. *O*-GlcNAcase= the enzyme that reverses *O*-GlcNAc protein modification.

Figure 2.

Panel A shows the cumulative energy intake of rats in Experiment 1 following a 3rd ventricle injection of PBS or 1.5 μ g leptin on Day 36 of the experiment. Values for a specific time point that do not share a common superscript are significantly different from one another. Panel B shows hypothalamic pSTAT3(Tyr705) and Panel D shows hypothalamic SOCS3 measured on tissue blocks collected at the end of the experiment (Day 44). Panel C shows pSTAT3(Tyr705) measured in tissue blocks of hindbrain. CS = Chow Saline, CL = Chow Leptin, SS = Sucrose Saline, SL = Sucrose leptin.

Figure 3.

Results from Western blot analysis of enzymes involved in the HBP measured in tissue collected from rats in Experiment 1 after 44 days of being offered chow and water (chow) or chow, water and 30% sucrose solution (sucrose). Rats were injected i.p. with either PBS or 2 mg leptin/kg exactly 30 minutes before tissue collection. Data are means + sem. Values on a specific axis that do not share a common superscript are significantly different at $P < 0.05$.

Figure 4.

Energy intake (A), body weight (B), fat depot weight (C) and leptin responsiveness (D and E) for rats offered chow and water (chow) or chow, water and 30% sucrose solution (sucrose) for 36 days. Data are means $\pm$ sem for groups of 7 rats. Asterisks indicate significant differences in total energy intake or fat depot weight. Values for energy intake or weight gain following leptin injection (D and E) that do not share a common superscript are significantly different ($P < 0.05$). Energy intakes of sucrose rats represent energy obtained from both chow and sucrose consumption.

Figure 5.

Serum glucose and insulin during a GTT performed on Day 12 (A and B) or an ITT performed on Day 35 (C and D). Leptin and TG (D and E) were measured on trunk blood collected at the end of the experiment. Data are means + sem for groups of 7 rats. An asterisk indicates a significant difference between treatment groups ($P < 0.05$). Values for ITT insulin that do not share a common superscript are significantly different.

Lateral and Medial Arcuate Nucleus, Plate 32

Figure 6.

Immunohistochemistry for pSTAT3 (Tyr705) in brain sections from rats offered chow and water or chow, water and 30% sucrose solution for 36 days in Experiment 2. The top panel shows quantification of pSTAT3 (Tyr705) nuclei in different brain areas the bottom panel shows images for the medial and lateral arcuate nucleus from a chow rat and a sucrose rat. Data are means + sem. Images have been adjusted for brightness and contrast.

Figure 7.

Panel A shows serum glucose in rats receiving i.v. infusions of saline or 30 $\mu\text{mol/kg/min}$ GlcN in Experiment 3. The rats were injected i.p. with 1 mg/kg leptin 30 minutes before the end of the experiment. Panel B shows hypothalamic pSTAT3 (Tyr705) measured by western blot on tissue blocks collected at the end of the experiment and Panel C shows SOCS3. Data are means + sem for groups of 8 rats. Asterisks indicate significant difference in blood glucose concentration ($P < 0.05$). SS = Saline Saline, SL = Saline Leptin, GS = Glucosamine Saline, GL = Glucosamine Leptin.

Figure 8.

Results from Western blot analysis of enzymes involved in the HBP measured in liver tissue collected from rats infused with saline or GlcN in Experiment 3. SS = Saline Saline, SL = Saline Leptin, GS = GlcN Saline, GL = GlcN Leptin. Data are means + sem. Superscripts indicate significant differences between treatment groups ($P < 0.05$).

Table 1

Food Intake, weight gain, fat pad weight and serum hormones and metabolites in rats from Experiment 1

	Chow	Sucrose
Start weight (g)	293 ± 2	291 ± 1
End weight (g)	392 ± 4	398 ± 9
Energy Intake (kcal/rat/6 days)	527 ± 7	582 ± 18*
<i>Fat pad weights</i>		
Inguinal (g)	4.0 ± 0.2	5.6 ± 0.6*
Epididymal (g)	3.0 ± 0.3	3.8 ± 0.3*
Retroperitoneal (g)	1.2 ± 0.1	2.3 ± 0.2*
Mesenteric (g)	1.3 ± 0.1	2.2 ± 0.3*
Total dissected fat (g)	9.5 ± 0.4	13.9 ± 1.4*
Liver weight (g)	12.3 ± 0.3	13.2 ± 0.4*
Liver lipid (g)	0.43 ± 0.01	0.45 ± 0.01

	Chow		Sucrose	
	<i>Saline</i>	<i>Leptin</i>	<i>Saline</i>	<i>Leptin</i>
Glucose (mg/dL)	133 ± 9	143 ± 10	154 ± 17	136 ± 8
Insulin (ng/ml)	0.56 ± 0.07 ^A	0.70 ± 0.16 ^A	0.97 ± 0.26 ^B	0.80 ± 0.09 ^{AB}
Leptin (ng/ml)	17 ± 4 ^A	1496 ± 168 ^B	13 ± 2 ^A	2674 ± 333 ^C
TG (mg/dL)	68 ± 9 ^{AB}	63 ± 6 ^B	79 ± 6 ^{AB}	90 ± 6 ^A
Glycerol (mg/ml)	0.22 ± 0.04 ^A	0.23 ± 0.04 ^{AC}	0.39 ± 0.05 ^B	0.36 ± 0.05 ^{BC}

Data are means ± sem for groups of 8 rats for serum measures and 16 rats for all other measures. Rats were killed after the sucrose rats had 44 days of access to 30% sucrose solution in addition to chow and water and exactly 30 minutes after an i.p. injection of 1 mg/kg leptin.

An asterisk indicates a significant difference between chow and sucrose rats.

Superscripts indicate significant differences between treatment groups for serum measures.

Table 2

Serum measures in GlcN-infused rats in Experiment 3

	Saline-Infused		Glucosamine-Infused	
	<i>Saline</i>	<i>Leptin</i>	<i>Saline</i>	<i>Leptin</i>
Glucose (mg/dL)	126 ± 7 ^A	126 ± 3 ^A	164 ± 7 ^B	175 ± 3 ^B
Insulin (ng/ml)	0.62 ± 0.09	0.73 ± 0.08	0.76 ± 0.11	0.62 ± 0.08
Leptin (ng/ml)	4 ± 2 ^A	1475 ± 124 ^B	9 ± 3 ^A	1239 ± 160 ^B

Blood was collected at the end of a 180 minute intravenous infusion of GlcN or saline and 30 minutes after an i.p. injection of 1 mg/kg leptin. Data are means ± sem for groups of 7 or 8 rats. Values that do not share a common superscript are significantly different at $P < 0.05$.