Changes in glucose tolerance and leptin responsiveness of rats offered a choice of lard, sucrose, and chow

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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 302: R1327–R1339, 2012. First published April 11, 2012; doi:10.1152/ajpregu.00477.2011.—Rats offered chow, lard, and 30% sucrose solution (choice) rapidly become obese. We tested metabolic disturbances in rats offered choice, chow + lard, or chow + 30% sucrose solution [chow + liquid sucrose (LS)] and compared them with rats fed a composite 60% kcal fat, 7% sucrose diet [high-fat diet (HFD)], or a 10% kcal fat, 35% sucrose diet [low-fat diet (LFD)]. Choice rats had the highest energy intake, but HFD rats gained the most weight. After 23 days carcass fat was the same for choice, HFD, chow + lard, and chow + LS groups. Glucose clearance was the same for all groups during an intraperitoneal glucose tolerance test (GTT) on day 12, but fasting insulin was increased in choice, LFD fed, and chow + LS rats. By contrast, only choice and chow + LS rats were resistant to an intraperitoneal injection of 2 mg leptin/kg on day 17. In experiment 2 choice rats were insulin insensitive during an intraperitoneal GTT, but this was corrected in an oral GTT due to GLP-1 release. UCP-1 protein was increased in brown fat and inguinal white fat in choice rats, and this was associated with a significant increase in energy expenditure of choice rats during the dark period whether expenditure was expressed on a per animal or a metabolic body size basis. The increase in expenditure obviously was not great enough to prevent development of obesity. Further studies are required to determine the mechanistic basis of the rapid onset of leptin resistance in choice rats and how consumption of sucrose solution drives this process.

dietary sucrose; leptin resistance; UCP-1; energy expenditure

DIET-INDUCED OBESITY is a commonly used rodent model that may be induced in a variety of ways including offering the animals a composite high-fat diet (HFD) (18), a cafeteria diet (68), or sucrose or fructose solution in addition to dry food (5, 31). A recent review by Panchal and Brown (46) concludes that rats fed high-fat, high-carbohydrate diets provide the best rodent model of human metabolic syndrome due to the development of obesity, hyperinsulinemia, hyperlipidemia, hepatic steatosis, and cardiovascular abnormalities. Hepatic and skeletal muscle insulin resistance is evident within 4 wk (12, 63), but it may take months for additional aspects of the metabolic syndrome to develop in mice (42, 47) and rats (48). We and others have demonstrated that rats offered lard and sucrose solution in addition to chow (choice) overeat and rapidly gain body fat (3, 36). This increase in adiposity has been associated with the development of glucose intolerance (35), disruption of central anorexigenic and orexigenic neuropeptide expression (36), and a suppression of the hypothalamic, pituitary, adrenal (HPA) axis response to stress (49). The adverse metabolic changes appear to occur more rapidly in choice rats than has been reported for rats fed a composite HFD (14, 40, 48).

Others have offered rats free access to sucrose or fructose solutions as a means of inducing hypertriglyceridemia and insulin resistance, typical of the metabolic syndrome (2). By contrast, when rats are offered dry diets containing high levels of fructose, they may or may not develop obesity, glucose intolerance, and hypertriglyceridemia (1, 9, 21–22, 54). Recently, we reported that composite high-fructose diets produced leptin resistance in rats, but that high levels of dietary monosaccharide reversed leptin resistance caused by a HFD (22). By contrast, Shapiro et al. (59) have proposed that consumption of high levels of both fat and fructose are required for the development of leptin resistance. Thus it is not clear whether leptin and insulin insensitivity in choice rats develops in response to consumption of large quantities of carbohydrate versus fat, or whether the combination of excess carbohydrate and fat calories is required. An additional consideration is that rats offered the choice diet, unlike animals offered a composite high-fat or high-fructose diet, have the opportunity to consume a large amount of carbohydrate or of fat unaccompanied by other dietary components. Thus it also is possible that the form in which macronutrients are offered influences how they are handled and the metabolic pathways that are disrupted in the early stages of diet-induced obesity.

The studies described here examined various aspects of the obesity phenotype of choice rats. The first experiment compared rats offered the choice diet with those offered a choice of chow and lard, a choice of chow and sucrose solution or a composite HFD. The objective of this study was to test whether choice rats became leptin resistant faster than rats fed a composite HFD and whether development of obesity, glucose intolerance, or leptin resistance was influenced by the consumption of macronutrients independently compared with consuming them in a fixed ratio from a formulated diet. The inclusion of groups offered chow plus lard or chow plus sucrose solution allowed us to test whether it was lard or sucrose consumption that was associated with the various metabolic disruptions in choice rats. The second experiment tested whether glucose intolerance of diet choice rats detected by intraperitoneal or intravenous glucose administration (36) remained significant when glucose was given by gavage, because that would indicate an impairment of incretin function in addition to development of insulin sensitivity in insulin-responsive tissues. It is well established that consumption of sucrose (31) or of fat (52) activates brown adipose tissue, with the potential to stimulate energy expenditure through uncoupling of mitochondrial oxidative phosphorylation. Therefore, the final study tested whether choice rats had higher energy expenditure than their chow-fed controls.

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METHODS

Male Sprague-Dawley rats (Harlan Laboratories, Indianapolis, IN) were housed individually in hanging wire mesh cages in a room maintained at 20–23°C with lights on for 12 h/day from 7:00 AM. They had free access to food and water unless stated otherwise. Each rat had a Nylabone (Nylabone Products, Neptune, NJ) in their cage for enrichment. All animal procedures were approved by the Institutional Animal Care and Use Committee of Georgia Health Sciences University. For each experiment rats were allowed to adapt to their environment for 1 wk before the initiation of baseline measures of food intake and body weight.

Experiment 1: comparing the effects of choice and HFDs on insulin and leptin sensitivity. A total of 60 rats in two cohorts of 30 were included in the study. The second cohort entered the study 4 days after the first. After 5 days of baseline measures of food intake and body weight, the rats were divided into six weight-matched groups. One group of rats remained on chow (Harlan Teklad Rodent Diet 8604; 24% minimum protein, 4% minimum fat), one group was offered free access to chow and to lard (Armour, ConAgra Foods, Omaha, NE), one group was offered free access to chow and to 30% sucrose solution (chow+LS: Kroger Sugar, Hood Packing, Hamlet, NC), one group was offered choice diet (free access to chow, lard, and 30% sucrose solution), one group was offered a composite low-fat diet (LFD: D12450B Research Diets, New Brunswick, NJ), and the last group was offered a composite 60% kcal fat diet (HFD: D12492 Research Diets). All rats had free access to water. Body weights and energy intakes, corrected for spillage, were recorded daily. On day 12 food was removed from the cages at 7:00 AM, and an intraperitoneal glucose tolerance test (GTT) delivering 1 g glucose/kg was initiated at 1:00 PM. Tail blood samples were used to measure blood glucose (EasyGluco blood glucose test strips, US Diagnostics) at 0, 15, 30, 45, 60, 90, and 120 min after glucose injection and insulin (Rat InsulinRIA kit, Millipore) at 0, 15, 30, 60, and 120 min. Food was returned to the cages at the end of the test. On day 17 the rats were tested for leptin responsiveness. Food was removed from the cages at 7:00 AM. At 5:00 PM half of the rats in each group were injected intraperitoneally with 2 mg leptin/kg (rat recombinant leptin, R&D Systems, Minneapolis, MN) and the other half was injected with an equivalent volume of PBS. Food, lard, and sucrose solution were returned to the cages 1 h later, and energy intakes and body weights were recorded 14, 24, and 36 h after the injection. The procedure was repeated on day 20, and the PBS and leptin treatments were switched so that the rats could act as their own controls.

On day 23 food was removed from the cages at 7:00 AM, and rats were euthanized between 10:00 AM and 12:00 PM. Trunk blood was collected for measurement of serum insulin, leptin (rat leptin RIA kit, Millipore), glucose (glucose assay kit GAGO20; Sigma-Aldrich, St. Louis, MO), glycerol (free glycerol reagent F6428; Sigma-Aldrich), free fatty acids (FFA; NEFA C kit, Wako Chemicals, Richmond, VA), and triglycerides (TG; L-Type TG H kit; Wako Chemicals). Inguinal (Ing), epididymal, retroperitoneal (RP), mesenteric white fat, intrascapular brown fat (IBAT), and the liver were dissected and weighed. (Ing), epididymal, retroperitoneal (RP), mesenteric white fat, intrascapular brown fat (IBAT), and the liver were dissected and weighed. 

Experiment 2: glucose clearance in choice rats. Twenty rats were housed as described above, and after 5 days of baseline measures of body weight, they were divided into two weight-matched groups. One group continued to receive ad libitum access to chow and the other was offered the choice diet. Body weights were recorded daily and energy intake, corrected for spillage, was measured from day 5 to day 11. On day 12 food was removed from the cages at 7:00 AM and an intraperitoneal GTT was performed as described above. On day 14 the procedure was repeated except that the glucose (1 g glucose/kg) was delivered by gavage and tail blood was collected for measurement of glucose and insulin in response to oral delivery of glucose. On day 16 rats were food deprived from 7:00 AM to 1:00 PM and gavaged again with 1 g glucose/kg. Tail blood samples were collected according to kit directions at 0 and 15 min for measurement of glucagon-like peptide (GLP-1, Active 7–36; ELISA, ALPCO, Salem, NH). On day 18 the rats were euthanized, and blood and tissue were collected as described for experiment 1. Serum hormone and metabolite assays and liver lipid and glycogen were measured. IBAT, Ing, and RP UCP-1 protein expression was measured by Western blot. Expression of UCP-1 mRNA was determined by real-time PCR in IBAT and Ing fat as described previously (50).

Experiment 3: energy expenditure of diet choice rats. Sixteen rats were divided into two weight-matched groups, one was offered chow and the other was offered the choice diet. Body weights were recorded daily. Energy expenditure was measured three times: during baseline (BL), during the first few days of exposure to diet choice, and after 12 days of diet choice. For each time point the rats were housed in calorimeter cages (TSE LabMaster-Metabolic Research Platform, TSE Systems International, Chesterfield, MO) for 3 days, and data from only the last day (BL, day 3 or day 12) was used to compare choice and chow-fed groups. Oxygen consumption, carbon dioxide production, and activity were recorded from each cage for 1 min every 39 min and used to calculate energy expenditure (expressed both as kcal h⁻¹ rat⁻¹ and per unit metabolic body weight (kcal h⁻¹wt⁻⁰.⁷⁵)), ventilation and respiratory exchange ratio (RER) as an index of macronutrient oxidation. Total activity was measured by InfraMOT (TSE Systems). Energy intakes of the rats were recorded for 3 days from day 15 when the rats were in their home cages.

Data analysis. Statistically significant differences between groups in experiment 1 were determined by one-way analysis of variance and post hoc Duncan’s multiple range test. Differences between single endpoint measures between chow and diet choice groups in experiments 2 and 3 were determined by unpaired Student’s t-test assuming equal variance. Differences in daily food intake, body weight, and of glucose and insulin during the intraperitoneal and oral GTTs in experiment 2 were determined by repeated measures analysis of variance. P < 0.05 was considered statistically significant. Data analysis was carried out using Statistica Version 9 software (StatSoft, Tulsa, OK).

RESULTS

Experiment 1. The objective of this experiment was to directly compare the effects of consumption of a high-fat composite diet with that of choice diet on glucose clearance, leptin sensitivity, and body composition of rats. Groups of rats offered chow plus lard or chow plus sucrose solution were also included to identify which aspect of the choice diet was responsible for development of different aspects of the phenotype of choice rats.
Weight gain during the experimental period was greater for HFD-fed rats than any other group, whereas the chow rats gained the least amount of weight (Fig. 1). These differences in growth did not correlate with energy intakes because choice rats had a higher total intake than any other group, and the energy intake of HFD rats was the same as that of chow + LS and chow + lard rats (Table 1). The total energy intake of LFD rats was higher than that of chow rats but lower than any other group. A majority of the fat in the HFD was lard (54% of kcal), and the total amount of energy consumed as fat by HFD rats was the same as that for chow + lard rats (Table 1). The LFD contained 34.5% kcal as sucrose and the LFD and choice rats consumed the same amount of energy as sucrose. Chow + LS rats consumed twice as much energy as sucrose solution as the choice rats (Table 1). The amount of sucrose contributed to the diet by chow could not be calculated because the specific composition of chow was unknown. Daily energy intakes of the rats are shown in Fig. 1. All rats offered a diet other than just chow ate more during the first 4 days of the study than during the rest of the experiment. Energy intakes of the rats had stabilized by day 12 when GTT was tested.

An intraperitoneal GTT performed on day 12 showed no differences in fasting glucose (Fig. 2, Table 1) or in area under the curve (AUC) for glucose calculated by the linear trapezoid method (data not shown). By contrast, fasting insulin was significantly increased in choice, LFD, and chow + LS rats compared with chow rats (Fig. 2, Table 1). Insulin was not different among HFD, LFD, choice, and chow + LS rats. Insulin was elevated during the first 15 min of the GTT in choice, LFD, and chow + LS rats compared with the other animals (Fig. 2; P < 0.01), but AUC for insulin was not significantly different between groups (data not shown). Similarly, the ratio of change in insulin to change in blood glucose concentration during the first 15 min of the GTT was not different between treatment groups (P < 0.07, data not shown). Peripheral injections of leptin did not have a significant effect on weight gain of any group of rats, but food intake was inhibited in all but the choice and the chow + LS rats at each time point tested. Figure 3 shows the cumulative intakes of the rats 14 and 36 h after the leptin or PBS injection.

At the end of the study rats offered chow + LS, chow + lard, choice, or HFD were equally fat and fatter than chow or LFD rats (Table 2). This was true whether carcass fat was expressed as grams per rat or as a percentage of carcass weight (data not shown). All of the fat depots that were weighed increased in association with an increase in total carcass fat. The percent increase in size of the RP pad was greater (100–150%) than that of any other depot (30–100%). Carcass protein was increased in HFD diet rats compared with chow rats, but was not different from any other group, lean body mass (water plus protein) was significantly higher in LFD and HFD rats compared with all other groups. Liver weight was lower in Chow + lard rats compared with all other groups (Table 2). Liver lipid content was higher in rats fed HFD than any other group, but there were no differences in liver glycogen (Table 2). UCP-1 protein was increased in the IBAT of choice, chow + lard, and chow + LS rats compared with other groups, but the difference was significant only for the chow + lard rats (Fig. 4). There were no differences in RP UCP-1. In Ing fat, UCP-1 protein was almost twice as high in chow + lard and choice rats as in chow rats, but these differences did not reach significance (P < 0.1; Fig. 4).

Serum TG were elevated in chow + LS and LFD rats compared with chow controls (Table 2). Serum leptin correlated with body fat mass, being highest in choice and HFD rats and lowest in chow and LFD animals. Serum FFA and glycerol were not different between groups (Table 2).

Experiment 2. Choice rats in experiment 1 showed an impaired glucose tolerance during an intraperitoneal GTT compared with their chow-fed controls. One objective of this study was to further evaluate glucose handling in choice rats by testing whether they showed a normal incretin response during an oral glucose load. A secondary objective was to confirm
that UCP-1 was increased in IBAT and Ing fat of choice rats compared with their controls. During days 5 to 11 of the experiment rats offered choice had the same caloric intake as those offered chow, but a large portion of their energy intake was derived from sucrose (22 ± 5%) or lard (52 ± 5%) (Fig. 5). These results differed from those in experiment 1, where choice rats had a higher energy intake than chow controls. One explanation for this may be that we measured intake for only 6 days during this experiment, as compared for the entire experimental period in experiment 1 where many of the rats ate more at the start of the experiment when the diet was novel. Despite the similarity in measured energy intake, the choice rats gained more weight than chow-fed animals, and the difference in body weight was significant from day 3 of the experiment (Fig. 5). AUC for glucose was greater in choice than control rats during the intraperitoneal GTT on day 12, indicating an impaired glucose clearance in choice rats as insulin release also tended to be increased (Fig. 6). Fasting glucose and insulin both showed a small, but statistically significant, increase in choice compared with chow rats (glucose: 122 ± 4 vs. 111 ± 3 mg/dl, insulin: 1.1 ± 0.1 vs. 0.7 ± 0.1 ng/ml). AUC for glucose was greater for the intraperitoneal GTT than for the oral GTT in choice rats, whereas they were the same in chow rats (Fig. 6: diet: P < 0.05, GTT: P < 0.03, interaction: not significant). As expected, AUC for insulin was greater for the oral than the intraperitoneal GTT, although this difference reached significance only for the chow rats (diet: not significant, GTT: P < 0.002, interaction: not significant). In nonstimulated conditions GLP-1 concentrations were lower in choice than chow rats, but they were identical in the two groups in response to a glucose gavage (Fig. 6).

Choice rats were significantly fatter than chow rats at the end of the experiment (Table 3). All of the measured fat depots, except the RP fat, increased by ~80%. RP fat showed an exaggerated enlargement of 220% (Table 3). Liver weight was not different between chow and choice rats, but liver lipid increased by about 35% and liver glycogen by ~40% in the choice rats (Table 3). The choice rats appeared to have an increased efficiency of energy utilization, in that they increased body fat despite appearing to maintain the same energy intake as chow-fed animals. Therefore, it was surprising that expression of the thermogenic protein UCP-1 was significantly increased in both IBAT and Ing white fat. There was no effect of diet on RP white fat UCP-1 protein levels. The increase in UCP-1 protein appeared to be due to a translational event because IBAT UCP-1 mRNA expression was not different between the two groups, whereas Ing UCP-1 mRNA was lower in choice than chow rats (Fig. 7). The increase in body fat content of choice rats was accompanied by a significant increase in serum FFA, TG, glycerol, and leptin concentrations (Table 3).

Experiment 3. Experiments 1 and 2 indicated that UCP-1 protein expression was increased in choice compared with chow rats. Because this protein is a marker of thermogenesis, this experiment measured the energy expenditure of choice rats to test whether elevated UCP-1 protein was associated with an increase in whole animal energy expenditure.

In this experiment choice rats had a higher energy intake than chow controls (316 ± 10 vs. 237 ± 4 kcal·rat⁻¹·3 days⁻¹), and this was associated with an increased weight gain (38 ± 4 vs. 22 ± 2 g/18 days). Choice rats consumed ~30% of their energy as sucrose solution and 45% as lard. Energy expenditure of both chow and choice rats was higher during the dark period than the light period at all times considered. The increase in night time expenditure was significant only in rats fed the choice diet and also was significantly higher in choice than chow rats. This was true whether expenditure was expressed on a per rat or a per metabolic body weight basis (Fig. 8). RER also was higher during the dark than the light period (Fig. 8). RER is an indirect measure of nutrient oxidation and is 1.0 when carbohydrate is oxidized and 0.7 when fat is oxidized (19). The RER of choice rats was not different from that of chow rats, although there was a trend (P < 0.06) for the daytime RER in choice rats to be lower at day 12 (Fig. 8). Activity of both chow and choice rats was increased during the dark compared with the light period of the day. There were no differences in activity during the light period on any of the days measured. Activity during the dark was not different between groups during the baseline period, was higher in chow than choice rats on day 3, but was higher in choice rats on day 12 (Fig. 8).

### DISCUSSION

Previous studies have shown that rats offered choice diet rapidly become obese and glucose intolerant (4, 35, 36). The objective of the first study described here was to determine whether glucose intolerance, obesity, and leptin resistance developed faster in rats offered choice diet than in those fed a composite HFD and whether this was associated with consumption of lard or of sucrose solution. Even though the choice and HFD rats were equally fat, only the choice rats showed an impairment of glucose clearance on day 12 and leptin responsiveness on day 17. Glucose intolerance was associated with sucrose intake because chow + LS, choice, and LFD rats were the three groups that had elevated insulin concentrations during

### Table 1. Energy Intakes of rats in experiment 1

<table>
<thead>
<tr>
<th></th>
<th>Chow</th>
<th>Chow + LS</th>
<th>Chow + Lard</th>
<th>Choice</th>
<th>LFD</th>
<th>HFD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy intake, kcal/23 days</td>
<td>1,532 ± 19a</td>
<td>2,091 ± 34b</td>
<td>1,959 ± 42c</td>
<td>2,220 ± 84d</td>
<td>1,737 ± 38e</td>
<td>1,972 ± 33f</td>
</tr>
<tr>
<td>% kcal Fat</td>
<td>4</td>
<td>1.6 ± 0.1</td>
<td>51 ± 2</td>
<td>37 ± 3</td>
<td>10</td>
<td>60</td>
</tr>
<tr>
<td>% kcal Sucrose</td>
<td>60 ± 1</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Fat, kcal/23 days</td>
<td>61 ± 1c</td>
<td>34 ± 1b</td>
<td>1,188 ± 46d</td>
<td>807 ± 73e</td>
<td>174 ± 4f</td>
<td>1,183 ± 20g</td>
</tr>
<tr>
<td>Sucrose, kcal/23 days</td>
<td>1,249 ± 45a</td>
<td>671 ± 88b</td>
<td>608 ± 13c</td>
<td>138 ± 2e</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are either based on information from manufacturers regarding the composition of chow or composite diet or are means ± SE for intakes of 10 rats/group during the 23 days of the experiment. LS, liquid sucrose; LFD, low-fat diet; HFD, high-fat diet. Values for intake over 23 days that do not share a common superscript are significantly different at P < 0.05.
the first 15 min of the GTT and also had the highest levels of sucrose consumption. The LFD rats consumed sucrose as part of a composite diet, whereas the other groups were drinking sucrose solution; therefore, the form and pattern of consumption of sucrose was not important in the development of glucose intolerance. Development of obesity also was not a requirement for development of glucose intolerance because the LFD rats were no fatter than the chow controls. These results are consistent with reports that hepatic insulin insensitivity occurs within 1 wk (13) in rats fed a dry, composite high-sucrose diet without any change in body fat content (63).

Adipose (51) and muscle tissue (13) also become insulin resistant in rats fed high-sucrose diets, but this takes 4 to 5 wk to develop. Therefore, although we did not measure hepatic glucose production or peripheral tissue insulin sensitivity, it is possible that the impaired glucose tolerance observed in our rats after only 12 days was caused by hepatic insulin insensitivity. The increase in serum concentrations of insulin 15 min after the glucose injection could have been due to either an increased release of insulin or a reduced rate of insulin clearance from the circulation in the three groups of rats that had the highest sucrose intake. The small, but significant, increase in fasting insulin of choice, chow+LS, and LFD rats compared with chow rats suggests, however, that circulating insulin concentrations are being regulated at a higher level in these rats. The glucose intolerance was more clearly defined in experiment 2, where there was a significant increase in AUC for glucose and a trend for an increase in AUC for insulin in choice rats compared with chow controls.

The results of experiment 1 differ from those reported by La Fleur et al. (35), who found that rats fed choice diet or chow plus beef tallow for 4 wk developed glucose intolerance, whereas those offered chow plus 30% sucrose solution had...
elevated fasting insulin but a normal glucose clearance. In the study described here we also found that elevated fasting insulin did not predict glucose intolerance because fasting insulin was higher in HFD rats than chow controls, but glucose clearance was not different. There are a number of differences between the study by La Fleur et al. (35) and experiment 1 described here including the strain of rats (Wistars vs. Sprague-Dawley rats in studies described here), the type of fat offered to the rats (beef tallow vs. pork fat used in studies described here), and the methods used to measure glucose tolerance. The intravenous delivery of glucose used by La Fleur et al. (35) caused a much greater, but abbreviated, rise in serum glucose compared with that obtained with an intraperitoneal injection of glucose. Blood glucose increased by 200 ng/ml but was back to basal levels within 15 min following an intravenous injection of glucose (35). By comparison, an intraperitoneal injection of the same dose of glucose increased blood glucose by only 100 mg/dl but took 2 h to return to baseline. Therefore, the glucose load experienced by insulin-responsive tissues was very different between experiments, and it is also possible that different aspects of β cell function were being compared in the two studies. The first phase of insulin secretion, which would be completed within 15 min following an intravenous injection of glucose, is reliant upon secretion of newly synthesized insulin.

Rats fed HFDs also develop insulin resistance (34), but it is unlikely that fat consumption caused insulin insensitivity in the choice rats because HFD rats and chow + lard rats had a higher fat intake than choice rats but showed a normal glucose clearance. Others have shown that consumption of composite HFDs impairs glucose clearance; however, this requires overconsumption of energy in addition to the proportionally high intake of calories as fat (60). As noted above, La Fleur et al. (35) found glucose intolerance in rats fed chow plus beef tallow for 4 wk and Sampay et al. (53) reported insulin insensitivity in rats fed either a 45% fat diet or a cafeteria diet for 11 wk compared with chow-fed controls. By contrast, we found normal glucose clearance in rats fed chow plus lard for 12 days in experiment 1. The duration of feeding may have been a significant factor in producing this difference in results because Sutherland et al. (65) found normal glucose tolerance in rats fed a 59% fat diet for 14 days but impaired glucose tolerance at 4 wk. Although the choice rats in experiment 1 were glucose intolerant when glucose was given as an intraperitoneal injection, the results of the oral GTT in experiment 2 indicate that GLP-1 release in response to glucose is normal in choice rats, and that glucose clearance and insulin sensitivity are normal when glucose is delivered orally. GLP-1 stimulates insulin secretion and increases tissue insulin sensitivity (25), therefore, although insulin-responsive tissues rapidly become insulin insensitive in choice rats, when the animal is consuming food orally the incretin response appears to maintain glucose homeostasis for at least the first 2 wk that the animals are consuming choice diet.

In experiment 1 we also found that rats fed choice diet or chow + LS were resistant to the effects of peripheral leptin administration on food intake. Rats that were fed a composite HFD remained leptin responsive at day 17, and these observations were consistent with the report that hypothalamic NPY was increased and proopiomelanocortin (POMC) was decreased in rats fed a choice diet, but not in those fed chow plus beef tallow (36), because leptin inhibits NPY but increases POMC expression (55, 56). There were many differences between the treatment groups in experiment 1, including diet composition, energy intake, and body fat content. Leptin resistance was not simply due to obesity in choice and chow + LS rats because chow + lard and HFD rats were equally fat but leptin responsive. Similarly, leptin resistance was not dependent on fat consumption because

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### Table 2. Body composition and serum hormones in rats in experiment 1

<table>
<thead>
<tr>
<th></th>
<th>Chow</th>
<th>Chow + LS</th>
<th>Chow + lard</th>
<th>Choice</th>
<th>LFD</th>
<th>HFD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carcass wt, g</td>
<td>313 ± 3a</td>
<td>332 ± 3b</td>
<td>332 ± 6b</td>
<td>331 ± 5b</td>
<td>339 ± 4b</td>
<td>357 ± 3c</td>
</tr>
<tr>
<td>Carcass fat, g</td>
<td>17 ± 1a</td>
<td>29 ± 2b</td>
<td>30 ± 2b</td>
<td>37 ± 2b</td>
<td>22 ± 2b</td>
<td>33 ± 2c</td>
</tr>
<tr>
<td>Carcass protein, g</td>
<td>69 ± 3a</td>
<td>78 ± 1ab</td>
<td>76 ± 1ab</td>
<td>77 ± 3ab</td>
<td>76 ± 6ab</td>
<td>83 ± 1b</td>
</tr>
<tr>
<td>Lean mass, g</td>
<td>283 ± 4a</td>
<td>291 ± 4a</td>
<td>289 ± 6a</td>
<td>284 ± 4a</td>
<td>305 ± 3b</td>
<td>311 ± 2b</td>
</tr>
<tr>
<td>Fat depot weights, g</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ing</td>
<td>4.7 ± 0.2a</td>
<td>7.0 ± 0.5b</td>
<td>7.4 ± 0.5bc</td>
<td>8.3 ± 0.5c</td>
<td>5.8 ± 0.2a</td>
<td>8.7 ± 0.3c</td>
</tr>
<tr>
<td>Epididymal</td>
<td>3.0 ± 0.1a</td>
<td>3.9 ± 0.2b</td>
<td>4.7 ± 0.3cd</td>
<td>4.6 ± 0.3c</td>
<td>3.9 ± 0.2b</td>
<td>5.2 ± 0.2d</td>
</tr>
<tr>
<td>RP</td>
<td>1.3 ± 0.1a</td>
<td>2.8 ± 0.3bc</td>
<td>2.6 ± 0.2c</td>
<td>3.3 ± 0.3b</td>
<td>2.0 ± 0.1bc</td>
<td>2.9 ± 0.3bc</td>
</tr>
<tr>
<td>Mesenteric</td>
<td>1.5 ± 0.1a</td>
<td>2.7 ± 0.2bc</td>
<td>2.9 ± 0.2bc</td>
<td>3.3 ± 0.3bc</td>
<td>2.4 ± 0.1bc</td>
<td>3.0 ± 0.2bc</td>
</tr>
<tr>
<td>IBAT</td>
<td>0.39 ± 0.02a</td>
<td>0.60 ± 0.06d</td>
<td>0.66 ± 0.05c</td>
<td>0.78 ± 0.06d</td>
<td>0.52 ± 0.04d</td>
<td>0.66 ± 0.01bc</td>
</tr>
<tr>
<td>Liver composition</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight, g</td>
<td>12.8 ± 0.3a</td>
<td>14.5 ± 0.4b</td>
<td>11.7 ± 0.3b</td>
<td>13.1 ± 0.4a</td>
<td>12.8 ± 0.4a</td>
<td>13.1 ± 0.2a</td>
</tr>
<tr>
<td>Lipid, mg</td>
<td>590 ± 26a</td>
<td>550 ± 40a</td>
<td>626 ± 40a</td>
<td>615 ± 30a</td>
<td>567 ± 30a</td>
<td>803 ± 50b</td>
</tr>
<tr>
<td>Glycogen, mg glucose</td>
<td>109 ± 8</td>
<td>154 ± 20</td>
<td>109 ± 9</td>
<td>132 ± 11</td>
<td>108 ± 9</td>
<td>121 ± 8</td>
</tr>
<tr>
<td>Serum leptin and lipids</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leptin, ng/ml</td>
<td>6 ± 1a</td>
<td>12 ± 2b</td>
<td>12 ± 2ab</td>
<td>17 ± 3c</td>
<td>8 ± 1b</td>
<td>15 ± 2bc</td>
</tr>
<tr>
<td>FFA, mmol/ml</td>
<td>472 ± 32</td>
<td>565 ± 42</td>
<td>634 ± 58</td>
<td>555 ± 76</td>
<td>484 ± 58</td>
<td>453 ± 26f</td>
</tr>
<tr>
<td>TG, mg/dl</td>
<td>61 ± 5a</td>
<td>132 ± 8a</td>
<td>62 ± 7a</td>
<td>78 ± 20ab</td>
<td>104 ± 6c</td>
<td>61 ± 5a</td>
</tr>
<tr>
<td>Glycerol, μM</td>
<td>35 ± 3</td>
<td>55 ± 6</td>
<td>57 ± 10</td>
<td>52 ± 9</td>
<td>45 ± 3</td>
<td>49 ± 6</td>
</tr>
<tr>
<td>Fasting glucose, mg/dl</td>
<td>101 ± 4</td>
<td>105 ± 2</td>
<td>107 ± 3</td>
<td>106 ± 4</td>
<td>111 ± 2</td>
<td>110 ± 3</td>
</tr>
<tr>
<td>Fasting insulin, ng/ml</td>
<td>0.46 ± 0.06a</td>
<td>1.06 ± 0.09b</td>
<td>0.57 ± 0.12ab</td>
<td>0.78 ± 0.11b</td>
<td>0.95 ± 0.14b</td>
<td>0.76 ± 0.11ab</td>
</tr>
</tbody>
</table>

Data are means ± SE for groups of rats killed at the end of 23 days exposure to the experimental diets. Fasting glucose and insulin values are for time 0 of the intraperitoneal glucose tolerance test (GTT) performed on day 12 when the rats had been food deprived for 5 h. Ing, inguinal; RP, retroperitoneal; IBAT, intrascapular brown tissue; FFA, free fatty acid; TG, triglyceride. Values for any parameter that do not share a common superscript are significantly different at $P < 0.05$. 

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chow + lard and HFD rats had the highest fat intake. It is possible that an undefined component of the HFD prevented development of leptin resistance that would be caused by fat consumption, but we were unable to control for this because we have previously found that rats offered the choice of LFD plus lard and sucrose solution do not become obese (4).

Finally, leptin resistance was not associated with sucrose consumption because the LFD rats consumed the same amount of sucrose as the choice rats, but these animals remained leptin responsive.

Others have reported the development of leptin resistance in rats fed a composite HFD, but for a majority of these experi-

**Energy Intake Experiment 2**

**Body Weight Experiment 2**

*Fig. 4. UCP-1 protein in intrascapular brown adipose tissue (IBAT), inguinal (Ing), and retroperitoneal (RP) fat of rats in experiment 1. The protein is expressed as a ratio of UCP-1 to actin determined by Western blot. Values for IBAT UCP-1 that do not share a common superscript letter are significantly different at $P < 0.05$. Data are means ± SE for groups of 10 rats.*

*Fig. 5. Energy intake and body weights of rats in experiment 2. Data are means ± SE for groups of 10 rats. *Days on which choice rats weighed significantly more than their chow controls.*
ments the rats were fed the diet for longer than 17 days before leptin resistance was detected. Many experiments testing for leptin responsiveness have administered leptin centrally, into either the third or fourth ventricle. Of those in which HFD-fed rats have received peripheral injections of leptin, Steinberg et al. (62) reported that rats developed leptin resistance in skeletal muscle after 4 wk on a 60% fat diet, and Fisher 344 × brown Norway (F344XBN) rats fed a 32% fat diet for 5 mo were resistant to the weight-reducing effects of peripheral infusions of leptin (57). de Lartigue et al. (15) found in vivo and in vitro leptin resistance in vagal afferent neurons from rats offered a 45% kcal fat diet for 8 wk, Shapiro et al. (59) found that rats fed a 30% kcal fat diet were leptin resistant after 65 days, and we found that Sprague-Dawley rats fed a 30% fat diet were resistant after 39 days (22). By contrast, Lin et al. (37) reported that Osbourne Mendel rats were resistant to peripheral injections of leptin after only 5 days on a 56% kcal fat diet (37). The reason for the rapid onset of resistance in this study may be associated with the obesity-prone Osbourne Mendel rats, which are relatively leptin resistant even on a LFD (26). The cause of leptin resistance in HFD-fed rats and mice has been associated with a downregulation of leptin receptors (39) and increased expression of suppressor of cytokine signaling 3 (15, 62) and protein tyrosine phosphatase 1B (69), both of which inhibit leptin receptor signaling. We did not measure any of these factors in our studies but found no differences in non-stimulated levels of phosphorylation of leptin signaling proteins in experiment 2.

Recently, Shapiro et al. (59) have proposed that both fat and fructose in the diet are required for rats to become leptin resistant and that obesity in animals fed a high-fat, high-sucrose diet is secondary to the development of leptin resistance. The results from experiment 1 are only partially consistent with this concept because the two groups of rats that became leptin resistant were consuming large amounts of sucrose and were gaining weight but were not necessarily consuming a large amount of fat. Therefore, it seems possible that leptin resistance develops when animals are consuming...
sucrose and are in a state of positive energy balance. The relatively early development of leptin resistance in experiment 1 was limited to the two groups of rats that were consuming sucrose solution, and it is possible that the form in which sucrose is consumed is important. When rats drink sucrose solution they are obtaining glucose and fructose in isolation from other macronutrients, whereas the LFD rats consumed the same amount of sucrose as choice rats, but the sucrose would have been part of a mixed meal. The results of experiment 1 do not allow us to separate the effect of consuming sucrose solution from consuming sucrose while in a state of positive energy balance.

In experiment 1 we did not test whether glucose, fructose, or any simple carbohydrate would have induced leptin resistance. Shapiro et al. (58) reported that rats fed a 67% kcal fructose diet were leptin resistant after 6 mo, and we (22) have previously reported that Sprague-Dawley rats fed a composite diet containing 60% kcal fructose for 25 days are resistant to peripheral injections of leptin but are responsive to central injections of leptin. In addition, rats fed diets containing either 40% kcal fructose and 10% kcal glucose or 50% kcal glucose become resistant to peripheral leptin injections after 65 days (22). In contrast to these data, we were surprised to find that the addition of fructose or glucose to a 30% kcal fat diet prevented development of leptin resistance caused by a 30% fat diet that had a low simple carbohydrate content (22). The studies described above, in which fructose was fed as part of a dry composite diet, suggest that fructose-induced leptin resistance would take longer than 17 days to develop. The rats offered choice diet in experiment 1 consumed ~30% of their calories as sucrose solution plus whatever sucrose was present in chow. Therefore, if the effect is specific to fructose, inclusion of 15% kcal fructose in solution is enough to induce leptin resistance within 17 days in rats that are overeating. It is clear from experiment 1 that the early onset of leptin resistance is associated with consumption of sucrose in solution, and it is possible that delivery of fructose and glucose as a bolus and

unaccompanied by other nutrients has a different metabolic impact than consumption of simple carbohydrate as part of a mixed diet.

Others have proposed that hyperleptinemia will induce leptin resistance, but that was not the case here because HFD rats had higher circulating leptin concentrations than chow+LS rats. Similarly, Banks et al. (7) demonstrated that high circulating concentrations of TG caused leptin resistance in mice, but serum TG were higher in LFD than in choice rats, and LFD rats remained leptin responsive. We did not measure leptin-stimulated phosphorylation of leptin signaling proteins in any of the studies described here, therefore, further studies are needed to determine the metabolic and molecular basis of leptin resistance in choice rats. Because both choice and chow+LS rats were consuming excess calories and a relatively large proportion of these calories were from sucrose solution, an obvious avenue for investigation of leptin resistance would be associated with metabolic pathways that respond to the presence of increased availability of glucose and fructose. One possibility is the hexosamine biosynthetic pathway that produces UDP-N-acetyl-glucosamine, a substrate for O-linked glycosylation of threonine and serine residues of a multitude of proteins (24). Normally only ~3% of glucose enters the hexosamine biosynthetic pathway (HBP), but this can increase when glucose load increases. Increased activity of the HBP in transgenic mice (14) or aging rats (17) results in insulin resistance, but serum TG were higher in LFD than in choice rats, and LFD rats remained leptin responsive. We did not measure leptin-stimulated phosphorylation of leptin signaling proteins in any of the studies described here, therefore, further studies are needed to determine the metabolic and molecular basis of leptin resistance in choice rats. Because both choice and chow+LS rats were consuming excess calories and a relatively large proportion of these calories were from sucrose solution, an obvious avenue for investigation of leptin resistance would be associated with metabolic pathways that respond to the presence of increased availability of glucose and fructose. One possibility is the hexosamine biosynthetic pathway that produces UDP-N-acetyl-glucosamine, a substrate for O-linked glycosylation of threonine and serine residues of a multitude of proteins (24). Normally only ~3% of glucose enters the hexosamine biosynthetic pathway (HBP), but this can increase when glucose load increases. Increased activity of the HBP in transgenic mice (14) or aging rats (17) results in insulin resistance.
resistance because multiple insulin signaling proteins are subject to O-linked glycosylation (6, 32, 33). Leptin and insulin have some signaling proteins in common (43, 66), and it is possible that consumption of sucrose solution leads to O-linked glycosylation of proteins and transcription factors that are critical for effective leptin signaling.

Measurement of UCP-1 expression in brown adipose tissue from the rats in experiment 1 indicated that consumption of sucrose solution leads to O-linked glycosylation of proteins and transcription factors that are critical for effective leptin signaling.

Fig. 8. Energy expenditure, respiratory exchange ratio (RER), and activity of rats housed in the calorimeter in experiment 3. Three different time points are represented: BL is baseline before rats were offered choice diet. Day 3 represents measurements from the third day that the rats were offered choice diet. Day 12 is the 12th day that the rats were offered choice diet. Open bars show average measures for the light phase (7:00 AM-7:00 PM); filled bars represent measures for the dark period. Rats were housed in the calorimeter but measurements were taken only from the third day for each of the time periods. Data are means ± SE for groups of 8 rats. Values on a specific axis that do not share a common superscript are significantly different at P < 0.05.

A dissociation between the levels of UCP1 mRNA and UCP1 protein levels is not uncommon (8, 27, 45) and has been attributed to large differences in the rate of turnover of the mRNA (hours) compared with the protein (days or weeks) (11, 28). It is well established that sucrose (30, 64) and dietary fat (20) increase BAT UCP-1 expression even though the increase in UCP-1 is not necessarily associated with increased thermogenesis (64). We also measured UCP-1 in white adipose tissue because activation of the sympathetic nervous system (44) or chronic cold exposure promotes expression of UCP-1 in white fat depots (67). In experiment 2 UCP-1 protein was significantly increased in Ing, but not RP fat of choice rats. Because uncoupling in brown adipose tissue is associated with increased fatty acid oxidation, it would be of interest to determine whether the exaggerated enlargement of RP fat depots in choice rats was due to a failure to increase expression of UCP-1.
Experiment 3 tested whether the increase in UCP-1 expression was associated with an increase in energy expenditure. There was a significant increase in energy expenditure of choice rats during the dark period, but it was not associated with a decrease in RER, which was not different from that of chow-fed controls during either the light or dark period, even though they were consuming ~45% of their energy intake as lard. This suggests that the choice rats were continuing to oxidize carbohydrate and were storing lipid. The sucrose and chow intake of choice rats most likely exceeded the carbohydrate needed for oxidation because liver glycogen content also increased in choice rats. Part of the increase in energy expenditure of choice rats may have been due to obligatory thermogenesis, which is the energy cost of moving a larger body and of digesting and processing extra calories (29). In rats that had been fed choice diet for 12 days there also was a significant increase in nocturnal activity, which would have contributed to the increase in energy expenditure. Therefore, either the increase in UCP-1 protein expression did not result in increased uncoupling of oxidative phosphorylation, or the effect was too small to detect by indirect calorimetry. So et al. (61) reported somewhat similar results for rats fed a 60% kcal fat diet for 8 wk. At the end of the study BAT UCP-1 protein was increased more than threefold in HFD-fed rats, but nocturnal activity decreased and there was no increase in energy expenditure after the first 2 wk of exposure to the diet. We did not measure energy expenditure of HFD rats in this study, but the results from experiment 1, in which choice rats consumed more energy, but gained less weight than HFD rats, would suggest that the HFD did not elevate expenditure to the same degree as the choice diet. In addition, unlike the choice diet, the HFD did not stimulate UCP-1 mRNA expression in IBAT. This would support the notion that the two diets had differential effects on factors that contributed to the metabolic state and energy balance of the rats.

Perspectives And Significance

The studies described here indicate that rats offered choice diet develop glucose intolerance and leptin resistance faster than rats fed a composite HFD, despite similar increases in fat intake and accrual of body fat mass. The metabolic impairments in choice rats appear to be associated with consumption of sucrose, and leptin resistance is specifically associated with consumption of sucrose solution by animals that are also consuming excess calories. Drinking sucrose solution results in delivery of boluses of simple carbohydrate in the absence of any other nutrient, and development of leptin resistance may be due to specific aspects of the metabolism of sucrose solution, or it may be due to the combination of the metabolic processing of sucrose in combination with an excess energy intake. There has recently been a significant interest in the association between consumption of sweetened beverages and risk for metabolic syndrome, cardiovascular disease, and obesity (10, 16, 41), and it is possible that the choice diet or chow+LS provide animal models that allow investigation of the mechanistic basis for this correlation. Choice diet also promotes expression of UCP-1 in both brown and white adipose tissue, but this does not seem to produce a significant level of diet-induced thermogenesis. If uncoupling is increased in choice animals, it is not sufficient to inhibit weight gain and obesity.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: R.B.H. and J.W.A. conception and design of research; R.B.H. and J.W.A. performed experiments; R.B.H. and J.W.A. analyzed data; R.B.H. and J.W.A. interpreted results of experiments; R.B.H. prepared figures; R.B.H. drafted manuscript; R.B.H. and J.W.A. edited and revised manuscript; R.B.H. and J.W.A. approved final version of manuscript.

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