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Fructose-induced leptin resistance exacerbates weight gain in response to subsequent high-fat feeding

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Shapiro A, Mu W, Roncal C, Cheng K-Y, Johnson RJ, Scarpace PJ. Fructose-induced leptin resistance exacerbates weight gain in response to subsequent high-fat feeding. *Am J Physiol Regul Integr Comp Physiol* 295: R1370–R1375, 2008. First published August 13, 2008; doi:10.1152/ajpregu.00195.2008.—It has been suggested that increased fructose intake is associated with obesity. We hypothesized that chronic fructose consumption causes leptin resistance, which subsequently may promote the development of obesity in response to a high-fat diet. Sprague-Dawley rats were fed a fructose-free control or 60% fructose diet for 6 mo and then tested for leptin resistance. Half of the rats in each group were then switched to high-fat diet for 2 wk, while the other half continued on their respective diets. Chronic fructose consumption caused leptin resistance, while serum leptin levels, weight, and adiposity were the same as in control rats that were leptin responsive. Intraperitoneal leptin injections reduced 24-h food intake in the fructose-free group (73.7 ± 6.3 vs. 58.1 ± 8 kcal, $P = 0.02$) but had no effect in fructose-fed rats (71.2 ± 6.6 vs. 72.4 ± 6.4 kcal, $P = 0.9$). Absence of anorexic response to intraperitoneal leptin injection was associated with 25.7% decrease in hypothalamic signal transducer and activator of transcription 3 phosphorylation in the high-fructose-fed rats compared with controls ($P = 0.015$). Subsequent exposure of the fructose-mediated, leptin-resistant rats to a high-fat diet led to exacerbated weight gain (50.2 ± 2 g) compared with correspondingly fed leptin-responsive animals that were pretreated with the fructose-free diet (30.4 ± 5.8 g, $P = 0.012$). Our data indicate that chronic fructose consumption induces leptin resistance prior to body weight, adiposity, serum leptin, insulin, or glucose increases, and this fructose-induced leptin resistance accelerates high-fat induced obesity.

obesity

IT HAS BEEN ONLY HALF A CENTURY since obesity was introduced into the international classification of diseases, and today obesity is epidemic (15, 21–24). Recently, obesity has been increasing rapidly among children and adolescents (1), suggesting that environmental factors are involved. While multiple factors likely contribute to the ongoing epidemic, including excessive energy intake and diminished physical activity, there is also increasing evidence that certain food items, such as fructose, may have a pathogenic role (5, 11, 16). Epidemiological studies indicate a correlation between the introduction and spread of fructose-enriched products, sugar consumption, and the increased rate of obesity (4, 5, 16). Rodents administered a high-fructose diet develop most of the features of metabolic syndrome, including hypertriglyceridemia, impaired glucose tolerance, hyperinsulinemia, insulin resistance, hyperuricemia, endothelial dysfunction, high blood pressure, and increased body weight (16). These changes are not observed in animals fed equivalent amounts of glucose (26).

Several mechanisms have been proposed to account for the increased weight gain associated with fructose intake. Fructose has been suggested to increase palatability of food with the consequence of overeating (5). Acute fructose ingestion fails to stimulate both insulin and leptin secretion, and attenuates postprandial ghrelin suppression (36); thus prolonged consumption of diets high in fructose could lead to increased caloric intake and contribute to weight gain and obesity. Studies, both in rats (13, 18, 25, 29) and humans (17), have reported that chronic fructose ingestion is associated with an increase in plasma leptin levels, and some studies report this increase precedes the obesity (18, 25, 29). Elevated plasma leptin is sometimes a sign of leptin resistance (33), but more importantly, the presence of leptin resistance could potentially be a major mechanism for inducing obesity. We therefore tested two hypotheses: *1*) that chronic high-fructose feeding induces leptin resistance and *2*) whether this leads to an increased susceptibility to weight gain.

RESEARCH DESIGN AND METHODS

Experimental Animals

Twenty-three male Sprague Dawley rats, obtained from Harlan Laboratories, were cared for in accordance with the principles of the "Guide to the Care and Use of Experimental Animals." Protocols were approved by the University of Florida Institutional Animal Care and Use Committee. Rats were housed individually with a 12:12-h light-dark cycle (07:00 to 19:00).

Experimental Design

Rats were raised on regular rodent chow diet by the breeder until 2.5 mo of age. Upon arrival, 11 rats were switched to a no-fructose control diet [5.2% fat (lard), 60% corn starch, 18.3% protein (mainly casein), 3.6 kcal/g, diet TD.05075; Harlan Teklad, Madison, WI], and 12 rats to a high-fructose diet [5.2% fat (lard), 60.4% fructose, 18.8% protein (mainly casein), 3.6 kcal/g, diet TD.89247; Harlan Teklad] ad libitum. Food consumption and body weight were recorded daily for the first 3 mo, weekly for the following 3 mo, and daily again upon introduction of the new high-fat diet. Fasting and nonfasting blood was collected by removal from tail vein upon arrival, at 12 and 24 wk, and at death. Every 30 days, body composition was measured by time domain nuclear magnetic resonance (TDNMR) using a Minispec lean fat analyzer (Bruker Optics, The Woodlands, TX). Validation of TDNMR methodology has been provided (37). After 6 mo on the

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respective diets, half of the rats in each group were switched to a high-fat diet [60% kcal from fat (lard) and 7% kcal from sucrose, 5.24 kcal/g diet D12492; Research Diets, New Brunswick, NJ]. The experiment was terminated 14 days after the initiation of the high-fat diet. There were six animals per group except for the control > high-fat group, where there were five animals.

Tissue Harvesting and Preparation

Rats were killed by thoracotomy under isoflurane anesthesia. The circulatory system was perfused with 20 ml of cold saline by injection into the right ventricle and epididymal, perirenal, and retroperitoneal white adipose tissues; and hypothalami were excised. For removal of the hypothalamus, an incision was made medial to the piriform lobes, caudal to the optic chiasm, and anterior to the cerebral crus, to a depth of 2–3 mm. The hypothalami were sonicated in 10 mM Tris·HCl (pH 6.8), 2% SDS, and 0.08 μg/ml okadaic acid + protease inhibitors. Protein concentrations were determined using the DC protein assay kit (Bio-Rad, Hercules, CA).

Western Blot Analysis

The methods for signal transducer and activator of transcription 3 (STAT3) and phosphorylated STAT3 (pSTAT3) assay were described in detail previously (20). Protein homogenates (20 μg) from processed tissues were boiled for 5 min, separated on a 10% agarose-Tris·HCl gel (Bio-Rad) and electrotransferred to polyvinylidene fluoride membranes (30). The membranes were probed using antibodies specific to the target protein (total STAT3, phosphotyrosine 705 of STAT3; Cell Signaling, Danvers, MA), or leptin receptor antibody (ABR, Golden, CO). Immunoreactivity was visualized by the ECL Plus detection system (Amersham, Piscataway, NJ) and quantified by ImageQuant TL (Amersham). When the values were quantified for each sample, the signal obtained for pSTAT3 was divided by the signal obtained for total STAT3. The average value in the control group was set to 100%, and all other values were normalized accordingly.

Leptin Responsiveness

Food was removed from the cages at 9:00. Rats received intraperitoneal injections of saline at 16:30. Food was returned immediately after the injections and food intake was recorded 24 h later. In the initial assessment of leptin responsiveness, food intake was also assessed at 2, 4, and 15 h after leptin administration. The next day, the same rats received intraperitoneal injections of 0.6 mg/kg rat leptin (PeproTech) following the same protocol. Food intake of each individual animal was compared between these 2 days using two-way ANOVA with repeated measures followed by the post hoc analysis.

Biochemical Measurements

Serum glucose, cholesterol, triglycerides, uric acid, blood urea nitrogen (BUN), and creatinine concentrations were determined by autoanalyzer (VetAce; Alfa Wassermann, West Caldwell, NJ).

RT-PCR

Expression levels of suppressor of cytokine signaling 3 (SOCS3) in the hypothalamus were identified by relative quantitative RT-PCR using the QuantumRNA 18S Internal Standards kit (Ambion, Austin, TX) as described previously (41).

Serum Insulin and Leptin

Fasting serum insulin levels were obtained at *week 20*. Serum insulin was determined with the rat insulin ELISA kit (Crystal Chem, Chicago, IL). Radioimmunoassay was used to determine serum leptin (Millipore, Billerica, MA).

Statistical Analysis

Data were analyzed by two-way ANOVA. When the main effect was significant, a Bonferroni post hoc test was applied to determine individual differences between means. A value of $P < 0.05$ was considered significant.

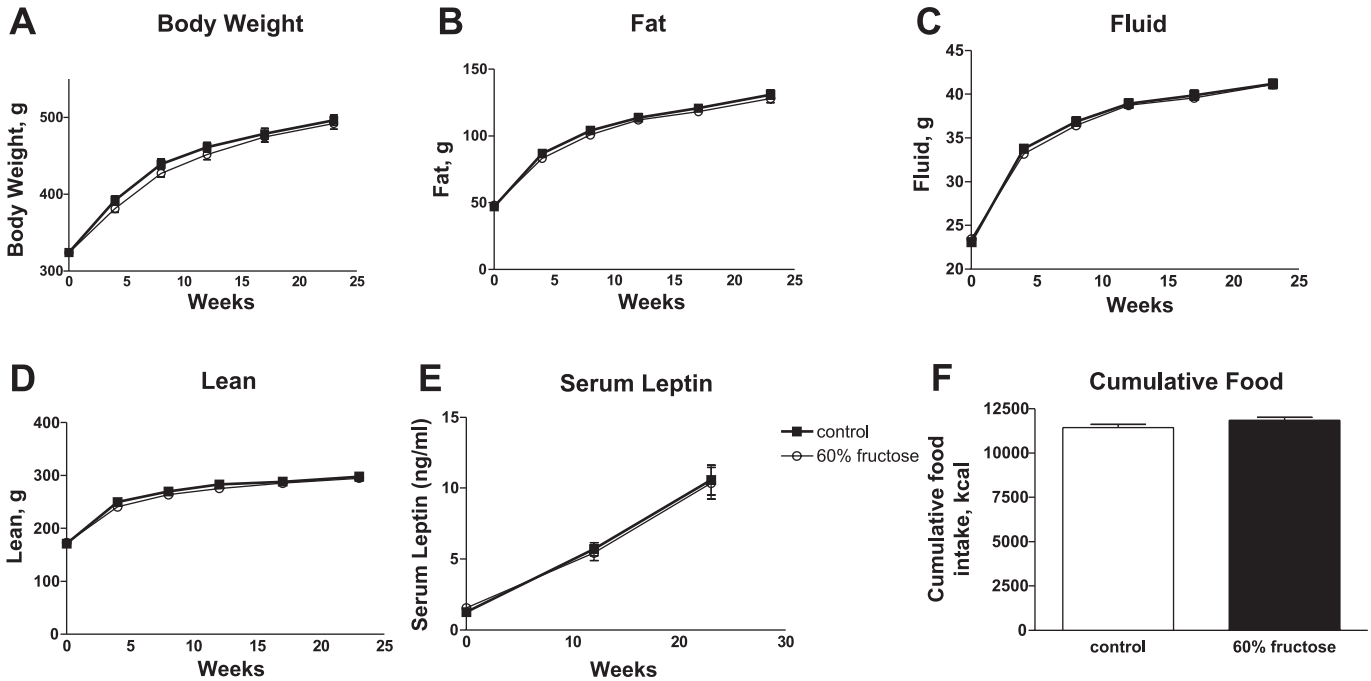

Fig. 1. Changes in body weight (*A*), body composition including fat (*B*), fluid (*C*), lean (*D*), serum leptin (*E*), and cumulative food intake (*F*) over 24-wk in control (fructose-free)-fed and 60% fructose-fed Sprague Dawley rats. Values are means ± SE of 11 fructose-free and 12 high-fructose-fed rats.

Table 1. *Biochemical measurements of serum and adiposity at the end of the experiment*

	Control	High Fructose	Control > High Fat	High Fructose > High Fat
Nonfasting triglycerides, mg/dl	143.3±12.3	280.7±32*	135.5±11.4	277.3±34.5*
Nonfasting uric acid, mg/dl	1.9±0.1	2.0±0.4	2.52±0.4	3.8±0.4†
Nonfasting leptin, ng/dl	5.4±1.7	5.0±1	10.1±2.6	10.4±2
Nonfasting glucose, mg/dl	194±11.8	193.5±6.9	209±23	214.2±21.5
Fasting glucose, mg/dl	161.8±12.5	160±26.7	145.4±16.3	146.5±10.7
Nonfasting cholesterol, mg/dl	113.3±9.1	146±12.8	118.6±12.5	144.7±15.6
Nonfasting BUN, mg/dl	19.2±0.7	18.5±1.3	18.8±1.8	19±1.7
Nonfasting creatinine, mg/dl	0.4±0.0	0.4±0.0	0.4±0.5	0.4±0.4
WAT, g	17.2±1.4	15.3±1.9	20.5±2.5	23.8±1.7‡
Total adiposity, g	129.8±6.2	129.1±3.4	138.9±10	143.3±3.5§

Data are means ± SE. Values are from nonfasting blood collection, except glucose, which is from either nonfasting or fasting blood collection. BUN, blood urea nitrogen; WAT, sum of perirenal, retroperitoneal, and epididymal white adipose tissues; total adiposity, total body fat assessed by time domain nuclear magnetic resonance. *$P < 0.0001$ for difference with high-fructose diet by two-way ANOVA. $P < 0.01$ for difference between control and control>high-fat diets by post hoc analysis. $P < 0.01$ for difference between high-fructose and high-fructose>high-fat diets by post hoc analysis. †$P = 0.0027$ for difference with high-fat diet by 2-way ANOVA. $P < 0.05$ for difference between high-fructose and high-fructose>high-fat diets by post hoc analysis. ‡$P = 0.0057$ for difference with high-fat diet by 2-way ANOVA. $P < 0.01$ for difference between high-fructose and high-fructose>high-fat diets by post hoc analysis. §$P = 0.016$ for difference between high-fructose and high-fructose>high-fat diets by *t*-test.

RESULTS

Response to High-Fructose Diet: Rats on Fructose-Free and 60% Fructose Diets

Body weight, adiposity, body composition, and food consumption. At an age of 2.5 mo and body weight of 324 ± 3.9 g, the rats were separated into two groups and provided either the fructose-free diet (control diet) or the high-fructose diet. Rats in both groups steadily gained weight with similar rates over the next 6 mo. High-fructose-fed rats were slightly lighter, though not significantly different than controls (Fig. 1*A*). Total body fat, assessed by TDNMR (Fig. 1*B*), as well as by the sum of the amount of epididymal, perirenal, and retroperitoneal white adipose tissues at death (17.2 ± 1.4 g vs. 15.3 ± 1.9 g), was similar in control and high-fructose groups. Changes in body fat as well as other measures of body composition (i.e., lean and fluid components assessed by TDNMR) paralleled those of total body weight (Fig. 1, *B, C,* and *D*). Food consumption was similar in both groups; cumulative food intake over 24 wk was virtually identical in control animals (11433.6 ± 206.2 kcal) compared with high-fructose-fed rats (11847.6 ± 179.6 kcal; Fig. 1*F*). Overall, control and high-fructose-fed rats demonstrated no differences in food intake or body weight gain over the course of 6 mo.

Serum leptin, triglycerides, glucose, uric acid, creatinine, BUN, and cholesterol. Despite the fact that high doses of fructose have been reported to induce metabolic syndrome in rats, in this study, the only significant difference was in serum triglycerides. In contrast, no differences were noted at the end of the experiment in serum cholesterol, uric acid, fasting glucose levels, BUN, or serum creatinine (Table 1, control and high fructose columns). Serum leptin levels were also unchanged between groups but rose in both groups in parallel with the steady increase in adiposity (Fig. 1*E*). Similarly, fasting serum insulin levels in fructose-fed vs. control rats (1.9 ± 0.2 vs. 2.4 ± 0.4 ng/ml) and blood glucose levels following 4-h fasting in fructose-fed rats vs. controls (158.8 ± 23.2 vs. 149.1 ± 31 mg/dl) obtained at *week 20* were also unchanged between groups.

Leptin responsiveness. Leptin responsiveness was assessed at 2.5 mo of age prior to initiating the fructose-free and high-fructose diets and at the end of the 6 mo on these respective diets prior to switching the rats to the high-fat diet. Initially, all rats responded to the leptin with a decrease in the 24-h food consumption (Fig. 2*A*). Food intake was also assessed at 2, 4, and 15 h after leptin administration; although food consumption was lower with leptin treatment (data not shown), the differences were more significant at 24 h. Interestingly, the reduced anorexic response was positively correlated with increased serum triglycerides levels ($r^2 = 0.35$, $P = 0.04$).

Subsequently, after 6 mo, rats fed control (fructose-free) diet remained responsive to exogenous leptin with a significant reduction in 24-h food intake (Fig. 2*B*). In contrast, rats that

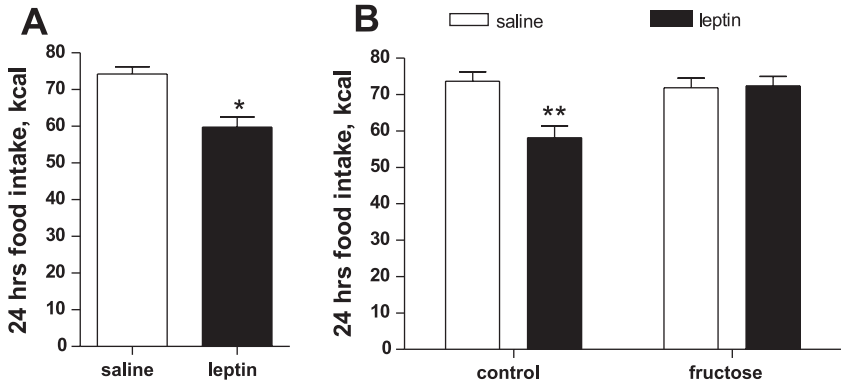

Fig. 2. Leptin responsiveness as measured by cumulative food intake 24 h after an injection of either saline or 0.6 mg/kg ip leptin prior to initiating the experimental diets (*A*) and after 6 mo on either the fructose-free control or 60% fructose diet (*B*). Values represent the means ± SE of 6 fructose-free and 6 high-fructose-fed rats. *A*: *$P = 0.03$ for difference with leptin administration by paired *t*-test. *B*: **$P < 0.04$ for difference with leptin; $P < 0.02$ for difference with diet; and $P < 0.04$ for interaction by 2-way ANOVA with repeated measures. $P = 0.02$ for difference with leptin among the control animals by post hoc analysis.

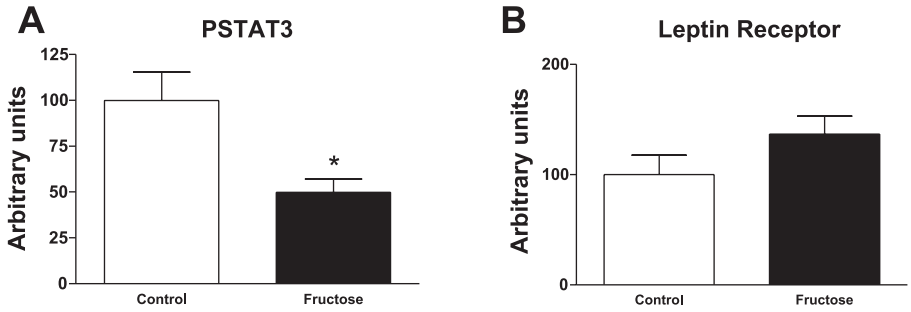

Fig. 3. Hypothalamic protein levels of phosphorylated signal transducer and activator of transcription 3 (pSTAT3; *A*) and leptin receptor (*B*) in fructose-free control and 60% fructose-fed rats. Values represent the means ± SE of 5 fructose-free- and 6 high-fructose-fed rats. *$P = 0.015$ for difference with high-fructose feeding by *t*-test.

were fed the high-fructose diet did not respond to leptin (Fig. 2*B*).

Hypothalamic signaling factors. Three markers of leptin signaling in the hypothalamus were evaluated: leptin receptor and pSTAT3 protein levels and SOCS3 expression levels. Basal hypothalamic pSTAT3 levels were significantly lower in the high-fructose-fed animals than in controls, while protein levels of leptin receptor were similar and not statistically different between groups (Fig. 3). Hypothalamic SOCS3 gene expression was decreased, although not significantly, in fructose-fed animals (100% vs. 74%, $P = 0.4$).

Response to High-Fat Diet: Rats on Fructose-Free and 60% Fructose Diets Switched to High-Fat Diet

The observation that fructose-fed rats were unresponsive to leptin suggests that they might be more susceptible to weight gain if provided a high-fat diet. To test this hypothesis, the high-fructose or control diets were discontinued in half of the rats in each group and these rats were switched to a high-fat Western-type diet, whereas the other half were maintained on their respective fructose-free or high-fructose diets.

Food consumption and body weight. Both control- and fructose-fed rats demonstrated increased food intake and increased weight gain when they were switched to the high-fat diet, but rats that had previously been fed fructose demonstrated a significantly higher energy intake along with a significantly higher weight gain (Fig. 4). Adiposity gain over the course of the high-fat diet was also significantly higher in fructose rats than in controls assessed by both whole body adiposity and the sum of three adiposity tissues at death (Table 1).

Serum factors. During the second week of high-fat feeding, fasted blood was collected for glucose assessment, but these values were unchanged across groups (Table 1). At the end of the experiment, nonfasted blood was collected for assessment of several metabolic parameters in the four groups of rats: controls, fructose-fed, and controls and fructose-fed rats switched to the high-fat diet, respectively (Table 1). Interestingly, uric acid levels increased in both groups of rats switched to the high-fat diet but were not elevated in those maintained on high fructose. Triglycerides, in contrast, were elevated with the high-fructose diet, but not with the high-fat diet: serum triglycerides levels were the same between controls and controls switched to high-fat diet, as well as between high-fructose-fed rats and those that were first fed high fructose and then switched to the high-fat diet. Thus, the high-fat diet did not affect serum triglycerides, while fructose feeding doubled their levels (Table 1). Moreover, elevated triglycerides levels were positively correlated with body weight gain following high-fat feeding ($r^2 = 0.88$, $P = 0.02$) among the fructose-fed rats switched to the high-fat diet. However, there was no correlation between these variables for the control-fed rats switched to the high-fat diet.

DISCUSSION

The adipocyte-derived hormone leptin has a critical role in energy balance and body weight regulation through interactions with hypothalamic nuclei to reduce food intake and increase energy expenditure (2, 10). Leptin circulates at levels proportional to body fat and is a key afferent signal linking adiposity level and nutritional status. However, with diet-induced obesity, even though leptin levels rise proportionally with adiposity, these elevated leptin levels fail to prevent weight gain (33). Such reduced response or unresponsiveness to the metabolic effects of endogenous or exogenous leptin is described as leptin resistance, and is characteristic of both obese humans (12, 28) and rodents (19, 40). Moreover, our experimental models of leptin resistance in rats suggest that such leptin resistance not only prevents the metabolic responses to leptin but also is one cause of obesity, including diet-induced obesity. For example, chronic exposure to elevated central leptin through rAAV-vector-mediated overex-

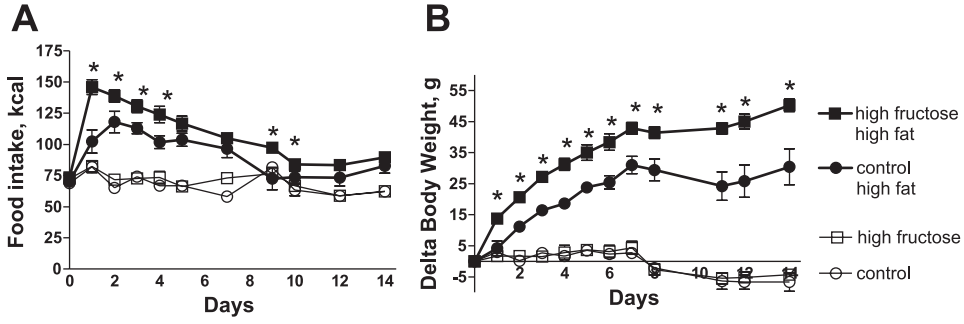

Fig. 4. Food intake (*A*) and change in body weight (*B*) in rats either maintained on the fructose-free (control; $n = 5$) or high-fructose diets ($n = 6$) or switched from the control to high-fat diet ($n = 6$) or from high-fructose to high-fat ($n = 6$) diets. Values represent the means ± SE. *$P < 0.01$ for difference with high-fat diet by *t*-test at individual days. Note that several assessments were performed on the rats during the 2nd week of high-fat feeding, including fasting prior to blood collection, which disturbed normal food intake and body weight.

pression results in a leptin resistance in the absence of obesity. Such rats are unresponsive to exogenously administered central leptin (32). When these rats are then exposed to a high-fat diet, they gain more weight and adiposity compared with weight-matched, leptin-responsive, high-fat fed counterparts (31). Conversely, if rats are first fed a high-fat diet and then chronically exposed to elevated central leptin (resulting in leptin resistance), such rats also gain greater body weight and adiposity compared with their high-fat fed counterparts without leptin resistance (34). Collectively, these data indicate that leptin resistance increases the susceptibility for diet-induced obesity.

In this study, we tested the hypothesis that chronic fructose feeding induces leptin resistance, which in turn could predispose animals to increased weight gain in response to a high-fat diet. Several salient findings were observed. First, chronic fructose feeding caused functional leptin resistance as demonstrated by an absent anorexic response to exogenous leptin. Interestingly, this leptin resistance occurred in the absence of either increased body weight or elevated serum leptin: fructose-fed and control-fed rats had the same body weight and serum leptin levels. Second, after the onset of leptin resistance, switching to a Western-type diet resulted in significantly increased energy intake, adiposity levels, and weight gain compared with control rats provided the same diet. Finally, we documented diminished pSTAT3 (one downstream component of the leptin receptor signaling cascade) in fructose-fed rats. This diminished pSTAT3 occurred despite equivalent expression of leptin receptors in the hypothalamus. In conclusion, these studies provide the first documentation that high-fructose diets can induce leptin resistance that subsequently predisposes the animal to the development of dietary obesity.

The mechanism underlying the fructose-induced leptin resistance is not known. Central leptin resistance appears to involve impaired leptin signal transduction, including reduced activation of JAK-mediated STAT3 phosphorylation, defective leptin receptor signaling, and/or a subsequent failure to stimulate downstream signaling events (8, 33). Because we observed reduced responses to peripherally administered leptin, another possibility is that the exogenous leptin failed to reach target sites in the brain, i.e., the resistance might be associated with impaired leptin transport across the blood-brain barrier (BBB). In the present study, basal levels of pSTAT3 were reduced in the hypothalamus. Many neuropeptides are known to signal through the JAK-STAT pathway, and basal pSTAT3 levels are likely the compilation of multiple signaling events, including those mediated by leptin. However, reduced basal pSTAT3 is consistent with a diminished anorexic response to leptin. Serum triglycerides are known to impair the ability of the BBB to transport leptin, and impaired transport can cause leptin resistance (3). In the present study, fructose feeding elevated serum triglycerides levels, suggesting that defective BBB transport could partially account for resistance to leptin in these fructose-fed animals. Consistent with this contention is that serum triglycerides levels positively correlated with the reduced anorexic response after acute peripheral leptin and with body weight gain when rats were switched to high-fat diet.

On the other hand, both defective signaling and an inability of leptin to cross the BBB may be contributing to the leptin resistance. For example, fructose feeding induces a hepatic leptin resistance characterized by deficient serine pSTAT3 within the nuclei of liver cells (29).

Interestingly, after 6 mo of high-fructose consumption there was only mild evidence of metabolic syndrome; rats on the 60% fructose diet had elevated serum triglycerides but displayed no changes in weight, adiposity, blood glucose, fasting insulin, or serum uric acid levels. These findings are consistent with some (9, 35, 38), but inconsistent with other reports in the literature (6, 14, 18, 25, 26). The variability in responses could be due to the differences in experimental design: administration of fructose diet at an earlier age than in our experiment (6, 18, 26), higher fructose percentile than in our experiment (6, 14, 25), or presence of sucrose in the diet (35). In addition, variability to fructose feeding has been observed with different rat strains (7, 39) and among identical rat strains supplied by different vendors (27, 35).

Perspectives and Significance

These studies demonstrate that chronic fructose feeding renders animals leptin resistant. A leptin-resistant state disrupts normal energy homeostasis, favors positive energy storage, and thus predisposes to obesity. As such, when fructose-fed, leptin-resistant animals are provided with a calorie-rich diet, the rate and extent of body weight and adiposity are augmented compared with control leptin-responsive rats switched to the calorie-rich diet. A key finding in this study is that a silent leptin resistance has occurred with almost no physiological or biochemical differences detected between the fructose- and control-fed groups. Thus, a high-fructose diet predisposes to obesity, and deleterious effects of chronic fructose consumption develop long before any visible signs of elevated leptin or the metabolic syndrome. The significance is that this mechanism might well explain why fructose ingestion is associated with obesity. While these studies were performed in rats exposed to high-fructose concentrations, it demonstrates a potential mechanism for the development of obesity in response to fructose that should be explored in future clinical studies.

GRANTS

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DISCLAIMERS

R. J. Johnson is listed as an inventor on several patent applications related to fructose and obesity and has a lay book on fructose that was published by Rodale, Inc. in 2008.

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