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Effect of High-Fat Diet on Body Composition and Hormone Responses to Glucose Tolerance Tests

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To determine potential hormonal mediators of the effect of high-fat diets on the development of insulin resistance, blood leptin, growth hormone (GH), glucose, and insulin responses to a 2 g/kg BW oral glucose challenge were evaluated in weanling female Sprague–Dawley rats that were randomly assigned to a high-fat (HF, 39% of calories, 20% fat by weight; *n* = 10) and moderate-fat (MF, 22% of calories, 10% fat by weight; *n* = 10) diets. Oral glucose challenges were administered following 5, 7, and 9 wk on the feeding trial. Animals were provided diet in excess of their requirements for growth. Body mass analysis was conducted by dual X-ray absorptiometry (DXA) on the 6th, 8th, and 10th weeks of the trial. HF animals gained more weight after 7 wk, had greater body fat than the MF animals, and similar glucose responses to the oral glucose challenges. HF rats secreted more insulin and leptin compared to MF animals. Lean body mass and serum GH and IGF-I concentrations were not different between the groups. Results of this study demonstrate that leptin but not GH or IGF-I is involved in the development of insulin resistance in growing rats as a result of excess energy intake in the form of dietary fat.

Key Words: Leptin; growth hormone; insulin resistance; dietary fat.

Introduction

Overweight and obesity are growing health concerns in industrialized nations such as the United States, where caloric intake is increasing and physical activity is decreasing, especially among the young *(1)*. Excess body weight and body fatness contributes to health conditions such as heart disease, dyslipidemias, and type 2 diabetes mellitus *(2)*. Obese individuals produce twice the amount of leptin compared to slender individuals, and there is evidence indicating this is due to increased production of leptin by subcutaneous adipocytes compared to visceral fat cells *(3–9)*.

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Body fat is positively correlated with leptin concentration, which is positively correlated to insulin resistance and negatively correlated with insulin-stimulated glucose uptake *(10,11)*. Frequently, type 2 diabetes, which is characterized by insulin resistance and impaired glucose tolerance, is not diagnosed until overt symptoms such as weight loss, increased appetite, and frequent urination develop *(12)*.

Diets high in fat are known to increase body weight and fat mass, induce alterations in carbohydrate and lipid metabolism, lead to insulin resistance, and increase production and release of leptin in humans, rodents, and other animals *(5,13–16)*. High-fat diets providing excess energy contribute to increased body weight and body fat accumulation, impaired glucose tolerance, and insulin resistance in laboratory animals and humans, but the hormonal mechanism by which this occurs is not clear *(17)*.

During puberty, growth hormone (GH) is associated with increased glucose-stimulated insulin secretion *(18)* and is inversely related to pubertal insulin sensitivity *(19)*. GH secretion is pulsatile in nature, with fluctuations occurring in rats every 2–4 h *(20)*. The present study was designed to determine if changes in leptin and/or GH secretion are associated with the effect of a high-fat (HF) diet in promoting impaired insulin sensitivity in female rats during rapid growth and sexual maturation. The HF diet was designed to mimic common HF diets consumed by humans, and this was compared to a moderate fat (MF) diet designed to mimic a dietary fat percentage lower than current recommendations *(1)*.

Results

Body weights of rats in both groups were not different until after the seventh week of diet treatment, at which time HF animals weighed significantly more than MF animals ($p < 0.05$, Fig. 1). HF rats had 42–49% greater ($p < 0.005$) body fat percent (Fig. 2), as well as 49–58% greater ($p < 0.005$) body fat mass (Table 1) than MF rats. Lean body mass and food energy intake was not significantly different between groups (Table 1).

No significant differences in blood glucose levels between groups at fasting or in response to the oral glucose challenges were observed at any time points (Fig. 3). Area under the curve (AUC) for blood glucose response was not different between groups. In the HF group, blood glucose concentrations peaked at 60 min after each glucose challenge, while

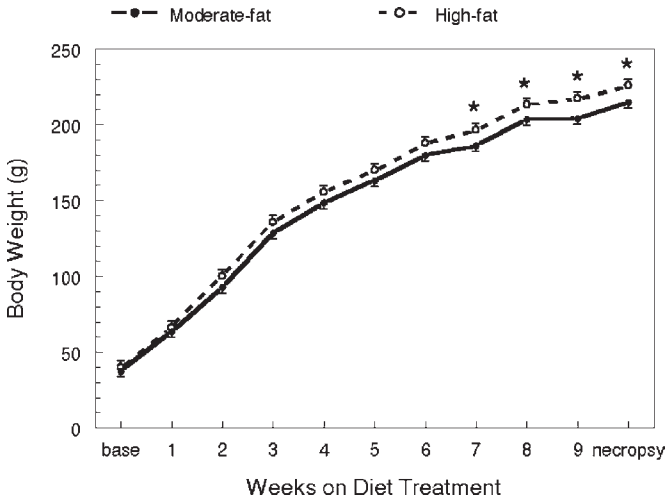

Fig. 1. Baseline and weekly body weights of female rats fed moderate-fat or high-fat diets. Asterisk indicates that body weight differs ($p < 0.05$) between groups; $n = 9$–10 rats per group.

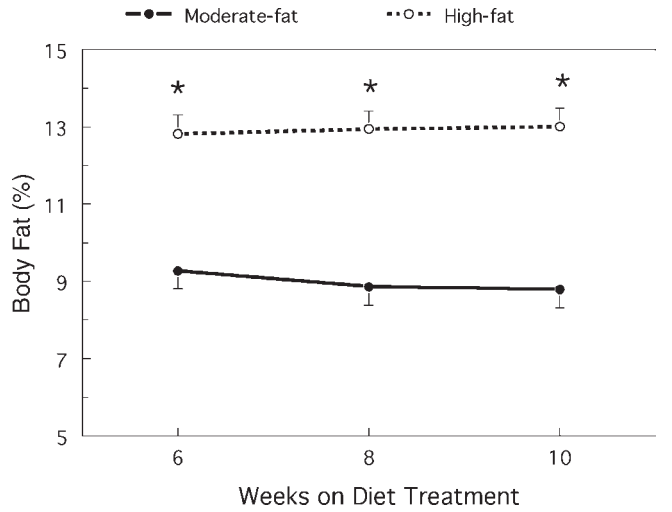

Fig. 2. Percentage of body fat in female rats fed moderate-fat (MF) or high-fat (HF) diets after 6, 8, and 10 wk of diet treatment, as measured by dual X-ray absorptiometry analysis. Asterisk indicates means of MF and HF groups differ ($p < 0.001$); $n = 8$–10 rats per group.

glucose concentrations seemed to plateau between 30 and 60 min postchallenge in the MF group. Rats in both groups exhibited a failure of blood glucose to return to fasting levels within 120 min after the glucose load (Fig. 3).

Fasting serum insulin concentrations at both the OGTT 2 (7 wk) and OGTT 3 (9 wk) were higher ($p < 0.05$) in the HF group than in the MF group (Fig. 4). Insulin levels were significantly higher ($p < 0.05$) in the HF group at 30 min during OGTT 1 (5 wk) and at 120 min during OGTT 2. During the OGTT 1, peak insulin response in MF rats was at 60 min compared to 30 min in HF rats, although this delay in insulin response did not persist to subsequent OGTTs. The insulin response AUCs for the OGTT 1 and OGTT 2 were significantly greater ($p < 0.05$) for HF rats than MF rats, with a tendency for significance ($p < 0.10$) at OGTT 3 (Fig. 4).

Mean serum leptin concentrations in response to OGTT 1 in HF rats were significantly greater ($p < 0.05$) at 30 min and 2 h after the glucose load was administered (Fig. 5). Serum leptin concentrations at OGTT 2 were higher in the HF animals 2 h after the glucose challenge ($p < 0.0001$).

Table 1
Body Mass Analyses and Food Intake of Rats Fed High-Fat (HF) and Moderate-Fat (MF) Diets at 6, 8, and 10 wk of Diet Treatment[a]

	HF	MF
	6 wk	
Rats per group	$n = 10$	$n = 10$
% Body fat	13.2 ± 0.7	9.3 ± 0.7*
Body fat (g)	25.6 ± 1.5	17.1± 1.5*
Lean body mass (g)	161.5 ± 3.1	162.2 ± 3.1
Food intake (kcal/d)	57.0 ± 1.5	56.5 ± 4.6
	8 wk	
Rats per group	$n = 9$	$n = 10$
% Body fat	13.0 ± 0.5	9.0 ± 0.5†
Body fat (g)	28.3 ± 1.3	18.9 ± 1.3†
Lean body mass (g)	183.5 ± 3.5	183.0 ± 3.5
Food intake (kcal/d)	52.2 ± 2.0	57.3 ± 2.8
	10 wk	
Rats per group	$n = 8$	$n = 8$
% Body fat	13.1 ± 0.5	8.7 ± 0.5†
Body fat (g)	30.1 ± 1.4	19.0 ± 1.5†
Lean body mass (g)	192.2 ± 3.8	193.6 ± 4.0
Food intake (kcal/d)	56.5 ± 8.0	51.6 ± 3.3

[a]Values given are mean ± SEM for body composition data, mean ± SD for food intake.
*$p < 0.0005$, †$p < 0.0001$ for significance between groups.

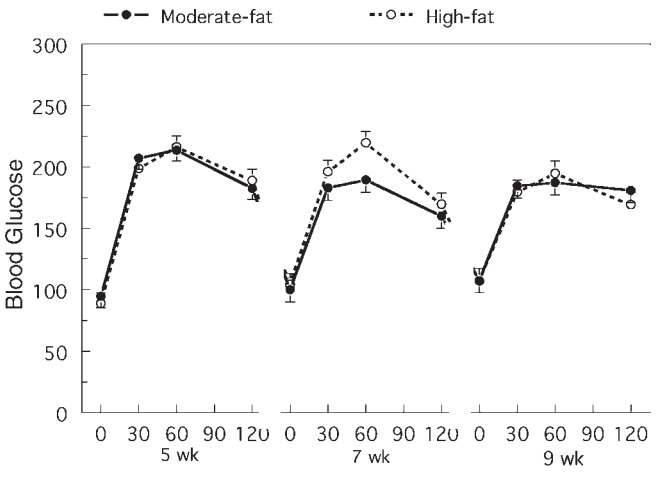

Fig. 3. Blood glucose concentrations of rats fed moderate-fat or high-fat diets at fasting (0 min) and in response to a 2 g/kg BW oral glucose challenge following 5, 7, and 9 wk of diet treatment; $n = 8$–10 rats per group per time point.

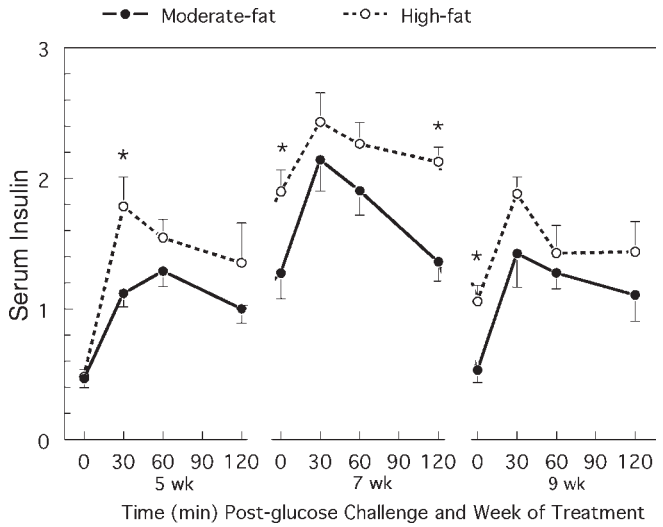

Fig. 4. Serum insulin concentrations of rats fed moderate-fat or high-fat diets at fasting (0 min) and in response to a 2 g/kg BW oral glucose challenge following 5, 7, and 9 wk of diet treatment; $n = 8–10$ rats per group per time point. Within week of treatment, asterisk indicates mean differs from its respective control (moderate-fat) group.

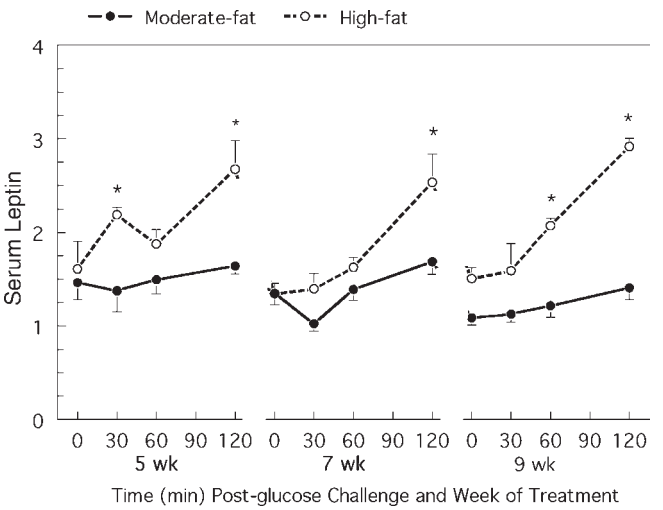

Fig. 5. Serum leptin concentrations of rats fed moderate-fat or high-fat diets at fasting (0 min) and in response to a 2 g/kg BW oral glucose challenge following 5, 7, and 9 wk of diet treatment; $n = 8–10$ rats per group per time point. Within week of treatment, asterisk indicates mean differs ($p < 0.05$) from its respective control (moderate-fat) group.

At OGTT 3, serum leptin levels of the HF rats were greater ($p < 0.001$) at 1 and 2 h after glucose administration. The serum leptin AUC was greater in HF rats at OGTT 1 and OGTT3 ($p < 0.05$ and $p < 0.001$, respectively). Leptin AUC was not significantly different between groups during OGTT 2 (Fig. 5).

No significant diet or time effects were observed for serum GH responses during any of the glucose tolerance tests. For MF rats, GH concentrations between 0 and 120 min averaged 6.20 ± 4.28 ng/mL, 7.96 ± 3.68 ng/mL, and 6.85 ± 4.02 ng/mL at OGTT 1, 2, and 3, respectively. For HF rats, GH concentrations between 0 and 120 min averaged 4.68 ± 3.81 ng/mL, 9.17 ± 3.99 ng/mL, and 4.49 ± 3.92 ng/mL at OGTT 1, 2, and 3, respectively.

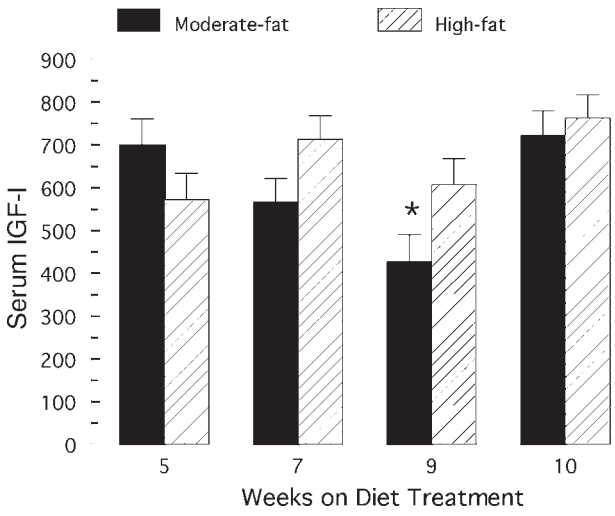

Fig. 6. Serum insulin-like growth factor-I (IGF-I) concentrations of rats fed moderate-fat or high-fat diets after overnight fasting at 5, 7, and 9 wk of diet treatment and at necropsy (10 wk); $n = 7–9$ rats per group per week. Asterisk indicates mean differs ($p < 0.05$) from the 5 and 10 wk moderate-fat group means.

Diet had no significant effect on serum IGF-I concentrations (Fig. 6). However, week ($p < 0.05$) and week × diet ($p < 0.07$) influenced serum IGF-I such that fasting IGF-I concentrations decreased ($p < 0.05$) in MF-fed rats between wk 5 and 9 but was unchanged in HF-fed rats between wk 5 and 10 of treatment (Fig. 6). Serum IGF-I concentrations were greater ($p < 0.05$) at necropsy (10 wk) than wk 9 in MF-fed rats only.

Discussion

This study was undertaken to determine the relationships between body composition and certain hormones in the development of prepubertal diet-induced insulin resistance. The HF diet was designed to resemble the total fat content of the typical American diet *(1)*. The majority of previous studies *(6,13,15,17)* evaluating the effect of HF diets on rats have used adult rats (200–300 g body weight at treatment start) and fed more total calories than control, whereas the present study utilized weanling, prepubertal rats fed an equivalent number of calories. The increased body weight and body fat of HF rats over MF rats in the present study is not explained by energy consumption, as the total cumulative difference in energy intake between groups, based on mean intake for each group, was calculated to be 22.4 calories per rat over 10 wk, approximately the equivalent of 2 g of body fat; HF rats contained 11 g more body fat than MF rats after 10 wk of treatment. Researchers have described HF-fed adult rats

that gain a disproportionately greater amount of body weight than would be expected based on energy intake *(13,21–23)*. This increased energy efficiency may be due to a lack of increased heat production postfeeding in HF rats, as was seen in a study conducted by Storlien and colleagues *(6)*. Others have suggested that storage of ingested fat requires less energy than conversion of consumed carbohydrate into fat stores *(24)*. Such differences in macronutrient partitioning may account for the increased body weight without evidence of increased energy consumption.

HF rats in this study accumulated significantly greater fat mass and body fat percentage than MF animals before significant increases in body weight were detected. Findings such as these were evident in male rats fed HF diets from 6 to 18 wk of age *(25)*. In the present study, the 40–60% increases in body fat percentage and total body fat of HF animals, coupled with no difference in lean body mass between groups, indicates that HF diets indeed caused increased body fat accretion in comparison with consumption of equivalent energy from primarily carbohydrates. If HF animals simply grew larger than MF animals in this study, we could not draw the same conclusion. Body fat percentages (8.7–13.2%) in the present study are within the range of those reported for Sprague–Dawley rats of similar age *(26,27)*.

Post-OGTT increases in blood glucose did not differ between HF and MF rats despite the fact that post-OGTT increases in serum insulin were greater and persisted for a longer period of time in HF-fed than MF-fed rats. Together, these results indicate that HF-fed rats were more insulin resistant than MF-fed rats. Similar to a previous study *(28)*, maximum blood glucose concentrations were achieved between 30 and 60 min postglucose challenge. Interestingly, rats receiving both diets displayed a failure to return to fasting blood glucose concentrations by 2 h postload following the 2 g/kg BW oral glucose challenge. Others *(28)* reported that in adult male Wistar rats consuming either 11% or 45% of calories from fat, the HF animals displayed a failure to return to fasting blood glucose within 2 h. It may be that 22% of calories from fat is greater than is easily tolerated by this strain of rat, or that in over-fed female rats the insulin resistance during puberty, also observed in humans *(29)*, lasts beyond 13 wk of age. Another possible explanation for the sustained elevation in blood glucose concentrations is that rats were fasted overnight before the OGTTs. Because the room the animals were housed in was not on a reverse light–dark cycle, the rats were fasted during their active cycle. Mid-pubertal insulin resistance may also account for the increased insulin secretion at all time points of the second OGTT over the first and third OGTTs for both the HF and MF groups. A postadolescent recovery of insulin sensitivity could also account for the overall decrease in insulin secretion at the third OGTT to levels similar to those seen at the first OGTT, when animals were 56 days old, the onset of sexual maturity in rats.

Researchers have shown that leptin responds to energy intake by initially declining slightly, if at all, then increasing steadily between the first postprandial hour and several hours postprandially *(30,31)*. With the exception of the HF group at the first OGTT, this same pattern was evident in both treatment groups at each OGTT. It is notable, however, that leptin concentrations significantly increased over fasting in HF but not MF rats at each OGTT, evidenced by the significant difference between groups in leptin concentrations at each 2-h postglucose load time point. The lack of a leptin rise in a lean versus an obese subject was also noted by Chapelot and colleagues *(30)*. This may be attributed to hypersecretion of insulin by HF rats, as leptin has been shown to be released in response to insulin both in vitro and in vivo *(32)*. However, in the present study, serum insulin increased in MF rats without a concomitant increase in serum leptin. This suggests that increased leptin observed in HF rats may not be due to increased insulin secretion, but perhaps due to some other unmeasured metabolic event associated with the glucose load. Levy and colleagues *(33)* observed that in male rats, increases in serum leptin concentrations were not different between HF and low-fat fed rats following an intravenous infusion of glucose (6.8 g/kg) or an 8 g chow meal at 3 h after administration. The difference between our findings and those of other studies may be due to differences in diets, age, body composition, and/or gender of animals among the various studies. Nonetheless, serum leptin concentrations measured at 9 wk of treatment and percentage of body fat measured at 8 and 10 wk of treatment were significantly correlated ($r = 0.50$ and 0.61, respectively; $p < 0.05$) as previously reported for humans *(10)* and rodents *(11)*.

In agreement with the present study, Tannenbaum et al. *(9)* sampled blood every 15 min over 6 h, and found no difference in mean GH levels between HF and MF rats. Consistent with this, animals in both groups in the present study experienced similar lean body mass accretion. Nonetheless, because GH is secreted in pulses *(20)*, a more frequent sampling schedule may be needed to discern differences in temporal GH secretory patterns. A lack of difference in mean GH levels indicates that the increased insulin resistance in the HF group cannot be ascribed to differences in mean GH levels. Instead, the present data indicate that increased leptin secretion may precipitate increased insulin resistance. In support of the latter suggestion, we observed positive correlations between plasma insulin and leptin concentrations after 9 wk of diet treatment ($r = 0.75$, $p < 0.001$). The precise mechanism by which leptin induces insulin resistance remains to be elucidated.

Serum concentrations of IGF-I were similar to those previously reported for female Sprague–Dawley rats *(34,35)* but were not dramatically influenced by diet in the present study. The only exception was that fasting caused a significant reduction in serum IGF-I concentrations in MF-fed rats but not in HF-fed rats. This suggests that HF-fed rats

were less susceptible to acute changes in feed intake. Chronic feed restriction in rats results in dramatic decreases in plasma IGF-I concentrations *(35)*. Lack of differences in serum IGF-I between groups are consistent with lack of differences in mean GH concentrations in the present study and further support the notion that the GH–IGF-I axis does not play a major role in the development of insulin resistance in HF-fed rats.

In conclusion, rats fed HF diets had significantly greater body mass, body fat mass, and percentage of body fat, and these changes were associated with increased serum leptin concentrations. Lean body mass was not different between groups, nor were blood glucose or serum GH concentrations. High-fat diet significantly increased serum insulin and leptin concentrations in response to oral glucose tolerance tests above that observed for MF-fed rats. Results of this study demonstrate that leptin but not GH may be involved in the development of insulin resistance in young growing rats as a result of excess energy intake from dietary fat.

Materials and Methods

Twenty female weanling Sprague–Dawley rats (Harlan Sprague-Dawley, Indianapolis, IN) were randomly assigned to one of two diets (Table 2). Animals were 21 d of age on arrival, and were housed individually in suspended wire bar-floor cages. Body weight (BW) in grams was recorded on arrival, and weekly thereafter. Animals were fed their assigned diets on the day of arrival and for the duration of the study. All animals had free access to water and were kept on a 12:12 light:dark cycle (lights on at 0700). Diet composition and energy value are outlined in Table 2. Diet treatments were a 10% fat by weight diet (MF) and a 20% fat by weight diet (HF). Both diets were prepared to provide equal vitamins and minerals per calorie, and contained equal percentages by weight of protein and fiber. Based on a projected weight of 100 g at 5 wk of age, the maximum expected energy requirement *(36)* during the first 2 wk of feeding trial was approx 40 kcal/d. Based on a projected BW of 250 g by the end of the study, maximum daily energy requirements for the animals was calculated to be 53 kcal/d. Animals were intentionally provided calories in excess of energy requirement for this study. For the first 2 wk, animals received 53 kcal/d. After 2 weeks of diet treatment, diet was increased to provide 65 kcal/d. Food intake was measured 24 h preceding each DXA measurement for three or four animals in each treatment group.

After 5, 7, and 9 wk of treatment diets, oral glucose tolerance tests (OGTT) were administered to animals following an overnight fast. Free access to deionized drinking water was allowed during the fast. On the morning of the OGTT, animals were weighed, then approx 1.0 mL of fasting blood was collected via the tail vein. Blood glucose was determined using a DEX glucometer (Bayer Corporation, Elkhart, IN).

Table 2
Composition and Energy Value of Experimental Diets

	Moderate-fat diet	High-fat diet
	g/kg Diet	
Casein	200	200
Cornstarch	100	100
Sucrose	500	400
Cellulose	50	50
Soybean oil	100	200
Mineral mix*	35	39.3
Vitamin mix†	10	11.2
L-cysteine	3	3
Choline bitartrate	2	2
	% kcal	
CHO	58	43
Fat	22	39
Protein	20	17
	kcal/g diet	
Energy density	4.10	4.57

*Mineral mix composition, g/kg mix: $CaCO_3$, 357; KH_2PO_4, 196; $K_3C_6H_5O_7 \cdot H_2O$, 70.78; NaCl, 74; K_2SO_4, 46.6; MgO, 24: $FeCl_2 \cdot 6H_2O$, 3.6; $ZnCO_3$, 1.65; $MnCO_3$, 0.63; $CuCO_3$, 0.3; KIO_3, 0.01; Na_2SeO_4, 0.01; $NH_4MoO_4 \cdot H_2O$, 0.008; Na_2SiO_2, 1.45; $LiCl_2$, 0.017; H_3BO_3, 0.08; NaF, 0.064; $NiCO_3$, 0.032; NH_4VO_3, 0.0066.

†Vitamin mix was obtained from Teklad, Madison, WI, cat. no. 40060.

Each animal then received 2 g glucose/kg BW by gavage; this dose was chosen based on a previous study *(28)*. Blood samples were taken from the tail vein at 30, 60, and 120 min after the glucose administration. The first drop of blood at each collection time was used to determine blood glucose concentration using the DEX. The glucometer was calibrated on each day of glucose tolerance testing, and all calibrations were within the acceptable range. The interassay coefficient of variation for glucose was 5%.

At 6, 8, and 10 wk of diet treatment (when rats were 9, 11, and 13 wk of age), animals were sedated with 58 mg/kg BW ketamine HCl and 2.9 mg/kg BW xylazine for body composition analysis. A model 4500 Elite Dual X-ray Absorptiometry machine (DXA, Hologic, Waltham MA) was then used to determine body fat mass, percentage of body fat, and lean body mass. Animals were necropsied following the last DXA, after 10 wk of diet treatment.

Serum insulin, leptin, and growth hormone (GH) concentrations were determined by radioimmunoassays (RIA, Linco Research, Inc., St. Charles, MO). Intra- and interassay coefficient of variations (CV) were 10.3% and 7.8% for insulin, and 7.1% and 7.4% for leptin, respectively. GH was analyzed in one assay in a subset ($n = 16$) of the animals studied. Intraassay CV for GH was 7.0%. Serum IGF-I concentrations were determined in one assay as previously described *(37)*. The intraassay CV for IGF-I was 12.9%.

SAS Version 8 Statistical Analysis Software for Windows was used to analyze all data *(38)*. The Mixed procedure was used to determine significant main effects and their interactions, with the Slice procedure used to determine differences between groups *(39)*. Pearson correlation coefficients were determined using the Corr procedure *(38)*. The level of significance was set at $p \leq 0.05$.

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