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Biometric Evidence of Diet-Induced Obesity in Lew/Crl Rats

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Although Lew/Crl rats are central to a classic model of renal transplantation and may provide a valid system for evaluating the effect of obesity on transplantation outcomes, their response to high-fat diet has not been evaluated sufficiently. The objective of this study was to evaluate biometric and basic metabolic data of Lew/Crl rats fed a 60% kcal, lard-based, very high-fat diet (HFD) compared with those fed a 10% kcal fat control diet (CD). Rats were maintained for 17 wk; body parameters and caloric intake were monitored weekly. Biometric data were collected and calculated before and after euthanasia. Serum was evaluated for liver enzyme activity and total bilirubin, glucose, triglyceride, cholesterol, insulin, leptin, and creatinine concentrations, and urine was evaluated for protein, glucose, specific gravity, and ketones. Tissues were harvested, weighed, and evaluated histologically. Compared with CD rats, HFD rats consumed more calories and weighed more after 3 wk. After 17 wk, HFD rats had significantly increased body weight, girth, volume, epididymal fat pad weight, omental weight, and body fat. In addition, HFD rats had mild elevations in some liver enzymes and a lower serum triglyceride concentration than did CD rats. Histologic assessment and other metabolic markers of disease were not different between the 2 groups. Lew/Crl rats fed a 60% kcal HFD become obese, but they lack significant metabolic abnormalities frequently associated with obesity in other rat strains.

Abbreviations: CD, control diet; HFD, high-fat diet.

Currently, about 70% of the population and 50% of renal transplant recipients in the United States are either overweight or obese, and these percentages continue to rise.[5] Obesity is a strong risk factor for developing chronic kidney disease. Further, the risk of renal disease increases directly with body mass index, even after adjustment for hypertension and diabetes.[13] Interestingly, the potential influence of obesity on renal transplant success remains inconclusive due to discord among a number of clinical reports.[3] For example, some clinical studies have reported no effect on the loss of allograft function in overweight recipients,[16,22] whereas others conclude obesity enhances risk for allograft dysfunction.[24,41] Therefore additional research is needed in this area to identify a valid animal model to help elucidate and define the pathophysiology associated with obesity and renal transplantation.

The Fisher-to-Lewis renal transplantation model is a classic model for renal transplantation research.[2,15,17,29,39] In this model, Fisher rats serve as renal donors, Lewis rats are transplant recipients, and renal allografts reliably develop lesions consistent with chronic allograft nephropathy, the leading cause of late allograft loss in people.[26] In addition, due to the inbred nature of this strain, Lewis rats frequently are used as donors and recipients to research allo-independent renal transplantation phenomena or as isogenetic controls.[7,28,34] Furthermore, Lewis rats have been used in numerous other research trials involving inflammation, including studies of autoimmune uveitis,[40] inflammatory bowel disease,[8,35] chronic colitis,[31] giant cell myocarditis,[9] CNS1 glioma,[4] cardiac transplantation,[32,43] and wound healing.[10] Because Lewis rats have a specific research niche as a transplantation and inflammation animal model, they present a tremendous opportunity to study the effect of obesity on various aspects of transplantation and inflammatory diseases.

Rodent strains are known to differ in their responses to high-fat diets, perhaps as a function in the variability of corticosteroid receptors.[23] The classic rat strains for studying diet-induced obesity are Wistar and Sprague–Dawley.[6] Although these outbred strains have a long and well-documented history in metabolic and physiologic obesity studies, the use of these strains for transplantation research is untenable due to their genetic variability. Organ transplant success is contingent on controlling host immune response against the transplant; this control is achieved largely through tissue typing and matching. Therefore, outbred animals that express high genetic variability in the genes controlling immunity, particularly tissue histocompatability genes, are poor transplant models. In addition, the rat strains currently used in obesity research have little to no history in trials studying renal transplantation. Finally, other models of diet-induced obesity are complicated by marked obesity-related comorbidities, including hypertension, insulin resistance, diabetes, and metabolic syndrome, which confound the influence of obesity alone and may complicate transplantation success and interpretation of data after transplantation. The extent to which obese Lewis rats succumb to these obesity-associated comorbidities is unknown.

Currently, few data are available regarding the effects of a high-fat diet on Lewis rats. In one study, hormone levels in Lewis

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rats were evaluated after feeding diets with either 35% or 60% of dietary energy (in kcal) derived from fat (% kcal fat); however, the biometric parameters were inconsistent.[21,27] In another study, neonatal Lewis rats were fed a high-fat diet (45% kcal fat) until 8 wk of age.[36] Although the rats fed the high-fat diet had increased epididymal fat pad weight, larger adipocytes, and increased blood glucose levels compared with those fed a standard diet, the 2 groups of rats did not differ in total body weight, or the level of energy consumption.[36] This result may suggest Lewis rats have some degree of obesity resistance.

In another study, the growth of weanling Lewis rats fed either a standard rodent diet or a variety of oils as a fat source was evaluated for 12 wk. Each high-fat diet contained 20% oil (hydrogenated coconut, olive, safflower, evening primrose, or menhaden).[42] The rats fed the control diet consumed more food by total mass, but not more energy, than did rats fed the high-fat diets. Unlike the results from the previous study,[36] rats fed the control diet weighed less than did those fed the fat-supplemented diets, whereas epididymal fat pad weight increased variably in the high-fat-diet rats.[42]

Clearly, the studies cited[36,42] differed in their conclusions regarding the propensity for Lewis rats to become obese on a high-fat diet. Potential contributors to these different interpretations are the experimental design and diet. Both percentage of dietary fat and trial duration varied between the studies. Control rats were fed standard rodent chow instead of a low-fat formulation of the custom formulated diet to which the fat was added. Therefore, data are lacking with regard to characterization of diet-induced obesity in Lewis rats for durations longer than 10 wk with a very high-fat diet. Given the variability in diet-induced obesity among some strains, knowledge of the chronic biometric and biochemical changes in a Lewis rat fed a lard-based high fat diet is warranted prior to conducting investigations of obesity on inflammation, immunity, or transplantation using this model.

In the present study, Lewis rats were fed a control diet (CD) or a defined high-fat diet (HFD) containing the same feed components as the CD; biometric and biochemical parameters were assessed to determine whether these conditions generated an obese rat that could be used in renal transplantation studies. Our hypothesis was that Lewis rats fed HFD would have an increased total body weight gain, serum glucose, liver enzymes, urine glucose, and serum triglyceride levels compared with those of Lewis rats fed CD. We further hypothesized that this information could be used as the basis for the development of a model to study the effect of obesity on the immunologic and physiologic processes leading to the loss of allogenic renal grafts.

Materials and Methods

Male Lewis rats (Lew/Crl; $n = 16$; age, 3 wk; weight, 50 g) were obtained from a commercial vendor (Charles River Laboratories International, Wilmington, MA). On arrival, rats were assigned sequential numbers and allocated into 2 groups by using a random number generator (www.random.org). One group was fed a very HFD (60% kcal fat; D12492, Open Source Diets, Research Diets, New Brunswick, NJ; Figure 1), and the other was fed a specific CD (10% kcal fat; D12450B, Open Source Diets). Diets and water were available ad libitum. Rats were housed individually and exposed to 12:12-h light:dark cycles. Rats were housed in accordance with the *Guide for the Care and Use of Laboratory Animals*.[14]

	High-fat diet (5.24 kcal/g)		Control diet (3.85 kcal/g)	
	g %	kcal %	g %	kcal %
Protein	26.2	20	19.2	20
Carbohydrate	26.3	20s	67.3	70
Fat	34.9	60	4.3	10
	g	kcal	g	kcal
Casein 80-mesh	200	800	200	800
L-Cystine	3	12	3	12
Cornstarch	0	0	315	1260
Maltodextrin 10	125	500	35	140
Sucrose	68.8	275.2	350	1400
Cellulose BW200	50	0	50	0
Soybean oil	25	225	25	225
Lard	245	2205	20	180
Mineral mix S10026	10	0	10	0
Dicalcium phosphate	13	0	13	0
Calcium carbonate	5.5	0	5.5	0
Potassium citrate	16.5	0	16.5	0
Vitamin mix V10001	10	40	10	40
Choline bitartrate	2	0	2	0
FD and C dye	0.05	0	0.05	0

Figure 1. Compositions of high-fat diets (Open Source Diets, D12492 (high fat) and D12450B (control), Research Diets, New Brunswick, NJ).

The University of Georgia Animal Resources program is fully AAALAC-accredited.

Rats were evaluated, weighed, and assigned a body condition score weekly. Body condition scoring was performed by the same person (AT) every week and was extrapolated from a published method for mice.[38] The quantity of food (in g) consumed by each rat was determined by subtracting the weight of uneaten food from the initial weight of food. The total number of kilocalories that each rat consumed was determined by multiplying the caloric content of 1 g of each diet by the total quantity eaten. At 17 wk, rats were euthanized by carbon dioxide asphyxiation followed by cervical dislocation. Two HFD rats were not euthanized at this time and were held for further studies. Therefore, posteuthanasia data were obtained from 6 HFD and 8 CD rats.

Biometric analysis. Immediately after euthanasia, rats were weighed on a top-loading scale (model S602, Denver Instruments, Bohemia, NY; precision, 0.01 g), blood was drawn by direct cardiocentesis, and urine was collected. Rodents were reweighed after blood and urine collection. Rat length and abdominal girth was measured by using a tape measure with a precision of 0.1 cm. Length was defined as the distance from the tip of the nose to the junction of the body and tail. The abdominal girth was measured midway between the xyphoid cartilage and pubis. Tracheotomy was performed, and the lungs were filled with saline by using a 22-gauge catheter. Rats were thoroughly soaked with tap water and allowed to float; the displacement of water was measured. The peritoneal cavities were opened with ventral midline incisions, and the greater omentum (removed from the spleen at its attachment and along the greater curvature of the stomach), bilateral epididymal fat pads, perirenal fat, spleen, liver, kidney, and stomach were removed. The omentum, epididymal fat pad, and spleen were weighed. Samples of all fatty tissue and all organs studied were removed and placed in 10% neutral buffered formalin for histologic examination.

Rat density was calculated by dividing carcass weight (weight of the rat after blood and urine collection less the weight of the

gastric contents) by the body volume (as measured by volume of water displaced). The fraction of carcass fat was estimated using the following densitometry-based equation:[25]

$$\text{Fraction of fat} = (0.0375 / \text{density} - 0.0334) \times \text{carcass weight}^{0.75}$$

After collection, blood was allowed to clot and then was centrifuged at 290 × *g* for 10 min.

Serum and urine biochemical analyses. Serum was collected and analyzed for alkaline phosphatase, alanine aminotransferase, aspartate aninotransferase, cholesterol, γ-glutamyl transpeptidase, total bilirubin, glucose, total triglyceride, and creatinine concentrations. All serum biochemical analyses and urine protein:creatinine ratios were performed by the Clinical Pathology Laboratory (University of Georgia Veterinary Teaching Hospital, Athens, GA) on a biochemistry analyzer (Modular P800, Roche Diagnostics, Indianapolis, IN). Urine specific gravity was determined by using a hand-held refractometer (J351, Jorvet, Loveland, CO). Urine glucose and ketones were determined by using a colorimetric reagent strip (Multistix, Siemens, Tarrytown, NY).

Serum insulin and leptin analysis. Serum insulin concentration was measured by using a commercial ELISA assay (Rat Insulin Enzyme Immunoassay Kit A05105, Caymen Chemical, Ann Arbor, MI). The samples were evaluated in triplicate and according to manufacturer's instructions. Briefly, rat serum and standard samples were mixed with guinea pig antirat insulin antibodies and acetylcholinesterase. This mixture was added to 96-well, flat-bottomed plates coated with goat antiguinea pig antibodies and incubated for 18.5 h at 4 °C. After washing and addition of Ellman reagent, plates were incubated on an orbital shaker at room temperature for 3.75 h prior to reading at 405 nm. The intensity of the color change was inversely proportional to the quantity of insulin. The samples were compared against a standard curve to determine concentration.

In addition, serum insulin and leptin concentrations were measured by using a multiple-analyte assay (Adipokine Assay RADPK-81K, Millipore, Bellerica, MA) according to manufacturer's instructions. Briefly, rat serum was mixed with antirat leptin and antirat insulin antibody-coated microspheres. Samples (evaluated in triplicate) were incubated with a reporter molecule (streptavidin–phycoerythrin conjugate), exposed to laser energy, and analyzed for emitted fluorescence (Luminex 100, Millipore). The values obtained for the samples were compared against a standard curve to determine concentration.

Histopathology. Tissues were allowed to fix in 10% neutral-buffered formalin for a minimum of 48 h prior to routine tissue processing (Tissue-Tek VIP, Sakura Finetek USA, Torrance, CA). Sections were cut at 3 µm (Shandon Finesse, Thermo Scientific, Holly, MI), and stained with hematoxylin and eosin (Autostainer XL, Leica, Bannockburn, IL). Tissue sections were evaluated by a pathologist who was blinded to diet groups. Structural and cellular abnormalities in each group were described.

Statistics. Commercial statistical software (version 5.00, GraphPad Prism for Windows, GraphPad Software, San Diego, CA) was used to analyze the data. The Kolmogorov–Smirnov test with Dallal–Wilkinson–Lillie for *P* value was used to determine normality. Unpaired, 2-tailed Student *t* tests with Welch correction or a Wilcoxon signed-rank test were used to evaluate data between the 2 groups, as appropriate. Data are reported as mean ± SEM or median and interquartile range as identified in the text, tables, or legends. Significance was set at a *P* value of less than 0.05.

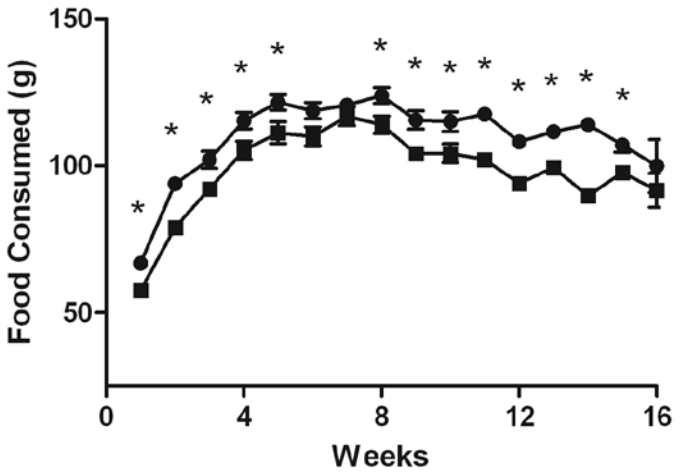

Figure 2. Food consumed per rat each week (g; mean ± SEM) in Lew/Crl rats fed a 60% kcal fat diet (squares) compared with those fed a control 10% kcal fat diet (circles). Rats fed a high-fat diet consumed significantly (*, $P < 0.05$) less food during all weeks except 6, 7, and 16.

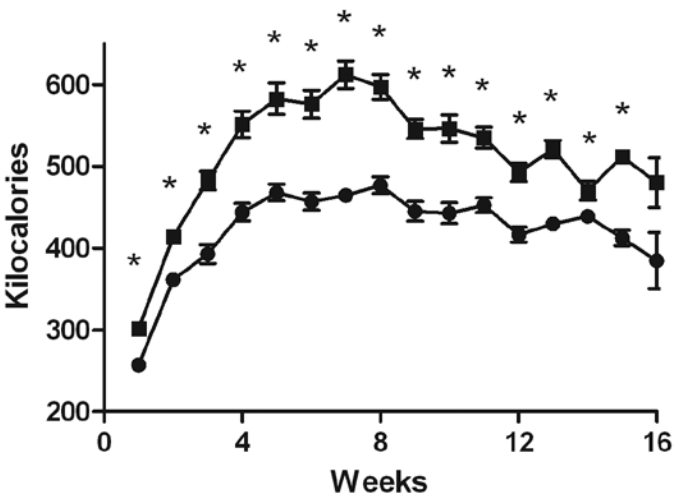

Figure 3. Food consumed per rat each week (kcal; mean ± SEM) in Lew/Crl rats fed a 60% kcal fat diet (squares) compared with those fed a control 10% kcal fat diet (circles). Rats fed a high-fat diet consumed a significantly (*, $P < 0.05$) greater amount of kilocalories every week with the exception of week 16.

Results

HFD rodents ate significantly ($P < 0.05$) fewer grams of food during weeks 1 through 5 and 8 through 15, compared with CD rats (Figure 2). However, HFD rats consumed a significantly ($P < 0.05$) higher number of kilocalories for every week, except week 16 (Figure 3). HFD rats weighed significantly ($P < 0.05$) more than did CD rats beginning at week 3 and continuing for the remainder of the experiment (Figure 4). After 8 wk of feeding, body condition scores generally were higher in HFD rats than CD rats (Figure 5).

Biometric analysis. After euthanasia, mean weight (HFD, 501.5 ± 10.6 g; CD, 409.0 ± 6.1 g; $P < 0.0001$, Figure 6), girth (HFD, 22.8

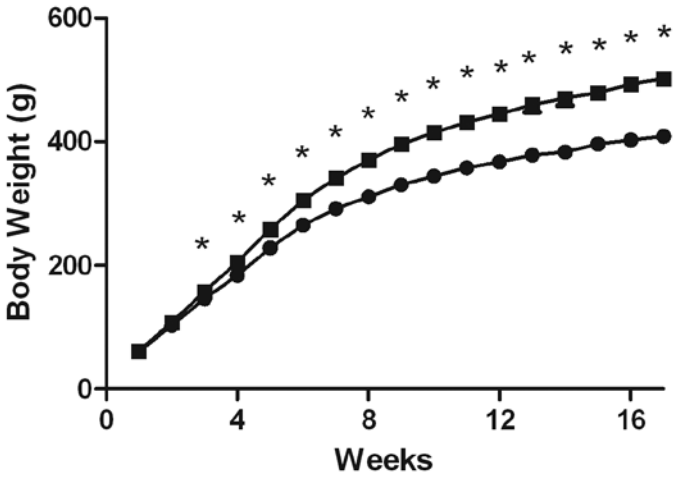

Figure 4. Body weight (g; mean ± SEM) of Lew/Crl rats fed a 60% kcal fat diet (squares) compared with those fed a control 10% kcal fat diet (circles). Error bars are present but are not visible. Beginning 3 wk after starting the experiment, rats fed a high-fat diet weighed significantly (*, $P < 0.05$) more than did those fed a control diet. The week 17 weight represents weights obtained immediately after carbon dioxide asphyxiation.

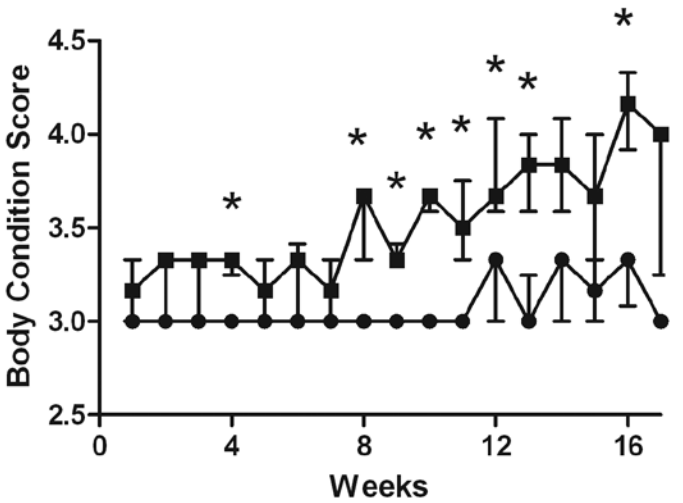

Figure 5. Median and interquartile range body condition scoring in Lew/Crl rats fed a 60% kcal fat diet (squares) compared with those fed a control 10% kcal fat diet (circles). Rats fed a high-fat diet had a significantly (*, $P < 0.05$) greater body condition score on weeks 4, 8 through 13, and 16. The week 17 body condition score represents data obtained immediately before carbon dioxide asphyxiation.

± 0.4 cm; CD, 19.8 ± 0.3 cm; $P < 0.001$), body volume (that is, water displacement; HFD, 491.7 ± 8.7 mL; CD, 398.8 ± 5.9 mL; $P < 0.0001$), epididymal fat pad weight (HFD, 11.3 ± 1.2 g; CD, 6.5 ± 0.4 g; $P = 0.01$), and omental weight (HFD, 3.0 ± 0.2 g; CD, 1.7 ± 0.1 g; $P < 0.01$) were significantly higher in HFD than CD rats. HFD rats had a higher estimated body fraction of fat (HFD, 0.40 ± 0.02 g; CD, 0.34 ± 0.02 g; $P < 0.05$). No significant differences were found in spleen weight, body density, or gastric content weight.

Although still heavier than the rats fed CD, 2 rats in the HFD group had body weights noticeably less than those of the other rats in the HFD group (Figure 6). Of these 2 rats, 1 began the study over the mean weight of the HFD group (61.6 g), and the other was under the mean weight of the group. However, when the mean amount of food consumed over the entirety of the study is evaluated, these 2 rats consumed less than the mean for the HFD group (mean of HFD group, 98.1 g; mean of 2 rats, 93.6 g).

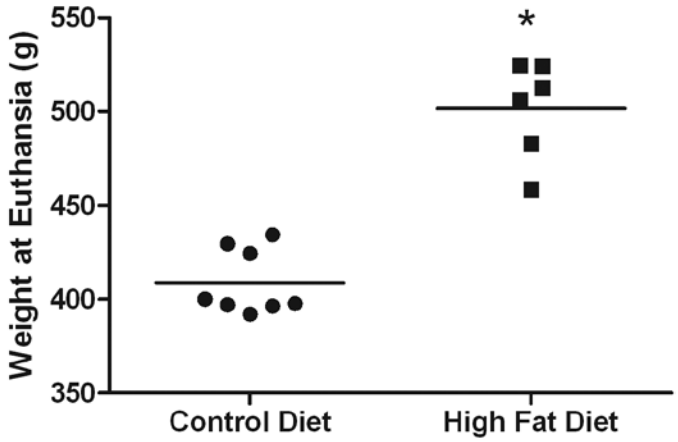

Figure 6. Scatter dot plot of posteuthanasia weight after 17 wk of feeding a 60% kcal fat diet compared with a 10% kcal fat control diet. Rats fed a high-fat diet weighed significantly (*, $P > 0.0001$) more than rats fed the control diet. The horizontal line is the mean.

Biochemical analysis. Compared with CD rats, HFD rats had significantly ($P < 0.05$) elevated alkaline phosphatase and alanine aminotransferase and reduced total triglyceride concentrations (Table 1). HFD rats showed a statistical trend ($P = 0.0625$) toward increased aspartate aminotransferase. There were no differences between groups in urinary parameters.

Insulin enzyme immunoassay. The standard curve was fitted to a linear line ($R^2 = 0.557$). At the time of reading the maximal binding wells had an absorption of 0.59 AU (manufacturer suggested range, 0.2 to 0.8). There was no difference in insulin concentration between HFD and CD rats (Table 1).

Insulin and leptin multi-analyte assay. Standard curves were fitted to a quadratic curve (standard curve R^2: insulin, 0.99; leptin, 1.00). Again, insulin concentration did not differ between diet groups (Table 1), nor did the leptin concentration.

Histopathology. No noteworthy abnormalities were found in the kidney, thymus, or spleen of any rat. In addition, 2 of the 6 HFD rats had rare hepatic lipid vacuoles, whereas 5 of the 8 CD rats had very mild to moderate periportal vacuolation, not associated with lipid. No abnormalities were noted in the perirenal fat, epididymal fat, or omentum.

Discussion

The results of the current study demonstrate that diet-induced obesity can be accomplished in Lew/Crl rats fed a 60% kcal fat diet having lard as the primary source of fat calories. HFD rats consumed more calories, gained more weight, and had a greater percentage of body fat than did CD rats. Obesity was achieved without modification of litter size, and a significant difference was detected after 3 wk of HFD feeding.

Obesity-induced organ dysfunction occurs in a variety of rodent strains. The extent of this dysfunction leads to overt signs in some strains, such as the Zucker (*fa/fa*) fatty rat which develops hypertension, diabetes, and metabolic syndrome.[19] In light of our current data, Lew/Crl rats appear to have a moderate response

Table 1. Biochemical data from rats fed high-fat or control diet

		Control diet	High-fat diet	*P*
Plasma				
	Alkaline phosphatase (U/L)	106.1 ± 2.7	201.7 ± 8.4[a]	<0.0001
	Alanine aminotransferase (U/L)	54 (29–502)	182 (47–407)[a]	0.0313
	Aspartate aminotransferase (U/L)	97 (66–756)	324 (111–711)	0.0625
	Cholesterol (mg/dL)	135.1 ± 6.0	121.7 ± 1.9	0.0687
	Triglyceride (mg/dL)	409.1 ± 31.9	190.3 ± 28.6[a]	0.0004
	Total bilirubin (mg/dL)	0.1 (<0.1–0.1)	0.1 (0.1–0.1)	—
	γ-glutamyl transpeptidase (U/L)	<3	<3	—
	Glucose (mg/dL)	300.3 ± 22.6	253.5 ± 21.1	0.1590
	Creatinine (mg/dL)	0.4 (0.3–0.4)	0.4 (0.3–0.5)	1.0000
	Insulin[b] (ng/mL)	3.87 ± 1.2	3.85 ± 0.8	0.9879
	Insulin[c] (ng/mL)	0.59 ± 0.1	0.58 ± 0.1	0.8894
	Leptin[c] (pg/mL)	3914 (2308–7737)	8423 (5190–11654)	0.0625
Urine				
	Specific gravity	1.050 (1.038–1.050)	1.043 (1.019–1.050)	0.5000
	Protein:creatinine	7.0 ± 3.2	5.3 ± 1.8	0.6721
	Glucose (g/dL)	0	0	—
	Ketones (mg/dL)	15 (0–40)	15 (5–50)	0.8227

[a]Values significantly ($P < 0.05$) different between high-fat and control diets.
[b]Data obtained by using a rat insulin immunoassay (see Methods for details)
[c]Data obtained by using a multiplex assay (see Methods for details)

to high-fat diet and therefore might model the human response to high fat more closely than do obesity-prone rodent strains, which tend to have an exaggerated metabolic response. Rats on the HFD diet had mild elevation in hepatic enzymes (alkaline phosphatase, alanine aminotransferase), suggesting ongoing mild hepatic injury and consequential leakage of hepatic enzymes. However, no structural pathologic changes were evident during histologic evaluation, and hepatic lipidosis was absent. Hepatic lipidosis can occur in animals as a result of obesity, liver or systemic disease, or a sudden demand for energy and mobilization of fat (anorexia, lactation, pregnancy). Hepatic lipidosis is a relatively simple histologic marker, and special stains are not needed to confidently diagnose its presence. Further, the serum chemistry values of HFD rats did not support reduced hepatic function, in that total bilirubin was not elevated nor was cholesterol reduced, both of which changes are indicative of decreased liver function.

Insulin resistance and diabetes are hallmarks of metabolic syndrome and occur in several classic rodent models of obesity including Zucker fatty rats, obese spontaneously hypertensive rats, and spontaneously hypertensive, stroke-prone fatty (*fa/fa*) rats.[11,19] When renal disease or renal transplantation is studied within the context of obesity, concomitant diabetes creates a significant confounding variable, given that diabetic renal disease is a common occurrence in these models.[37] In the present study, HFD rats had no evidence of diabetes or insulin resistance. In addition, serum and urine glucose concentrations and urine ketone concentrations did not differ between diet groups, nor was the serum insulin concentration different. The insulin concentration was confirmed in 2 independent assays. These results suggest that, as in the human scenario, Lew/Crl rats can become obese without acute systemic collapse into a state of metabolic dysregulation. The protective genetic or molecular mechanism of this characteristic in Lew/Crl rats requires additional investigation.

The cause for the reduction in serum triglycerides in HFD rats compared with CD rats was not consistent with other studies in different strains.[6] Most of the caloric fat in the high-fat diet originated from lard (Figure 1). Generally, rats fed diets high in lard had hypertriglyceridemia, whereas rats fed diets high in fish oils had reductions in serum triglyceride concentrations.[6] The sources of fat in the 2 diets in the current study were the same (lard and soybean oil) and only varied in quantity. The difference between the 2 diet groups likely relates to a more severe elevation of the triglycerides in the CD rats, resulting in a relative reduction in the triglycerides of HFD rats compared with CD rats. Triglyceride concentrations in normal, 11-wk-old Lewis rats have been reported to be approximately 49.6 mg/dL,[18] a concentration markedly lower than that of either of our groups, suggesting that both the HFD and CD rats had elevations in triglycerides, but the CD elevation was significantly greater. The major caloric differences in the diet were from the corn starch and sucrose in the control diet (Figure 1). A high-sucrose diet has been shown to cause hypertriglyceridemia and elevated liver triglyceride concentrations in male Wistar rats.[42] However, in another study, 20 d of fructose loading of Wistar and Lewis rats only nominally increased serum triglycerides.[18] In future studies, altering the control diet to eliminate sucrose as a caloric source may alter insulin and triglyceride concentrations.

In the present study, Lew/Crl rats fed a high-fat diet consumed less food but significantly more calories than did rats on a control diet. These results are in discordance with previous studies, where Lew/Crl rats consumed less food and equivalent calories relative to their controls.[36,42] However one of these studies[42] reported that Lewis rats fed a high-fat diet had less energy expenditure to account for weight gain despite equivalent energy consumption. This discordance may have emerged because the current study is the first to evaluate diet-induced obesity in Lew/Crl rats by using

a specific, component-matched control diet; this variation may account for the observed difference in both energy consumption and weight gain.

Obese rodents of various strains have exhibited variable degrees of adipose inflammation and structural alterations in adipocyte morphology in both hepatic and renal tissue.[1,12,30,33] In the present study, the HFD rodents did not demonstrate any significant structural histologic changes. However, detailed morphometric data (glomerular size, adipocyte size, and others) and immunohistochemical staining were not obtained. Because Lew/Crl rats appear to offer a promising and potentially germane model of diet-induced obesity, the next step in the characterization of this model likely will be evaluation of systemic changes in inflammation.

Not only do rats differ between strains in their propensity to become obese when fed a high-fat diet, they often respond to high-fat diets differently, even within the same strain.[20] Some rats will become obese on a high-fat diet, whereas others are obesity-resistant. In the current study, 2 of 6 Lew/Crl rats weighed markedly less than did the other 4 rats (Figure 6). This difference may reflect normal variation within the obese group or an obesity-resistant subgroup within the HFD population. However, these 2 rats fell outside the weight range of the rats in the control group. This finding suggests that these rats likely are not completely obesity-resistant, given that they did become obese as compared with controls, but they may still have a degree of obesity resistance. Given that Lew/Crl rats are an inbred strain, genetic differences reflect a minor source of variation, and the prevalence of obesity resistance may also be lower due to genetic consistency.

Sources of error in the current study include failure to account for food that may have been dropped in the cage during food weight. This oversight likely had minimal effect on data, because a clear separation in food weights was apparent. In addition, during the water displacement test, air may have added buoyancy to the rats and thereby altered the calculation of body fat percentage, despite the fact that rats were soaked thoroughly before measurement of displacement.

In summary, this study is the first to document the biometric effects on male Lew/Crl rats fed a very high-fat, lard-based, open-source diet. The major clinical observation was that male Lew/Crl rats consistently achieved diet-induced obesity without significant effects on blood glucose or basic renal function for the study duration. HFD rats showed mild biochemical evidence of hepatic injury. Whether these results are similar in female rats is unknown and warrants further study, as does other systemic inflammatory manifestations specific to the Lew/Crl rat. As a model, the methods reported here appear to be suitable for inducing and studying obesity in Lew/Crl rats.

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