Cardiovascular and renal responses to a high-fat diet in Osborne-Mendel rats

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A close association between obesity and hypertension has been well established from studies in experimental animals and humans. Excessive weight gain caused by feeding a high-fat (HF) diet increases arterial pressure in rabbits and dogs (5, 10). Clinical studies have also shown that weight loss is an effective way of reducing arterial pressure in most overweight hypertensive patients (26). And finally, population studies have shown a close correlation between obesity and hypertension. Risk estimates from the Framingham Heart Study, for example, suggest that ~65–75% of the risk for essential hypertension can be attributed to obesity (12).

Although obesity is now recognized as a major cause of essential hypertension, there are important variations in blood pressure responses to weight gain. For example, some obese subjects are not hypertensive, and there also are significant variations among different individuals in the amount of weight gain associated with a high caloric diet (11). These differences in weight gain and blood pressure responses to high caloric intake have been postulated to be due, at least in part, to genetic variability, but the contribution of genetic and environmental factors in causing human obesity and hypertension is still unknown.

In recent years, there have been significant advances in our understanding of the genes that control body weight, especially in monogenetic models of murine obesity. Many of the monogenetic models of obesity, however, do not appear to closely mimic the cardiovascular and neurohumoral profile observed in obese humans. For example, leptin-deficient ob/ob mice are extremely obese but have reduced arterial pressure compared with their lean littermates (17). Obese Zucker rats, which have a mutation in the leptin receptor gene, have decreased sympathetic activity and decreased plasma renin activity (PRA) compared with obese humans who often have increased PRA and sympathetic activation (2).

On the other hand, dietary models of obesity, especially those produced by feeding an HF diet, have many of the neurohumoral and cardiovascular characteristics of obese humans (9, 10). The importance of diet in causing obesity in humans is also suggested by the fact that the prevalence of obesity has risen dramatically during the past 10–15 years in most industrialized countries where there is an abundance of energy-dense food (7). These observations point toward the importance of interactions between dietary and genetic factors in human obesity and hypertension. However, there are few animal models available to study these interactions. The Osborne-Mendel (OM) rat is a potential model for studying interactions between dietary and genetic factors in obesity. These rats are more susceptible to the development of obesity when fed an HF diet, compared with other rat strains such as S5B/PI rats, which only increases their weight slightly when fed similar HF diets (8). The OM rat also demonstrates several metabolic abnormalities such as insulin resistance and decreased expression of hypothyroidism.
laric leptin receptors when fed an HF diet (16). However, there have been no studies, to our knowledge, that have characterized the cardiovascular responses to dietary-induced obesity in OM rats. Therefore, the present study was designed to examine the cardiovascular, renal, and hormonal responses in OM rats fed either a low-fat (LF) or HF diet.

METHODS

The experiments were conducted in 21 male OM rats obtained at 6 wk of age and averaging 168 ± 6 g body weight (National Institutes of Health Genetic Resource, Bethesda, MD). The rats were randomly assigned to one of two experimental groups: LF (8% fat, n = 10) or HF (74% fat, n = 11). The OM strain was chosen for its susceptibility to develop obesity when exposed to an HF diet; however, when fed an LF diet, OM rats had only modest increases in body weight (16, 23). OM rats were fed the selected diets (see Table 1 for dietary composition) for 17 wk. During the first 15 wk of the study, systolic blood pressures were measured once a week in conscious rats using the indirect tail-cuff method. Thereafter, arterial pressures were measured directly (24 h/day) from the chronically indwelling catheters, until the end of the study. The rats received either the LF or HF diet and water ad libitum throughout the study. Surgery and care of the rats were conducted in accordance with National Institutes of Health Guide for the Care and Use of Laboratory Animals using protocols that had prior approval from the Institutional Animal Care and Use Committee of the University of Mississippi Medical Center.

Systolic blood pressure measurements. The rats were handled repeatedly and preconditioned to the restraint chamber before the actual measurements of systolic blood pressure were conducted. Briefly, each rat was placed in a plastic restrainer, and a pressure cuff was slipped onto the tail. The rats were then placed in a preheated chamber (32 ± 1°C) to dilate peripheral blood vessels and stimulate blood flow to the tail. After the rats were allowed to adjust to the restraint and tail apparatus, the systolic blood pressure was measured using an electrophysmomanometer (ITC Life Science, Woodland Hills, CA). With the use of this method, the reappearance of pulsations after gradual deflation of the blood pressure cuff were detected by a photoelectric sensor, amplified, and recorded digitally as the systolic blood pressure. An average of three such readings was taken to obtain the individual systolic blood pressure.

Table 1. Composition of the low- and high-fat diets

<table>
<thead>
<tr>
<th>Component</th>
<th>Low-Fat Diet</th>
<th></th>
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<th>High-Fat Diet</th>
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<tr>
<td></td>
<td>g/100 g</td>
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<td>%kcal</td>
<td>g/100 g</td>
<td>%kcal</td>
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<tr>
<td>Vitamin-free casein</td>
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<td>23</td>
<td>32.6</td>
<td>22</td>
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<td>Sucrose</td>
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<td>22</td>
<td>5.7</td>
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<tr>
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<td>47</td>
<td>0</td>
<td>0</td>
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<td>Lard</td>
<td>2.3</td>
<td>5</td>
<td>33.8</td>
<td>50</td>
<td></td>
<td></td>
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<tr>
<td>Corn oil</td>
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<td>5</td>
<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vegetable shortening</td>
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<td>1.3</td>
<td>17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium chloride</td>
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<td>0.5</td>
<td></td>
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<tr>
<td>Mineral mix</td>
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<tr>
<td>Vitamin mix</td>
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<td></td>
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<tr>
<td>Choline bitartrate</td>
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<tr>
<td>DL-Methionine</td>
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<td></td>
<td>0.3</td>
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<td></td>
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<tr>
<td>Kilocalories per gram</td>
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<td></td>
<td>6.03</td>
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</table>

Both diets from Purina Test Diets (Richmond, IN).

Direct arterial pressure measurements (24 h/day). Rats were anesthetized and chronically instrumented with an arterial catheter for the direct measurement of arterial pressure. Gaseous anesthesia was induced and maintained using isoflurane (Isoflo, Abbott Laboratory, Chicago, IL). In addition, atropine (40 μg ip per rat) was administered to ensure an unobstructed airway. Body temperature was maintained at ~37°C using a servo-controlled heating pad. Under aseptic conditions, a laparotomy was performed and a nonocclusive, polyvinyl catheter was implanted in the abdominal aorta through a puncture wound in the aortic wall made with the tip of an L-shaped 18-gauge needle. The insertion point was sealed with cyanoacrylate adhesive, and the catheter was exteriorized through the lateral abdominal wall. The incision was infiltrated with penicillin G procaine (300,000 U/ml) and 0.25% bupivacaine HCl solution (Sensocaine-MPF, AstraZeneca, Wilmington, DE) at closure, and the catheter was routed subcutaneously to the scapular region and exteriorized through a Dacron-covered stainless steel button sutured subcutaneously over the scapulae. The rats were allowed to recover from surgery in a warmed cage for 1–2 h. Thereafter, rats were placed in individual metabolic cages in a quiet, air-conditioned room with a 12:12-h light-dark cycle. The catheter was connected to a single-channel infusion swivel (Instech) mounted above the cage and was protected by a stainless steel spring that also served as a tethering device. The arterial catheter was filled with heparin solution (1,000 USP U/ml) and connected, via the hydraulic swivel, to a pressure transducer mounted on the cage exterior at the level of the rat. The amplified pulsatile arterial pressure signals were sent to an analog-to-digital converter and analyzed by computer using customized software. The analog signals were sampled at 500 samples/s for 4 s each minute, 24 h/day. Food and water intake and urine volumes were monitored daily after recovery from surgery, and no experimental measurements were begun until these variables had stabilized.

Analytical methods. On the final day of the study, 2.5 ml arterial blood were withdrawn after a 4-h fast and 1 drop was placed on a test strip for analysis of blood glucose with an Accuchek III blood glucose analyzer. The remainder of the blood was placed into chilled potassium-EDTA tubes for measurement of PRA and plasma concentrations of insulin and sodium. Hematocrit was measured in microcapillary tubes, and plasma protein concentration was measured by refractometry. On the same day, 24-h urine samples were collected for the measurement of sodium, potassium, and albumin. PRA and plasma insulin concentrations were measured by radioimmunoassay (Diagnostic Products). Plasma and urinary sodium and potassium concentrations were determined using ion-sensitive electrodes (Nova, Waltham, MA). Urinary albumin was measured using a Nephrit II, rat albumin ELISA kit (Exocell, Philadelphia, PA).

Histological analysis. After the study was completed, the rats were killed and the heart and kidneys were rapidly removed, dissected free from connective tissue, and immediately placed in ice-cold PBS. Weights were recorded for the heart and right kidney, and the left and right ventricles of the heart and the right renal medulla were also isolated and weighed. The organs were then fixed either in neutral buffered formalin and embedded in paraffin or frozen in liquid N2.

Sections (5 μm thick) of formalin-fixed, paraffin-embedded tissue were mounted on glass slides and stained with the following: 1) hematoxylin-eosin for general histology, 2) periodic acid schiff for mesangial matrix and basement membrane in the kidney slices, or 3) proliferating cell nuclear
antigen (PCNA) for immunohistochemistry. All tissues were evaluated without investigator knowledge of the group from which it originated.

**Cell proliferation.** Glomerular cell proliferation was quantified by counting the number of PCNA-positive cells within a glomerulus. Twenty glomeruli per rat were chosen for cell counting. For PCNA immunohistochemistry, sections were deparaffinized, rehydrated, and endogenous peroxidase activity was quenched with 3% H₂O₂ solution for 30 min.

Nonspecific binding was prevented by incubating the sections with horse serum. The sections were then incubated with 150 μl of anti-PCNA (1:2,000, Dako, Carpinteria, CA) antibody. After three rinses with PBS, the sections were incubated with biotinylated secondary antibody, rinsed with PBS, then incubated with avidin-labeled peroxidase (VectaStain Elite ABC Kit, Vector Laboratories, Burlingame, CA). Positive labeling was visualized using diaminobenzidine. Another set of slides was run simultaneously as negative controls with all the same steps as above but no primary antibody incubation.

**Statistics.** Comparisons between rats fed an HF or LF diet were performed using unpaired t-tests. Data obtained on a weekly basis (body wt, systolic arterial pressure, and caloric intake) were analyzed by ANOVA with repeated measures. A value of P < 0.05 was considered statistically significant, and data are presented as means ± SE.

**RESULTS**

The two groups of rats were of similar weights before commencement of the HF or LF diet. Both groups continued to gain weight during the 17 wk on the different diets; however, there was a 34% greater increase in body weight in the rats fed an HF vs. LF diet (Fig. 1, P < 0.001). Despite a significantly greater increase in body weight in the rats fed an HF diet, systolic pressures, as measured by tail cuff during weeks 3–14 of the study, were not significantly different than those measured in rats on an LF diet. When arterial pressure was measured directly (24 h/day) via arterial catheterization, we observed a slight increase in the HF diet group (88 ± 1 mmHg in HF diet-fed rats vs. 85 ± 1 mmHg in the LF diet-fed rats, P < 0.05). However, there was no difference in resting heart rate between the two groups of rats (332 ± 4 vs. 332 ± 5 beats/min).

**Organ weights.** Total heart weights, left and right ventricular weights, as well as kidney and renal medulla weights were significantly greater in the rats fed an HF diet (Table 2, P < 0.05). Total heart weight was ~24% greater in the HF compared with the LF diet-fed rats. Likewise, left and right ventricular weights were 21% and 36% greater, respectively, in HF compared with LF diet-fed rats. Total kidney weight was ~33% greater in H rats (Table 2).

**Plasma and urinary data.** Despite a reduced food intake in the rats fed an HF diet (13 ± 1 vs. 17 ± 1 g/day in the rats fed an LF diet), caloric intake, averaged over the 15 wk of the study, was 20% greater in the rats fed an HF vs. LF diet (Table 2, P < 0.05) due to the higher caloric content of the HF diet. Urinary potassium excretion was greater in the rats fed an HF diet (1.33 ± 0.07 vs. 1.00 ± 0.05 mmol/day; P < 0.05). Because the only source of potassium intake in these rats was the rat chow, a higher potassium intake in the rats on the HF diet likely explains the higher potassium excretion; although the rats fed an HF diet ate less food, the potassium content was higher in the HF diet (0.14 vs. 0.09 meq/g food in the LF diet). The HF diet was formulated with a higher potassium content in anticipation that the rats would eat less food and with the goal of maintaining a relatively constant potassium intake in the two groups. There was no difference in urinary sodium excretion between the rats fed

![Fig. 1. Body weight of male Osborne-Mendel rats during 14 wk of a high (n = 11)- or low-fat diet (n = 10). Body weight of Osborne-Mendel rats fed a high-fat diet was significantly higher than Osborne-Mendel rats fed a low-fat diet (P < 0.001, ANOVA with repeated measures).](http://ajpregu.physiology.org/)
an HF vs. LF diet (Table 2). Urinary albumin excretion tended to be higher in the rats fed an HF diet, and in two of the HF diet-fed rats (not included in the statistical analysis), urinary albumin excretion was >20 mg/day.

OM rats fed the HF diet had a threefold increase in plasma insulin, a 16 ± 4% higher blood glucose, and a 40 ± 6% reduction in PRA compared with rats fed an LF diet (Table 2). Hematocrit was slightly reduced in rats fed an HF diet (41 ± 1 vs. 43 ± 1%, P < 0.05), whereas there were no significant differences in plasma protein or sodium between the two groups (Table 2).

Histology. There was no evidence of major renal or cardiac pathology in either group of OM rats based on observational analysis at the level of the light microscope. However, a significantly greater number of cells within the glomerulus stained for PCNA in the HF (34 ± 2%) vs. the LF diet-fed rats (29 ± 2%, P < 0.05), indicating increased proliferation of glomerular cells.

DISCUSSION

The main finding of this study is that ingestion of an HF diet in male OM rats resulted in cardiac and renal hypertrophy, hyperinsulinemia, and a slight increase in mean arterial pressure along with increased body weight. Furthermore, OM rats fed an HF diet had higher blood glucose and lower PRA compared with OM rats fed an LF diet.

There is a strong association between obesity and hypertension and metabolic disorders such as hyperinsulinemia and type II diabetes (29). To investigate these interactions, several animal models of obesity have been developed. These include genetic models of obesity such as the Zucker fatty rat (2), Wistar fatty rats (25), and Koletsky spontaneously hypertensive rats (6). However, many of the genetic models of obesity may not mimic the cardiovascular, renal, and neurohormonal changes found in obese humans (14, 27).

Another obvious difference between some of the genetic rodent models of obesity and human obesity is that most of the rodent models become obese even when fed an LF diet in contrast to obese humans in which excess dietary fat intake appears to play a major role. Therefore, models of obesity induced by fat intake may mimic the human condition more closely (20). Indeed, there is a direct relationship between the fat content in the diet and body fat (7, 22). In most diet-induced models of obesity, the quantity of fat in the diets is usually in the range of 44–64% (1, 8, 28, 30).

In the current study of OM rats on the HF diet, 74% of the energy intake was provided by fat to ensure that these rats had a significant weight gain. Therefore, even though the rats fed an HF diet actually decreased their food intake, as has been previously shown (23), they still consumed 20% more calories per day than rats fed an LF diet. When expressed as calories consumed per day per kilogram body weight, the HF OM rats had a slightly lower intake (162 kcal/kg) compared with the LF OM rats. However, this observation is difficult to interpret because we did not measure relative body composition (lean and adipose tissue mass) or metabolic rate in these studies.

The OM strain of rats was chosen for this study because they have been shown to have a greater tendency to become obese when fed an HF but not an LF diet (23). The mechanisms for the increased susceptibility of OM rats to develop obesity are not known but have been hypothesized to involve reduced transport of ketone bodies into the brain (3), type II glucocorticoid receptor activation (21), and insulin resistance (4). There is also evidence for a role of leptin resistance in the development of diet-induced obesity in OM rats (16).

In the current study, OM rats fed an HF diet for 17 wk had a greater increase in body weight (46%) but no change in systolic blood pressure measured weekly via the tail-cuff method. This is in apparent contrast to the findings of Wilde and colleagues who reported that OM rats fed an HF diet had a significantly greater body weight (40%) and a modest elevation in systolic blood pressure, as measured by tail cuff at the end of the feeding study (30). The difference in blood pressure responses may be due to the method of measurement. Acute measurements of arterial pressure, such as those obtained using the indirect tail-cuff method, are more susceptible to variability caused by handling and restraining stress. Blood pressure and heart rate in rodents are elevated by restraint, even when they have been conditioned to a restrainer (18). Indeed, we found in OM rats fed an HF diet that systolic blood pressure, assessed by the tail-cuff method, was significantly greater in the first week of measurements. However, after subsequent measurements and further acclimation of the rats to handling, there were no differences in systolic arterial pressure between the two groups of OM rats. Direct measurement of arterial pressure (24 h/day), with the use of chronically indwelling catheters, revealed only a slight increase (3 mmHg) in mean arterial pressure in OM rats fed an HF diet.

The marked increase in body weight in OM rats fed an HF diet was associated with greater left and right ventricular weights compared with OM rats fed an LF diet. These findings are consistent with previous studies that have shown that obesity results in left ventricular hypertrophy (5, 15, 19). However, our studies suggest that, in OM rats, the cardiac hypertrophy associated with obesity can occur even in the absence of marked hypertension. The hypertrophy of the right ventricle as well as the left ventricle also suggests that factors other than increased arterial pressure play an important role in obesity-induced cardiac hypertrophy. Increased blood volume, elevated cardiac output, and increased cardiac work may be important, but other neuroendocrine factors have also been suggested to contribute to growth of cardiac muscle in obesity (24). Further studies are needed to address this issue.

Obesity also causes hypertrophy of other organs, including the kidney. The greater increase in kidney and renal medulla weights in OM rats fed an HF diet...
may be related to increased work of the kidney, secondary to increased glomerular filtration rate (GFR) and increased tubular sodium reabsorption associated with obesity (2, 10). The glomerular hyperfiltration may help to maintain sodium balance despite increased tubular reabsorption (2), but in the long term, it may also contribute to injury of the glomerulus. Although we did not measure GFR in the present study, this speculation is consistent with our findings of increased proliferation of glomerular cells in OM rats fed an HF diet. However, the mechanisms of glomerular proliferation and renal growth in obesity are still unclear. Although there was no evidence of major renal pathology after 17 wk of an HF diet in the OM rats, urinary albumin excretion tended to be higher in OM rats fed an HF diet, possibly reflecting early, mild injury to renal glomeruli.

One of the major similarities between diet-induced obesity in OM rats and human obesity is the presence of hyperinsulinemia. Fasting plasma insulin concentrations were threefold greater in OM rats fed an HF diet compared with OM rats fed a normal diet. The elevated insulin levels observed in obesity are thought to be necessary to maintain normal glucose levels in the presence of impaired insulin action resulting from insulin resistance (9). Indeed, in rats fed an HF diet, there is reduced insulin-mediated glucose in skeletal muscle and liver (13). Our findings that OM rats have hyperinsulinemia along with mild increases in plasma glucose are consistent with decreased insulin sensitivity.

In the current study, PRA was significantly lower in OM rats fed an HF diet vs. OM rats fed an LF diet. To our knowledge, this is the first report of a difference in PRA due to the fat content of the diet in OM rats and is consistent with the finding of reduced PRA in some rodent models of obesity, such as obese Zucker rats (2).

**Perspectives**

Although obesity has reached epidemic proportions in the United States and other industrialized countries, the mechanisms by which excess weight gain causes hypertension and increases cardiovascular risk are still unclear. One major problem in this field has been the lack of animal models that closely mimic the cardiovascular, neurohumoral, and metabolic changes associated with obesity in humans. Multiple studies have suggested that an HF diet plays a key role in human obesity, but genetic factors also appear to be important. Therefore, development and characterization of animal models in which both genetic and dietary factors contribute to their obesity will likely provide a better understanding of the mechanisms of obesity-induced cardiovascular disease in humans. The OM rat used in the present study is a possible model for studying interactions of dietary and genetic factors in obesity. When fed an HF diet, these rats gain greater amounts of weight than many other rat strains and have some of the cardiovascular and hormonal changes associated with human obesity, including cardiac and renal hypertrophy, a slight increase in mean arterial pressure, and hyperinsulinemia. Additional effort, however, is needed to develop other animal models of obesity that more closely mimic the cardiovascular changes observed in obese humans.

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