Development of Hypertension in a Rat Model of Diet-Induced Obesity

Anca D. Dobrian, Michael J. Davies, Russell L. Prewitt, Thomas J. Lauterio

Abstract—Although obesity is a risk factor for hypertension, the relationship between these 2 conditions is not well understood. Therefore, we examined some parameters of hypertension and cardiovascular disease in a dietary model of obesity. Male Sprague-Dawley rats were provided either a control diet (C) or a diet containing 32% kcal as fat (similar to a Western diet) for 1, 3, or 10 weeks. Rats in the latter group diverged based on body weight gain into obesity-prone (OP) and obesity-resistant (OR) groups. Systolic blood pressure in OP rats was significantly higher after 10 weeks of the diet (149±4.8 mm Hg) compared with both OR and C groups (131±3.7 and 129±4.5 mm Hg, respectively). The aortic wall area of OP rats was significantly increased, indicating arterial hypertrophy, and a 2-fold increase in plasma renin activity was found in OP rats compared with OR and C rats. The lipid profile showed a significant increase in plasma and VLDL triglycerides of OP versus OR and C groups as early as 3 weeks on the diet. Plasma and LDL-cholesterol levels were increased in the OP group versus the OR and C groups after 3 weeks of the diet, but the difference was blunted after 10 weeks. Lipid peroxidation (thiobarbituric acid–reactive substances) in OP rats was increased 2-fold in LDL and 1.5-fold in aortic wall compared with OR rats, suggesting an increased oxidative stress in these animals. Periodic acid–Schiff staining of the kidney showed mesangial expansion and focal sclerosis that were more prominent in OP rats than in OR rats. The results suggest that hypercholesterolemia, but not hypertriglyceridemia, is linked to the diet; that hypertension and renin-angiotensin system activation are associated with obesity; and that lipid peroxidation and renal damage are the results of both factors. (Hypertension. 2000;35:1009-1015.)

Key Words: renin ■ aorta ■ kidney ■ lipoproteins ■ lipids ■ diet

Obesity is a complex, multifactorial disease that is often associated with diabetes, cardiovascular diseases, and stroke. Among other factors, obesity is an important contributor to essential hypertension in humans. Data from the Framingham Heart Study suggest that ~78% of essential hypertension in men and ~65% of essential hypertension in women can be directly attributed to obesity.1 However, the mechanisms that link obesity with high blood pressure and altered renal function have not been fully elucidated. One problem in the study of the mechanisms of obesity hypertension has been the lack of a suitable animal model. The ideal model would not only have the features of human hypertension but also allow the study of sequential changes in cardiovascular and kidney function that occur with weight gain. The genetic models of obesity may or may not develop hypertension or do not mimic the changes observed in humans. For example, Zucker rats have decreased plasma renin activity (PRA)2 as opposed to the high PRA observed in humans.3 In contrast, diet-induced obese animal models appear to be the most relevant with regard to human obesity. Some of these models, such as the obese dog4 or obese rabbit5 fed a high-fat diet, are used extensively to study obesity hypertension because they closely mimic some of the cardiovascular and kidney function that occur with weight gain. A particularly interesting model of diet-induced obesity is one originated by Levin et al6 and currently developed into a purified diet model.7 In this model, Sprague-Dawley (SD) rats fed a purified moderately high-fat (MHF) diet exhibit a bimodal pattern in body weight (BW) gain similar to that observed in humans. Approximately half of the rats gain weight rapidly compared with chow-fed rats (obesity prone [OP]), whereas the other half gain BW at a rate similar to or lower than that of the chow-fed animals (obesity resistant [OR]).7 This model enables one to dissociate between the factors related to a high-fat diet and obesity per se.

Some of the major characteristics associated with obesity hypertension in humans are the activation of the renin-angiotensin system (RAS),8-9 high levels of circulating leptin,10 reduced growth hormone (GH) concentration,11 and an activation of the sympathetic nervous system.12 Moreover, obesity hypertension is often associated with dyslipidemia, indicating low levels of HDL-cholesterol13 and higher levels of triglycerides.14 In addition, hyperlipidemia associated with hypertension may induce glomerulosclerosis in the kidney and eventually alter kidney function.15 There also are data that indicate increased oxidative stress in human essential hypertension because they closely mimic some of the cardiovascular and kidney function that occur with weight gain.
hypertension,16,17 as well as in obese hypertensive patients,18 that may further contribute to the development of atherosclerosis or other cardiovascular diseases.

In the present study, we used a diet-induced obesity model in the rat7,19 that displays some of the characteristics of human obesity hypertension, such as increased plasma norepinephrine response to intravenous glucose,6,20 increased plasma leptin concentration,21 and decreased GH secretion and synthesis.22,23 The purpose of the present study was to document the development of hypertension in this animal model and to partially characterize the model with respect to some factors likely to be involved in the cardiovascular and renal changes associated with obesity hypertension. We consider this model particularly useful in the assessment of the causal relationship among hypertension, obesity per se, and diet.

Methods

Animals

All procedures that involved animals were approved by the Animal Care and Use Committee of Eastern Virginia Medical School. Two separate sets of animals were used to measure short-term and long-term responses to diet-induced obesity. Animals were housed individually in stainless steel cages in a temperature-controlled room (22±2°C) with a 12-hour light/dark cycle. Food and water were provided ad libitum throughout the acclimation and experimental periods.

Short-Term Responses

Forty-two male SD rats (175 to 200 g) were randomly assigned to receive either an MHF (32% kcal as fat; Research Diets) or a purified low-fat diet (LF) (10.6% kcal as fat; Research Diets) for either 1 or 3 weeks. Six rats were sacrificed as baseline controls for blood pressure and blood lipid profile values. BWs and body lengths were measured initially and then weekly together with food intake. At each time point (1 and 3 weeks), the rats with the greatest and lowest weight gain were selected along with LF-fed animals, and blood pressures were measured.

Long-Term Responses

Of 32 male SD rats (300 to 350 g), 24 were randomly selected to be fed the MHF diet, whereas the remaining 8 rats (controls) were fed a standard rat chow diet (6.5% kcal fat; Harlan Teklad) for 10 weeks. BW and food intake (corrected for spillage) were measured weekly. After 10 weeks, rats fed the MHF diverged into distinct groups based on BW gains. BW gains were analyzed for frequency distribution with χ2 and Student’s t test analyses as previously described.19,24 Rats with the greatest BW gains were referred to as OP (n=8), whereas those with the lowest BW gains were referred to as OR (n=8). This analysis demonstrated a significant difference in BW gain between OP and OR rat populations. At the end of the study, rats were decapitated, and trunk blood was collected in EDTA-coated tubes. Plasma was immediately separated through centrifugation and used for PRA determination, lipid assays, and lipoprotein isolation. Thoracic aorta, kidney, and fat depots (retroperitoneal and epididymal) were harvested, weighed, and either used immediately or snap frozen in liquid nitrogen.

Systolic Blood Pressure

The onset and development of hypertension were assessed with the tail-cuff method with a Narco Biosystems Electro-Sphygnomanometer after the rats were warmed at 35°C for 5 minutes, while under slight restraint. Blood pressure was measured under conscious conditions at the beginning of the experiment and at 1, 3, 8, and 10 weeks of the diet. The average of 3 pressure readings was recorded for each measurement.

Lipid Profile

Blood was collected after decapitation, and plasma was subjected to density gradient ultracentrifugation.25 VLDL was isolated at the plasma density of d<1.006 g/mL, and LDL was collected at d=1.018 to 1.050 g/mL. Both VLDL and LDL fractions were washed through centrifugation and extensively dialyzed at 4°C in the dark against multiple changes of Tris-buffered saline, pH 7.4, with 0.01% EDTA and 0.22 mmol/L BHT (Sigma Chemical Co.). Cholesterol and triglyceride levels in total plasma and lipoprotein fractions were assayed with enzymatic kits from Sigma Chemical Co.

Lipid Peroxidation

Lipid peroxidation in LDL fraction and tissues (thoracic aorta and kidney) was determined with spectrophotometric measurement of the amount of malondialdehyde equivalents with thiobarbituric acid and was expressed as thiobarbituric acid-reactive substances (TBARS; nmol malondialdehyde/mg protein), according to the method of Fogelman et al.26 LDL was dialyzed against Tris-HCl buffer, supplemented with 0.01% EDTA and 0.33 mmol/L BHT, and assayed within 48 hours on isolation. The thoracic aorta and kidneys were collected on ice, washed in 0.9% NaCl, and immediately homogenized in 20 mmol/L Tris-HCl, pH 7.4, supplemented with 5 mmol/L BHT.27 LDL protein was assayed according to a modification of the Lowry method,28 and tissue protein was assayed according to the BCA method29 with BSA as a standard.

Morphological Analysis

To determine the wall area of thoracic aorta as an index of arterial hypertrophy, thin sections (5 µm thick) of the paraffin-embedded tissue were dehydrated and stained for 1 minute with toluidine blue to visualize the intima-media of the vessel wall. The internal and external circumferences of each vessel were measured with a video-based image system with edge-tracking software (JAVA; Jandel Scientific). The mean of 3 different measurements was used to calculate the internal and external diameters and the intimal-medial area.

Histology

Kidneys were fixed in 10% buffered formalin for 4 hours and embedded in paraffin. Thin (4- to 5-µm) sections were stained with periodic acid–Schiff (PAS) reagent and hematoxylin for counterstaining. To evaluate the degree of segmental sclerosis, 3 independent investigators examined the slides in a blinded fashion, mixing the slides after covering the protocol numbers. In each case, 10 to 20 glomeruli were examined for each slide and individually graded on a scale of 0 to 2+ according to the degree of glomerular sclerosis. Grade 0 indicated a normal-appearing glomerulus; grade 1+ was characterized by mild expansion of mesangial matrix, no occlusion in the glomerular capillaries, or adhesion to Bowman’s capsule; and grade 2+ included expansion of the mesangial matrix, usually focal with adhesion to Bowman’s capsule, and some degree of capillary occlusion. A score representing the sum of grades was obtained for each rat.

Other Assays

PRA was measured through radioimmunoassay with a kit in which [125I]-angiotensin (DiaSorin Inc) was used according to the manufacturer’s instructions.

Statistical Analysis

All data were analyzed for statistical significance with ANOVA for repeated measures and Student’s t test. The null hypothesis was rejected at P<0.05.

Results

BW, Food Intake, and Composition Data

The BWs for OP, OR, and C rats were measured weekly. Initial mean BWs of the 3 groups did not differ significantly.
(Table 1). At week 3, OP rats had significantly higher BWs than OR rats, and the BWs remained higher throughout the 10-week dietary period. At week 7, the BWs of OP rats were significantly greater than those of C rats. In terms of the BW gain, the short-term (1- and 3-week) study showed a significant difference after the first week of MHF diet between the OP and OR rats (Table 1). After 3 weeks of the diet, a 25% increase in mean BW was observed for the OP rats versus the OR and C rats, and in the long-term study, an even higher (34%) increase was found after 10 weeks of the diet (Table 1). Epididymal and perirenal fat depot weights were higher in OP than in OR rats after 10 weeks of the diet (Table 1). The obesity index (the equivalent of the body mass index for humans) after 10 weeks was significantly increased in OP compared with both OR and C rats, reflecting the increased body fat content (Table 1). Feed efficiency of weight gain calculated as BW gain (g) divided by energy consumed (MJ) was 25% higher for OP than for resisters, suggesting a difference in the metabolic response to the diet between the 2 groups (Table 1).

Blood Pressure, PRA, and Thoracic Wall Area in OP, OR, and C Rats
The average baseline systolic blood pressure, measured 1 day before the start of the MHF diet, was 114±0.8 mm Hg for all of the rats used in the experiment. After 1 and 3 weeks of diet, the blood pressure increased slightly in all 3 groups, but it did not differ among groups (Figure 1). Starting with week 8, the systolic blood pressure of the OP rats was significantly increased compared with C rats (Figure 1). After 10 weeks of the diet, the mean blood pressure of OP rats was 149±4.8 mm Hg, which was significantly higher than that of either OP (131±3.7 mm Hg) or chow-fed C (129±4.5 mm Hg) groups (Figure 1). PRA was in the normal range for all groups after both 1 and 3 weeks of diet (Table 2). After 10 weeks, PRA was increased 2-fold in the OP group compared with both OR and C

Table 1. Comparison of Weight Gain, Obesity Index, and Feed Efficiency for OP, OR, and Control Rats

<table>
<thead>
<tr>
<th>Variable</th>
<th>OP/MHF</th>
<th>OR/MHF</th>
<th>Control/Chow Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body weight, g</td>
<td>389±17.5</td>
<td>367.6±14.9</td>
<td>386.1±2.9</td>
</tr>
<tr>
<td>Total weight gain, g</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 wk</td>
<td>37.5±0.6*</td>
<td>20.4±0.4</td>
<td>ND</td>
</tr>
<tr>
<td>3 wk</td>
<td>72.7±1.6†</td>
<td>55.9±1.3</td>
<td>59.4±2.3</td>
</tr>
<tr>
<td>10 wk</td>
<td>282.1±15.7†</td>
<td>199.3±4.9</td>
<td>213.5±11.6</td>
</tr>
<tr>
<td>Total fat at 10 wk, g§</td>
<td>51.3±4.3†</td>
<td>40.1±3.4</td>
<td>35.7±3.8</td>
</tr>
<tr>
<td>Obesity index at 10 wk, g†</td>
<td>333.4±1.8†</td>
<td>325.1±2.0‡</td>
<td>315.2±2.7</td>
</tr>
<tr>
<td>Feed efficiency at 10 wk, g/MJ</td>
<td>9.1±0.4*</td>
<td>7.5±0.1</td>
<td>8.4±0.3</td>
</tr>
</tbody>
</table>

ND indicates not determined. Data are presented as mean±SE; n=8 for 10-wk group and n=6 for 1- and 3-wk groups. *Significant vs OR. †,‡Significant vs control animals. §Total fat represents the sum of epididymal and perirenal fat depots. ¶Obesity index was calculated by dividing the cubic root of the body weight (g) by the nasoanal length (mm)×10^4.

Table 2. Plasma Renin Activity and Thoracic Wall Area of OP, OR, and Control Rats

<table>
<thead>
<tr>
<th>Rat Group</th>
<th>Plasma Renin Activity, ng·mL⁻¹·h⁻¹</th>
<th>Wall Area, mm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-wk diet</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OP</td>
<td>4.9±1.1</td>
<td>0.44±0.02</td>
</tr>
<tr>
<td>OR</td>
<td>5.1±1.3</td>
<td>0.45±0.03</td>
</tr>
<tr>
<td>3-wk diet</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OP</td>
<td>5.1±1.3</td>
<td>0.43±0.03</td>
</tr>
<tr>
<td>OR</td>
<td>5.0±1.2</td>
<td>0.44±0.02</td>
</tr>
<tr>
<td>Control</td>
<td>5.2±1.2</td>
<td>0.45±0.03</td>
</tr>
<tr>
<td>10-wk diet</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OP</td>
<td>11.2±1.3*</td>
<td>0.59±0.03‡</td>
</tr>
<tr>
<td>OR</td>
<td>5.7±1.4</td>
<td>0.48±0.03</td>
</tr>
<tr>
<td>Control</td>
<td>5.8±1.2</td>
<td>0.52±0.02</td>
</tr>
</tbody>
</table>

Plasma renin activity was assayed at the end of the experiment, after decapitation and blood collection on 0.01% EDTA. Wall area was measured on paraffin-embedded sections that were stained with toluidin blue, with the use of a video-based image system with edge tracking software. Data represent mean±SE of 6 to 8 rats per group. *Significant vs OR. †Significant vs control rats.
rats (Table 2). The result parallels the increase in blood pressure observed in the OP group versus the OR and C rats. Moreover, there is a good correlation in all 3 groups between blood pressure and PRA ($r = 0.794, P = 0.05$), suggesting that activation of the RAS is important in the development of hypertension in this model. To assess whether the high blood pressure in the OP group of animals had any effect on the morphology of the arteries, we measured the wall area of thoracic aorta in the 3 groups of rats after 1, 3, and 10 weeks of the diet. The results showed no differences among the 3 groups after 1 and 3 weeks of the diet (Table 2). After 10 weeks of the diet, the OP rats displayed a 30% increase in the aortic wall compared with OR rats (Table 2), suggesting that structural changes already occurred in the arteries of hypertensive rats. In addition, there is a good correlation between wall area and blood pressure in OP, OR, and C rats ($r = 0.861, P < 0.01$), suggesting that the structural arterial changes observed are in close connection to the blood pressure in these animals.

**Plasma Lipid Profile in OP, OR, and C Rats**

After 1 week of the diet, there were no differences in the cholesterol and triglyceride levels in both plasma and lipoprotein (LDL and VLDL) fractions among the OP, OR, and C groups. After 3 weeks of the diet, the cholesterol values for the OP group were significantly increased compared with OR and C groups in plasma, LDL, and VLDL fractions, whereas only the LDL-cholesterol level was higher in OR than in C rats (Figure 2A). The difference in the total cholesterol and LDL- and VLDL-cholesterol levels was blunted between OP and OR rats after 10 weeks of the MHF diet but remained significantly increased in both groups compared with the chow-fed C group (Figure 2B). The total, LDL, and VLDL triglyceride levels were significantly increased after 3 weeks of the MHF diet in OP compared with both OR and C groups (Figure 2C). The same differences for plasma and VLDL triglycerides were also observed after 10 weeks of the diet, with a further increase in the VLDL triglycerides in the OP group and a correspondent decrease in LDL triglycerides that abolished the difference among the OP, OR, and C groups in this latter fraction (Figure 2D). The data suggest that hypercholesterolemia in OP and OR rats was a direct consequence of the MHF diet, whereas the elevated level of triglycerides in OP group may be attributable to the obese state and not to the high-fat diet.

**Lipid Peroxidation in Plasma and Tissues of OP, OR, and C Rats**

The level of lipid peroxides expressed as TBARS was assessed in LDL fraction (known to be the most prone to oxidative modification) and in the homogenates of thoracic
**TABLE 3. Lipid Peroxides Expressed as TBARS in LDL Fraction and Thoracic Aorta of OP, OR, and Control Rats**

<table>
<thead>
<tr>
<th>Rat Group</th>
<th>TBARS/LDL, nmol MDA/mg Protein</th>
<th>TBARS/Aorta, nmol MDA/mg Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-wk diet</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OP</td>
<td>0.3±0.02</td>
<td>ND</td>
</tr>
<tr>
<td>OR</td>
<td>0.25±0.01</td>
<td>ND</td>
</tr>
<tr>
<td>3-wk diet</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OP</td>
<td>0.9±0.03†</td>
<td>0.1±0.03</td>
</tr>
<tr>
<td>OR</td>
<td>0.8±0.05†</td>
<td>ND</td>
</tr>
<tr>
<td>Control</td>
<td>0.4±0.02</td>
<td>ND</td>
</tr>
<tr>
<td>10-wk diet</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OP</td>
<td>2.8±0.32†</td>
<td>3.3±0.44†</td>
</tr>
<tr>
<td>OR</td>
<td>1.1±0.43</td>
<td>2±0.24†</td>
</tr>
<tr>
<td>Control</td>
<td>0.9±0.3</td>
<td>1.1±0.15</td>
</tr>
</tbody>
</table>

ND indicates not detectable.

Lipid peroxides were expressed as TBARS in the LDL fraction and thoracic aorta of OP, OR, and control rats. All of the working buffers for LDL and tissue homogenates were supplied with EDTA and BHT as indicated in the text. Data represent mean±SE of 6 to 8 rats per group.

*Significant vs control rats.
†Significant vs OR.

Renal Morphology Changes in OP, OR, and C Rats

To address the possible morphological changes in the kidney, we used PAS-hematoxylin staining and morphometric analysis of the kidneys of OP, OR, and C rats after 10 weeks of their respective diets. Figure 3 illustrates the mild sclerosis observed in the OP (Figure 3C) and OR (Figure 3B) rats compared with C rats (Figure 3A). The lesions were in various stages of development, with most of them in a relatively early stage, displaying capillary loop collapse, moderate mesangial expansion, and sometimes adhesion between the affected segments and Bowman’s capsule (Figure 3C). Morphometric analysis indicated a mean±SE mesangial score of 11.8±0.9 for the OP group, which was higher than that for the OR (9.2±1.2) and C (8.4±1.2) groups. Still, the difference was significant only between the OP and C groups. The data suggest a combined role of diet and obesity per se in the kidney injury in this rat model.

**Discussion**

This model of diet-induced obesity developed mild hypertension accompanied by vascular and renal changes similar to those observed in obese hypertensive humans. The MHF diet that we used contains 32% kcal fat, a value similar to the average Western diet, as opposed to many other models that have very high levels of fat.30 In addition, similar to humans, not all rats fed the MHF diet become obese, with the BW displaying a bimodal distribution. The increased BW reflects an increase in the adipose mass in the OP rats versus the C rats as measured with the obesity index. This mimics the increased body mass index associated with human obesity. The experiments enabled us to assess early (1 to 3 weeks after the beginning of the MHF diet) and late (10 weeks) changes induced by diet and obese state. One of the earliest changes measured is a drastic modification in the lipid profile among OP, OR, and C groups after 3 weeks of diet. Total plasma cholesterol is significantly increased in OP rats only, because of a cholesterol enrichment in both LDL and VLDL fractions (Figure 2A). Results are consistent with other reports of diet-induced obesity but only after 8 weeks of a hypercholesterolemic diet.31 After 10 weeks of the diet, both OP and OR rats had significantly higher levels of total and LDL-cholesterol but not VLDL-cholesterol. These data suggest that the clearance of cholesterol is impaired in OP rats, whereas in OR rats the cholesterol loading of LDL is decreased. Therefore, OP rats are exposed to high cholesterol levels for a longer time period, which may lead to vascular complications that may appear later in time. Some preliminary data from our group indicate that the OP rats have a lower HDL-cholesterol level compared with the OR and C groups. This may explain the moderate increase in HDL-cholesterol observed in the OR group. However, the HDL-cholesterol values were not significantly different among the 3 groups. Further studies are needed to investigate this further.

Figure 3. Representative micrographs showing PAS staining of the kidney in OP, OR, and C rats. Mild glomerulosclerosis may be observed in the OP and OR kidney compared with the C kidney (bar=25 μm).
level throughout the diet compared with OR and C groups (unpublished data). This is consistent with other reports showing a decrease in HDL-cholesterol level in hypercholesterolemic rats.\textsuperscript{32} Triglycerides are also increased in OP rats after 3 weeks of the MHF diet in plasma, LDL, and VLDL fractions (Figure 2C). As opposed to cholesterol content, this difference is even greater after 10 weeks of the diet, indicating that factors other than diet are also responsible for the high levels of triglycerides in OP rats. One possible factor may be the reduced GH secretion reported in OP rats.\textsuperscript{22,23} Impaired GH secretion may lead to an abnormal lipid profile in rats, with high levels of triglycerides and low levels of HDL-cholesterol.\textsuperscript{33} Dyslipidemia, which occurs in this model of diet-induced obesity, is also a common feature of obesity in humans and is frequently associated with hypertension.\textsuperscript{34}

Another early detectable parameter in our model is lipid peroxidation. Free radicals are known to be involved in a variety of human pathologies, including atherosclerosis,\textsuperscript{35} obesity,\textsuperscript{18} and hypertension.\textsuperscript{16,17,36} In addition, increased oxidative stress was reported for various animal models of hypertension, such as spontaneously hypertensive rats,\textsuperscript{37} the Dahl hypertensive rat,\textsuperscript{38,39} or angiotensin-induced hypertension in the rat.\textsuperscript{40} In this study, peroxides associated with the native LDL fraction were elevated in both OP and OR rats compared with C rats by 3 weeks. This may be due to the increase in the overall cholesterol loading of LDL particles caused by the MHF diet. However, the values do not exceed the normal values reported for the physiological levels of peroxides associated with LDL particles. The aortic homogenates of OP rats displayed a low level of TBARS compared with OR and C rats, in which the peroxides, if present, were not detectable with our method (Table 3). After 10 weeks of the diet, the level of lipid peroxides in the LDL fraction was significantly increased in OP rats compared with both OR and C rats, and the TBARS associated with LDL indicated the presence of a minimally modified LDL level. This was shown to be a potentially atherogenic state of LDL that is also common with other diseases, such as diabetes.\textsuperscript{41} The level of lipid peroxides in the aortic wall was also significantly increased in OP versus OR and C rats and in OR versus C rats, suggesting that both the diet and the obese state are responsible for the induction of the increased oxidative stress. A potential mechanism for the generation of free radicals may be the activation of $\beta$-adrenergic receptors reported for OP rats.\textsuperscript{19} This could increase lipolysis to yield free fatty acids that are able to uncouple the mitochondrial phosphorylation and further generate free radicals.\textsuperscript{42} Another possibility is that increased leptin reported for OP\textsuperscript{21} is able to generate oxidative stress, as was recently reported for endothelial cells in culture.\textsuperscript{43}

The most important associated pathology that was detected late in the diet was hypertension. A significant increase in blood pressure that exceeds the normal threshold (systolic blood pressure $>$140 mm Hg) was measured only after 8 weeks of the diet and became significant in OP versus OR and C rats only after 10 weeks of the diet (Figure 1). Hypertension developed in OP, but not OR, rats, suggesting that diet is not the major factor that causes the high blood pressure in this model. The hypertension is also well correlated to PRA, indicating that activation of the RAS may be one of the major causes of the increase in blood pressure in OP rats. There is a consensus that sympathetic overactivity in obese subjects may predispose to cardiovascular disease in general and to hypertension in particular.\textsuperscript{44}

The hypertension and obesity in this rat model are also accompanied by changes in the vascular and renal morphology. The aortic wall area of the OP rats is significantly increased compared with OR and C rats. In consideration of the significant correlation between blood pressure and wall area in all 3 groups, arterial hypertrophy is most likely caused directly by hypertension. High blood pressure is known to induce vascular hypertrophy in animal models of experimental hypertension,\textsuperscript{35,46} and the elevated pressure may initiate a growth response through elevations in wall stress.\textsuperscript{47} The hypertrophy of the wall and the increased lipid peroxidation detected are potential candidates to contribute to further complications in OP rats, such as atherosclerosis. The kidney morphology indicated a moderate glomerulosclerosis in OP rats and some mild changes in the glomerular structure in OR rats compared with C rats (Figure 3). The alterations in kidney structure are apparently a combined effect of diet and other aggravating factors associated with the obese state. Hypertriglyceridemia present in the OP rats may contribute to glomerulosclerosis, as shown in other animal models.\textsuperscript{48–50} In addition, reactive oxygen species, which are apparently increased in our model, were shown to induce glomerular sclerosis and altered tubular cell function.\textsuperscript{51} Although we did not measure any renal functional parameters, we expect the sclerosis in the OP rat kidney to increase tubular reabsorption, which may raise glomerular filtration rate and stimulate renin secretion. On the other hand, the increased perirenal fat depots measured in OP rats may generate medullary pressure, which is also known to cause renin release.\textsuperscript{4} Further investigation is needed to assess the renal function in our model. One possible scenario, based on the present results and supported by data reported by other authors, is that increased perirenal fat and hypertriglyceridemia associated with obesity, together with the increased oxidative stress generated by both the obese state and diet, may induce augmented renin release by the kidney due to glomerulosclerosis or other mechanisms, which ultimately induce the increase in blood pressure.

In conclusion, we partially characterized a rat model of diet-induced obesity that also develops hypertension. The advantages over other similar animal models are the close similarity to human obesity hypertension, namely activation of RAS, dyslipidemia, increased oxidative stress, and associated vascular and renal pathology, and the ability to dissociate between the effect of MHF diet (specific differences between OP, OR, and chow-fed C rats) and other factors specifically related to obese state (differences between OP and OR rats). With the latter differential analysis, we can conclude that diet induces hypercholesterolemia, but not hypertriglyceridemia; that obesity is responsible for the development of hypertension and RAS activation; and that lipid peroxidation and the renal changes are apparently the result of the combined effect of dietary factors and obesity state per se. The model may also be useful to study other pathologies associated with obesity, such as atherosclerosis.

Acknowledgments

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References


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