Oxidative Stress in a Rat Model of Obesity-Induced Hypertension

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

Abstract—The mechanisms underlying the development of hypertension in obesity are not yet fully understood. We recently reported the development of hypertension in a rat model of diet-induced obesity. When Sprague-Dawley rats (n=60) are fed a moderately high fat diet (32 kcal% fat) for 10 to 16 weeks, approximately half of them develop obesity (obesity-prone [OP] group) and mild hypertension (158±3.4 mm Hg systolic pressure), whereas the other half (obesity-resistant [OR] group) maintains a body weight equivalent to that of a low fat control group and is normotensive (135.8±3.8 mm Hg). We examined the potential role of oxidative stress in the development of hypertension in this model. Lipid peroxides measured as thiobarbituric acid-reactive substances showed a significant increase in the LDL fraction of OP rats (2.8±0.32 nmol malondialdehyde/mg protein) compared with OR and control rats (0.9±0.3 nmol malondialdehyde/mg protein). Also, aortic and kidney thiobarbituric acid–reactive substances showed a significant (3- and 5-fold) increase in OP rats after 16 weeks of diet. In addition, superoxide generation by aortic rings, measured by lucigenin luminescence, showed a 2-fold increase in the OP group compared with both the OR and control groups. In addition, free isoprostane excretion and nitrotyrosine in the kidney showed an increase in OP rats only. The urine and plasma nitrate/nitrite measured by the LDH method showed a 1.8-fold decrease in OP rats compared with OR rats. However, endothelial NO synthase expression in the kidney cortex and medulla assessed by reverse transcriptase–polymerase chain reaction showed a strong increase in the OP rats versus OR and control rats (endothelial NO synthase/β-actin ratio 1.3±0.04 in OP rats versus 0.44±0.02 in OR rats), suggesting a possible shift toward superoxide production by the enzyme. Collectively, the data show a decreased NO bioavailability in OP animals that is due in part to the increased oxidative stress. (Hypertension. 2001;37[part 2]:554-560.)

Key Words: diet, nitric oxide, obesity, oxidative stress, kidney, lipids

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% in women can be directly attributed to obesity. However, the mechanisms that link obesity with high blood pressure (BP) have not been fully elucidated. There are data that indicate increased oxidative stress in human essential hypertension as well as in obese hypertensive patients. Also, the involvement of the superoxide radical in BP regulation has been reported for several animal models of hypertension, such as the spontaneously hypertensive rat, Dahl rat, and angiotensin II–infused rat. Romero’s group (Haas et al9 and Reckelhoff et al10) has recently reported that suppressor doses of angiotensin II are able to generate oxidative stress in pigs and rats, which, in turn, is able to induce chronic elevations in BP. The increase in superoxide production in hypertension has been shown to have an impact on the production and bioavailability of endogenous vascular NO. The ability of NO to interact quickly with superoxide, leading to the formation of peroxynitrite,11 is a probable cause for the impairment of endothelium-dependent relaxation in rats after the experimental elevation of BP12,13 or in patients with essential hypertension. Much less is known about the involvement of oxidative stress in obesity-induced hypertension. The only animal studies concern obese Zucker rats, a genetic model of obesity that also displays type II diabetes and develops hypertension only when fed a high salt diet. In this model, endothelium-dependent vasodilation is not affected, unless a pro-oxidant prodiabetic challenge is induced in vivo. Also, the endothelial hyperreactivity in Zucker rats can be reversed by vitamin E. However, in this rat model, one cannot rule out the confounding effect of type II diabetes on the development of hypertension and its relation to oxidative stress. We have recently reported that diet-induced obesity in Sprague-Dawley rats also leads to the development of mild to moderate hypertension. We hypothesize that oxidative stress might be a common link that underlies both obesity and hypertension. Therefore, we investigated the oxidative status in this model and its effects on renal function. Also, we consider this model particularly useful in assessing the role of hyperlipidemia, which is known to be involved in the generation of free radicals and increased vascular...
reactivity,20 and diet in a direct causal relationship with hypertension, obesity, and oxidative stress.

Methods

Animals

All procedures involving animals were approved by the Institutional Animal Care and Use Committee of Eastern Virginia Medical School. Thirty-two male Sprague-Dawley rats (300 to 350 g) were randomly selected to be fed a moderately high fat (MHF) diet (32 kcal% as fat, Research Diets), and 8 rats (control group) were fed a purified low fat diet (10.6 kcal% as fat, Research Diets) for 16 weeks. Food and water were provided ad libitum throughout the experiment. Body weights (BWs) and lengths were measured initially and then weekly together with food intake. Rats fed the MHF diet diverged into distinct groups based on BW gains. The assignment of rats into obesity-prone (OP, n = 8) and obesity-resistant (OR, n = 8) groups was performed by construction of BW gain histograms at the end of the study for MHF and low fat diet groups, respectively. This gave a bimodal distribution for the former and a unimodal distribution for the latter, as has been previously described.21,22 The result was confirmed by Pearson χ2 analysis of MHF diet-fed rats. OR rats were defined as those with weight gains equal to or less than the heaviest control rats, and OP rats were defined as those with greater weight gains.21 At the end of the study, the rats were decapitated, and trunk blood was collected in EDTA-coated tubes. Plasma was immediately separated by centrifugation and used for different assays. Thoracic aorta, kidney, and fat depots were harvested, weighed, and either used immediately or snap-frozen in liquid nitrogen. The week before euthanasia, the rats were transferred to metabolism cages over a 24-hour period was supplemented with 0.05% butylated hydroxytoluene, and assayed within 48 hours on isolation. The tissues were collected on ice, washed in 0.9% NaCl, and immediately homogenized in 20 mmol/L Tris-HCl (pH 7.4) with 5 mmol/L butylated hydroxytoluene.26

Nitrate/Nitrite

Nitrate/nitrite was assayed in plasma and urine (diluted 1:50 in PBS) by use of an LDH colorimetric method with a kit from Cayman Chemicals.

\[ \text{Enzyme Linked Assay} \]

\[ \text{Nitrate} + \text{Nitrite} \rightarrow \text{Nitric Oxide} \]

\[ \text{Nitric Oxide} \rightarrow \text{Nitrite} + \text{Nitric Oxide}^- \]

\[ \text{Nitric Oxide}^- \rightarrow \text{Nitrate} + \text{Nitric Oxide} \]

Statistical Analysis

Data are shown as mean ± SE. To determine the significance between different groups, 1-way ANOVA was performed, followed by the Tukey post hoc test. A value of P < 0.05 was considered statistically significant.

Results

BW, BP, and PRA

The BWs for OP, OR, and control rats were measured weekly and did not differ significantly at the beginning of the study (Table 1). After 16 weeks of diet, both the BW and the BW gain in the OP group were significantly higher than those in the OR and control groups (Table 1). Results also show that all 3 fat depots measured, as well as the adiposity index (the
equivalent of the body mass index for humans), were all significantly higher in the OP rats compared with OR and control rats, reflecting the increased body fat content (Table 1). Systolic BP measured in the conscious rats at the beginning of the diet indicated an average of 121 ± 3.1 mm Hg. Starting with week 8, the OP rats had a higher BP compared with both control and OR rats (Figure 1). The increase in BP after week 8 was minimal for OP rats, whereas the BP of OR and control rats remained unchanged until the end of the study (Figure 1). With a systolic BP averaging 157.8 mm Hg, the OP group was considered to be mildly to moderately hypertensive, whereas the OR rats exhibited only borderline hypertension (Table 2). The increase in BP was paralleled by an increase in heart rate, which became significantly different after 16 weeks of diet in the OP group compared with the OR and control groups (Table 2). Also, in accordance with our previous data,18 OP rats showed an activation of the renin-angiotensin system, as reflected by a doubling in PRA, compared with OR and control rats (Table 2).

### Table 1. BW and Adiposity in OP, OR, and Control Rats After 16 Weeks of Diet

<table>
<thead>
<tr>
<th>Variables</th>
<th>OP (n=8)</th>
<th>OR (n=8)</th>
<th>Control (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW Baseline, g</td>
<td>298.0 ± 1.2a</td>
<td>293.8 ± 2.6c</td>
<td>298.2 ± 2.0f</td>
</tr>
<tr>
<td>Week 16, g</td>
<td>473.8 ± 6.2a</td>
<td>417.0 ± 8.7d</td>
<td>429.6 ± 8.7d</td>
</tr>
<tr>
<td>Nasal length (week 16), cm</td>
<td>24.8 ± 0.2e</td>
<td>24.0 ± 0.3f</td>
<td>24.1 ± 0.2g</td>
</tr>
<tr>
<td>BW gain, g</td>
<td>175.9 ± 5.5i</td>
<td>123.2 ± 7.6j</td>
<td>131.5 ± 7.2k</td>
</tr>
<tr>
<td>Epidydimal fat weight, g</td>
<td>8.3 ± 0.5a</td>
<td>5.5 ± 0.3b</td>
<td>4.9 ± 0.4c</td>
</tr>
<tr>
<td>Retroperitoneal fat weight, g</td>
<td>7.9 ± 0.5a</td>
<td>4.6 ± 0.5ab</td>
<td>4.7 ± 0.4ab</td>
</tr>
<tr>
<td>Mesenteric fat weight, g</td>
<td>2.9 ± 0.2a</td>
<td>2.1 ± 0.1b</td>
<td>2.3 ± 0.2c</td>
</tr>
<tr>
<td>Visceral fat, g</td>
<td>19.1 ± 1.2c</td>
<td>12.2 ± 0.9d</td>
<td>11.8 ± 0.8e</td>
</tr>
<tr>
<td>Adiposity index, %</td>
<td>4.45 ± 0.25a</td>
<td>3.18 ± 0.2b</td>
<td>3.01 ± 0.18c</td>
</tr>
</tbody>
</table>

Values are mean ± SE. Differences between groups designated by different letters are considered statistically significant (P < 0.05). The adiposity index was calculated by dividing the sum of the fat pads by (body weight – fat pad weight) × 100.

### Table 2. Cardiovascular and Renal Parameters in OP, OR, and Control Rats After 16 Weeks of Diet

<table>
<thead>
<tr>
<th>Variable</th>
<th>OP (n=8)</th>
<th>OR (n=8)</th>
<th>Control (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic BP, mm Hg</td>
<td>157.8 ± 4.21a</td>
<td>135.8 ± 3.8b</td>
<td>129.7 ± 2.1c</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>440 ± 8.7a</td>
<td>408 ± 4.5b</td>
<td>397 ± 10.3d</td>
</tr>
<tr>
<td>PRA, ng Ang I · ml L⁻¹ · h⁻¹</td>
<td>8.5 ± 0.8c</td>
<td>4.9 ± 0.9b</td>
<td>4.6 ± 0.5c</td>
</tr>
<tr>
<td>Urine creatinine, mmol/24 h</td>
<td>1.65 ± 0.04a</td>
<td>1.53 ± 0.08b</td>
<td>1.18 ± 0.05e</td>
</tr>
<tr>
<td>Urine protein, mg/24 h</td>
<td>42.4 ± 7.2a</td>
<td>33.2 ± 5.8b</td>
<td>39.2 ± 2.9b</td>
</tr>
<tr>
<td>Albumin excretion, mg/24 h</td>
<td>2.3 ± 0.16a</td>
<td>1.8 ± 0.41b</td>
<td>2.9 ± 0.38c</td>
</tr>
<tr>
<td>Na⁺ excretion, mmol/24 h</td>
<td>0.84 ± 0.18a</td>
<td>0.80 ± 0.29b</td>
<td>1.01 ± 0.06c</td>
</tr>
</tbody>
</table>

Values are mean ± SE. Differences between groups that are considered statistically significant (P < 0.05) are designated by different letters.

### Oxidative Stress in OP, OR, and Control Rats

The ability of isolated aortic rings from OP, OR, and control rats to generate superoxide anion as measured by lucigenin chemiluminescence is illustrated in Figure 2A. The results showed a 1.8-fold increase in superoxide production by the thoracic aortas from OP rats compared with both OR and control rats, suggesting an increased in vivo production of the...
radical. Also, the excretion of free 8-isoprostane F\textsubscript{2alpha}, measured by EIA in 24-hour urine samples, indicated a 3-fold increase, to 22.3 ± 2.9 ng over 24 hours, compared with both OR and control rats (Figure 2B). Together, superoxide generation and isoprostane excretion suggest an increase in local and systemic oxidative stress in the OP rats. In accordance with these data, lipid peroxidation in the LDL fraction and thoracic aorta was elevated by ~3-fold in the OP group, whereas the values for thiobarbituric acid–reactive substances (TBARS) were increased ~7-fold in the kidney samples of the OP rats compared with both OR and control rats (Figure 3A). The augmentation in lipid peroxides mirrors the increased ability of the thoracic aorta of OP rats to generate superoxide anions. Figure 3B illustrates the modest but still significant increase in the nitrotyrosine content of the OP rat kidney samples compared with OR and control rat samples. The OP rats had an average amount of 12.8 ± 2.3 ng nitrotyrosine/mg tissue protein compared with 9.1 ± 0.87 and 8.4 ± 0.12 ng nitrotyrosine/mg tissue protein for OR and control rats, respectively. The elevated amount of nitrotyrosine in the OP rats may reflect the increased interaction between superoxide and NO in the obese hypertensive animals. Figure 4 illustrates a 1.8-fold decrease in plasma and urine nitrate/nitrite content, suggesting a decreased production or bioavailability of NO in OP rats compared with OR and control rats. The expression of eNOS mRNA normalized to β-actin expression and representative micrographs of the
gels are shown in Figure 5. A substantial increase in eNOS expression seems to occur in the OP rats and occurs to a much lower extent in the OR rats compared with control rats. This rules out the role of the high fat diet in the upregulation of eNOS expression in this animal model. The data so far pointed toward a decreased NO availability in the OP rats that was due, at least in part, to the increased superoxide production. Finally, we measured some parameters to assess the renal excretory function, as shown in Table 2. Except for the creatinine values, which are moderately increased in both OP and OR rats, the protein, albumin, and Na⁺ excretion are all within the normal values reported for rats, indicating a normal kidney excretory function for all 3 groups (Table 2).

**Discussion**

This model of diet-induced obesity, extensively described previously, developed hypertension subsequent to the development of visceral adiposity, suggesting a role for metabolic factors associated with obesity in the development of hypertension. In a recent study, we showed that in both OP and OR rats there is increased peroxidation in the LDL fraction. The present study aimed to extend the data regarding oxidative stress in this model. Our data show that in OP rats, but not in OR or control rats, there is a 2-fold increase in the PRA. This indirectly suggests that the circulating levels of angiotensin II are increased in obese hypertensive animals. It is extensively reported that angiotensin II in either high doses or subpressor doses is able to induce oxidative stress. One way is through NADH/NADPH activation, which is believed to be the major source of superoxide anion in the arterial wall. Therefore, it is reasonable to assume that angiotensin II is likely to be involved in the increased generation of superoxide radicals by the aortic rings of OP rats. However, the cause for the increased PRA is more difficult to explain. One possible explanation might be related to the sympathetic activation in OP rats. Increased plasma norepinephrine response to intravenous glucose was previously reported by Levin and colleagues in OP rats. Also, our results showing an increased heart rate in OP rats may reflect a certain degree of sympathetic activation. As previously reported, the lipid peroxides, measured as TBARS, were increased in the LDL fraction and thoracic aorta of OP rats compared with OR and control rats. In the present study, we showed the same 3-fold increase in both LDL and aortic TBARS and, in addition, an even higher elevation of 7-fold in the kidney samples of OP rats compared with OR and control rats. This elevation is observed without a significant increase in total lipid content of the kidney (data not shown), suggesting that only the peroxidation state, and not the amount of lipids, varies in the kidneys of OP rats. A more accurate method to assess oxidative stress (and in particular, lipid oxidation) is the formation of 8-isoprostaglandin F₃α, a stable product generated from arachidonic acid in phospholipids and subsequently released in free form. Our data showed that the increase in kidney TBARS is paralleled by a 3.5-fold elevation of the free isoprostanes in urine. The level of 24-hour urine isoprostanes presumably provides an integrated assessment of isoprostane production with time and, because the lipids in urine are negligible, is less subject to artifacts because of ex vivo generation of isoprostanes. It is not yet clear whether the urinary isoprostanes are derived exclusively by filtration from the circulation, by formation in the kidney, or by a combination of both. Nevertheless, there is substantial evidence indicating that isoprostanes are potent vasoconstrictors in the aortic, renal, and pulmonary beds in vitro. Of particular importance is the observation that administration through intrarenal infusion of low nanomolar concentrations of isoprostanes in rats produces a potent renal vasoconstriction, reducing glomerular filtration rate and renal blood flow. Moreover, enhanced isoprostane production was observed in spontaneously hypertensive rats and in rats infused with angiotensin II. Also, an increased level of isoprostanes was reported in hypercholesterolemic pigs and in human atherosclerotic plaques. However, in our model, hypercholesterolemia does not seem to be the cause of the increased level of isoprostanes, because in OR rats, which are hypercholesterolemic, the isoprostanes are at the same levels as in control normolipidemic rats. Apart from the increased production of vasoconstrictor isoprostanes, in the plasma and urine of OP rats, the level of nitrate/nitrite was decreased 8-fold in OP rats, suggesting a reduced production of NO compared with that in OR and control rats. However, semiquantitative RT-PCR showed that eNOS in the thoracic aorta, kidney cortex, and medulla was increased 8-fold in OP rats compared with OR and control rats, suggesting the potential that more NO could be generated in these animals. An increase in eNOS expression was also recently reported in subcutaneous adipose tissues of obese humans. There are several possible explanations for the apparent discrepancy of the 2 results. First, under certain conditions, eNOS can produce superoxide rather than NO. This was mainly noticed in hypercholesterolemia, in which a tetrahydrobipterin deficiency seems to be responsible for the superoxide production by eNOS, but also in other pathological conditions, such as insulin resistance. In our model, hypercholesterolemia does not seem to have a major role, because both OP and OR rats are hypercholesterolemic, but only OP rats have increased eNOS expression. Another possible explanation is the ability of superoxide to quench NO, with the formation of the potent oxidant peroxynitrite. One major product of peroxynitrite attack on proteins is the nitration on the 3 position of tyrosine. Although other reactions can form nitrotyrosine, the rates and yields of the alternative nitration mechanisms seem to be rather small, and it is still considered a good footprint left by peroxynitrite in tissues in vivo. Our data showed that there is a modest, although significant, increase in the nitrotyrosine levels in the kidneys of OP rats compared with OR and control rats. This result, together with the increase in superoxide production and eNOS expression, may suggest that we indeed have an increased formation of peroxynitrite in obese hypertensive animals that reduces the NO bioavailability and hence may induce vasoconstriction of the kidney vasculature. This adds to the potential vasoconstrictor effect of the increased level of isoprostanes in OP rats. Although peroxynitrite can also induce vasodilation (and hence counteract, at least in part, the reduced NO bioavailability in certain vascular areas), at high concentrations its damaging action is likely to overcome the protective vasodi-
atory response. Moreover, Villa et al.\(^4\) have demonstrated that under a broad range of concentrations, peroxynitrite may induce tachyphylaxis to its own vasodilator action and may impair the response to other vasodilators in coronary rat vessels. The apparent reduction in NO bioavailability may also explain the increased level of PRA in OP rats. Although the precise effect of NO on renin synthesis and secretion is still a matter of controversy, several authors have reported a significant increase in PRA in animals made hypertensive by chronic NO blockade.\(^45\) Also, the chronic infusion of N\(^\circ\) nitro-L-arginine methyl ester seems to activate both PRA and sympathetic functions,\(^6\) which were also reported to be elevated in the OP rats.\(^2\) Additionally, our data suggest that diet is not the critical factor in the evolution of hypertension, inasmuch as both OP and OR rats are fed a similar MHF diet, but only OP rats are hypertensive and have a significantly increased adiposity index. Moreover, hypercholesterolemia, which is manifest in both the OP and OR rats, does not seem to have a crucial role in the development of hypertension in this model. On the other hand, the increased levels of circulating renin, and possibly angiotensin II, seem to be related to the oxidative stress measured in the model. Although we cannot conclude whether oxidative stress is the cause or consequence of hypertension obesity, it seems possible that it mediates the effect of angiotensin II on BP regulation in this model.

Acknowledgments

This study was supported by National Institutes of Health grant HL-54810, a Grant-in-Aid from the American Heart Association, and a postdoctoral fellowship from the MidAtlantic Affiliate of the American Heart Association to Dr Dobrian.

References


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Hypertension. 2001;37:554-560
doi: 10.1161/01.HYP.37.2.554

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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