Effect of salt on hypertension and oxidative stress in a rat model of diet-induced obesity

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Dobrian, Anca D., Suzanne D. Schriver, Terrie Lynch, and Russell L. Prewitt. Effect of salt on hypertension and oxidative stress in a rat model of diet-induced obesity. Am J Physiol Renal Physiol 285: F619–F628, 2003.—High-salt diet is known to induce or aggravate hypertension in animal models of hypertension and in humans. When Sprague-Dawley rats (n = 60) are fed a moderately high-fat diet (32% kcal fat, 0.8% NaCl) for 10 wk, about one-half develop obesity [obesity prone (OP)] and mild hypertension, whereas the other half [obesity resistant (OR)] maintain body weight equivalent to a low-fat control (C) and are normotensive. The aim of this study was to test the effect of high-NaCl diets (2 and 4% NaCl) on the development of hypertension and obesity, oxidative stress, and renal function. Both 2 and 4% NaCl induced an early increase in systolic blood pressure of OP but not OR or C rats. High-salt intake induced an increase in the size and reduction in number of adipocytes, concomitant to a twofold increase in circulating leptin in OP rats. Aortic superoxide generation indicated a 2.8-fold increase in the OP high-salt vs. normal-salt groups, whereas urine isoprostanes were not significantly increased. Also, hydroxynonenal protein adducts in the kidney were highly increased in OP rats on 2 and 4% NaCl, indicating oxidative stress in the renal tissue. Urine albumin was increased threefold in the OP on 2% NaCl and fourfold in the same group on 4% NaCl vs. 0.8% NaCl. Kidney histology indicated a higher degree of glomerulosclerosis in OP rats on high-salt diets. In summary, high-salt diet accelerated the development but did not increase the severity of hypertension; high salt increased oxidative stress in the vasculature and kidney and induced kidney glomerulosclerosis and microalbuminuria. Also, the OP rats on high salt displayed adipocyte hypertrophy and increased leptin production.

glomerulosclerosis; kidney; leptin; sodium dietary

OBSERVATIONS

OBESITY IS A COMPLEX, multifactorial disease that is associated with essential hypertension in ~78% of men and ~65% of women, as indicated by the data from the Framingham Heart Study (25). Another important contributor to hypertension in humans is the excessive consumption of dietary salt, and epidemiological studies have demonstrated a significant but weak relation between salt intake and hypertension (32, 33). Some, but not all, interventional studies have shown that salt restriction may lower blood pressure (BP) (19, 33).

Some recent studies report correlation among hypertension, salt sensitivity, and insulin resistance in obese humans (38), whereas others fail to observe a significant relationship (8). Animal models of obesity, hypertension, and insulin resistance display differences with respect to salt sensitivity. In Zucker rats, there is a clear correlation between salt intake and the severity of hypertension (4, 47), whereas in chronic hyperinsulinemic Sprague-Dawley (SD) rats, hypertension is not salt sensitive, albeit a shift in pressure-natriuresis relationship was reported (2). One important contributor to hypertension in salt-sensitive animal models and humans seems to be the endothelial dysfunction, in particular the altered vascular reactivity due to an impairment in nitric oxide (NO) production (22, 31, 36). High-salt intake is able to decrease both plasma levels and urinary excretion of nitrates (3, 16). One possible explanation is a reduced availability rather than decreased production of NO. The ability of NO to quickly interact with superoxide anion, forming the potent oxidant peroxynitrite, is well documented (43). Increased superoxide production in both vasculature and kidney was extensively reported in various forms of hypertension in experimental models and humans (40–42, 46). Moreover, we reported that obese hypertensive rats on high-fat diet also display increased oxidative stress and reduced NO bioavailability (12). Also, salt sensitivity was associated with increased oxidative stress in rats (5, 49). Apart from the effects on BP regulation, elevated salt intake was associated with cardiovascular and renal changes leading to end-organ damage (6). Moreover, a recent report connects salt intake with oxidative stress and nephrosclerosis in Dahl-sensitive hypertensive rats (48). Another important factor involved in BP regulation in obesity is leptin (24). Leptin was shown to have both a vasopressor effect at peripheral level and, infused in high doses, a hypertensive effect acting at central level (24). However, a recent report suggests that leptin may not contribute to arterial pressure sensitivity to salt in hyperleptinemic obese rats (7). The aim of our study was to assess the effect of high-fat, high-salt diets on the development of hypertension and oxidative stress in a rat model of diet-induced obesity. Moreover, the effect on vascular hypertrophy and kidney sclerosis

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was assessed. Additionally, the effect of salt on adiposity and leptin production was also measured.

METHODS

Animals

All procedures involving animals were approved by the Institutional Animal Care and Use Committee of Eastern Virginia Medical School. Eighteen male SD rats (300–350 g) individually caged were randomly selected to be fed a moderately high-fat diet (MHF) with 0.8% sodium (32% kcal as fat, Research Diets, New Brunswick, NJ), whereas six rats (controls) were fed purified low-fat (LF) diet with 0.8% sodium (10.6% kcal as fat, Research Diets) for 10 wk. An identical number of rats was placed on M HF and LF diets, each containing either 2 or 4% sodium (high-salt diets). Food and water were provided ad libitum throughout the experiments. Body weights (BW) and lengths were measured initially and then weekly together with food intake. Rats fed the M HF diet on both low and high salt diverged into distinct groups based on BW gains. Assignment of rats into obesity-prone (OP) (n = 8) and obesity-resistant (OR) (n = 8) groups was performed as described previously (12).

Systolic BP

The onset and development of hypertension were assessed by using the tail-cuff method with a Narco Biosystems Electro-Sphygomanometer (Houston, TX). BP was measured under consciousness at the beginning of the experiment and at 1, 5, 8, and 10 wk of diet. The average of five pressure readings was recorded for each measurement.

Assessment of Oxidative Stress

Superoxide anion production was measured in isolated aortic rings using a method previously described (12, 21). Briefly, 5-mm aortic rings were preincubated in Krebs-bicarbonate buffer, at 37°C, for 30 min and then transferred to a cocktail containing 5 μmol/l lucigenin and immediately measured, every 2 min, for 15 min total, using a scintillation counter set in the out-of-coincidence mode. The readings were plotted and the area under the curve was integrated. Results were normalized per milligram of DNA measured using the Hoechst 33258 dye as described (27). The specificity of the reaction was tested by the ability of 50 U/ml of SOD to quench the chemiluminescence at the end of the measurement.

Free 8-isoprostane F2\textsubscript{iso}. Isoprostanes were measured by EIA using a kit from Cayman Chemicals as previously described (12). Urine collected in metabolic cages over a 24-h period was supplemented with 0.05% butylated hydroxytoluene and spiked with 8-[^3H]isoprostane. The samples (1 ml) were passed on an affinity column (Cayman Chemicals) and only the free isoprostanes were eluted using 95% methanol. The eluate was evaporated to dryness under a stream of N\textsubscript{2} and the pellet was resuspended in a 1-ml assay buffer. Each sample was assayed in duplicate at two different dilutions and corrected for individual recovery of 8-[^3H]isoprostane, and the results were averaged. Nitrate/nitrite was assayed both in plasma and urine (diluted 1:50 in PBS) using a LDH colorimetric method with a kit from Cayman Chemicals.

Immunohistochemistry for 4-hydroxy-2-nonenal. Kidneys were fixed in 10% buffered formalin for 3 h and paraffin embedded. The sections were incubated with a polyclonal antibody recognizing 1:1 Cys, His, Lys-4-hydroxy-2-nonenal “Michael” adducts (Calbiochem, dilution 1:750). The slides were then reacted with biotinylated secondary goat anti-rabbit antibody (1:500 dilution; Vector Laboratories, Burlingame, CA), with the ABC-Elite avidin reagent (Vector Laboratories), and finally with the ImmunoPure Metal Enhanced DAB Substrate kit (Pierce, Rockford, IL).

Vascular Hypertrophy and Kidney Sclerosis

Aortic wall area. Thin sections of the paraffin-embedded tissue were stained for 1 min with toluidine blue and analyzed as described previously (10).

Kidney histology. Kidneys were fixed in 10% buffered formalin for 4 h and embedded in paraffin. Sections were stained using the periodic acid-Schiff (PAS) reagent and counterstained with hematoxylin. To evaluate the degree of segmental sclerosis, three independent investigators examined the slides in a blind fashion, mixing the slides after covering the protocol numbers. In each case, 80–100 glomeruli were examined for each slide and individually graded on a scale of 0 to 2+ according to the degree of glomerular sclerosis. 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.

Adipocyte Morphometry

Adipose tissues from the same depot and group were pooled and collagenase was digested according to Rodbell and Krishna (39). Adipocytes were washed several times to remove collagenase and centrifuged to separate adipocytes from preadipocytes, stromal cells, and vascular membranes. Cell diameter of ~1,200 cells was measured with the Image 1 Analysis System (Universal Image, West Chester, PA). Mean cell diameter was used to estimate mean cell volume. Cell size (μg lipid/cell) was calculated by multiplying cell volume (μl) by lipid density (~0.915 g/ml). Cell lipid content was determined according to the method of Dole (14). Cell lipid content and cell size were used to calculate cell number.

Statistics

Data are means ± SE. To determine the significance between different groups, two-way or three-way ANOVA was performed followed by Tukey’s post hoc test. P < 0.05 was considered statistically significant.

RESULTS

Effect of Salt on BW, Adiposity, Adipocyte Morphometry, and Leptin

After 10 wk of diet, BW in the OP groups on both high- and regular-salt diets was significantly higher than those in the corresponding OR and control (C) groups (Table 1). In addition, no significant difference in BWs was detected between each of the OP, OR, and C groups on 0.8, 2, and 4% NaCl, respectively (Table 1). The result is in accordance with daily food intake data, indicating that high-salt diets did not result in increased food consumption in OP, OR, or C rats on the respective diets vs. their counterparts on the low-salt diet (Fig. 1, A and B). However, from the beginning of the experiment until week 8, the OP rats ate significantly more than OR rats on a similar diet (Fig. 1, A
SALT AND OXIDATIVE STRESS IN OBESITY HYPERTENSION

Table 1. **BW and adiposity in OP, OR, and C rats on 0.8, 2, and 4% NaCl diets**

<table>
<thead>
<tr>
<th>Group</th>
<th>BW, g</th>
<th>Length, cm</th>
<th>Epididymal Fat Weight, g</th>
<th>Retroperitoneal Fat Weight, g</th>
<th>Obesity Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>OP 0.8%</td>
<td>660.0 ± 9.3*</td>
<td>27.0 ± 0.25</td>
<td>30.3 ± 3.0*</td>
<td>25.9 ± 1.1*</td>
<td>323.4 ± 2.3*</td>
</tr>
<tr>
<td>OR 0.8%</td>
<td>540.0 ± 8.4</td>
<td>26.4 ± 0.20</td>
<td>17.1 ± 2.5</td>
<td>14.7 ± 1.1</td>
<td>308.2 ± 2.4</td>
</tr>
<tr>
<td>C 0.8%</td>
<td>566.1 ± 22.1</td>
<td>26.0 ± 0.27</td>
<td>15.7 ± 2.5</td>
<td>18.6 ± 1.6</td>
<td>316.9 ± 2.2</td>
</tr>
<tr>
<td>OP 2%</td>
<td>673.8 ± 18.1*</td>
<td>26.9 ± 0.27</td>
<td>32.2 ± 1.5*</td>
<td>26.0 ± 1.4*</td>
<td>325.6 ± 2.1*</td>
</tr>
<tr>
<td>OR 2%</td>
<td>539.2 ± 18.1</td>
<td>25.9 ± 0.20</td>
<td>20.8 ± 2.8</td>
<td>18.7 ± 2.9</td>
<td>312.4 ± 1.7</td>
</tr>
<tr>
<td>C 2%</td>
<td>559.9 ± 16.1</td>
<td>26.4 ± 0.32</td>
<td>16.7 ± 4.6</td>
<td>19.2 ± 1.5</td>
<td>310.4 ± 1.6</td>
</tr>
<tr>
<td>OP 4%</td>
<td>681 ± 10.2*</td>
<td>27.1 ± 0.28</td>
<td>31.8 ± 2.3*</td>
<td>27.2 ± 1.8*</td>
<td>324.6 ± 2.2*</td>
</tr>
<tr>
<td>OR 4%</td>
<td>537.5 ± 14.7</td>
<td>26.1 ± 0.30</td>
<td>17.2 ± 3.1</td>
<td>16.7 ± 1.9</td>
<td>311.5 ± 4.3</td>
</tr>
<tr>
<td>C 4%</td>
<td>558 ± 12.6</td>
<td>25.8 ± 0.27</td>
<td>15.9 ± 5.1</td>
<td>17.6 ± 5.4</td>
<td>319.0 ± 2.7</td>
</tr>
</tbody>
</table>

Data are presented as means ± SE; n = 6 rats/group. Obesity index was calculated by dividing the cubic root of the body weight (BW; g) by the nasoanal length (mm) × 10^4. *Significant vs. obesity resistant (OR) and control (C). OP, obesity prone (P < 0.05).

and B). Also, the average food intake in all experimental groups reaches a peak after 3 wk on the respective diets, followed by a decrease by week 5 and a subsequent relatively stable level until the end of the experiment (Fig. 1, A and B), indicating that the highest salt intakes occurred in the first 3–5 wk on the diet. The increased BWs in the OP groups compared with OR and C groups were also mirrored by the elevated adiposity. Both the epididymal and retroperitoneal fat depots were significantly increased in the OP groups compared with OR and C, but no significant differences were recorded between the high- and low-salt groups (Table 1). Furthermore, the obesity index was higher in the OP groups compared with OR and C and was not influenced by the salt intake (Table 1). In contradiction, the adipocyte morphometry and number were different among the OP, OR, and C groups placed on low- vs. high-salt diets. For all OP, OR, and C, the 2% NaCl diet induced an increase by 12–20% in cell volume and 12–15% in cell size with the highest effect on OP adipocytes (Table 2). Also, a decrease in adipocyte number was measured for OP (~30%) and OR (~12%) groups on 2% vs. 0.8% NaCl diet, with no difference for C adipocytes (Table 2). This indicates hypertrophy of adipocytes from the OP rats, significantly exacerbated by the high-salt intake. In accordance with previous findings (29), our results indicate an increase in circulating leptin for OP rats compared with OR and C after 10–14 wk of diet (Table 2). Interestingly, both the 2 and 4% NaCl diets significantly increased, by ~40%, plasma leptin in the OP rats, and only the 4% NaCl diet induced a significant increase in the leptin levels in OR and C rats, compared with their counterparts on 0.8% NaCl (Table 2). The latter result suggests that obesity and high salt are both important in regulation of leptin levels. Moreover, the finding that the 4% NaCl, but not 2% NaCl, diet increased leptin levels in both OR and C lean groups of rats suggests that even in the absence of obesity, and independently of the amount of fat in the diet, a high enough content of NaCl (in our particular experiment 4 vs. 2%) could modulate the leptin levels.

**Effect of High-Salt Diet on BP and Plasma Renin Activity in OP, OR, and C Rats**

Systolic BP measured in the conscious rats at the beginning of the diet indicated an average of 122 ± 3.9 mmHg. Starting with week 5, the OP rats on both 2 and 4% NaCl displayed a significant increase in BP with an average of 160.2 ± 5.2 and 156.5 ± 4.4 mmHg, respectively, as opposed to all the other groups that were either normotensive or borderline hypertensive (Fig. 2, **A** and **B**).
A and B). At week 8, the OP rats on 0.8% NaCl diet were moderately hypertensive with an average BP of 154 ± 3.2 mmHg, whereas the OP rats on both 2 and 4% NaCl did not show a further significant increase in their systolic BP compared with week 5 (Fig. 2, A and B). By the end of the experiment (week 10), all three OP groups (on 0.8, 2, and 4% salt) had a similar increase in BP that averaged 158 mmHg. Also, the OR and C groups on high- and normal-salt diets were normotensive (Fig. 2, A and B). In the OP rats on 0.8% NaCl, the increase in systolic BP was paralleled by an approximately twofold increase in plasma renin activity (PRA), as measured at the end of the experiment (Fig. 2C). The 2% NaCl diet induced a ~40% reduction in PRA in the OP rats and slightly decreased PRA in the OR and C rats (Fig. 2C). In addition, the 4% NaCl diet induced a significant reduction in PRA in OP, OR, and C groups compared with their respective counterparts on 0.8 and 2% NaCl diets (Fig. 2C). The ability of the OP rat groups to adequately respond to the different increase in dietary salt at week 10 may explain the lack of difference in the systolic BP between the three OP groups at that time point.

**Oxidative Stress in Rats Fed Regular- and High-Salt Diets**

The systemic oxidative stress measured as the excreted free 8-isoprostan F₂α, in 24-h urine samples indicated an ~2.5-fold increase in the OP groups on both normal (0.8% NaCl)- and high (2 and 4% NaCl)-salt diets, compared with the respective OR and C groups (Fig. 3A), indicating that high salt does not further increase systemic oxidative stress in the obese rats. However, the ability of thoracic aortic rings to generate superoxide anions, measured as lucigenin chemiluminescence, is double in OP rats on both 2 and 4% NaCl vs. OP rats on regular salt, indicating an increase in oxidative stress in the large vessels of obese animals (Fig. 3B). Also, a significant increase induced in response to high salt was measured in C rats and the same trend was present in the OR rats (Fig. 3B). The latter result indicates that high salt increased superoxide formation independent of obese state and the amount of dietary fat. In addition, the high-salt intake and obesity, but not dietary fat, seem to have a synergistic effect on superoxide generation. Also, the urinary nitrate/nitrite is four- to fivefold decreased in OP rats on both regular and salt-supplemented diets, compared with the OR and C counterparts (Fig. 3C). The result indicates that salt intake does not further decrease nitrite/nitrate excretion, despite its significant effect on superoxide generation in the vasculature. Therefore, nitrite/nitrate formation seems to be modulated mainly by the obese state per se and not critically by the high-fat or high-salt content in the diets. Kidney immunohistochemistry using a polyclonal antibody for 2-hydroxy-4-nonenal protein adducts indicates a similar staining pattern in all groups on both regular- and high-salt diets; however, the intensity of the staining is much higher in the OP, OR, and C rats on 4% vs. regular-salt diets (Fig. 4, G–L). The most intense staining is noticed in the distal tubules, thick ascending limb, and to a lesser extent in the cortical proximal tubules, whereas it is virtually absent in the glomeruli. As shown in Fig. 4, G–L, the staining is more prominent in all OP, OR, and C rats on high salt (Fig. 4, G–I) vs. regular salt (Fig. 4, J–L), suggesting an increased local free radical production in the kidney cortex induced by high-sodium intake. The control in which the primary antibody was replaced with nonimmune serum shows no staining (Fig. 4M).

**Effect of Salt on Vascular Hypertrophy, Kidney Sclerosis, and Excretory Function**

Vascular hypertrophy was measured as aortic cross-sectional wall area. Results indicated that in all the OP groups (0.8% NaCl, 2% NaCl, and 4% NaCl), there is a significant increase in wall area compared with the respective OR and C groups (Fig. 5A). However, high salt did not induce a further increase in wall area in OP rats, suggesting no additional effect on vascular hypertrophy (Fig. 5A). To address the possible morphological changes in the kidney, we used PAS-hematoxylin staining followed by morphometric analysis. In accordance with our previous data (11), in OP rats on regular-salt diet, a mild sclerosis with most of the lesions

<table>
<thead>
<tr>
<th>Group</th>
<th>Cell Volume, pl</th>
<th>Cell Size, µg lipid/cell</th>
<th>Cell Lipid, g/ml</th>
<th>Cell Number, ×10⁵/cell</th>
<th>Plasma Leptin, ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>OP 0.8%</td>
<td>620.8 ± 20.2†</td>
<td>0.576 ± 0.06†</td>
<td>0.033 ± 0.007†</td>
<td>0.07 ± 0.006†</td>
<td>32.7 ± 4.1‡</td>
</tr>
<tr>
<td>OP 2%</td>
<td>732.4 ± 15.4†</td>
<td>0.670 ± 0.08†</td>
<td>0.045 ± 0.005†</td>
<td>0.05 ± 0.007‡</td>
<td>52.1 ± 7.8‡</td>
</tr>
<tr>
<td>OP 4%</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>55.6 ± 6.6‡</td>
</tr>
<tr>
<td>OR 0.8%</td>
<td>346.3 ± 19.6</td>
<td>0.317 ± 0.07</td>
<td>0.033 ± 0.003</td>
<td>0.12 ± 0.005</td>
<td>18.8 ± 5.5</td>
</tr>
<tr>
<td>OR 2%</td>
<td>393.3 ± 11.3‡</td>
<td>0.360 ± 0.08‡</td>
<td>0.036 ± 0.003‡</td>
<td>0.10 ± 0.005‡</td>
<td>29.2 ± 4.4</td>
</tr>
<tr>
<td>OR 4%</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>32.4 ± 4.7†</td>
</tr>
<tr>
<td>C 0.8%</td>
<td>610.3 ± 12.3</td>
<td>0.558 ± 0.08</td>
<td>0.036 ± 0.004</td>
<td>0.07 ± 0.006</td>
<td>23.1 ± 2.7</td>
</tr>
<tr>
<td>C 2%</td>
<td>664.9 ± 13.5‡</td>
<td>0.608 ± 0.07‡</td>
<td>0.038 ± 0.005‡</td>
<td>0.07 ± 0.004‡</td>
<td>29.0 ± 7.3</td>
</tr>
<tr>
<td>C 4%</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>34.4 ± 4.7‡</td>
</tr>
</tbody>
</table>

Data are means ± SE of 3–4 rats/group for adipocyte morphometry and n = 6 rats/group for the leptin data. Cell diameter of ~1,200 cells (~200 cells·fat pad⁻¹·rat⁻¹) was measured and used to calculate cell volume and cell size; cell lipid content was used to calculate cell number. Mean cell diameter was used to estimate mean cell volume. Cell size (µg lipid/cell) was calculated by multiplying cell volume (pl) by lipid density (~0.915 g/ml). n.d., Not determined. *Significant compared with 0.8% NaCl. †Significant compared with OR. ‡Significant compared with C. All P < 0.05.
Fig. 2. Systolic blood pressure (BP) in OP, OR, and C on 2% NaCl (A) and 4% NaCl (B) compared with 0.8% NaCl diets. Systolic BP was monitored from the beginning of the study using the tail-cuff method. OP rats on high salt have significantly increased BP starting with week 5, whereas OP rats on regular salt are hypertensive starting with week 8 on the diet. Plasma renin activity (C) was measured in terminal plasma samples from OP, OR, and C rats on 0.8% NaCl, 2% NaCl, and 4% NaCl, using a RIA method. Data represent means ± SE of 6 rats/group. * Significant compared with OR and C groups; † significant compared with counterpart on 2% salt; ‡ significant compared with counterpart on 4% salt (P < 0.05).

Fig. 3. Free urinary isoprostanes (A), aortic superoxide generation (B), and nitrate/nitrite excretion (C) in OP, OR, and C rats on 0.8% NaCl, 2% NaCl, and 4% NaCl diets. Urinary free isoprostanes were measured using an EIA method. In both OP groups on high- and regular-Na diet, the isoprostanes are significantly increased compared with the respective OR and C groups. Superoxide anion generation by aortic rings was measured by lucigenin chemiluminescence as described under METHODS. High-Na intake induces an increase in superoxide production in the OP group but not in the OR and C. Nitrate/nitrite excretion, measured by a colorimetric LDH method, indicates an approximately fourfold reduction in OP rats on both regular- and high-salt diets, compared with OR and C groups. Salt intake does not further reduce nitrate/nitrite excretion. * Significant compared with OR and C; † significant vs. 2% NaCl counterpart; ‡ significant vs. 4% NaCl counterpart (P < 0.05).
in a relatively early stage was noticed, as opposed to OR and C rats that displayed a normal kidney histology (Fig. 4, D–F). The OP rats on 4% NaCl displayed numerous and more advanced lesions of the glomeruli as well as significant matrix deposition throughout the cortex (Fig. 4A). The glomerular lesions displayed capillary loop collapse, mesangial matrix expansion, and sometimes adhesion to Bowman’s capsule. In addition, interstitial fibrosis and glomerular membrane thickening were noticed (Fig. 4A). The changes noticed in the 2% NaCl groups were somewhat intermediate between the 4 and 0.8% NaCl counterparts (not shown). In OR and C rats, a normal histological appearance was observed regardless of the amount of NaCl in the diets. Morphometric analysis indicated a mean ± SE mesangial score of 16.9 ± 1.4 for the OP rats on 4% NaCl compared with 14.2 ± 0.8 and 10.9 ± 1.2 for the OP rats on 2% NaCl and 0.8% NaCl, respectively. The scores for the OR rats on 4, 2, and 0.8% NaCl were 9.4 ± 1.1, 8.7 ± 1.2, and 8.4 ± 1.3, respectively, and the scores for the C rats on 4, 2, and 0.8% NaCl were 8.8 ± 1.4, 8.2 ± 1.2, and 8.4 ± 1.3, respectively. To test the possible changes in the renal function, protein, creatinine, and albumin excretion were measured. OP rats on both high-salt diets did not display overt proteinuria or significantly increased protein excretion compared with OP rats on low salt. Also, the creatinine values were similar among all groups. However, OP rats on 2% NaCl had mildly increased albuminuria (5.6 ± 0.42 mg/24 h) compared with OP on regular salt (2.12 ± 0.47 mg/24 h) (Fig. 5B). In addition, the OP group on 4% NaCl had a significantly higher albumin excretion (7.8 ± 0.53 mg/24 h) compared with both 2% NaCl and 0.8% NaCl counterparts (Fig. 5B). The results indicate that the changes in renal morphology, paralleled by albuminuria, are dependent on the salt content in OP rats only, suggesting a synergistic effect for salt and obesity but not for the dietary fat.

DISCUSSION

This rat model of diet-induced obesity was shown to develop moderate hypertension subsequent to the accentuation of visceral adiposity, suggesting a role for metabolic factors associated with obesity in the development of hypertension (11, 12). We also reported increased oxidative stress in the vasculature, plasma, and urine of obese animals at both early (3 wk) and late (10 wk) stages of the diet (11, 12). Oxidative stress was documented in a variety of animal models, such as the spontaneously hypertensive (42, 44), Dahl-sensitive (45, 46), or ANG II-infused rat (28). In addition, an important role for free radicals in BP regulation was shown in a model of lead-induced hypertension (9, 50), in 1K1C renal hypertension (13), in chronic renal failure (51), and in a model of glutathione-depleted rats (52). Several mechanisms were proposed for explaining the effect of free radicals production on BP regulation. It was recently demonstrated that endogenously produced superoxide anion can decrease NO bioavailability in the thick ascending limb and therefore increase NaCl reabsorption and induce hypertension (37). Also, chronically increased oxidative stress induced in the medulla of uninephrectomized Sprague-Dawley rats was shown to lower medullary blood flow leading to hypertension (34). Hypertension in spontaneously hypertensive rats seems to involve reduced NO availabil-
It is associated with obesity, are influenced by an increase in the amount of dietary salt intake (17, 53). The aim of the present study was to assess the effect of increased salt intake on the development of hypertension and associated mechanisms involving oxidative stress and end-organ damage in obese rats. The data showed that the increase in dietary sodium up to 2 or 4% induces a more rapid elevation in BP, after only 5 wk of diet, instead of 8 wk in OP rats on a regular-salt diet. Although we do not have PRA data at week 5, it is reasonable to assume that the latter result could be explained by the inability of OP rats to adequately respond to an increase in the dietary salt early (at week 5) on the diet. Conversely, the lack of difference in the BP between OP rats on the three different diets at the end of the experiment is reflected in the ability of OP rats to reduce their PRA according to the different levels of salt intake. Also, the similar daily food intake for OP rats on 0.8, 2, and 4% NaCl at any time point throughout the experiment rules out the possibility of a higher salt intake in the early (up to week 5) as opposed to late part of the diet, which could have accounted for the earlier increase in BP in the 2 and 4% NaCl vs. 0.8% NaCl OP group. Another possible explanation for the earlier increase in BP in OP rats on a high-salt diet may be related to the pressor effect of leptin, as recently reported (23). Our own data or data reported by others (29) indicate that OP rats are hyperleptinemic by the end of the diet. Data showed that high-salt diets induced a significant 40% increase in plasma leptin in OP rats. Interaction between high-salt intake and obese state significantly increased circulating leptin in the OP groups on high- vs. regular-salt diet. Also, there was a lower, although significant, increase in plasma leptin in lean OR and C groups on 4% NaCl, compared with 0.8% NaCl, suggesting that high-salt intake could contribute to elevated plasma leptin independently of obesity and high dietary fat. A recent report by Correia et al. (7) demonstrated that high amounts of circulating leptin can act centrally to increase BP in rats. Increased leptin may act centrally as a pressor agent in the initial stages of the diet, before full onset of obesity, but it is unable to have any effect later, possibly due to the onset of leptin resistance.

Also, high-salt diet induced a significant increase in the adipocyte size, especially in the OP rats. Adipocyte hypertrophy may potentiate the insulin resistance in OP rats, due to the increased fatty acids efflux and increased circulating triglycerides. It was shown that salt increases circulating levels of fatty acids (17), and our data indicating adipocyte hypertrophy suggest a possible increase in circulating fatty acids.

Oxidative stress was reported previously for this animal model in both prehypertensive (11) stage and after the development of moderate hypertension (12). In the present study, we tested whether salt has an effect on free radicals formation in the obese rats. Data indicated that 2 and 4% NaCl diets did not enhance free isoprostanes excretion in OP, OR, or C rat groups compared with their counterparts on 0.8% NaCl diet. However, the superoxide production by aortic rings is significantly increased in OP rats on both high-salt diets vs. 0.8% NaCl group. Urine isoprostanes are considered a reliable marker to quantify systemic oxidative stress (30). However, recent reports indicate that in rats, under certain conditions such as increased oxygen tension (26) or NADPH-stimulated free radical production (15), F2 \( \text{iso} \) isoprostanes were not increased, although other oxidative stress parameters were elevated. Although this study does not provide data to support this hypothesis, it is possible that increased vascular superoxide production mainly originates from a vascular NAD(P)H oxidase (18, 35) and hence isoprostanes F2 \( \text{iso} \) could not accurately reflect the increased aortic oxidative stress. Nevertheless, increased superoxide production in the aorta does not seem to affect vascular hypertrophy. The wall area in OP rat groups on high- and regular-salt diets is increased vs. the OR and C, but no differences were measured among the three OP groups on 4, 2, and 0.8% NaCl. The results suggest that vascular remodeling is due to the elevation in BP rather than directly related to free radicals production in rats on high-salt diet. The presence of increased hydroxynonenal protein adducts in the kidneys of OP rats on high-salt vs. normal-salt diets indicates elevated free radicals production in the renal tissue in the former. One possible source of free radicals in the kidney may originate from high-leptin production by the local infiltrates of adipose tissue. Leptin was shown to induce oxidative stress in the endothelial cells in culture (1). Therefore, it is possible that increased local leptin production may contribute to reactive oxygen species generation. High dietary fat does not appear to have a direct effect, because both the OR and C groups displayed similar levels of lipid peroxidation. Conversely, high-salt intake (4% NaCl) induced increased renal lipid peroxidation in all study groups (OP, OR, and C), suggesting a role for high salt independent from obesity and dietary fat. However, the higher lipid peroxidation in the OP group on 4% NaCl vs. 0.8% NaCl and the higher peroxidation in all OP groups compared with their OR and C counterparts on similar diets indicate a possible synergistic effect of obesity and salt on renal lipid peroxidation.

A direct or indirect effect of high-salt intake, possibly via free radicals production, could be responsible for the kidney glomerulosclerosis in the OP rats. Salt was shown to induce smooth muscle cells and myoblasts hypertrophy in vitro (20). Also, oxidative stress seems to be directly involved in the renal dysfunction in Dahl salt-sensitive rats (48). Therefore, it is reasonable to assume that the higher degree of renal damage in the OP rats on high salt vs. normal salt is likely to be independent of a pressor effect and rather due to the production of local excess leptin and/or free radicals. In conclusion, our results indicate that 1) high-salt diet induces an earlier increase in systolic BP in OP rats (5 wk on 4 and 2% NaCl vs. 8 wk on 0.8% NaCl), possibly due to the inability of OP rats to reduce their renin production in response to increased NaCl intake early.
in the diet; 2) salt does not affect fat accretion, but it induces adipocyte hypertrophy and increased leptin production, independently from dietary fat and in synergy with obese state; 3) high-NaCl intake induces increased vascular and renal oxidative stress, independently from dietary fat and synergistically to obesity; and 4) high-salt diet accelerates kidney sclerosis, which correlates with renal oxidative stress, but it is, at least in part, independent of a directpressor effect and does not affect vascular hypertrophy, which is probably the direct result of high arterial pressure. In this model, the concurrent effect of metabolic factors related to obese state and high-salt intake seems to induce kidney sclerosis and moderate hypertension. The finding could be relevant for human pathology, indicating that increased salt intake in obese individuals with moderate hypertension may lead to accelerated end-organ damage.

DISCLOSURES

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