Aldosterone infusion with high-NaCl diet increases blood pressure in obese but not lean Zucker rats

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Aldosterone infusion with high-NaCl diet increases blood pressure in obese but not lean Zucker rats. Am J Physiol Renal Physiol 291: F597–F605, 2006. First published April 4, 2006; doi:10.1152/ajprenal.00508.2005.—Insulin-resistant, obese Zucker rats have blunted pressure natriuresis and are mildly hypertensive. This may involve inappropriate regulation of the renin-angiotensin-aldosterone system. To evaluate mechanisms underlying this defect, we employed the model of aldosterone escape. Male lean (L) and obese (O) Zucker rats were infused with aldosterone (2.8 μg/g body wt3/4) via osmotic minipump while being fed a 0.02% NaCl diet (LS). After 4 days, six rats of each type were switched to a high-NaCl (HS) diet (4%) for 4 additional days. Mean arterial blood pressure measured by radiotelemetry was significantly increased by the HS diet only in obese rats (final mean mmHg): 104 (LLS), 99 (LHS), 103 (OLS), and 115 (OHS). Obese rats had relatively increased renal cortical abundance of the bumetanide-sensitive Na-K-2Cl cotransporter (NKCC2) and whole kidney α- and β-ENaC (epithelial sodium channel) relative to lean rats. However, band density for the thiazide-sensitive (Na-Cl) cotransporter (NCC) was similarly reduced by HS in lean and obese rats (~50%). Obese rats had relatively reduced creatinine clearances and plasma renin activities, effects exacerbated by HS. Furthermore, HS resulted in a 129% increase in urinary nitrates plus nitrites excretion in lean rats and led to, in contrast, a 46% reduction in obese rats. Plasma sodium and potassium concentrations were increased by HS in obese but not lean rats. Thus we demonstrate an impaired response to aldosterone infusion in obese relative to lean Zucker rats. This impairment may involve increased sodium reabsorption via NKCC2 or ENaC, decreased glomerular filtration rate, and/or nitric oxide bioavailability.

THE MECHANISMS UNDERLYING the relationship between obesity and hypertension are likely complex. Peripheral insulin resistance, which often accompanies obesity, may play a role in this relationship. Insulin resistance in humans and animal models has been associated with increased activity of the renin-angiotensin-aldosterone system (RAAS) (10, 12, 19, 24). Specifically, in the obese Zucker rat, a model characterized by morbid obesity coupled to severe insulin resistance and mild hypertension, increased renal cortical Ang II AT1 receptor mRNA and protein (35), AT1 binding activity (4), and losartan-mediated blood pressure decrease (1) have been demonstrated. Increased activity of Ang II would predict increased aldosterone activity in these animals, although this has not been adequately examined.

Aldosterone, a mineralocorticoid, is the key regulatory hormone in day-to-day sodium balance. In the kidney, aldosterone acts primarily on the distal tubule, i.e., distal convoluted tubule, connecting tubule, and collecting duct system, to increase sodium reabsorption (11). However, aldosterone may also increase sodium reabsorption in renal proximal brush border by increasing the abundance of the sodium/hydrogen exchanger (NHE3) (23). Most genomic actions of aldosterone are mediated through the mineralocorticoid receptor (MR), which has highest expression in the distal tubule (7). In addition, the distal tubule cells express 11β-hydroxysteroid dehydrogenase-2 (11-β-HSD-2) (7) which protects the MR from glucocorticoids, which can also bind the MR but circulate in >100-fold greater concentrations than aldosterone. 11β-HSD-2 converts glucocorticoids such as corticosterone into MR-inactive metabolites. How aldosterone action is mediated in the proximal tubule is not as clear. Nevertheless, inappropriate high aldosterone activity has been associated with a variety of cardiovascular diseases such as essential hypertension, congestive heart failure, and myocardial infarction (11). Therapy to antagonize aldosterone such as spironolactone or eplerenone treatment has been shown to successfully control and attenuate cardiovascular disease, primary aldosteronism, and associated end-organ damage (8, 16, 28).

Nevertheless, when plasma aldosterone levels are inappropriately high, relative to NaCl intake, NaCl reabsorption and retention do not continue at the same level, indefinitely. Humans and animals undergo a physiological adaptive process known as “aldosterone escape” in which a natriuresis occurs and distal tubular NaCl reabsorption is attenuated despite high or even clamped aldosterone levels (3, 30). This process has been characterized at the renal level in young, healthy, male rats and involves downregulation of the renal protein abundances of one major sodium transporter, i.e., the thiazide-sensitive Na-Cl cotransporter (NCC) of the distal convoluted tubule (34). In addition, the lower band (70 kDa) of the γ-subunit of the epithelial sodium channel (ENaC) was downregulated (34). This band region has been shown to be increased by low-NaCl diets or by aldosterone infusion (27). However, α-ENaC, a protein also strongly upregulated in abundance by aldosterone, was not changed in escape. Thus aldosterone escape involves downregulation of some but not all aldosterone-regulated proteins of the distal tubule. Nevertheless, the role of changes in proximal and thick ascending limb...
implanted with aldosterone-infusing osmotic minipumps to infuse 2.8 μg/g body wt/day into all rats. After 4 days, six rats of each body type were switched to a high-NaCl (HS) diet (4% NaCl was added to the above agar-based diet during preparation). Rats were euthanized after 4 additional days. Diets and additional drinking water were provided ad libitum throughout the study. Rats were housed in metabolic cages during the course of the study to facilitate daily collection of urine and measurement of feed and water intake. Urine was collected daily with addition of 30 μl of an antibiotic cocktail containing (8.2 mg/ml penicillin G, 261 mg/ml streptomycin, and 0.5 mg/ml amphotericin, Sigma) on day 5 to better preserve urine for analysis of nitrates plus nitrates (NOx). All protocols were approved by the Georgetown Animal Care and Use Committee, an Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC)-approved facility.

Sample preparation. Rats were euthanized by decapitation and trunk blood was collected into both heparinized- and K3-EDTA tubes (Vacutainer, Becton-Dickinson, Franklin Lakes, NJ). Whole blood was centrifuged at 1,500 × g (Sorvall RT 6000 D, Sorvall, Newtown, CT) at 4°C for 20 min to separate plasma. Both kidneys were rapidly removed. The left kidney was homogenized whole, and the right kidney was dissected into cortex, inner stripe of outer medulla, and inner medulla and each region was individually prepared for blotting as previously described (14, 15).

Plasma and urine analyses. Plasma aldosterone and renin activity were measured by radioimmunoassays (Coat-a-Count, Diagnostic Products, Los Angeles, CA and Gammaincoat Plasma Renin Activity RIA kit, DiaSorin, Stillwater, MN, respectively). Plasma and urine NOx was measured by colorimetric assay (Nitrate/Nitrite Colorimetric Assay Kit, Cayman Chemical, Ann Arbor, MI). Urinary sodium and potassium were measured by an ion-selective electrode system (EL-ISE Electrolyte System, Beckman Instruments, Brea, CA) and osmolality by freezing-point depression (The Advanced Osmometer, model 3900, Advanced Instruments, Norwood, MA).

Immunoblotting. Initially, Coomassie-stained “loading gels” were done on all sample sets to assess the quality of the protein by sharpness of the bands and to confirm equality of loading, as previously described (13, 15). For immunoblotting, 2–30 μg of protein from each sample were loaded into individual lanes of minigels of 7, 10, or 12% polyacrylamide (precast, Bio-Rad, Hercules, CA). We used our own polyclonal antibodies against NCC, α-, β-, and γ-ENaC (31). Polyclonal antibodies against NKCC2 were a kind gift of Dr. J. Klein, Emory University. Commercial polyclonal antibodies were used against endothelial NO (eNOS or NOSIII; BD Transduction Laboratories, San Diego, CA), NHE3, and sodium-phosphate cotransporter (NaPi-2; Alpha Diagnostic International, San Antonio, TX). Mouse monoclonal antibody to the α1-subunit of Na-K-ATPase was obtained from Upstate Biotechnology (Lake Placid, NY).

Statistics. Data were analyzed by two-way ANOVA (body type × treatment) to determine overall effects of body type or treatment. Also, to determine differences between specific mean pairs, data were also analyzed by one-way ANOVA followed by Tukey’s multiple comparisons test or Kruskal-Wallis ANOVA on ranks followed by Dunn’s multiple comparisons test (when data were not normally distributed or variance was different between groups). Multiple comparison tests were only applied when a significant difference was determined in the ANOVA analysis, P < 0.05.

RESULTS

Blood pressure. Mean arterial blood pressure (MAP) is plotted in Fig. 2A. There was a small but significant difference in MAP between lean and obese rats at the outset of the study,
Tukey’s multiple comparisons test.

As we previously showed (5, 6, 21), however, after rats were switched to a LS diet and were infused with aldosterone, in the second period, only obese rats on high-NaCl diet. Aldosterone infusion. Obese rats, but not lean, had an increase in blood pressure. This difference was eliminated during the period of low-NaCl diet and was maintained with aldosterone infusion or the high-NaCl diet. In general, there were no differences between lean and obese except for the final day, where there was a slight but significant increase in the obese relative to lean. Similarly, absolute urinary potassium excretion was, in general, higher in the obese rats (Fig. 3C). All rats underwent a relative kaliuresis between “day 0” and “day 1” likely due to the aldosterone infusion. This kaliuresis (relative to prealdosterone infusion day 0) was transient in the lean rats but seemed to persist in the obese rats. A second kaliuresis occurred after initiation of the HS diet (day 5). This was relatively blunted in the obese rats. When urinary potassium was normalized to dietary intake (Fig. 3D), the stark, spiked, kaliuresis in the lean rats both after initiation of the aldosterone infusion or the high-NaCl diet is even more apparent. As a result of this, lean rats had a significantly lower Na⁺-/K⁺ ratio, in their urine on day 1 (Fig. 3E), the first day after the beginning of aldosterone infusion; i.e., the ratio in lean rats was 0.015 ± 0.001 and in obese rats it was 0.019 ± 0.005 (n = 12). P = 0.026 (this is difficult to appreciate from the figure due to the scale). In contrast, on day 6 (2 days after initiation of the HS period) lean HS rats had a significantly higher Na⁺-/K⁺ ratio relative to obese HS rats. Urinary volume was higher in the obese rats over the course of the experiment and was increased in both lean and obese rats by HS (Fig. 3F).

Physiological parameters. Dietary treatment did not affect final body weight (Table 1). Similarly, plasma sodium and potassium levels were not affected by diet in the lean rats; however, for obese rats, the obese HS rats were relatively hyperkalemic and hypernatremic. In contrast, the obese LS rats were relatively hypokalemic, although not significantly different from the lean rats. Plasma aldosterone levels were not significantly different among groups, as expected due to the high rate of infusion in all groups. They were an order of magnitude higher than normal physiological levels on moderate NaCl intake (6, 20). Plasma renin activity was reduced by both HS and in obese rats by two-way ANOVA, so that mean plasma renin activity in obese HS rats was only 2.3% of lean LS rats. Creatinine clearance (CCl), expressed on a per body weight basis, was significantly reduced in obese rats with a slight exacerbation of this reduction with HS. Furthermore, when not corrected for body weight, CCl was still significantly reduced in obese rats (ml/min): 1.5 ± 0.1, 1.1 ± 0.2, 0.8 ± 0.2, and 0.7 ± 0.2 in lean LS, lean HS, obese LS, and obese HS groups, respectively, P = 0.009. This was partly due to significantly decreased urine creatinine, which was 28% lower in obese rats than lean, on the final day of the study, irrespec-

Fig. 2. Mean arterial blood pressure (MAP) in lean and obese rats during aldosterone escape. A: daily mean absolute MAPs over time. Obese rats had marginally but significantly increased blood pressure at the start of the study. This difference was eliminated during the period of low-NaCl diet and aldosterone infusion. Obese rats, but not lean, had an increase in blood pressure on high-NaCl diet. B: delta MAP from baseline (average of day −4 and −3) for the low-NaCl (LS) period of the study (5 days, days 0 to +4) and for the next 4 days (HS or LS, days +5 to +8). There was a significant fall in blood pressure in the obese rats in the first period, when all rats received low-NaCl diet and were infused with aldosterone. In the second period, only obese rats responded to the high-salt diet with an increase in blood pressure. *Significant (P < 0.05) difference between lean and obese rats by 2-way ANOVA. †Significant difference from the other 3 groups by 1-way ANOVA followed by Tukey’s multiple comparisons test.
tive of treatment, despite increased body size (data not shown). Plasma creatinine was variable within the treatment groups, so there were no significant differences by one- or two-way ANOVA. Mean plasma creatinine concentrations were \( \mu \text{mol/l} \) 18 ± 2, 31 ± 4, 35 ± 11, and 58 ± 23 in lean LS, lean HS, obese LS, and obese HS groups, respectively \( (P = 0.11) \). In addition, plasma NOx did not differ significantly among groups. However, urine NOx excretion was increased in obese rats relative to lean, but HS caused a 129% increase in excretion in lean rats, but resulted in a 46% decrease in the obese rats. Final urine osmolality was not different among groups. Remarkably, fractional excretion of sodium (clearance of sodium relative to creatinine) was significantly increased in the obese HS rats \( (P < 0.04) \), relative to the lean.

Proximal tubule sodium transporters. In Fig. 4, left, we show immunoblots of cortex homogenates (which are enriched
Table 1. Physiological data

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<tr>
<th>Parameter</th>
<th>Lean LS</th>
<th>Lean HS</th>
<th>Obese LS</th>
<th>Obese HS</th>
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<tr>
<td>Final body weight, g</td>
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<td>275±5B</td>
<td>374±8A</td>
<td>355±15A</td>
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<td>Plasma sodium, mmol/l</td>
<td>143±2B</td>
<td>140±1B</td>
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<td>Plasma potassium, mmol/l</td>
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<td>5.5±0.3AB</td>
<td>4.6±0.3B</td>
<td>6.6±0.6A</td>
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<td>Plasma aldosterone, mmol/l</td>
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<td>16±2</td>
<td>18±3</td>
<td>21±3</td>
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<tr>
<td>Plasma NOx, μmol/l</td>
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<td>8.2±1.3</td>
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<td>Creatinine clearance, ml/min 1-kg body wt -1</td>
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<td>4.1±0.9AB</td>
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<td>2.0±0.5B</td>
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<td>Urine NOx, (day 5), μmol/kg body wt -1</td>
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<td>2.20±0.3AB</td>
<td>6.01±1.5A</td>
<td>3.24±0.6AB</td>
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<tr>
<td>Urine osmolality, (day 8), mosmol/kgH2O</td>
<td>690±54</td>
<td>671±55</td>
<td>711±40</td>
<td>883±140</td>
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<tr>
<td>Fractional excretion of Na+ (day 8), %</td>
<td>0.08±0.04C</td>
<td>2.07±0.55AB</td>
<td>0.15±0.03BC</td>
<td>15.4±6.2A</td>
</tr>
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Values are means ± SE, n = 6/group. NOx, nitrates plus nitrates. A,B,C Letters are assigned based on the outcome of Tukey’s multiple comparison’s test following a significant (P < 0.05) one-way ANOVA. The letter “A” is assigned to the highest mean in the row. Means with letters in common within a row are not significantly different from each other.

in proximal tubules) probed with antibodies against NHE3, the NaPi-2, and the α1-subunit of Na-K-ATPase. In Fig. 4, right, are bar graph summaries of the densitometry. (Na-K-ATPase is also expressed in the TAL and in the distal tubule also found in the cortex of the kidney, but these segments of the kidney make up a much lower proportion of the cells in a cortex homogenate; thus the signal for Na-K-ATPase can be mainly attributed to the proximal tubule.) There was no significant difference in protein abundance for NHE3 or the α1-subunit of Na-K-ATPase; however, NaPi-2 (84-kDa band) was markedly reduced in the obese rats relative to lean, by two-way ANOVA (body type × treatment) but not affected by treatment (HS or LS).

**TAL.** We utilized the outer medullary homogenate (OMH) as an enriched fraction of TAL cells to examine the abundance of key sodium transport proteins expressed in the TAL (Fig. 5). However, NKCC2 was also examined in the cortex homogenate because the TAL extends into the cortex and is the only renal tubule cell type (other than the macula densa) that expresses NKCC2. Two-way ANOVA revealed a significant decrease in the density of NKCC2 in the OMH in the HS rats, relative to LS. However, no differences were found due to body type. In the cortex, however, NKCC2 abundance was significantly reduced in the lean but not the obese HS rats (by one-way ANOVA). NHE3 abundance was not significantly different among any of the groups. However, α1 Na-K-ATPase was significantly reduced in the lean LS group relative to the other three groups in outer medulla and increased in both HS and in obese rats (by two-way ANOVA).

**Distal tubule and collecting duct.** Distal sodium transporters and channel subunits were examined in whole kidney homogenates. The abundance of NCC was significantly reduced in both lean and obese rats by HS (Fig. 6), with no significant differences between body types. The α- and β-subunits of ENaC were increased in obese rats and not affected by diet. The major band of γ-ENaC (85-kDa) was reduced in the lean LS rats relative to the obese LS. Finally, HS decreased the abundance of the 70-kDa band of γ-ENaC in both lean and obese rats.

**eNOS.** We examined eNOS abundance in cortex, outer medullary, and inner medullary homogenates (Fig. 7). Abundance of eNOS was significantly increased in obese rats by LS (by one-way ANOVA). There was a marked increase in eNOS abundance in response to body type. In the cortex, however, eNOS abundance was significantly increased in obese rats by LS (by one-way ANOVA). There were no differences between body types. The 70-kDa band of α1-ENaC (85-kDa) was reduced in the lean but not the obese HS rats (by one-way ANOVA).
to HS in both lean and obese rats in outer medulla by two-way ANOVA ($P < 0.01$). However, no differences were found due to body type. In the inner medulla, eNOS abundance was not significantly different between groups.

**DISCUSSION**

Pressure natriuresis is likely invoked during natriuretic and blood pressure escape from aldosterone infusion with HS

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**Fig. 5. Thick ascending limb-associated sodium transporters.** Immunoblot (left) and bar graph summaries of densitometry (right) are shown for bumetamide-sensitive Na-K-2Cl cotransporter (NKCC2), NHE3, and $\alpha$-Na-K-ATPase in outer medulla and/or cortex homogenates. Within each blot, all lanes are loaded with equal amounts of total protein. Each lane contains a sample from a different rat. Preliminary coomassie-stained gels confirmed equality of loading. *Significant ($P < 0.05$) difference between lean and obese rats, $\Psi$ between HS and LS rats by 2-way ANOVA. $\tau$Significant difference from the other 3 groups by 1-way ANOVA followed by a multiple comparisons test.

**Fig. 6. Distal tubule-associated sodium transporters.** Immunoblot (left) and bar graph summaries of densitometry (right) are shown for Na-Cl cotransporter (NCC) and epithelial sodium channel (ENaC) subunits in whole kidney. Within each blot, all lanes are loaded with equal amounts of total protein. Each lane contains a sample from a different rat. Preliminary coomassie-stained gels confirmed equality of loading. *Significant difference between lean and obese rats, $\Psi$ between HS and LS rats by 2-way ANOVA. $\delta$Significant difference from lean LS as determined by 1-way ANOVA followed by a multiple comparisons test.
In general, in both lean and obese rats, renal NKCC2 was reduced in abundance by HS treatment. However, in the obese rats, a significant decrease was only observed in outer medulla, whereas in the lean rats, this reduction in abundance extended into the cortical homogenates, presumably cortical TAL. Thus it is possible that this difference in the downregulation of NKCC2 contributed to relative NaCl retention in the obese rats. Inappropriately high renal expression of NKCC2 has been reported in at least two hypertensive rat strains (2, 9).

The reduction in NKCC2 during aldosterone escape disagreed somewhat with the study of Wang et al. (34) in which NKCC2 abundance was not changed in young, male Sprague-Dawley rats undergoing aldosterone escape. However, a subsequent study by this group (33) revealed that when L-NAME, a NOS inhibitor, was superimposed on the aldosterone escape, the obese rats showed a greater sensitivity of blood pressure and plasma electrolyte concentrations with aldosterone clamp, the obese rats showed a reduced suppression of the NKCC2 abundance by aldosterone (27), as is the activity of the ENaC multimeric channel (29). This may suggest a greater sensitivity to the same circulating level of aldosterone in the obese rats, relative to lean with regard to α-ENaC expression. Thus increased ENaC activity in conjunction with the HS would be expected to result in greater sodium retention and increased blood pressure, in these rats, relative to lean.

In addition, α- and β-ENaC abundances were increased in the obese rats, relative to lean, regardless of the level of dietary NaCl. Previously, we (6) found no significant differences in α-ENaC abundance between lean and obese rats, when aldosterone levels were not clamped, although we found increased β-ENaC abundance. α-ENaC abundance is strongly upregulated by aldosterone (27), as is the activity of the ENaC multimeric channel (29). This may suggest a greater sensitivity to the same circulating level of aldosterone in the obese rats, relative to lean with regard to α-ENaC expression. Thus increased ENaC activity in conjunction with the HS would be expected to result in greater sodium retention and increased blood pressure, in these rats, relative to lean. However, in agreement with findings of Wang et al. (34) in the Sprague-Dawley rats, the abundance of this subunit did not seem to be highly regulated by the level of NaCl in either the lean or obese rats, suggesting that the downregulation of this protein is not normally a mechanism via which escape is mediated.

However, marked downregulation of the NCC has been associated with aldosterone escape (34). Moreover, previously we (6, 20) showed an increase in NCC abundance in young, male obese Zucker rats relative to lean age mates. Nonetheless, we did not detect any difference in the downregulation of renal NCC abundance between lean and obese rats treated with HS. Band density for the major band of NCC (165 kDa) was decreased to between 45 and 55% of body type LS controls in both lean and obese rats (Fig. 6). This suggests that differential regulation of this protein, at least its abundance, did not play a role in reduced “escape” capability in the obese rats.

However, decreased glomerular filtration rates (GFR), as assessed by reduced CCl, may have contributed to impaired escape in the obese rats. CCl was reduced in obese rats, relative to lean, irrespective of level of dietary NaCl (Table 1). In fact, obese LS rats had 50% lower CClS than did lean LS rats, suggesting that the infusion of aldosterone alone resulted in a fall in GFR. Furthermore, HS on top of the clamp led to a further reduction in CCl in both lean and obese rats of ~15–30%. Previously, we (6) and others (1) observed no differences or even increased GFR or CCl in obese relative to lean Zucker rats, when aldosterone levels weren’t clamped and rats were fed more normal levels of NaCl. Thus we suggest that this relatively decreased GFR may have increased salt sensitivity of the obese rats with regard to the ability to normalize blood pressure.

The effect of this fall in GFR on sodium excretion may have been partly ameliorated by activation of glomerular tubular balance. Because sodium entry into the proximal tubule would be less, sodium reabsorption would be proportionally reduced. This might occur from or result in decreased abundance of proximal tubule sodium transporters. We observed a marked decrease in NaPi-2 protein, a sodium-coupled phosphate co-

**Fig. 7. Endothelial nitric oxide synthase.** Immunoblot (left) and bar graph summaries of densitometry (right) are shown for eNOS in cortex (CTXH), outer medulla (OMH), and inner medulla (IMH) homogenates. Within each blot, all lanes are loaded with equal amounts of total protein. Each lane contains a sample from a different rat. Preliminary Coomassie-stained gels confirmed equality of loading. \*Significant \( P < 0.05 \) difference between HS and LS groups by 2-way ANOVA. \$Significant difference from lean LS as determined by 1-way ANOVA followed by a multiple comparisons test.
transporter of the proximal tubule in the obese rats relative to lean. This, similar to CCl, was reduced independently of dietary NaCl level. We previously observed reduced NaPi-2 abundance in the cortex of older (6-mo-old) diabetic, obese Zucker rats that, similarly, had reduced CCl, relative to lean age mates. However, it is also possible that the fall in GFR and the fall in NaPi-2 protein are not causally related. There was no similar reduction in NHE3 or in the α1-subunit of Na-K-ATPase in the cortex.

Both plasma sodium and potassium levels were elevated in the obese rats on HS, whereas HS had no bearing on plasma Na+ and K+ in the lean rats. The hypernatremia in obese rats we suspect may have resulted from inappropriate sodium retention, although we could not clearly show, using classic balance techniques, a defect in the natriuresis in the obese rats. However, there may have been some blunting in the speed of onset of the natriuretic response in the obese rats that in that on day 5, the second day after initiation of the HS diet, the lean rats had peaked at the level of 440 ± 40 μmol Na+/g diet, whereas urine Na+ was still climbing in the obese rats (309 ± 51; P = 0.07), between these two groups by unpaired t-test. Decreased GFR could result in potassium as well as sodium retention. Collecting duct flow rate is a major determinant of potassium secretion (26), and in support of this we found a relative blunted kaliuresis in the obese rats, when offered the HS diet.

Despite lowered GFR in the obese rats, renin activity was suppressed, relative to lean. This confirms our (20) and previous findings of Alonso-Galicia et al. (1) of relatively reduced renin activity in nonaldosterone clamped obese vs. lean Zucker rats. In fact, this pattern was exacerbated in the obese rats on the HS diet. That is, renin activity was reduced in the lean rats undergoing escape from 69 ± 14 to 20 ± 11 ng·ml⁻¹·h⁻¹, a drop of 70%. However, in the obese rats it was reduced from 25 ± 5 to 1.6 ± 0.6, a drop of 93%. This suggests higher, rather than lower, macula densa NaCl load in the obese relative to lean rats. This possibly could explain the reduction in GFR in these rats as the tubuloglomerular feedback response to high macula densa NaCl load would result in the release of vasoconstrictors acting on the afferent arteriole (25).

Finally, there may be a role for impaired NO bioavailability in determining the relative inability of the obese rats, relative to lean, to escape with regard to blood pressure. Fujiwarawara et al. (17) demonstrated impaired renal NOX production in response to elevated renal perfusion pressure in obese Zucker rats. In fact, we found that urine NOX, even when normalized by body weight, was 626% higher in the obese LS rats, relative to lean LS rats. The addition of high NaCl in the diet resulted in a twofold increase in urinary NOX in the lean, but actually an ~46% drop in the obese. Thus the obese rats were unable to respond to the HS diet with an increase in NO, perhaps because the system was already maximally stimulated in response to the aldosterone infusion alone. Furthermore, urine NOX correlated with cortex eNOS protein in that it was clearly lowest in the lean LS rats and highest in the obese LS rats. This inability to produce additional NO to respond to the high-NaCl challenge may have reduced proximal tubule sodium reabsorption in these rats, shifting more sodium to the TAL and distal tubule, which may have reduced GFR, further confounding the situation.

Therefore, in summary, we demonstrate an impaired response to aldosterone infusion with regard to blood pressure and the normalization of serum sodium and potassium levels in obese relative to lean Zucker rats. This impairment may involve increased sodium reabsorption via NKCC2 or ENaC in the cortex, which were increased in the obese rats on HS relative to lean. In addition, decreased GFR and NO bioavailability may have a role in sodium retention.

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