Blunted Natriuretic Response to a High-Sodium Meal in Obese Dogs
Role of Renal Nerves

Salah Kassab, Steve Patterson, F. Clayton Wilkins, H. Leland Mizelle, Glenn A. Reinhart, Joey P. Granger

Abstract Although the relation between body weight and arterial pressure is well established, the mechanisms involved in the pathogenesis of obesity-related hypertension are unclear. However, recent studies suggest that abnormalities in renal function may be involved. The purpose of this study was to test the hypothesis that obese animals have a reduced ability to excrete a sodium load as a result of abnormal renal nerve function. To quantify the role of renal nerves, we examined changes in renal hemodynamics and sodium excretion in response to a high-sodium meal (200 mmol Na) in separate innervated and denervated kidneys simultaneously within the same conscious dog. Two surgically designed hemibladders with indwelling catheters were used to collect urine from innervated and denervated kidneys of the same dog. Body weight averaged 19.9±1.0 kg in the control lean dogs and 25.1±1.1 kg in the obese dogs. Arterial pressure averaged 101±4 mm Hg in the obese dogs and 90±4 mm Hg in the lean dogs. In response to the high-sodium meal in lean dogs, urinary sodium excretion increased from 20.8±4.2 to 189.7±21.2 μmol/min in the innervated kidneys and from 25.3±5.9 to 194.8±26.9 μmol/min in the denervated kidneys. In contrast, urinary sodium excretion in obese dogs increased from 9.6±1.4 to 129.9±34.3 μmol/min in the innervated kidneys and from 18.4±3.7 to 125.2±30.5 μmol/min in the denervated kidneys. Cumulative sodium excretion over 140 minutes was significantly lower in the obese dogs (innervated, 8.4±2.8 mmol; denervated, 9.8±2.7 mmol) than in the lean dogs (innervated, 19.1±3.3 mmol; denervated, 21.2±4.2 mmol) in response to the high-sodium meal. These data indicate that the natriuretic response to a high-sodium meal is markedly attenuated in obese dogs. Furthermore, the renal nerves do not appear to play a major role in mediating this abnormal response. (Hypertension. 1994;23[part 2]:997-1001.)

Key Words • obesity • natriuresis • sodium • kidney

Although the association between body weight and arterial pressure has been documented in a number of epidemiologic and experimental studies, the mechanisms underlying the pathogenesis of obesity-induced hypertension are unclear.1-3 Recent studies, however, have indicated that the development of obesity-induced hypertension may be related to abnormal renal handling of sodium.4-6 Development of hypertension in dogs fed a high-fat diet is associated with significant sodium retention and extracellular fluid volume expansion.6-8 The pressure-natriuresis relation has also been reported to be abnormal in obese hypertensive dogs.5 Furthermore, we have recently demonstrated that the natriuretic response to an acute sodium load is significantly attenuated in obese dogs.7 Although the exact mechanism responsible for the blunted natriuretic response to an acute sodium load in obesity hypertension is unknown, abnormalities in sodium-retaining systems such as renin-angiotensin and renal sympathetic nervous systems may be involved.8 A possible role for renal nerves is supported by studies indicating that obesity is associated with increased activity of the sympathetic nervous system.8,10 Furthermore, previous studies have demonstrated that the blunted diuretic and natriuretic responses to an acute oral or intravenous sodium load in sodium-retaining states such as congestive heart failure, liver cirrhosis, and nephrotic syndrome are ameliorated by prior bilateral renal denervation.11 These observations support the notion that the impaired ability to excrete an acute saline load in these sodium-retaining states is partially dependent on an increase in basal efferent renal sympathetic nerve activity that fails to suppress normally in response to an acute saline load.12,13 The importance of this mechanism in the blunted natriuresis of obesity is unclear, since a recent study failed to demonstrate a role for renal nerves in the blunted renal response to an acute saline load in obese Zucker rats.14 Therefore, the goal of this study was to test if the attenuated renal excretory response to an acute sodium load in obese dogs is due to abnormal renal sympathetic nerve activity. To quantify the role of renal nerves in response to an acute sodium load in obese dogs, we studied the changes in renal hemodynamics and sodium excretion in separate innervated and denervated kidneys simultaneously within the same dog. This model is extremely powerful in detecting any effect of renal nerves because each kidney is exposed to the same arterial pressure and circulating hormones. Any difference in renal excretion between innervated and denervated kidneys can then be attributed solely to a direct or indirect effect of the renal nerves.15

Methods

Experiments were conducted on female mongrel dogs (n=14) with an average weight of 19.9±1.0 kg. Seven dogs (obese-dog group) were given a daily diet consisting of 2 lb of cooked beef fat in addition to their regular diet of 2 cans of dog
Renal Hemodynamic and Excretory Responses to a High-Sodium Meal In Innervated and Denervated Kidneys of Lean and Obese Dogs

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| RPF, mL/min | 64.9±2.3 | 65.6±4.4 | 63.1±8.8 | 79.9±11 | 70.8±11 | 79.6±12 | 81.6±6.4* | 92.9±5.8
| Lean   |         |       |       |       |       |       |       |
|        |         |       |       |       |       |       |       |
| Obese  | 66.1±6.5 | 79.8±8.6 | 67.4±7.0 | 86.7±9.8 | 71.2±4.2 | 65.4±7.8 | 97.8±9.7* | 88.3±3.9
| GFR, mL/min | 21.9±1.9 | 22.5±2.7 | 21.7±3.0 | 28.4±4.1 | 23.6±3.9 | 27.3±4.3 | 29.8±2.7* | 34.5±2.3*
| Lean   |         |       |       |       |       |       |       |
|        |         |       |       |       |       |       |       |
| Obese  | 24.2±3.9 | 28.3±3.3 | 24.7±3.9 | 30.5±3.4 | 25.4±3.0 | 26.4±3.3 | 40.2±9.2* | 32.9±3.2
| FEun, % | 0.69±0.1 | 0.82±0.2 | 0.70±0.2 | 0.86±0.2† | 0.89±0.2 | 1.20±0.2 | 1.34±0.3 | 1.58±0.3
| Lean   |         |       |       |       |       |       |       |
|        |         |       |       |       |       |       |       |
| Obese  | 0.27±0.04† | 0.48±0.1† | 0.27±0.06 | 0.57±0.2 | 0.36±0.09 | 0.69±0.2 | 0.60±0.2 | 0.83±0.3
| FEUL, % | 13.8±4.7 | 14.2±4.8 | 13.1±4.2 | 15.3±4.1 | 15.8±5.2 | 18.0±3.6* | 21.1±5.8* | 22.1±5.5*
| Lean   |         |       |       |       |       |       |       |
|        |         |       |       |       |       |       |       |
| Obese  | 15.6±1.1 | 20.6±4.5‡ | 17.1±2.1 | 22.4±3.7 | 21.3±3.7 | 22.1±3.8 | 23.1±2.9 | 27.9±3.2

INN indicates innervated kidney; DNX, denervated kidney; RPF, renal plasma flow; GFR, glomerular filtration rate; FEun, fractional excretion of sodium; and FEUL, fractional excretion of lithium. Values are expressed as mean±SEM. *P < .05 was considered significant. *Significant compared with control; †significant in obese vs lean dogs; ‡significant in INN vs DNX kidneys within each dog.
sodium provided in food. Approximately 12 to 14 hours before each experiment, the animals were given 300 mg lithium carbonate to allow estimation of proximal tubule sodium reabsorption by the lithium-clearance technique.16

On the day of the experiment, 125I-iothalamate (Glofil, Isotex Diagnostics) and 131I-iodohippurate (CIS) were added to the saline infusion for measurement of glomerular filtration rate and renal plasma flow, respectively. A priming dose of 0.45 μCi/kg of iothalamate and 1.0 μCi/kg of iodohippurate was given, followed by a sustained infusion of 0.005 and 0.010 μCi/kg per minute of iothalamate and iodohippurate, respectively.

After a 1-hour equilibration period, two 20-minute control clearances were obtained. The dogs were then fed a meal (2 cans of Ken-L-Ration) containing a total of 200 mmol of sodium. The dogs ate all the food within 5 to 10 minutes. Seven 20-minute clearances were obtained after ingestion of the food. Each clearance period consisted of 20 minutes of urine collection, measurement of hemodynamic parameters, and withdrawal of 3 mL of arterial blood for electrolyte and isotope determination. During the experiment, mean arterial pressure and heart rate were monitored continuously using a Statham amplifier were then sent to a digital computer to be analyzed. The average blood pressure and heart rate data were calculated from the values recorded every 20 minutes. Whole-kidney proximal tubule reabsorption of sodium was determined by the lithium-clearance technique.18 Many studies have demonstrated that lithium is reabsorbed almost exclusively in the proximal tubule in parallel with sodium.16 Therefore, lithium can be used as a reliable marker of proximal tubule handling of sodium. Plasma and urine sodium, potassium, and lithium were determined by flame photometry (IL-943, Instrumentation Laboratory). Concentrations of 125I-iothalamate and 131I-iodohippurate in plasma and urine were measured by liquid scintillation, and data were used to calculate the clearances of iothalamate (glomerular filtration rate) and hippurate (renal plasma flow).

Data in the text and Figure are expressed as mean±SE. Dunnett’s t test was used to compare the average control with the values obtained after administration of the meal. The data were subjected to ANOVA. Comparisons between innervated and denervated kidneys were made by using paired t test. Probability values less than .05 were considered statistically significant.

Results

The obese dogs weighed an average of 25.1±1.1 kg, and the lean dogs weighed an average of 19.8±1.0 kg. Before the start of high-fat feeding, dogs in the obese group weighed an average of 19.1±1.0 kg. Associated with the increase in body weight were significant increases in heart rate. The heart rate averaged 153±7 beats per minute (bpm) in the obese group and 90±6 bpm in the lean dogs. During the control phase of the experiment, arterial pressure averaged 91±3 mm Hg in the lean dogs and 101±4 mm Hg in the obese dogs. Heart rate and arterial pressure did not change significantly in response to the high-sodium meal in either lean or obese dogs.

The Figure illustrates the postprandial changes in sodium excretion in both innervated and denervated kidneys of lean and obese dogs. In response to a high-sodium meal, a significant increase in urinary sodium excretion (UNaV) occurred in both kidneys of lean and obese dogs. UNaV increased in lean dogs from 20.8±4.2 to 189.7±21.2 μmol/min in the innervated kidneys and from 25.3±5.9 to 194.8±26.9 μmol/min in the denervated kidneys. In the obese-dog group, UNaV increased from 9.6±1.4 to 129.9±34.3 μmol/min in the innervated kidneys and from 18.4±3.7 to 125.2±30.5 μmol/min in the denervated kidneys. Fractional excretion of sodium (FENa) also increased in the lean dogs from 0.69±0.1% to 3.8±0.6% in the innervated kidneys and from 0.82±0.2% to 4.0±0.6% in the denervated kidneys (Table). FENa increased from 0.27±0.04% to 2.63±0.7% in the innervated kidneys and from 0.48±0.1% to 2.82±0.9% in the denervated kidneys of the obese dogs. Increases in UNaV and FENa were both significantly attenuated in obese dogs. Under basal

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conditions, $FE_N$ was significantly lower in the innervated kidneys of obese dogs than in the innervated kidneys of lean dogs. However, there were no significant differences in the $U_{NaV}$ or $FE_N$ responses to the high-sodium meal between innervated and denervated kidneys of both groups.

The Figure also illustrates the cumulative $U_{NaV}$ over 140 minutes after the ingestion of a high-sodium meal in lean and obese dogs. Cumulative $U_{NaV}$ averaged 19.1±3.3 mmol in the innervated kidneys and 21.2±4.2 mmol in the denervated kidneys of the lean dogs. In the obese dogs, cumulative $U_{NaV}$ was only 8.4±2.8 mmol in the innervated kidneys and 9.8±2.7 mmol in the denervated kidneys. The cumulative $U_{NaV}$ was significantly lower in obese dogs. After ingestion of a high-sodium meal (200 mmol), obese dogs excreted only 9% of the sodium load compared with 20% in lean dogs. However, there was no significant difference in cumulative $U_{NaV}$ between innervated and denervated kidneys in either the lean or obese dogs.

In response to a high-sodium meal, fractional excretion of lithium ($FE_Li$) increased significantly in lean and obese dogs (Table). In lean dogs, $FE_Li$ increased from 13.8±4.7% to 28.7±3.5% in the innervated kidneys and from 14.2±4.8% to 28.8±3.8% in the denervated kidneys. In the obese dogs, $FE_Li$ increased from 15.6±1.1% to 35.8±4.9% in the innervated kidneys and from 20.6±4.5% to 38.1±6.1% in the denervated kidneys. There were no significant differences in the response of $FE_Li$ to the high-sodium meal between the lean and obese dogs or between innervated and denervated kidneys of lean dogs. Although the $FE_{Li}$ was significantly higher in the denervated kidneys of obese dogs under basal conditions, this difference disappeared after the ingestion of the high-sodium meal.

The Table also depicts the renal hemodynamic responses to a high-sodium meal in lean and obese dogs. Sixty minutes after the ingestion of the high-sodium meal, glomerular filtration rate and renal plasma flow were significantly higher than basal levels in both groups. Glomerular filtration rate in lean dogs significantly increased from 21.9±1.9 to 29.8±2.7 mL/min in the innervated kidneys and from 22.5±2.7 to 34.5±2.3 mL/min in the denervated kidneys. In the obese dogs, glomerular filtration rate significantly increased from 24.2±3.8 to 40.2±9.1 mL/min in the innervated kidneys and from 28.3±3.3 to 33.0±3.2 mL/min in the denervated kidneys. Renal plasma flow in lean dogs increased from 64.9±2.3 to 90.6±6.4 mL/min in the innervated kidneys and from 65.6±4.4 to 92.9±5.8 mL/min in the denervated kidneys. In obese dogs, renal plasma flow increased from 66.1±6.5 to 97.8±9.7 mL/min in the innervated kidneys and from 79.8±8.6 to 88.3±3.9 mL/min in the denervated kidneys. There were no significant differences in glomerular filtration rate or renal plasma flow between lean and obese dogs or between the innervated and denervated kidneys of both groups during all periods of the experiment.

Discussion

A number of studies have indicated that the development of obesity-induced hypertension may be related to abnormal renal handling of sodium.7,8 We have recently demonstrated that the natriuretic response to an acute sodium load is significantly attenuated in obese dogs. Although the exact mechanism responsible for the blunted natriuretic response to an acute sodium load in obesity-hypertension is unknown, abnormalities in sodium-retaining systems such as renin-angiotensin and renal sympathetic nervous systems may be involved.4 To quantify the role of renal nerves in the blunted response to an acute sodium load in obese dogs, we examined the changes in renal hemodynamics and sodium excretion in separate innervated and denervated kidneys simultaneously within the same dog. This model is extremely powerful in detecting any effect of renal nerves because each kidney is exposed to the same arterial pressure and circulating hormones. Any difference in renal excretion between innervated and denervated kidneys then can be attributed solely to a direct or indirect effect of the renal nerves.15 The results of the present study confirm our previous finding that the sodium excretory response to a sodium load is markedly attenuated in obese dogs (9.1% of sodium load) in comparison to lean controls (20.2% of sodium load). Furthermore, our data suggest that removal of the renal nerves does not ameliorate the blunted natriuretic response to a high-sodium meal in obese dogs.

A role for renal nerves in the blunted response to a sodium load in obesity is supported by studies indicating that obesity is associated with increased activity of the sympathetic nervous system.8,10 Furthermore, previous studies have demonstrated that the blunted diuretic and natriuretic responses to an acute oral or intravenous saline load in several sodium-retaining states are ameliorated by prior bilateral renal denervation.11-13 In the present study, however, renal denervation had no effect on the natriuretic response to a high-sodium meal in the obese or lean dogs. Cumulative excretion of sodium in the innervated kidney of obese dogs averaged approximately 4.2% of the sodium load in comparison to 4.9% in the denervated kidney of obese dogs. Our findings in the obese dogs are in agreement with a recent study that also failed to demonstrate a role for renal nerves in the blunted renal response to an acute saline load in obese Zucker rats.14 Thus, it appears that the renal nerves play a relatively unimportant role in the blunted natriuretic response to sodium loading in obesity.

The fact that renal denervation does not correct the blunted natriuretic response to ingestion of a high-sodium meal does not necessarily mean that the renal sympathetic nervous system is not important in the sodium retention observed during the development of obesity-related hypertension. Before the ingestion of the high-sodium meal, $U_{NaV}$ was significantly lower in the innervated kidneys of lean dogs than in the innervated kidneys of obese dogs. Renal denervation in the obese dogs almost completely abolished the differences in $U_{NaV}$ between the lean-dog innervated kidney and obese-dog denervated kidney under basal conditions. In contrast, renal denervation in the control lean dogs had no effect on $U_{NaV}$ under basal conditions. These data support the notion that the renal sympathetic nervous system may be activated as well as be responsible in part for the sodium retention observed during the development of obesity-related hypertension.4,15 Ongoing studies in our laboratory are attempting to further assess the importance of renal nerves in mediating the sodium retention and hypertension in dogs fed a high-fat diet.
The exact mechanism responsible for the blunted natriuretic response to a high-sodium meal in obese dogs remains unresolved. It seems unlikely that the attenuated sodium excretory response to an acute sodium load in obese dogs was due to differences in renal hemodynamics. Glomerular filtration rate and renal plasma flow increased similarly in the obese and lean dogs after ingestion of the high-sodium meal. Furthermore, our data indicate that renal denervation had no effect on the renal hemodynamic response to the high-sodium meal in both the lean and obese dogs. These findings suggest that the attenuated natriuretic response to a high-sodium meal in obese dogs is due to abnormalities in renal tubular reabsorption of sodium.

Changes in proximal reabsorption of sodium, as estimated by the lithium-clearance technique, appear to be normal in the obese dog. In response to the high-sodium meal, FE\textsubscript{\textit{Na}} increased similarly in the obese and lean dogs. Renal denervation had no significant effect on the proximal tubule response to the high-sodium meal in the obese or lean dogs. These data indicate that abnormalities in renal handling of sodium beyond the proximal tubule may be responsible for the blunted natriuretic response to sodium loading in the obese dog.

Our finding that renal denervation does not correct the blunted natriuretic response to sodium loading in obese dogs indicates that abnormalities of other sodium regulatory mechanisms may be involved. We have previously reported that plasma renin activity is 150% higher in dogs fed a high-fat diet than in lean dogs. An inability to suppress renin in obese dogs could possibly account for the abnormal sodium handling. A recent preliminary report also suggests that obese patients have an inability to enhance atrial natriuretic factor secretion in response to volume expansion. Abnormalities in renal interstitial pressure dynamics have also been observed in obesity. The relative importance of these various sodium-regulatory derangements in accounting for the abnormal response to extracellular fluid volume expansion is unknown and requires further investigation.

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References

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