Reduced carotid baroreceptor distensibility-induced baroreflex resetting contributes to impairment of sodium regulation in rats fed a high-fat diet

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1Department of Physiology, Gifu University Graduate School of Medicine, Gifu, Japan; 2Department of Tissue and Organ Development, Regeneration, and Advanced Medical Science, Gifu University Graduate School of Medicine, Gifu, Japan; 3Department of Cardiovascular Dynamics, National Cerebral and Cardiovascular Center, Osaka, Japan; and 4Department of Cardiac Physiology, National Cerebral and Cardiovascular Center, Osaka, Japan

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Abe C, Nagai Y, Yamaguchi A, Aoki H, Shimizu S, Akiyama T, Kawada T, Sugimachi M, Morita H. Reduced carotid baroreceptor distensibility-induced baroreflex resetting contributes to impairment of sodium regulation in rats fed a high-fat diet. Am J Physiol Heart Circ Physiol 308: H942–H950, 2015. First published February 13, 2015; doi:10.1152/ajpheart.00697.2014.—Decreased carotid arterial baroreflex is thought to be important for the short-term control of arterial pressure (AP). However, because the renal sodium excretion rates are controlled by arterial pressure, the involvement of the renal sodium excretion in the long-term control of AP might be also considered. Therefore, we hypothesized that sodium retention and circulatory volume expansion seen in the early phase of obesity are not accompanied by renal vasocostriction or decreased glomerular filtration rate. To examine this hypothesis, we conducted the following experiments in rats fed a normal-fat diet or a high-fat diet: 1) we determined mean AP, water and sodium balance, and renal function in response to chronic hyperosmotic NaCl infusion in rats fed a high-fat diet. To examine this hypothesis, we used rats fed a high-fat (Fat) or normal (NFD) diet, and measured mean AP, water and sodium balance, and renal function in response to chronic hyperosmotic NaCl infusion in a venous catheter. Furthermore, we examined arterial baroreflex characteristics with static open-loop analysis and distensibility of the common carotid artery. Significant positive water and sodium balance was observed on the 1st day of 9% NaCl infusion; however, this disappeared by the 2nd day in Fat rats. Mean AP was significantly higher during 9% NaCl infusion in Fat rats compared with NFD rats. In the open-loop analysis of carotid sinus baroreflex, a rightward shift of the neural arc was observed in Fat rats compared with NFD rats. Furthermore, distensibility of the common carotid artery was significantly reduced in Fat rats. These results indicate that a reduced baroreceptor distensibility-induced rightward shift of the neural arc might contribute to impairment of sodium regulation in Fat rats.

High-fat diet; renal function; sodium excretion; arterial pressure; renal sympathetic nerve activity

METABOLIC SYNDROME, which is characterized by the clustering of obesity, dyslipidemia, hyperglycemia, and hypertension, is a major public health problem (7, 17, 33). Although the relationship between body weight and arterial pressure (AP) is well established, the mechanisms involved in the pathogenesis of obesity-induced hypertension are still unclear. The following mechanisms have been proposed: 1) increased renal sympathetic nerve activity (SNA), 2) activation of the renin-angiotensin-aldosterone system, and 3) physical compression of the kidney by fat, each of which is known to increase renal sodium reabsorption and impair pressure natriuresis in obese subjects (23). These mechanisms might participate in elevation of extracellular fluid and plasma volumes in experimental animals fed a high-fat diet (21) and in obese humans (32). Sodium retention and circulatory volume expansion seen in the early phase of obesity are not accompanied by renal vasocostriction or decreased glomerular filtration rate (22). Glomerular filtration rate and renal plasma flow are maintained in obese animals (18) and humans (38). Sodium retention is therefore mainly due to increased renal tubular reabsorption before glomerular injury and loss of nephron function in obesity (22).

Arterial baroreflex is thought to be important for the short-term control of AP. However, because the renal SNA is under control of arterial baroreflex, the involvement of the renal SNA in the long-term control of AP might be also considered (5, 25, 26, 30, 36). Carotid baroreceptors are located at the bifurcation of the common carotid artery, and respond to distension of the arterial wall (19). A decrease in arterial compliance is observed in obese subjects and animals (2, 8, 39, 42); thus, it is possible that higher AP is required to activate the baroreceptors. Because infusion of hyperosmotic NaCl solution is known to increase plasma volume and AP (1, 43), we hypothesized that sodium retention via the baroreflex might be impaired in response to chronic hyperosmotic NaCl load in rats fed a high-fat diet. To examine this hypothesis, we conducted the following experiments in rats fed a normal-fat diet or a high-fat diet: 1) we determined mean AP, water, and sodium balance, and performed open-loop analysis of baroreflex characteristics using static open-loop analysis, and 3) we determined distensibility of the common carotid artery.

MATERIALS AND METHODS

Animals used in the present study were maintained in accordance with the “Guiding Principles for Care and Use of Animals in the Field of Physiological Science” set by the Physiological Society of Japan. The experiments were approved by the Animal Research Committee of Gifu University. Male 3-wk-old Sprague Dawley rats weighing 50–60 g (n = 24) were used for the experiment.

Rats were fed with a normal-fat diet (n = 6, 344.9 kcal/100 g, CE-2; CLEA, Tokyo, Japan) or a high-fat diet (n = 18, 415.1 kcal/100 g, QuickFat; CLEA) for 10 wk. We recorded body weight data for 10 wk. On the 13th wk, rats fed the high-fat diet were divided into three groups based on their body weight, i.e., higher, middle, and lower body weight. In the present study, we employed three groups of rats: rats fed the normal-fat diet (NFD, n = 6, 433 ± 5 g), rats fed the high-fat diet with higher body weight (Fat, n = 6, 521 ± 4 g), and rats...
fed the high-fat diet with lower body weight (Lean, n = 6, 474 ± 8 g). All rats were fed ad libitum and were maintained on a 12:12-h light-dark cycle at a temperature of 24 ± 1°C.

On the 13th wk, a telemetric transmitter (PA-C40; Data Science International, St. Paul, MN) was implanted in all rats to measure AP. Through a midline laparotomy, the catheter part of the telemetric transmitter probe was inserted in the abdominal aorta. The tip of the catheter was placed distal to the renal artery bifurcation. The probe was then sutured to the abdominal wall, and the incision was closed. Penicillin G potassium (6,000 U/day) and buprenorphine (3 μg/kg) were injected intramuscularly for 3 days after surgery. Seven days after surgery, rats were anesthetized with isoflurane (Escain; Mylan, Tokyo, Japan) inhalation. A polyethylene catheter (PE-50; Becton-Dickinson, Sparks, MD) was inserted in the inferior vena cava via the left femoral vein to infuse NaCl solution. The catheter was exteriorized from the back of the neck, covered with a spring, and connected to a swivel.

Three days after venous catheter implantation, rats were moved to individual metabolic cages and maintained there for the remainder of the experiment. Rats were fed with a low-sodium diet (0.005%; CLEA) and tap water ad libitum and were maintained on a 12:12-h light-dark cycle at a temperature of 24 ± 1°C. NaCl solution was infused at a rate of 0.5 ml/h via the venous catheter for 22 h (8:00 A.M.-6:00 A.M.). The concentration of the NaCl solution was set at 0.9% (days 1 and 2), 9% (days 3 and 4), 3% (days 5 and 6), 6% (days 7 and 8), and 0.45% (days 9 and 10). Cages were refreshed every day between 6:00 A.M. and 8:00 A.M., during which period urine volume and water intake for the preceding 22 h were measured. Daily AP was also measured during this 2-h period using a PhysioTel Receiver (RLA 1020; Data Science International, Minneapolis, MN). The output was relayed through a calibrated pressure output adapter (RI1CPA; Data Science International) and a dual ambient pressure monitor (C11PR; Data Science International). The AP was recorded using an analog-to-digital converter (PowerLab; AD Instruments, Dunedin, New Zealand) at a sampling rate of 100 Hz. Sodium concentration was measured as above except for an experimental period. The solution was administered continuously (5 ml·kg⁻¹·h⁻¹). AP was measured using a chemistry analyzer (VetTest; IDEXX Laboratories, Westbrook, ME) through a tracheal tube with oxygen-enriched air. A mixed solution [1 mol·kg⁻¹·h⁻¹ NaCl plus sorbitol (Wako Pure Chemical Industries, Osaka, Japan)] was administered continuously (5 ml·kg⁻¹·h⁻¹). AP was measured using a telemetry system described above. Heart rate (HR) was calculated from the AP waveform. To record renal SNA,

Table 1. Parameters representing the static characteristics of the carotid sinus baroreflex

<table>
<thead>
<tr>
<th>Parameters</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
<th>P4</th>
<th>Gain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neural arc:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lean</td>
<td>74 ± 4</td>
<td>0.14 ± 0.01</td>
<td>111 ± 1</td>
<td>25 ± 4</td>
<td>-2.1 ± 0.2</td>
</tr>
<tr>
<td>Fat</td>
<td>88 ± 3</td>
<td>0.14 ± 0.01</td>
<td>128 ± 3*</td>
<td>12 ± 2*</td>
<td>-3.1 ± 0.5*</td>
</tr>
<tr>
<td>NFD</td>
<td>73 ± 3</td>
<td>0.12 ± 0.01</td>
<td>115 ± 1</td>
<td>26 ± 4</td>
<td>-2 ± 0.2</td>
</tr>
<tr>
<td>Peripheral arc:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lean</td>
<td>0.8 ± 0.08</td>
<td>43 ± 4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat</td>
<td>0.8 ± 0.02</td>
<td>41 ± 7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NFD</td>
<td>0.9 ± 0.06</td>
<td>22 ± 4†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total arc:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lean</td>
<td>62 ± 7</td>
<td>0.1 ± 0.01</td>
<td>115 ± 3</td>
<td>60 ± 6z</td>
<td>-1.5 ± 0.1</td>
</tr>
<tr>
<td>Fat</td>
<td>75 ± 2</td>
<td>0.1 ± 0.01</td>
<td>126 ± 4*</td>
<td>51 ± 6</td>
<td>-2.0 ± 0.3</td>
</tr>
<tr>
<td>NFD</td>
<td>69 ± 4</td>
<td>0.1 ± 0.01</td>
<td>121 ± 5</td>
<td>40 ± 2</td>
<td>-1.6 ± 0.1</td>
</tr>
</tbody>
</table>

Values are means ± SE. Lean, rats fed a high-fat diet with lower body weight; Fat, rats fed a high-fat diet with higher body weight; ND, rats fed a normal-fat diet; a, slope of the peripheral arc; b, intercept of the peripheral arc; P1, response range of the output; P2, slope coefficient; P3, midpoint pressure of the input; P4, minimum value for the output; X, y-axis value of the intersection between the neural and peripheral arcs; Y, y-axis value of the intersection between the neural and peripheral arcs; SNA, sympathetic nervous system; CHP, carotid sinus pressure; AP, arterial pressure. *P < 0.05 vs. Lean and NFD. †P < 0.05 vs. Lean and Fat. ‡P < 0.05 vs. Lean.

Table 2. Internal area, length, and volume of the right common carotid artery in NFD and Fat rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>NFD</th>
<th>Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area, mm²</td>
<td>0.24 ± 0.03</td>
<td>0.23 ± 0.01</td>
</tr>
<tr>
<td>Length, mm</td>
<td>6.99 ± 0.1</td>
<td>6.84 ± 0.08</td>
</tr>
<tr>
<td>Volume, mm³</td>
<td>1.73 ± 0.02</td>
<td>1.6 ± 0.05</td>
</tr>
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</table>

Values are means ± SE.

Fig. 1. Body weight alteration from the 3rd to the 13th wk in rats fed a high-fat diet with lower body weight (Lean), rats fed a high-fat diet with higher body weight (Fat), and rats fed a normal-fat diet (NFD). *P < 0.05 vs. NFD. †P < 0.05 vs. Lean.
postganglionic renal sympathetic nerve was isolated through a right or left flank incision, and two stainless-steel electrodes (AS633; Cooner Wire, Chatsworth, CA) were placed around it. The nerve and electrodes were covered and fixed with silicone gel (Kwik-Sil; World Precision Instruments, Sarasota, FL).

The vagal and aortic nerves were sectioned bilaterally at the neck, avoiding the reflexes from the cardiopulmonary region and the aortic arch. The carotid sinus regions were isolated bilaterally from the systemic circulation. Briefly, the external carotid artery was ligated with a 6-0 silk surgical suture close to the carotid bifurcation. Because two arteries branch off from the internal carotid artery just before entering the cranial base, these two arteries were ligated using a 6-0 silk surgical suture just below the cranial base. The isolated carotid sinuses were filled with warmed Ringer’s solution through catheters inserted in the common carotid arteries. Carotid sinus pressure (CSP) was regulated using a servo-controlled piston pump. Heparin sodium (100 U/kg) was given intravenously to prevent blood coagulation. Body temperature was maintained at 37°C with a heating pad. All signals were recorded using an analog-to-digital converter at a rate of 1,000 Hz. To estimate the static input-output relationship of the carotid sinus baroreflex, the right common carotid artery was ligated with a 6-0 silk surgical suture just below the cranial base. Because the absolute voltage of renal SNA varied among the animals, depending on the recording conditions, the average renal SNA during the last 20 s at a CSP level of 60 mmHg was defined as 100%. To quantify the open-loop static characteristics of the carotid sinus baroreflex, the mean renal SNA and AP were obtained during the last 20 s at each CSP level of the stepwise input protocol.

The static characteristics of the baroreflex neural arc (relationship between CSP and renal SNA) and the total baroreflex (relationship between CSP and AP) were described by fitting four-parameter logistic functions to the input-output data as follows:

\[
y = \frac{y_{max}}{1 + \exp\left(\frac{-P_2 \times (x - P_3)}{P_1}\right)} + P_4,
\]

where \(x\) and \(y\) denote the input (CSP) and output (renal SNA or AP), respectively; \(P_1\) is the response range of the output; \(P_2\) is the slope coefficient; \(P_3\) is the midpoint pressure of the input; and \(P_4\) is the minimum value for the output. For convenience, the maximum gain of the logistic function is reported by a positive value as \(P_1 \times P_2/4\).

The static characteristics of the baroreflex peripheral arc (relationship between renal SNA and AP) were quantified from a scatter plot. A linear regulation line is represented as follows:

\[
AP = a \times SNA + b
\]

where \(a\) and \(b\) represent the slope and intercept, respectively.

After measurement of the open-loop static characteristics of the carotid sinus baroreflex, the right common carotid artery was ligated proximal to the bifurcation with 5-0 silk in Fat and NFD rats. A catheter inserted in the right common carotid artery was connected to a syringe pump (NE-300; New Era Pump Systems, Farmingdale, NY).

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**Fig. 2.** A: summarized data of the alteration in arterial pressure in Lean, Fat, and NFD rats housed in metabolic cages for 10 days. The concentration of the NaCl solution via the venous catheter was set at 0.9% [days (D) 1 and 2], 9% [days 3 and 4], 3% [days 5 and 6], 6% [days 7 and 8], and 0.45% [days 9 and 10]. *P < 0.05 vs. NFD. †P < 0.05 vs. days 1 and 2 in Fat rats. B: summarized data of the alteration in sodium balance in Lean, Fat, and NFD rats housed in metabolic cages for 10 days. *P < 0.05, Fat vs. Lean and NFD. †P < 0.05 vs. days 1 and 2 in Lean, Fat, and NFD rats. C: summarized data of the alteration in cumulative sodium content of Lean, Fat, and NFD rats housed in metabolic cages for 10 days. *P < 0.05. Fat vs. Lean and NFD. †P < 0.05 vs. days 1 and 2 in Fat rats. D: summarized data of the alteration in water balance in Lean, Fat, and NFD rats housed in metabolic cages for 10 days. *P < 0.05, Fat vs. Lean and NFD. †P < 0.05 vs. days 1 and 2 in Fat rats.
Ringer’s solution was infused at a rate of 5 µl/min for 1 min, and internal pressure of the right common carotid artery was then measured for 30 s through a three-way stopcock. The infusion was repeated until the internal pressure exceeded 180 mmHg. The right common carotid artery was cut at the ligatures after the experiment, and a tissue preparation was made (CCMount tissue mounting medium; Sigma-Aldrich, St. Louis, MO). After photographs were taken with a fluorescence microscope (BZ-9000; Keyence, Osaka, Japan), internal area, length, and volume of the right common carotid artery were evaluated using ImageJ software (http://rsbweb.nih.gov/ij/). Infusion volume was normalized to internal volume of the right common carotid artery.

After the experiment of sorbitol infusion, the rats were anesthetized using pentobarbital sodium (50 mg/kg ip) and transcardially perfused using a phosphate-buffered saline containing heparin followed by a 4% paraformaldehyde solution for fixation. The common carotid artery and carotid sinus were removed, and 3-μm-thick sections were cut and stained using hematoxylin and eosin. The vascular wall of the common carotid artery and carotid sinus was examined under an optical microscope. All data are presented as means ± SE. For the data presented in Table 1, one-way ANOVA was applied. Repeated-measures two-way ANOVA was used for the data presented in Figs. 1, 2, 3B, and 5. If the F ratio indicated statistical significance, the Tukey-Kramer post hoc test was applied for between-group comparisons. A simple linear regression line was calculated using the least-squares method for Figs. 3A and 7, and the analysis of covariance method was applied. An unpaired t-test was used in Table 2. In all tests, P < 0.05 was considered statistically significant.

RESULTS

Body weight increased gradually from the 3rd wk to the 13th wk in Lean, Fat, and NFD rats (Fig. 1). At the 13th wk, a significant difference in body weight was observed between these groups.

In the metabolic cage experiment, basal AP on days 1 and 2 was significantly higher in Fat (95 ± 1 mmHg) and Lean (95 ± 1 mmHg) rats compared with NFD rats (89 ± 2 mmHg). A further increase in AP was observed in Fat rats on days 3 and 4, when infusion solution was changed from 0.9 to 9% NaCl, but not in Lean or NFD rats (Fig. 2A). The increased AP in Fat rats recovered on day 5, when the infusion solution was changed from 9 to 3% NaCl, and no further increase in AP was observed thereafter, although the infusion solution was changed to 6% NaCl on day 7.

In all groups, sodium balance increased in response to the increase in infused NaCl concentration on days 3, 7, and 8 (Fig. 2B). The magnitude of increased sodium balance on day 3 in Fat rats was significantly larger than that in Lean and NFD rats. Cumulative sodium balance was significantly larger in Fat rats on days 3 and 4 compared with Lean and NFD rats; this difference was not observed thereafter (Fig. 2C). In contrast, water balance did not correlate with sodium balance, i.e., water balance was maintained at a constant level in Lean and NFD rats regardless of infused solution, and in Fat rats except on the 1st day of 9% NaCl infusion (Fig. 2D).

In the additional experiment, there was no difference in baseline (day 1) sodium and water balance between Fat and NFD rats (sodium balance, 0.52 ± 0.07 mmol in NFD rats and 0.61 ± 0.08 mmol in Fat rats; water balance 15.5 ± 1.3 g in NFD rats and 16.3 ± 1.7 g in Fat rats). Neither sodium nor water balance was influenced by the sorbitol infusion in either Fat or NFD rats. In the AP data, higher baseline AP were observed in Fat rats (84 ± 1.2 mmHg in NFD rats and 92 ± 2.4 mmHg in Fat rats). Sorbitol solution increased AP in Fat rats at day 2 (87 ± 0.6 mmHg in NFD rats and 102 ± 1.3 mmHg in Fat rats) and day 3 (84 ± 0.7 mmHg in NFD rats and 101 ± 1.9 mmHg in Fat rats).

The role of the kidney in long-term control of AP was assessed by plotting sodium excretion against AP (renal function line). Regression lines between AP and sodium excretion were significantly different between the groups (Fig. 3A); the regression line in Lean rats shifted right compared with NFD rats, and the regression line in Fat rats became blunt. Urinary norepinephrine excretion in Lean and Fat rats was significantly greater than that in NFD rats on days 1 and 2 (Fig. 3B). Norepinephrine excretion was decreased by 9% NaCl infusion in all groups; however, in Fat rats it was greater than in NFD or Lean rats on day 3 but not on day 4. There was no difference in daily urinary albumin contents between the groups (0 g in NFD, 2.1 ± 2.1 g in Fat, and 1.1 ± 0.8 g in Lean).

Typical recordings of open-loop analysis in the carotid sinus baroreflex are shown in Fig. 4. Stepwise increases in CSP reduced AP, renal SNA, and HR in all groups. Summarized data of the open-loop static characteristics of the carotid sinus baroreflex are shown in Fig. 5. Each parameter is summarized in Table 1. In Fat rats, a significantly higher midpoint pressure of the CSP (P3) and a significantly lower minimum value of

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**Fig. 3.** A: summarized data of the correlation between arterial pressure and sodium excretion in Lean, Fat, and NFD rats. *P < 0.05 vs. NFD. †P < 0.05 vs. Lean. ‡P < 0.05 vs. value at days 1 and 2 in all groups.
renal SNA (P4) were observed compared with Lean or NFD rats (Table 1 and Fig. 5A). At 100 and 120 mmHg in CSP, the value of renal SNA was significantly higher in Fat rats compared with Lean or NFD rats (Fig. 5A). A significantly larger value of the intersection of the peripheral arc in Lean and Fat rats indicates an upward shift of the peripheral arc in these groups (Table 1 and Fig. 5B). In the total arc (Fig. 5C), a significantly higher midpoint pressure of the AP (P3) was observed in Fat rats compared with Lean or NFD rats (Table 1). The baroreflex equilibrium diagram was drawn by plotting the neural and peripheral arcs on the pressure-SNA plane (Fig. 6). The vertical line is either CSP (the neural arc) or AP (the peripheral arc). The intersection between the neural and peripheral arcs gives the closed loop operating points. The AP of the operating point was significantly higher in Fat rats compared with Lean or NFD rats (Fig. 6 and Table 1).

Distensibility was determined by compliance (volume/pres- sure) divided by internal volume of the right common carotid artery. There was no difference in internal volume in the common carotid artery (Table 2). However, the distensibility was significantly smaller in Fat rats compared with NFD rats (Fig. 7C). In the histology, no difference was observed in the aortic wall structure in either carotid sinus or common carotid artery between NFD and Fat rats (Fig. 8).

DISCUSSION

The major findings of the present study were as follows. 1) Significantly larger increases in AP, water balance, and sodium balance were observed during 9% NaCl infusion in Fat rats compared with Lean and NFD rats. 2) A high-fat diet modified the correlation between AP and sodium excretion: the renal function curve shifted to the right in Lean rats, and the slope decreased in Fat rats. 3) The neural arc of the carotid sinus baroreflex shifted to the right in Fat rats; higher CSP was required to suppress the renal SNA. 4) Distensibility of the common carotid artery was significantly reduced in Fat rats compared with NFD rats.

Obesity is known to induce impairment of renal function (12, 15, 28, 45). In the present study, the ability to excrete sodium was impaired in Fat rats during 9% NaCl infusion. On the 1st day of a 9% NaCl infusion (day 3), significantly larger positive sodium and water balances were seen in Fat rats. Although this difference had disappeared by the 2nd day (day 4) of the 9% NaCl infusion, the accumulated sodium had not compensated by day 4. In contrast, because of a tendency to a more negative sodium balance in Fat rats on day 5, there was no longer a significant difference of cumulative sodium content on day 5 and thereafter. These results indicate that renal sodium excretion in response to an acute sodium load was impaired and delayed in Fat rats. Because sodium balance was not influenced by sorbitol solution, hyperosmolality itself did not affect sodium handling. Long-term rearing on a high-fat diet has been reported to induce renal injury and delay sodium excretion in mice (12, 28). The observed delayed sodium excretion in the present study might not be due to
nephropathy, because albuminuria was not observed. Accordingly, another malfunction of the sodium excretion system during hyperosmotic NaCl infusion might participate in the delayed sodium excretion observed in the present study.

Fig. 5. Static characteristics of the average carotid sinus baroreflex for Lean, Fat, and NFD rats. Static characteristics of the baroreflex neural arc (A), baroreflex peripheral arc (B), and total baroreflex (C) are shown. Values are means ± SE. *P < 0.05 vs. Lean or NFD.

Fig. 6. Baroreflex equilibrium diagram constructed using the fitted logistic function for the neural arc and the regression line for the peripheral arc in Lean, Fat, and NFD rats. The intersections indicated by the circle, square, and triangle show the operating point of the Lean, Fat, and NFD rats, respectively.

Fig. 7. A: a typical picture of the right common carotid artery in a NFD rat. The bar indicates 1.2 mm. B: a typical representation of internal pressure in the right common carotid artery in response to intermittent infusion. Each bar indicates a 5-μl infusion, with an infusion rate of 5 μl/min. C: summarized data of the correlation between infusion volume and internal pressure of the right common carotid artery in Fat and NFD rats. *P < 0.05 vs. NFD.
A rightward shift (Fig. 5A, or upward shift in Fig. 6) of the neural arc of the carotid sinus baroreflex might contribute to this impaired sodium excretion because a decrease in renal SNA has a major role in controlling renal sodium excretion during NaCl load (34). Dobrian et al. reported that the aortic wall thickness in rats fed a high-fat diet was significantly increased compared with the control group (13), suggesting that arterial distensibility around the carotid baroreceptor might be reduced. Although we did not estimate distensibility of the carotid sinus itself, distensibility of the common carotid artery was significantly reduced in Fat rats compared with NFD rats in the present study. Histology data of the present study revealed no difference in the wall structure of the carotid sinus and common carotid artery between NFD and Fat rats. It is reported that more elastin fragmentation, which impairs the cushioning effect of the aorta, was observed in obese animals although there was no difference in elastin and collagen contents (8). Accordingly, it is possible that altered characteristic of the arterial wall rather than structure might participate in reduced arterial distensibility.

If the mechanoneural transduction of the baroreceptor was not altered, the reduced distensibility induces a rightward shift of the neural arc, because higher CSP is required to suppress the renal SNA. However, the neural arc maximum gain was not reduced, rather it was significantly increased, in Fat rats compared with the other groups. This suggests that, once the baroreceptor is stimulated, the renal SNA will decrease enough to increase urinary sodium excretion. In other words, higher CSP is required in Fat rats compared with Lean or NFD rats to achieve the maximum gain of the neural arc (Table 1). Taken together, these results suggest that, because a longer time is required to obtain the CSP which is sufficient to suppress the renal SNA, a delay of sodium regulation might be observed in Fat rats in the metabolic cage experiment.

The unit of the abscissa of the baroreflex equilibrium diagram is the percentage change of renal SNA, which is not an absolute value; thus, absolute values of renal SNA cannot be compared among the three groups of rats. However, SNA in Lean and Fat groups might be larger than that in the NFD group because daily urinary norepinephrine excretion in Lean and Fat rats was significantly greater on days 1 and 2. Nine percent NaCl infusion significantly decreased urinary norepinephrine excretion on day 3 in all three groups; however, this was significantly larger in Fat rats than in the other two groups. On the 2nd day of 9% NaCl infusion (day 4), this difference was no longer observed. These results indicate that basal sympathetic tone in Fat and Lean rats was higher than that in NFD rats, and the 9% NaCl-induced suppression of SNA was delayed in Fat rats but not in Lean rats.

Intravenous infusion of hyperosmotic solution has a two-step impact on muscle SNA in humans: hyperosmolality-induced sympathoexcitation followed by volume load-induced sympathoinhibition (16). Thus, the delayed suppression of SNA is attributable to an augmented hyperosmolality-induced sympathoexcitation and/or a restrained volume load-induced sympathoinhibition. Hyperosmolality-induced sympathoexcitation has also been observed in animal studies (6, 9, 41), and the angiotensin type 1 (AT1) receptor in the paraventricular nucleus is a candidate to participate in this response (9). Furthermore, gene targeting ablation of AT1 receptors significantly decreased AP in mice fed a high-fat diet (11), suggesting that the possibility of higher AT1 receptor activity in Fat rats should be considered. Accordingly, it is possible that hyperosmolality-induced sympathoexcitation via AT1 receptors might also participate in the delayed sodium excretion.

The mean baseline AP was significantly higher in Fat and Lean rats compared with NFD rats. Weight gain is known to induce an increase in SNA (3, 20, 23, 27). Increased leptin...
concentration induced by a rise in adipocytes has been reported to participate in SNA augmentation (3, 14, 23, 24, 29, 37). Indeed, higher urine norepinephrine excretion was observed on days 1 and 2 in Lean and Fat rats compared with NFD rats in the present study. In contrast, a significant increase in AP was observed on days 3 and 4 in Fat rats. Because a significant increase in AP is consistent with the observed cumulative sodium content, insufficient sodium excretion might contribute to the mean AP increase in Fat rats. From the data of sorbitol infusion, sympathoexcitation and/or water shift from the intracellular to the extracellular space might also participate in AP increase in Fat rats. A blunted slope of the renal function curve was observed only in Fat rats. The blunted slope was mainly due to reduced sodium excretion ability during the 9% NaCl infusion; in other words, sodium excretion ability was maintained under 6% NaCl infusion in Fat rats. Accordingly, it is possible that Fat rats do not have a sufficient urinary sodium excretion ability to compensate for the chronic infusion of 9% NaCl solution, and this then results in a mean AP increase in Fat rats.

There was no difference in the slope of the SNA-AP relationship, but the intercept was significantly higher in Fat and Lean rats compared with NFD rats (Fig. 5 and Table 1). Increased circulating blood volume and/or venous elastance are known to shift the peripheral arc to a higher AP level (40). In a human experiment, the total blood volume was significantly higher in obese subjects than in lean subjects; however, there was no difference in venous compliance (35). In an animal experiment, a modest level of collagen α1 type 1 protein was detected in surrounding blood vessels in rats fed a standard diet; in contrast, although no increase in the left ventricular end-diastolic pressure was observed, the perivascular deposition of collagen was markedly higher in rats fed a high-fat diet (4). These results indicate that an increase in circulating blood volume and/or deposition of collagen might contribute to the peripheral arc shifting to a higher AP level.

The intersection AP of the fitting curve was significantly higher in Fat rats compared with Lean rats (Fig. 6 and Table 1). However, there was no difference in baseline AP between Fat and Lean animals in the metabolic cage experiment. These results indicate that the system that lowers AP might work more in conscious Fat rats. Leptin is known to activate nitric oxide synthase; measurement of both leptin and the metabolites of nitric oxide (NO$_3$-NO$_2$) in plasma from conscious rats revealed that NO$_3$-NO$_2$ increased linearly with leptin (31). Although plasma leptin was not measured in the present study, if the plasma leptin is higher in Fat rats compared with Lean rats, the NO synthetic pathway might be more active in Fat rats in a conscious state.

The present study has shown that the distensibility of the common carotid artery was significantly reduced in Fat rats. This means that volume expansion-induced higher AP is required to activate the carotid baroreceptors. Indeed, the baroreflex open-loop experiment showed a rightward shift of the neural arc. In conclusion, a reduced distensibility-induced rightward shift of the neural arc might contribute to an impairment of sodium regulation in Fat rats.

Limitations. The present study showed that a significant positive sodium and water balance had disappeared on the 2nd day of 9% NaCl infusion (day 4), whereas cumulative sodium contents were still high on day 4, in Fat rats. AP was consistent with cumulative sodium contents, suggesting that volume expansion might participate in the higher AP on days 3 and 4 in Fat rats. Because total blood volume was not measured, the significantly higher AP during 9% NaCl infusion could not be regarded as the result of volume expansion. The present study has shown that the decrease in arterial compliance-induced rightward shift of the neural arc partially contributes to both the delay in, and insufficient level of, sodium and water regulation during higher NaCl infusion in Fat rats. However, a malfunction in the regulation of endogenous factors and systems, including antiuretic hormone, atrial natriuretic peptide, and the renin-angiotensin-aldosterone system, should also be considered in the context of volume control. In obese humans, acute hypo- or hyperosmolarity-induced vasopressin secretion is maintained (10). Although the responses against acute hyperosmotic stimulation are unclear, a decrease in atrial natriuretic peptide (44) and activation of the renin-angiotensin-aldosterone system (23) are observed in obese humans or animals. Thus, it is possible that malfunction of the latter two factors might also contribute to higher AP during 9% NaCl infusion in Fat rats. This possibility needs to be examined in future studies.

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DISCLOSURES
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AUTHOR CONTRIBUTIONS

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