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〔報　　文〕

Effects of a High-NaCl Diet on Pressure-Natriuresis in Young Zucker Fatty Conditions

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Background: Renal mechanisms of sodium homeostasis are reportedly impaired in obese subjects. However, the precise contribution of a high-sodium diet to pressure-natriuresis in Zucker fatty rats (ZFR) remains unclear. The purpose of this study was to evaluate whether a high-sodium diet affects the blunted pressure-natriuretic response in ZFR.

Methods: This study examined responses of pressure-natriuresis using laser Doppler flowmetry techniques when the NaCl diet was altered (0.4% or 4%) in young ZFR and estimated cortical and medullary blood flow (MBF) distributions.

Results: Systolic arterial pressure was significantly higher for ZFR＋high NaCl than for ZFR＋low NaCl. The slope of the pressure-natriuresis curve was significantly lower in ZFR＋high NaCl than in ZFR＋low NaCl. In ZFR＋high NaCl, MBF was also significantly lower than in Zucker lean rats (ZLR) at a renal perfusion pressure of 140 mmHg without any substantial changes in total renal blood flow or cortical blood flow. Urinary excretion of nitrites and nitrates was lower in ZFR＋high NaCl than in ZLR.

Conclusion: These findings suggest that high-NaCl diet, by itself, eliminated the slope of the pressure-natriuresis curve in young ZFR due to decreased MBF, resulting in salt-sensitive hypertension.

Key words: *pressure-natriuresis, renal medullary blood flow, ZFR, high NaCl, nitric oxide*

INTRODUCTION

Hyper-insulinemia may play a role in the development of hypertension[1]. Obesity-induced hypertension is associated with important hemodynamic, cardiac, and renal alterations[2]. Renal mechanisms of sodium homeostasis are reported to be impaired in obesity, in which insulin resistance may conceivably develop. Obese patients commonly exhibit salt-sensitive hypertension[3]. However, how insulin resistance is associated with impaired natriuresis remains controversial[4].

The kidneys appear to play an important role in the long-term regulation of extracellular fluid volume and blood pressure[5]. The recent development of laser Doppler flowmetry and videomicroscopic techniques has led to heightened interest in the role of medullary hemodynamics in the control of sodium reabsorption[6, 7]. A previous study in our laboratory showed that pressure-natriuresis is blunted in Zucker fatty rats (ZFR), the best animal model of metabolic syndrome[8, 9]. Sodium excretory mechanisms have also been demonstrated to be impaired in obesity[10]. Furthermore, Fujiwara *et al.* demonstrated that pressure-natriuresis was impaired in male ZFR compared with Zucker lean rats (ZLR), and that this deranged mechanism was associated with reduced renal nitric oxide (NO) production[11]. However, some reports have found no differences in blood pressure between ZFR and ZLR due to salt loading, using variations in salt sensitivity under different experimental

systems[12–14]. The precise contribution of a high-sodium diet to different areas of regional renal blood flow and the pressure-natriuretic response of ZFR remains unclear. The purpose of this study was to evaluate whether high-sodium feeding deteriorates the blunted pressure-natriuretic response in ZFR using laser Doppler flowmetry techniques.

METHODS

All of the procedures followed the guidelines approved by the Toho University Animal Care and Use Committee.

Animals

Six-week-old male ZFR (n＝24; Charles River Japan, Tokyo, Japan) were divided into the following groups: 1) ZFR＋low NaCl (n＝6); 2) ZFR＋high NaCl (n＝6); 3) ZLR＋low NaCl (n＝6); and 4) ZLR＋high NaCl (n＝6). Animals were placed in metabolic cages for urine collection, and were given formulated diets (Oriental Yeast Co., Ltd. Tokyo, Japan) that contained 0.4% and 4.0% (wt/wt) NaCl for 4 weeks under pair feeding. Weight was recorded and systolic arterial blood pressure and heart rate were measured in all conscious rats using the indirect tail-cuff method (BP-98A; Softron, Tokyo, Japan) on a 37℃ preheated cloth jacket for about 10 min. Averages of three such recordings were taken as the individual systolic arterial blood pressure and heart rate. All urine for each rat was collected to determine sodium concentrations. Plasma glucose and insulin were also measured. Urine nitrite and nitrate (uNOx) excretion were determined using the Greiss reaction with automated NO detector high-performance liquid chromatography. The right kidney and right adrenal gland were removed 7 days before the experiment with laser Doppler flowmetry.

Pressure-natriuresis and laser Doppler flowmetry techniques

All rats were anesthetized with intraperitoneal thiobutabarbital (Inactin, 100 mg/kg; Sigma, Tokyo, Japan) and placed on a heated surgical table to maintain a body temperature of 37℃. Cannulas were placed in the femoral arteries for measurement of arterial blood pressure and collection of blood and in the jugular vein for infusions. Fluids lost during surgery were replaced by continuous intravenous infusion of 2% bovine serum albumin (Sigma, Tokyo, Japan) in a 0.9% NaCl solution at 1 ml/h/100 g body weight throughout the procedure. The left kidney was exposed through a midline incision, isolated, and placed in a holder. The tip of the arterial catheter was advanced to just below the level of the left renal artery to permit continuous measurement of renal perfusion pressure (RPP).

Aortic clamps were placed above and below the left renal artery, and ties were loosely placed around the mesenteric and celiac arteries to allow manipulation of renal arterial pressure by adjusting peripheral resistance. The left kidney was denervated by stripping all visible nerves from the renal artery coating the hilar region of the kidney with a 10% solution of phenol ethanol. A cannula was placed in the left ureter to collect urine. An ultrasonic transit-time flow probe was placed around the left renal artery to measure renal blood flow (RBF). Signals were transmitted to a transit-time flow meter (PDV-20; Crystal Biotech, Northborough, MA, USA). This device measures absolute RBF in milliliters per minute.

For the measurement of changes in cortical blood flow (CBF) and medullary blood flow (MBF) in rats, two 500-μm diameter optical fibers were implanted in the left kidney and

exteriorized for blood flow measurement by laser Doppler flowmetry (ALF21D, ADVANCE Co, Ltd., Tokyo, Japan). Fibers were implanted in the renal cortex and medulla by inserting directly into the kidney tissue through a small hole in the renal capsule made with a 26-gauge needle. Fiber tips were inserted to a depth of 2 mm beneath the surface of the renal cortex to allow measurement of the net flux of red blood cells in the renal cortex, and to a depth of 5 mm to monitor changes in the outer medulla. After study completion, the kidneys were removed for morphological examination and determination of the precise point of tip placement of the optical fibers. Data from animals with incorrectly placed fibers or extensive renal damage were excluded from the study.

Experimental protocols

Urine flow, sodium excretion, RBF, CBF, MBF, and arterial pressure were measured in a 30-min control period. RPP was then reduced to 60 mmHg by aortic occlusion. After a 10-min equilibration period, urine flow, sodium excretion, RBF, CBF, and MBF were measured for 30 min. RPP was then increased by 40 mmHg by releasing the clamp on the abdominal aorta and tying off the mesenteric and celiac arteries. After another 10-min equilibration period, urine flow, sodium excretion, RBF, CBF, and MBF were again measured for 30 min. Finally, RPP was further increased by tightening the clamp below the renal artery. Ten minutes later, urine flow, sodium excretion, RBF, CBF, and MBF were measured for 30 min.

Statistics

Values are presented as means±standard deviation. One-way repeated-measures analysis of variance was performed for each group, followed by the Kruskal-Wallis test to assess the significance of results. Values of $p<0.05$ were considered significant. The Tukey test was used to assess differences between each group.

RESULTS

Body weight, mean arterial blood pressure, and laboratory data

Body weights were higher for ZFR+low NaCl (449±15 g) and ZFR+high NaCl (551±41 g) than for ZLR+high NaCl (324±23 g) (Table 1). Systolic arterial pressure in ZFR+high NaCl (148±9 mmHg) was higher than in ZLR+high NaCl (119±11 mmHg) and ZFR+low NaCl (119±6 mmHg) (Table 1). Plasma glucose concentrations in both ZFR+low NaCl and ZFR+high NaCl were higher than in ZLR (Table 2). Plasma insulin levels were increased in ZFR, but no significant differences were seen between groups. Excretion of uNOx in ZLR+high NaCl (1.9±0.9 μmol/kg/day), but not in ZFR+low NaCl (3.4±1.6 mmHg), was lower than that in ZLR+high NaCl (8.5±2.4 μmol/kg/day) (Table 2).

Table 1 Physiological findings

Rats	BW (g)	SAP (mmHg)
ZFR+Low Na	449±15*	119±6
ZFR+High Na	551±41*	148±9* **
ZLR+Low Na	298±10	118±12
ZLR+High Na	324±23	119±11

*significant difference from ZLR+Low and from ZLR+High
**significant difference from ZFR+Low
BW; body weight, SAP; systolic arterial pressure

Table 2 Laboratory data

Rats	PG (mg/dl)	uNOx (μmol/kg/day)
ZFR+Low	147±12*	3.4±1.6
ZFR+High	152±12*	1.9±0.9*
ZLR+Low	107±8	9.1±2.8
ZLR+High	114±13	8.5±2.4

*significant difference from ZLR+Low and fron ZLR+High
PG; plasma glucose

Pressure-natriuresis and regional renal blood flow

Data for urinary volume and urine NaCl excretion are shown in Figure 1, which illustrates the pressure-natriuresis relationship. Urinary volume and NaCl excretion were significantly lower in ZFR+low NaCl than in ZLR+low or either +high NaCl group when compared at 140 mmHg RPP. The slope of the pressure-natriuresis curve was significantly lower in ZFR +low NaCl than in ZLR+low or +high NaCl. In addition, urinary volume and NaCl excretion were significantly lower in ZFR+high NaCl than in ZFR+low NaCl when compared at 140 mmHg RPP. The slope of the pressure-natriuresis curve was significantly lower in ZFR+high NaCl than in ZFR+low NaCl.

RBF, CBF, and MBF data are shown in Figure 2. Both RBF and CBF were autoregulated and showed no significant differences in the four groups between RPP at 60 mmHg or 140 mmHg. MBF was significantly lower in ZFR +high NaCl than in ZLR+low or high NaCl when compared at 140 mmHg RPP.

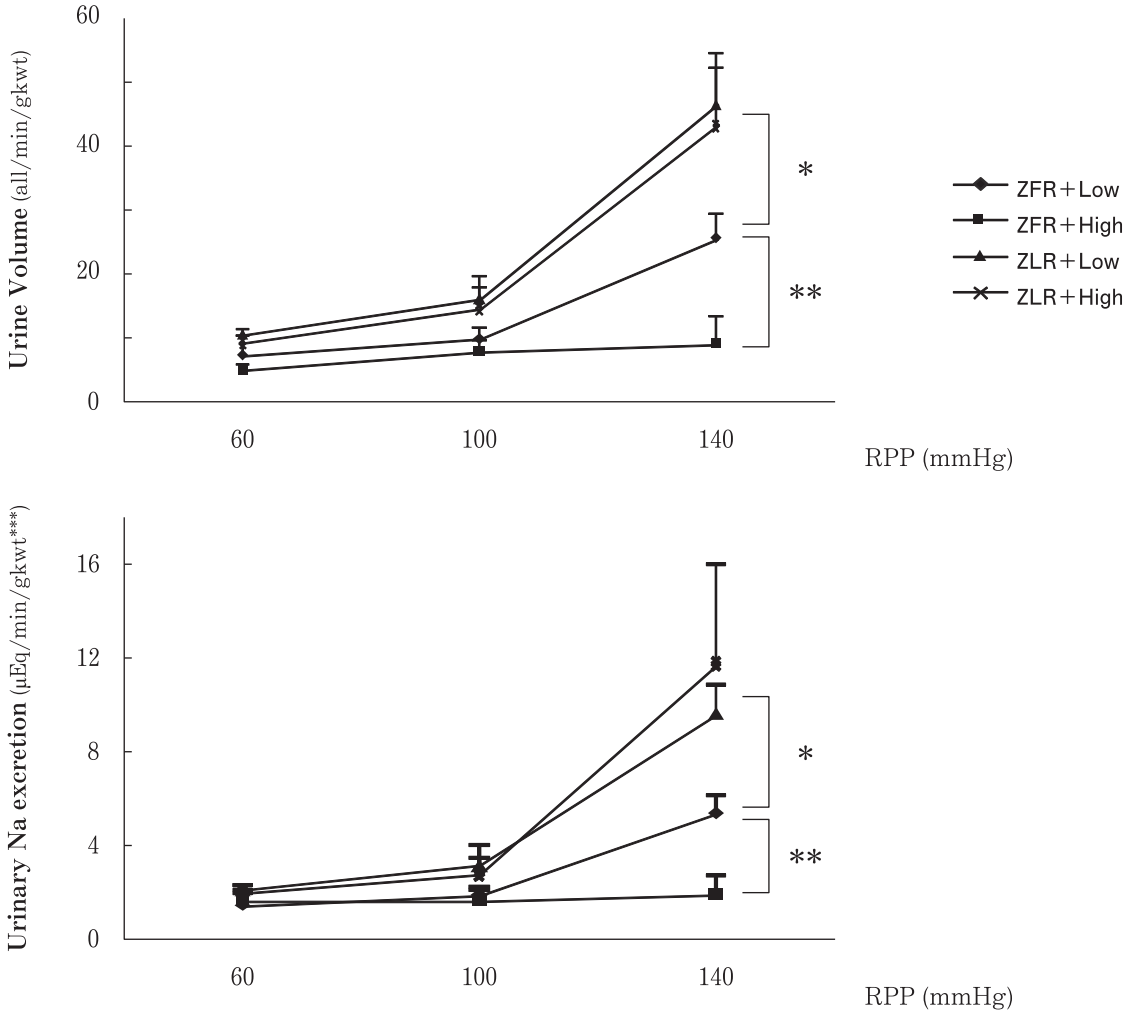

Fig 1. Comparison of the pressure-natriuretic response in each group of rats. Line graphs show the relationship between urinary volume and urinary Na excretion and RPP.

*Significant difference between ZLR+low and high vs ZFR+low.
**Significant difference between ZFR+low vs ZFR+high.
gkwt, kidney weight (g); RPP, renal perfusion pressure

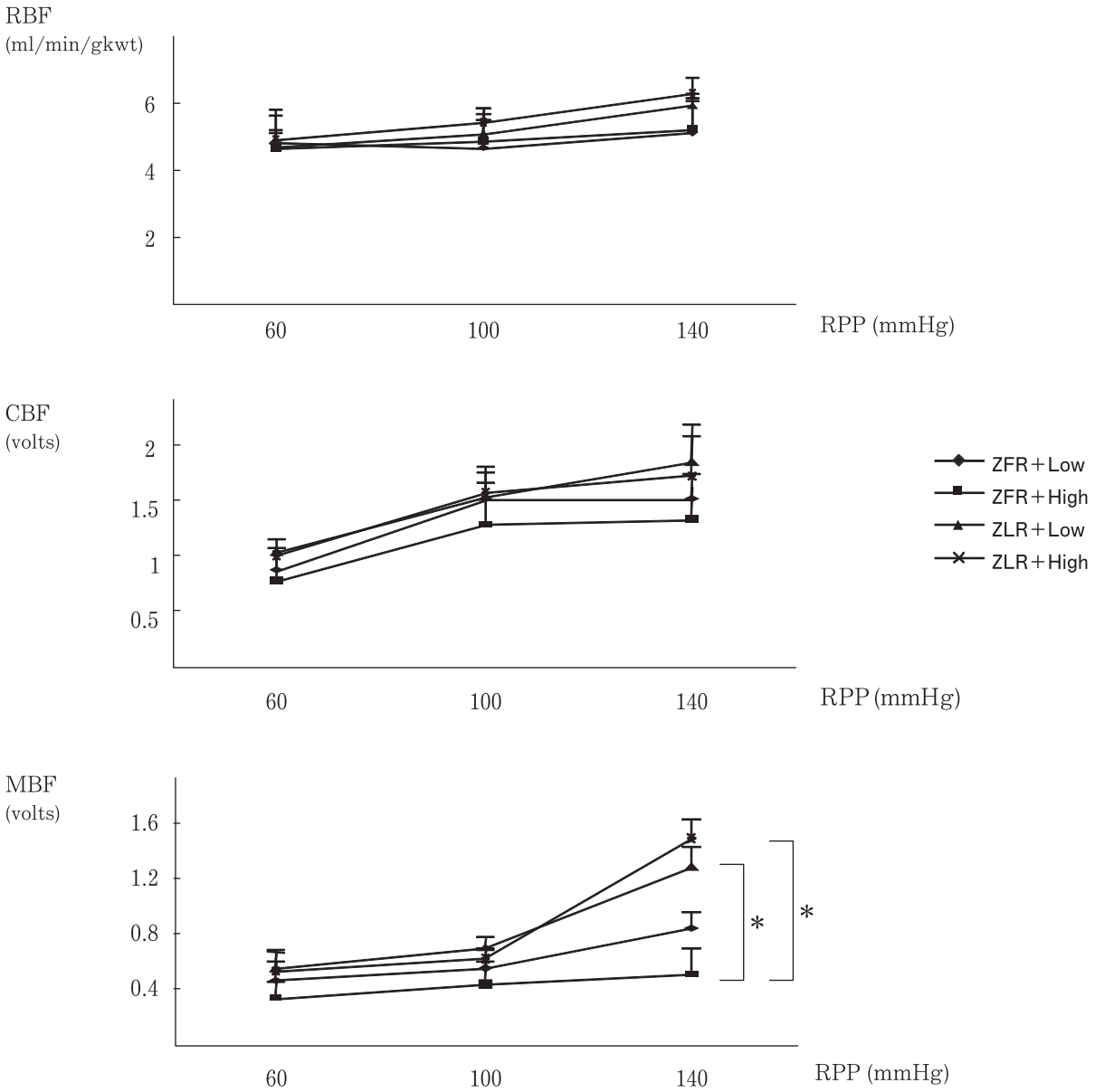

Fig 2. Line graphs showing the relationship between RBF, CBF, MBF and RPP in each group of rats.
*Significant difference between ZLR+low and high vs ZFR+high.
RBF, renal blood flow; CBF, renal cortical blood flow; MBF, renal medullary blood flow

DISCUSSION

The kidney plays an important role in blood pressure regulation and the development of hypertension. Recent reports have demonstrated the importance of the renal medullary region in mediating the renal pressure-natriuresis response[6]. As MBF and blood pressure are closely linked, shifts in MBF are likely to contribute to the development of hypertension. We have previously reported that pressure-natriuresis is blunted in ZFR with a normal NaCl diet, and this was attributed to decreases in renal MBF[8]. In the present study, we aimed to clarify how high-NaCl diet affects the relationship of sodium excretion and MBF in response to elevation of RPP. The novel finding in this study is that high-NaCl diet, by itself, decreased MBF and deteriorated pressure-natriuresis in young ZFR. The slope of the

pressure-natriuresis curve in ZFR is diminished by high NaCl diet. The effect of high NaCl on blood pressure is thus associated with decreased MBF and NOx excretion in ZFR.

Many facets of the pathophysiology of hypertension associated with obesity remain unclear. However, some reports have found that the majority of hypertension cases associated with obesity involve salt-sensitive hypertension, so failures in the renal mechanisms of sodium excretion seem likely to be deeply involved in the pathological condition. Some reports have noted that decreased mechanisms of pressure-natriuresis cause hypertension in ZFR[11]. On the other hand, it has also become clear that renal medullary levels of reactive oxygen and nitrogen species are capable of modulating tubular reabsorptive responses to increased arterial pressure and that levels of outer-medullary reactive oxygen and nitrogen species are unbalanced in salt-sensitive hypertension[15]. We showed significantly decreased NOx excretion in high-NaCl diet-fed ZFR in the present study. Decreased NO activity by high-NaCl diet may thus constitute one cause of salt-sensitive hypertension in young ZFR.

Recently, NO and O_2- were clearly shown to act as opposing forces altering the redox state of the renal medulla and the set point of the renal pressure-natriuresis curve[15]. Abe *et al.* have previously demonstrated that O_2- production limits cellular NO availability in the perfused medullary thick ascending limb[16]. Renal medullary O_2- likely modulates renal medullary perfusion and the pressure-natriuresis response through its ability to inactivate NO[17]. O_2- levels in the renal outer medulla were elevated in Dahl salt-sensitive rats fed 0.4% sodium when compared with levels in salt-resistant control animals, and such O_2- levels were further exacerbated by high-NaCl feeding (4%, the same concentration used in the present study)[18, 19].

Although we made no observation of renal expression or activity of NO in this experiment, high-NaCl diet could have decreased NO activity and MBF, deteriorated the blunted pressure-natriuresis, and resulted in the development of salt-sensitive hypertension in young ZFR. To the best of our knowledge, this is the first study to demonstrate that chronic high-NaCl diet deteriorates the pressure-natriuresis curve in rats. We emphasize that high-NaCl diet, by itself, eliminated the slope of the pressure-natriuresis curve and decreased MBF in young ZFR. The present results indicate that a low-NaCl diet may have potential for preventing hypertension in young obese individuals.

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