

# Natriuretic Response to Renal Medullary Endothelin B Receptor Activation Is Impaired in Dahl-Salt Sensitive Rats on a High-Fat Diet

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## Summary

Renal medullary endothelin B receptors (ET<sub>B</sub>) mediate sodium excretion and blood pressure (BP) control. Several animal models of hypertension have impaired renal medullary ET<sub>B</sub> function. We found that 4-week high-caloric diet elevated systolic BP in Dahl salt-sensitive (Dahl S) rats (126±2 vs. 143±3 mm Hg, p<0.05). We hypothesized that renal medullary ET<sub>B</sub> function is dysfunctional in DS rats fed a high-caloric diet. We compared the diuretic and natriuretic response to intramedullary infusion of ET<sub>B</sub> agonist sarafotoxin 6c (S6c) in DS rats fed either a normal or high-caloric diet for 4 weeks. Urine was collected during intramedullary infusion of saline for baseline collection followed by intramedullary infusion of either saline or S6c. We first examined the ET<sub>B</sub> function in DS rats fed a normal diet. S6c increased urine flow (2.7±0.3 µl/min during baseline vs. 5.1±0.6 µl/min after S6c; p<0.05; n=5) and sodium excretion (0.28±0.05 vs. 0.81±0.17 µmol/min; p<0.05), suggesting that DS rats have renal medullary ET<sub>B</sub> function. However, DS rats fed a high-caloric diet displayed a significant increase in urine flow (2.7±0.4 vs. 4.2±0.4 µl/min, baseline vs. S6c infusion, respectively; p<0.05, n=6), but no significant change in sodium excretion in response to S6c (0.32±0.06 vs. 0.45±0.10 µmol/min). These data demonstrate that renal medullary ET<sub>B</sub> function is impaired in DS rats fed a high-caloric diet, which may be contributed to the elevation of blood pressure during high-caloric feeding in this model.

## Key words

High fat diet • Obesity • ET<sub>B</sub> receptor • Sarafotoxin 6c • Renal medulla • Natriuresis

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## Introduction

Renal control of sodium excretion involves a wide range of factors that control tubular reabsorption of filtered sodium. Our lab and others have developed a long line of evidence that endothelin (ET) is important for blood pressure control by promoting the kidney's ability to excrete sodium in response to high salt intake (Kohan *et al.* 2011). These actions are primarily *via* ET-1 stimulating the ET<sub>B</sub> receptor in the collecting duct of the kidney. The inability of renal ET<sub>B</sub> receptors to inhibit sodium reabsorption contributes to salt-sensitive hypertension (Kohan 2013). Consistent with this idea, Speed *et al.* (2011) demonstrated that the ET-1/ET<sub>B</sub> natriuretic pathway is impaired in the Dahl salt-sensitive (Dahl S) rat.

Obesity is a major risk factor for the development of cardiovascular disease that is closely associated with hypertension (Hall *et al.* 2015). While it is generally agreed upon that the root cause of obesity is due to taking in more calories than are being used, this can occur due to a number of genetic and environmental stressors. They include sedentary lifestyle, impaired leptin production or signaling, and others such as high salt intake. Of note, high salt intake is a lifestyle factor that is a powerful stimulus for ET-1 production by the kidney, but also in extra-renal tissues including skin and vasculature (Speed *et al.* 2015). However, less studied is the observation that high fat diets can increase renal

tissue ET-1 content (Barton *et al.* 2000).

Thus, the present study was designed to test the hypothesis that a high fat diet is associated with an impairment of ET<sub>B</sub> receptor dependent natriuresis in a model of salt-sensitivity. Our hypothesis predicts that the natriuretic response to an ET<sub>B</sub> receptor agonist infused into the renal medulla of anesthetized Dahl S rats will be reduced in rats on a high fat compared to a normal fat diet.

## Methods

### Animals

Studies used male Dahl S rats between 8 and 10 weeks old purchased from Harlan Laboratories (Indianapolis, IN, USA). All rats were allowed to acclimate to their cages for at least one week prior to experimentation and continuously housed at a constant temperature and humidity and a 12:12-h light-dark cycle. All experiments and procedures were approved and monitored by the Augusta University Institutional Animal Care and Use Committee in accordance with the NIH Guide for the Care and Use of Laboratory Animals. Rats were placed on a normal fat diet (16.6 % kcal fat, Harlan Teklad, Madison, WI, USA) or high fat diet (58.7 % kcal fat, Harlan Teklad, Madison, WI, USA) for a period of 4 weeks. Both diets have what is typically considered normal salt content (0.4 % NaCl).

### Metabolic and renal function

Each week while on normal or high fat diet, rats were placed in metabolic cages to assess food and water intake and urine collection. Each week, rats were allowed a 24 h acclimation to the metabolic cage followed by a 24-h urine collection. Systolic arterial pressure was also measured by tail cuff plethysmography as previously described (D'Angelo *et al.* 2005).

### Analysis of ET<sub>B</sub>-dependent natriuresis

At the end of 4 weeks on each diet, rats were anesthetized with inactin (100 mg/kg i.p.) and placed on a temperature controlled surgical table and surgically prepared as previously described (Nakano *et al.* 2008, Kittikulsuth *et al.* 2012). A catheter was placed in the jugular vein for infusion of saline (0.9 % NaCl) containing 4 % BSA at 30  $\mu$ l/min during surgery and reduced to 15  $\mu$ l/min after surgery to maintain euvoemia. A similar catheter was placed in the femoral artery to monitor arterial pressure. A midline incision was used to

access the left kidney and insert a stretched PE-10 catheter 5 mm into the kidney from the lateral side to reach the inner/outer medullary junction. Saline was infused into the catheter at a rate of 0.5 ml/h and the position of the catheter was verified at the end of each experiment by dissecting the kidney. A ureteral catheter was used to collect urine from each kidney. Animals were allowed a 60-min equilibration period followed by a baseline urine collection of 40 min. The intramedullary infusion was then changed to saline containing sarafotoxin 6c (S6c, American Peptide Co., Sunnyvale, CA, USA) at 0.45  $\mu$ g/kg/h for an additional 80 min. Separate groups of rats were maintained on saline (0.9 % NaCl) infusion into the renal medulla throughout the protocol to serve as time controls. Urine collected during the final 20 min of the baseline and experimental periods were used for analysis.

### Receptor-binding assay to endothelin-1 and endothelin-3

In a separate set of identically treated rats (normal and high fat diets), rats were anesthetized with Na pentobarbital (65 mg/kg i.p.) and blood drawn from the abdominal aorta followed immediately by removing the kidney. The inner medulla was then removed before being snap frozen for receptor-binding analysis. In brief, tissue was homogenized and plasma membrane fractions prepared for binding studies as previously described (Kittikulsuth *et al.* 2011). Plasma membrane and wheat germ agglutinin polyvinyltoluene beads (PerkinElmer, Boston, MA, USA) were added into each well and incubated for 3 h. Then, [<sup>125</sup>I]-ET-1 (PerkinElmer, Boston, MA, USA) at the final concentration of 0.01-1.0 nM or [<sup>125</sup>I]-ET-3 (PerkinElmer, Boston, MA, USA) at the final concentration of 0.06-1.0 nM was added and incubated for 18 h. Total binding of [<sup>125</sup>I]-ET-1 or [<sup>125</sup>I]-ET-3 was determined using 0.3 or 1  $\mu$ g plasma membrane protein, respectively. Nonspecific binding was determined in the presence of 10  $\mu$ M unlabeled ET-1 or ET-3 ligand (American Peptide, Inc., Sunnyvale, CA, USA). All measurements were performed in duplicate.

### Assays

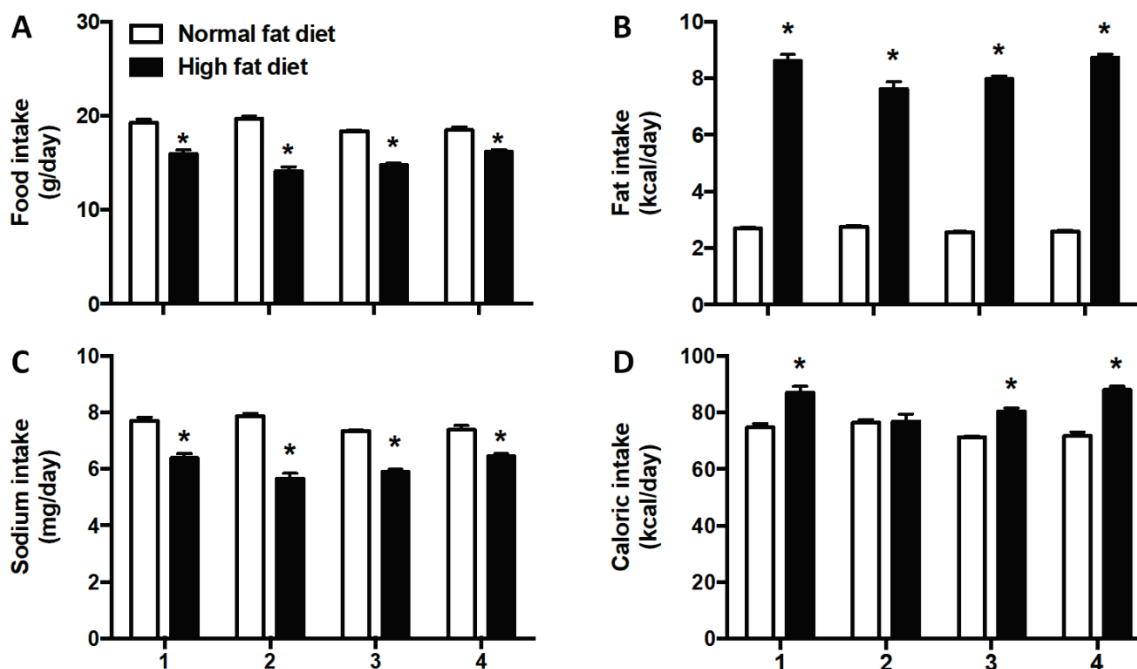
Urinary sodium concentration was measured by atomic absorption. Urinary ET-1 excretion was measured by radioimmunoassay via the manufacturer's protocol (Peninsula Laboratories, San Carlos, CA, USA).

### Statistical analysis

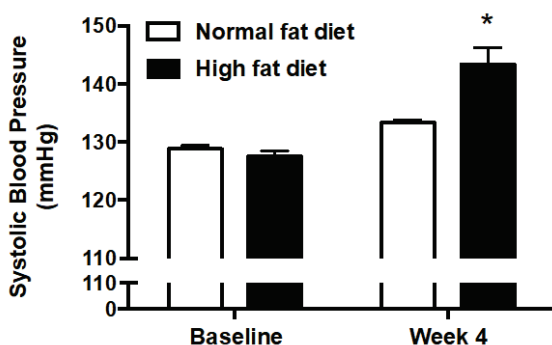
Urine flow rate, urinary sodium excretion and

mean arterial pressure in response to intramedullary infusion of saline or S6c were analyzed by two-way repeated-measures ANOVA followed by Bonferroni *post hoc* tests among rats receiving a normal or high fat diet. Similarly, metabolic parameters were analyzed by

two-way ANOVA for repeated measures followed by Bonferroni *post hoc* tests. Binding data were analyzed using Student's t-test (two-tailed). Results are expressed as mean  $\pm$  SE with  $p < 0.05$  being considered statistically significant.



**Fig. 1.** Intake of food (A), fat (B), sodium (C), and calories (D) in Dahl S rats given a normal ( $n=8$ ) or high fat ( $n=5$ ) diet. \* denotes  $p < 0.05$  vs. normal fat group at each week.



**Fig. 2.** Systolic blood pressure (tail cuff) measured in Dahl S rats before and after 4 weeks of a normal ( $n=6$ ) or high fat ( $n=5$ ) diet. \* denotes  $p < 0.05$  vs. normal fat group.

## Results

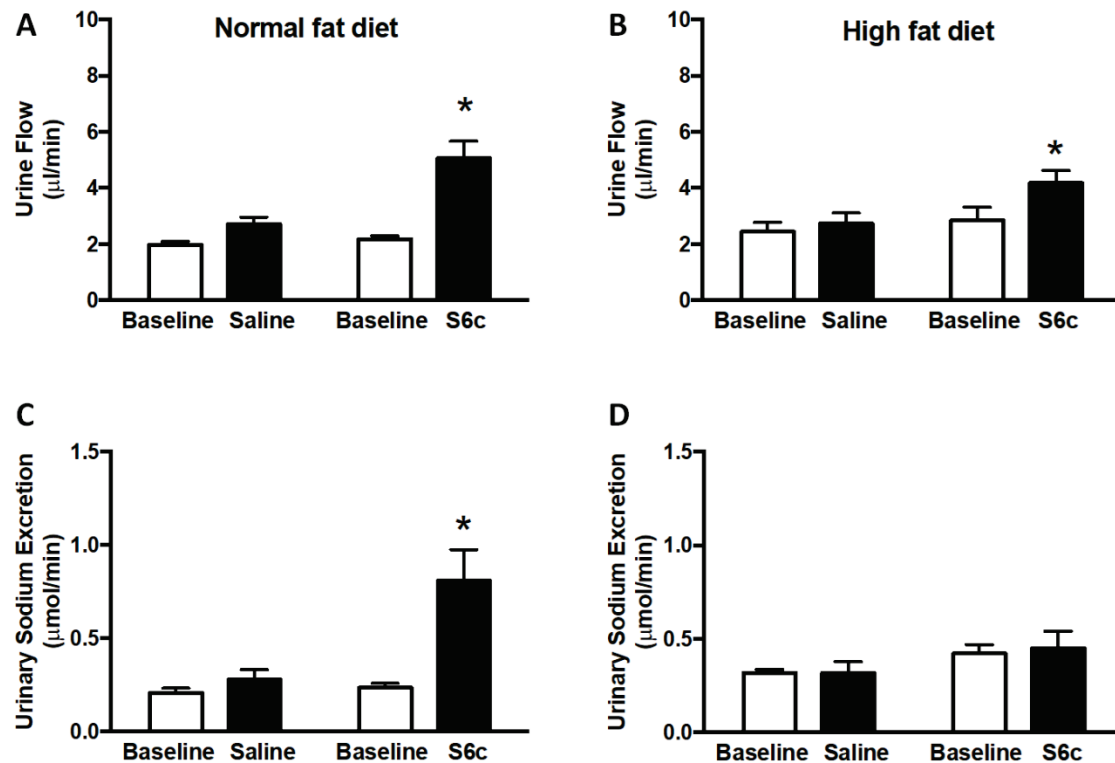
### 24-h measurements

Figure 1 displays food and related intake data in Dahl S rats from 1 to 4 weeks of normal or high fat feeding. Rats eating the high fat diet ate less food than those eating the normal fat diet, although by design, the amount of high fat ingested was much higher (Fig. 1B). However, caloric intake was significantly higher in the

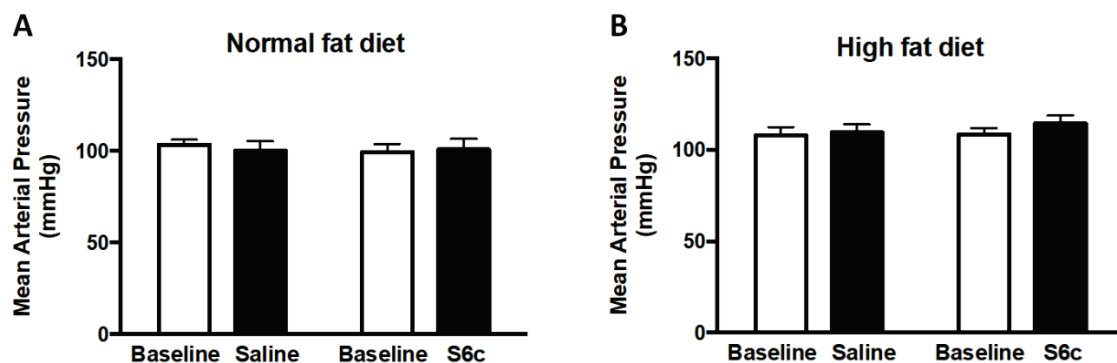
group eating the high fat diet due to the greater energy content in the high fat diet. Thus, since the NaCl content was identical in both groups, the total sodium intake was roughly 20% lower in rats eating the high fat diet (Fig. 1C). As previously reported, we observed that the high fat diet produced a significant increase in systolic blood pressure in the Dahl S rat (Fig. 2). While urinary ET-1 was lower in rats on a high fat diet ( $3.4 \pm 0.5$  ng/day,  $n=5$ ) compared to normal fat ( $4.7 \pm 0.5$  ng/day,  $n=6$ ), this difference did not reach statistical significance.

### Intramedullary $ET_B$ receptor function

As previously reported, intramedullary infusion of the  $ET_B$  receptor agonist, S6c, in anesthetized rats produced a significant increase in urine flow rate (Fig. 3A) and urinary sodium excretion (Fig. 3C) in rats on a normal fat diet. In contrast, intramedullary infusion of S6c did not significantly increase urine flow rate (Fig. 3B) or urinary sodium excretion (Fig. 3D) in rats on a high fat diet. Mean arterial pressure remained unchanged during intramedullary infusion of saline or S6c on either diet (Figs 4A and 4B).



**Fig. 3.** Urine flow rate (**A, B**) and sodium excretion (**C, D**) in anesthetized Dahl S rats during baseline and intramedullary infusion of either saline or sarafotoxin 6c (S6c). Separate groups of animals were maintained for 4 weeks on a normal (**A, C**; n=6) or high fat (**B, D**; n=5) diet. \* denotes  $p < 0.05$  vs. corresponding baseline value.

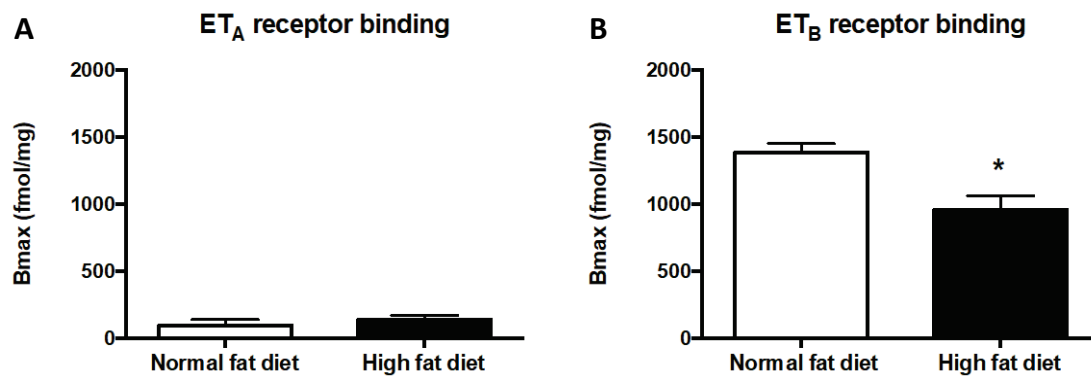


**Fig. 4.** Mean arterial pressure in anesthetized Dahl S rats during baseline and intramedullary infusion of either saline or sarafotoxin 6c (S6c). Separate groups of animals were maintained for 4 weeks on a normal (**A**; n=6) or high fat (**B**; n=5) diet. There was no statistically significant effect of S6c and baseline or diet groups.

#### *ET<sub>A</sub> and ET<sub>B</sub> receptor binding*

As in previous studies, we took advantage of the differential binding of ET-1 and ET-3 peptides to discern maximum binding to ET<sub>A</sub> and ET<sub>B</sub> receptors. Using membrane preparations from inner medullary tissue, Scatchard analysis was used to determine maximum binding (B<sub>max</sub>) values of [<sup>125</sup>I]-ET-1 binding sites that represent total ET<sub>A</sub> and ET<sub>B</sub> receptor binding, and B<sub>max</sub> values of [<sup>125</sup>I]-ET-3 binding sites represent specific ET<sub>B</sub> receptor binding as previously described. The difference between B<sub>max</sub> values of [<sup>125</sup>I]-ET-1 and [<sup>125</sup>I]-ET-3 binding was used to determine ET<sub>A</sub> receptor

binding, which was not significantly different between tissues taken from rats on a normal and high fat diet (Fig. 5A). B<sub>max</sub> for ET<sub>B</sub> receptor binding in renal inner medullary tissue from rats on a high fat diet was significantly lower than values obtained from tissue taken from rats on a normal fat diet (Fig. 5B). Dissociation constants (K<sub>d</sub>) for [<sup>125</sup>I]-ET-1 were comparable between inner medullary preparations from rats on a normal or high fat diet (0.063±0.009 and 0.062±0.011, respectively). In contrast, the K<sub>d</sub> for [<sup>125</sup>I]-ET-3 binding was significantly lower in the high fat vs. normal fat groups (0.065±0.002 nM and 0.114±0.007 nM, respectively).



**Fig. 5.** Maximum binding for ET<sub>A</sub> receptors (**A**; total ET-1 binding minus ET-3 binding) and ET<sub>B</sub> receptors (**B**; ET-3 binding) in Dahl S rats on normal or high fat diets (n=3 each; p<0.05).

## Discussion

There is growing evidence that high salt diets contribute to obesity-dependent hypertension (Hall *et al.* 2015). However, the possible intrarenal mechanisms that could account for this form of salt-sensitivity are not known. The current study demonstrates that one of the more influential natriuretic pathways, the ET<sub>B</sub> receptor, loses its ability to produce a natriuresis in a susceptible strain of rat when placed on a high fat diet. Physiologically, the kidney plays a key role in blood pressure regulation through control of salt and water balance. Loss of the ET<sub>B</sub> receptor, whether through genetic deletion or pharmacological blockade, results in hypertension that is very sensitive to dietary salt intake (Kohan *et al.* 2011). More specifically, these receptors are in greatest abundance within the renal medulla and in particular the inner medullary collecting duct. The current findings support the hypothesis that the contribution of high fat diets towards hypertension could be related to a loss of ET<sub>B</sub> receptor function in the renal medulla. Of course, further studies are needed to provide a more definitive cause and effect, but these studies show a clear, non-salt-dependent effect of dietary changes that can impact this important natriuretic system.

We know from the work of Speed and colleagues that the Dahl S rat already has reduced renal medullary ET-1 and ET<sub>B</sub> receptor activity since salt-induced hypertension in this model was attenuated by chronic intramedullary infusion of ET-1 (Speed *et al.* 2011). The current study provides rationale for further investigation into the potential role for ET<sub>B</sub> receptor dysfunction in obesity-dependent hypertension. However, the mechanism for how ET<sub>B</sub> receptor function is lost in the Dahl S rat is not known. Recent findings from our group demonstrate that both exogenous and endogenous

angiotensin II causes a loss of ET<sub>B</sub>-dependent natriuresis and a down-regulation of inner medullary ET<sub>B</sub> receptor expression (Kittikulsuth *et al.* 2012, Kittikulsuth *et al.* 2013). Thus, the renin-angiotensin system could account for this loss of function since Spradley *et al.* (2013) have shown that high fat diet increases plasma renin activity in the Dahl S rat. Our study was powered to assess the natriuretic response to S6c, however, further studies will be needed to determine whether adiposity impairs renal ET-1 production.

High fat feeding is often used as a surrogate for obesity in animal models. While rodents often do not get obese, the Dahl S model on a high fat diet has an obese phenotype (Spradley *et al.* 2013). Our group has previously reported that 4 weeks of high fat diet results in a significant increase in body weight and fat distribution as well as many other factors associated with obesity such as elevated triglycerides and free fatty acids (Spradley *et al.* 2013). This study also used telemetry to show that this diet regimen causes hypertension in the Dahl S model as we confirmed by tail cuff even though we did not see this blood pressure difference when the animals were anesthetized.

Because the high fat chow has a greater caloric content, we observed that Dahl S rats consuming a high fat diet actually consumed less food, although they consumed more calories. Since the high fat diet has identical sodium content compared to the matched normal fat diet, the animals actually ate about 20% less sodium. Therefore, we cannot rule out the possibility that even this small reduction in sodium intake could reduce ET<sub>B</sub> receptor expression/function. This will need to be examined in future studies. Spradley *et al.* (2013) also showed that the high fat diet exacerbates renal inflammation in the Dahl S rat, which is a known stimulus for excess ET-1 production and tubular

dysfunction. Again, future studies will need to explore whether these inflammatory mechanisms contribute to ET<sub>B</sub> receptor dysfunction.

### Conflict of Interest

There is no conflict of interest.

### Acknowledgements

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