Original Paper

Effect of Salt Intake on Serum Glucagon-Like Peptide-1 Levels in Normotensive Salt-Sensitive Subjects

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Key Words

Dietary interventions • GLP-1 • Blood Pressure • Sodium excretion

Abstract

Background/Aims: Excess dietary salt is a critical risk factor of salt-sensitive hypertension. Glucagon-like peptide-1 (GLP-1), a gut incretin hormone, conferring benefits for blood pressure by natriuresis and diuresis. We implemented a randomized trial to verify the effect of altered salt intake on serum GLP-1 level in human beings. **Methods:** The 38 subjects were recruited from a rural community of Northern China. All subjects were sequentially maintained a baseline diet period for 3 days, a low-salt diet period for 7 days (3.0g/day of NaCl), and a high-salt diet period for additional 7 days (18.0g/day of NaCl). **Results:** Serum GLP-1 level increased significantly with the change from the baseline period to the low-salt diet period and decreased with the change from the low-salt to high-salt diet in normotensive salt-sensitive (SS) but not salt-resistant (SR) individuals. There was a significant inverse correlation between the serum GLP-1 level and the MAP in SS subjects. Inverse correlation between the serum GLP-1 level and 24-h urinary sodium excretion was also found among different dietary interventions in SS subjects. **Conclusions:** Our study indicates that variations in dietary salt intake affect the serum GLP-1 level in normotensive salt-sensitive Chinese adults.

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Introduction

Excess dietary salt is strongly correlated with cardiovascular disease and is considered to be a critical contributor to the pathogenesis of hypertension [1, 2]. Salt sensitivity refers to a heterogeneous blood pressure change in response to dietary salt intake, which is found in

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hypertensive patients and normotensive individuals [3]. Studies reported that normotensive salt-sensitive (SS) individuals not only are more likely to develop hypertension than salt-resistant (SR) subjects, but also have an increased incidence of cardiovascular complications or death [4].

Genetic and environmental factors are involved in salt-sensitivity and high salt intake is a major risk factor. Epidemiologic and clinical studies demonstrate that excessive dietary salt intake is strongly associated with salt-sensitive hypertension [3, 5]. On the contrary, salt reduction can effectively control blood pressure [5, 6]. High salt intake can promote the development of hypertension through different pathways in salt-sensitive subjects, including enhanced sympathetic activity, endothelial dysfunction, vascular abnormalities and water/sodium retention [3, 7].

Glucagon-like peptide-1 (GLP-1), a gut-derived hormone which is produced in enteroendocrine L cells and released into the blood in response to nutrient intake, can specifically bind to GLP-1 receptor that is widely expressed in endothelial cells and vascular smooth muscle cells and organs such as heart, brain, and kidney [8, 9] and enhance glucose-dependent insulin secretion. Interestingly, GLP-1 confers additional benefits for the cardiovascular system, such as inhibiting the development of hypertension, reducing cardiac fibrosis and hypertrophy [10]. Early studies have indicated that GLP-1R agonists and dipeptidyl peptidase-IV (DPP-4) inhibitors –based [11] therapy was a promising treatment option in the setting of hypertension and other disorders of sodium retention [12]. GLP-1 decreases blood pressure in vivo by natriuresis and diuresis [13]. The natriuretic and diuretic effects of GLP-1 are mediated by the increase in renal plasma flow and downregulation of Na⁺/H⁺ exchanger isoform 3 (NHE3) activity in the renal proximal tubule [13]. Moreover, Kim et al. reported that GLP-1 receptor activation decreases blood pressure by promoting the secretion of atrial natriuretic peptide (ANP) [14]. High salt intake attenuates the protective effect of GLP-1 on blood pressure [10, 15]. However, little about the relationship between circulating GLP-1 level and dietary salt intake in human beings has been elucidated.

In this study, our aim was to examine the changes of serum GLP-1 level in salt-sensitive and salt resistant subjects. The correlation between serum GLP-1 level and blood pressure (BP) was also investigated.

Materials and Methods

Subjects

A total of 38 subjects (aged 18 to 65 years) with similar dietary habits were enrolled from a rural community of Northern China. Data of demographic characteristics (age, physical activity, personal cardiovascular disease-related history, and physical examination findings) were collected with a standard questionnaire. Hypertension was defined as the mean systolic blood pressure (SBP) ≥140mmHg and/or the mean diastolic blood pressure (DBP) ≥90 mmHg.

The exclusion criteria were hypertension, use of antihypertensive medication, clinical cardiovascular disease, chronic liver disease, chronic kidney disease, or diabetes mellitus, pregnancy, or high alcohol consumption.

The Institutional Ethics Committee of Xi'an Jiaotong University Medical School approved the study protocol, and each subject provided written informed consent. The study adhered to the principles of the Declaration of Helsinki, and all study procedures were performed in accordance with institutional guidelines.

Protocol

The protocol consisted of questionnaire survey, physical examination (height, weight, BP, waist circumference and brachial-ankle artery pulse wave velocity), and biochemical examination (creatinine, glucose, total cholesterol, total bilirubin, urinary sodium, urinary potassium). The subjects were given habitual salt diet for three days (3-day baseline observation period), low salt diet (3 g or 51.3 mmol of NaCl per day) for seven days (7-day low-salt diet period), and high salt diet (18 g or 307.8 mmol of NaCl

Fig. 1. Trial design (A) and the effect of different doses of salt intake on MAP in SS(B) and SR(C) subjects. NS means No Significance.

per day) for another seven days (7-day high-salt diet period) (Figure 1A). During the baseline investigation, each subject was given detailed dietary instructions to avoid table salt, cooking salt, and high-sodium food for the subsequent 7-day low-salt diet period and 7-day high-salt diet period. To ensure the compliance of participants with the intervention program, all of the meals (breakfast, lunch and dinner) were prepared in the research kitchens without salt and then added prepackaged salt to the meals. The meals were consumed onsite under the supervision of the study staff during the entire study period.

Blood Pressure Measurement and Salt Sensitivity Definition

BP was measured in the morning by 4 trained and certified staff members using a standard mercury sphygmomanometer after the subjects had rested quietly for ≥ 5 minutes. BP was measured 3 times at 1-min intervals during the 3-day baseline observation period as well as on day 6 and day 7 of the 7-day low-salt diet and the 7-day high-salt diet intervention periods, and the mean value was recorded. BP observers were blinded to the participants' dietary interventions. The subjects were instructed to avoid alcohol, cigarette smoking, coffee/tea, and exercise for at least 30 min prior to their BP measurement. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were determined as the first and fifth phases of the Korotkoff sounds, respectively. Mean arterial pressure (MAP) was calculated as $1/3\text{SBP} + 2/3\text{DBP}$. For the lack of an authoritative consensus on the definition of salt sensitivity using blood pressure, subjects who demonstrated at least a 10% increase in MAP going from a low-salt to a high-salt diet were classified as salt sensitive (SS), and the others were defined as salt resistant (SR) [16].

Biochemical Analyses

Blood samples from all of the subjects were obtained in the morning on day 3 of the 3-day baseline observation period and day 7 of the 7-day low-salt diet and high-salt diet intervention periods by peripheral venous puncture. Those blood samples were centrifuged at 3,000 g for 10 min immediately, and stored at -80°C until analysis. The serum creatinine, blood glucose, total cholesterol, triglyceride, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol were measured using an

automatic biochemical analyzer (model 7600; Hitachi, Ltd., Tokyo, Japan). Human serum GLP-1 enzyme-linked immunosorbent assay (ELISA) kits were purchased from Abcam (Cambridge, MA, USA) and used to measure serum active GLP-1 peptide [GLP-1 (7-37) and GLP-1 (7-36)NH₂] levels following the manufacturer's instructions.

24-Hour Urinary Sodium and Potassium Determination

24-hour urine samples were collected on day 3 of baseline and on day 7 of each intervention period and were kept frozen at -40°C until analysis. Urinary concentrations of sodium and potassium were determined using ion-selective electrodes (Hitachi, Ltd., Tokyo, Japan). The 24-hour urinary excretion of sodium and potassium was calculated by multiplying the concentrations of sodium and potassium with the 24-h total urine volume.

Statistics Analyses

Continuous data are reported as means ± standard error. Categorical data are shown as frequency and percentage. Differences in baseline characteristics between SS and SR groups were analyzed by an independent t-test for continuous variables when adequate, otherwise analyzed by Mann Whitney U test. Differences in repeated measures were analyzed by repeated-measures analysis of variance. Correlations were determined with Pearson's correlation coefficient, if the residuals were normally distributed, and with Spearman's correlation coefficient otherwise. Statistical analyses were performed with SPSS for Windows (SPSS Inc., USA). A two-tailed P value of <0.05 was considered to be statistically significant.

Results

Profiles of Studied Subjects

Table 1 shows the basic characteristics of the subjects. 13 of the 38 subjects that were recruited showed an increase in mean arterial pressure (MAP) by 10% going from a low-salt to a high-salt diet were classified as salt sensitive (group Salt-Sensitive (SS)), and the other 25 showed little or no response to salt loading (group Salt-Resistant (SR)), (Table 1). No significant difference was found in age, body mass index and sex ratio between SS and SR groups, but the SBP and brachial-ankle pulse wave velocity (baPWV) of SS subjects were significantly higher than those of SR subjects (Table 1; *P<0.05).

Effects of Salt Intake on BP

Figure 1 shows the effect of different doses of salt intake on mean arterial pressure (MAP) in SS and SR subjects. The MAP significantly decreased with the change from the baseline to the low-salt intervention (P<0.05) and increased with the change from the low-salt to high-salt intervention (P<0.05) in SS subjects (Figure 1A and 1B), whereas no significant change was observed in the MAP of SR subjects with the same intervention (Figure 1A and 1C).

Table 1. Baseline Clinical Characteristics of SS and SR Subjects

Parameters	Salt-Sensitive Subjects	Salt-Resistant Subjects
N, %	13, 34.2	25, 65.8
Age, y	52.2±1.8	50.8±2.4
Body mass index, kg/m ²	25.2±0.4	24.4±0.8
Smoking (n, %)	2, 12.5	4, 13.3
SBP, mmHg	118.1±3.5*	106.2±2.2
DBP, mmHg	79.2±2.4	76.8±1.3
MAP, mmHg	92.2±2.2	86.5±1.5
Glucose, mmol/L	5.49±0.16	5.16±0.12
Total cholesterol, mmol/L	4.76±0.22	4.52±0.16
Total bilirubin, umol/L	13.0±0.92	11.6±0.86
Serum creatinine, umol/L	54.25±1.69	54.93±1.73
Uric acid, umol/L	253.1±12.0	269.7±15.3
Triglycerides, mmol/L	1.31±0.12	1.40±0.18
LDL-cholesterol, mmol/L	2.71±0.17	2.52±0.11
HDL-cholesterol, mmol/L	1.53±0.09	1.44±0.08
baPWV, cm/s	1615.1±58.5*	1398.4±53.3

Continuous values are shown as means±SE. SBP means systolic blood pressure, DBP means diastolic blood pressure, MAP means mean arterial pressure, LDL means low-density lipoprotein, HDL means high-density lipoprotein, baPWV means brachial - ankle artery pulse wave velocity. *P<0.05 vs SR

Fig. 2. The effect of low salt intake and high salt intake on serum GLP-1 in SS(A) and SR(B) subjects. # $P < 0.05$ vs. Baseline, & $P < 0.05$ vs. Low-Salt. The correlation between serum GLP-1 levels and mean arterial pressure in SS(C) or SR(D) subjects on a low-salt diet and on a high-salt diet.

Effects of Salt Intake on Serum GLP-1

Figure 2 shows the effect of low salt intake and high salt intake on serum GLP-1 in SS and SR subjects. Serum GLP-1 concentration was significantly increased when the diet changed from the baseline to low-salt ($0.802 \pm 0.040 \mu\text{g/mL}$ versus $0.542 \pm 0.039 \mu\text{g/mL}$; $P < 0.01$; Figure 2) and decreased when the diet change from low-salt to high-salt ($0.591 \pm 0.049 \mu\text{g/mL}$ versus $0.802 \pm 0.040 \mu\text{g/mL}$; $P < 0.01$; Figure 2) in SS subjects but not in SR subjects.

In the SS subjects, further analyses showed that there was significantly inverse correlation between serum GLP-1 level and MAP on a low-salt diet or on a high-salt diet ($r = -0.621$; $P < 0.01$) after adjusting to the age and sex ratio factors. However, no significant correlation between serum GLP-1 level and MAP was found in SR subjects (Figure 2).

Effects of Salt Intake on 24-hour Urinary Sodium and Potassium Excretion

The 24-hour urine samples were collected on day 3 of baseline and on day 7 of each intervention period. The 24-hour sodium and potassium excretion in the urine samples was determined to ensure the subjects' sodium intake was in full compliance with the study protocol. Table 2 shows the 24-h urinary sodium and potassium excretion (mmol/day) at baseline and during dietary interventions. As shown in Table 2, the 24-hour urinary sodium excretion significantly decreased with the change from the baseline to the low-salt treatment ($P < 0.05$), but increased with the change from the low-salt treatment to high-salt treatment

Table 2. 24-h Urinary Sodium and Potassium Excretion (mmol/day) at Baseline and During Dietary Interventions

	24-h Urinary Na ⁺ (mmol/day)		24-h Urinary K ⁺ (mmol/day)	
	SS	SR	SS	SR
Baseline	156.2±13.9*	175.3±12.9	29.0±2.4	32.0±1.6
Low-salt diet	73.6±7.0*#	84.3±4.1#	26.6±2.2	29.2±1.5
High-salt diet	269.8±19.7#&	286.9±14.4#&	34.4±2.5&	36.5±1.9

* $P < 0.05$ vs. SR, # $P < 0.05$ vs. Baseline, & $P < 0.05$ vs. Low-salt diet.

Fig. 3. The correlation between serum GLP-1 levels and 24-h urinary sodium and potassium excretion in SS(A and B) and SR(C and D) subjects on a low-salt diet and on a high-salt diet.

in both SS and SR subjects ($P < 0.05$, Table 2). These results proved the subjects' compliance with the dietary administration protocol.

Further analyses showed that the serum GLP-1 concentration was inversely correlated with the 24-h urinary sodium excretion ($r = -0.767$, $P < 0.01$) but not correlated with the 24-h urinary potassium excretion ($r = -0.368$, $P = 0.059$) during both low-salt and high-salt diet intervention periods in SS subjects (Figure 3). However, no correlation between the serum GLP-1 concentration and the 24-h urinary sodium ($r = -0.356$, $P > 0.05$) and potassium excretion ($r = -0.349$, $P > 0.05$) during both low-salt and high-salt diet intervention periods was found in SR subjects (Figure 3).

Discussion

Our present study demonstrates variations in dietary salt intake significantly affect the serum GLP-1 concentration in SS Chinese adults. High salt intake induces the increase in BP and the decrease in serum GLP-1 level in normotensive SS but not SR Chinese adults. In addition, an inverse correlation not only between serum GLP-1 concentration and MAP but also between the 24-h urinary sodium excretion and serum GLP-1 level is demonstrated in the normotensive SS but not SR Chinese adults.

In this study, we quantitatively measured the active peptides GLP-1(7-37) and GLP-1(7-36) NH₂ in human serum. GLP-1 is a gut incretin hormone produced in enteroendocrine L cells and released into the blood in response to food intake [17]. It has two active peptide forms, GLP-1(7-36)amide and GLP-1(7-37)amide [10]. The former is the major circulating form in human beings. The active peptides can bind to the GLP-1 receptor (GLP-1R) to act on numerous pathways [18]. They are rapidly degraded into GLP-1(9-37)/GLP-1(9-36)NH₂ by DPP-4 at N-terminal in the blood physiologically [11].

Interestingly, some studies indicate that serum active GLP-1 has anti-hypertension effect. GLP-1 receptor is expressed in the kidney and mediates the diuretic and natriuretic actions [12]. Further studies have suggested an inhibition effect of GLP-1 on the renin-angiotensin aldosterone [19]. Asmar et al. reported that plasma renin activity was decreased in response to GLP-1 infusion [20]. Furthermore, cardio-renal mechanism also contributes to the anti-hypertensive action of GLP-1. GLP-1 ameliorates hypertension by promoting natriuresis and smooth muscles relaxation via cyclic guanosine mono phosphate [15, 21]. GLP-1 promotes concentration-dependent relaxation of rat aorta through a K_{ATP} and cAMP pathway [22].

In this study, we found that different salt interventions can significantly alter the level of GLP-1 in SS individuals but not in SR individuals. Early studies have shown that chronic administration of recombinant glucagon-like peptide-1(7-36) amide (rGLP-1) attenuated the development of hypertension, endothelial dysfunction, and hypertension-induced renal and cardiac damage compared with the purely high salt (8%NaCl) -treated Dahl SS rats [10]. Hirata K et al reported that exendin-4, a GLP-1 analog, attenuated the development of hypertension in SS mice after high-salt loading [16]. Similarly, the GLP-1 receptor agonist exenatide analogue AC3174 ameliorated hypertension and renal dysfunction in Dahl SS rats with high salt (HS, 8% NaCl) diets [23]. Furthermore, DPP-4 inhibition increased plasma GLP-1 level and attenuated the development of salt-induced hypertension [24]. Another human study shows significantly increased renal excretion of sodium during GLP-1-infusion in both intravenous salt loading (26.7 ± 0.9 g NaCl) intervention and oral salt loading (27.7 ± 0.5 g NaCl) when compared with placebo-infusion [25].

These studies in salt-sensitive models have shown that infusion with rGLP-1 or treatment with GLP-1 analog or GLP-1 receptor agonist or DPP-4-specific inhibitor can reverse high-salt induced hypertension. These evidence strongly validate that GLP-1 or GLP-1 analog treatment promotes urinary sodium excretion and reduces blood pressure in SS subjects with high salt intervention. Unfortunately, none of these studies directly measured the serum GLP-1 levels at different concentrations of salt interventions. We infer that delayed urinary sodium excretion and increased blood pressure in SS subjects may be attributed to the deficiency of GLP-1 caused by high salt diet intervention in some respects.

So far, the molecular mechanisms through which salt regulates serum GLP-1 concentration are still unknown. However, there are several potential explanations. Firstly, inhibition of sodium glucose co-transporter SGLT1 augment the secretion of gastrointestinal incretins GLP-1 [26, 27]. SGLT1 activity was increased with the increasing amount of phosphorylated serine residues in SGLT1 after high-salt loading (8% NaCl) in the Dahl salt sensitive rats compared with Dahl salt-resistant rats [28]. This may be explained by the fact that high salt intervention lowers and delays the secretion of GLP-1 due to the increase of SGLT1 activity in salt sensitive individuals. Secondly, incretin hormone secretion could be elicited by autonomic nerves. For example, a transient increase of GLP-1 level was observed

in the anticipation of food in rodents [29]. Niijima A reported that high-salt intake (5% NaCl) resulted in the attenuation of autonomic nerve activity [30]. Therefore, high-salt intake induced attenuation of autonomic nerve activity may result in decreased secretion of GLP-1. Thirdly, ATP sensitive K^+ channels (K^+_{ATP}) was involved in glucose- triggered GLP-1 release [31]. However, Matthew J found that K^+_{ATP} function is in fact impaired in DOCA-salt rats [32]. We infer that the impaired K^+_{ATP} may lead to reduced release of GLP-1. These studies together revealed that high salt-treatment can reduce the secretion and release of GLP-1 in SS subjects. On the contrary, low salt intervention may reverse these changes. However, further studies are necessary to better define these hypotheses.

It is worth noting that sodium retention is an important mechanism of salt-sensitive hypertension [33]. Sodium metabolism is through an intricate interaction between signals of antinatriuretic factors and natriuretic factors. Atrial natriuretic peptide (ANP) is a natriuretic factor. Its natriuretic effect is exerted by inhibiting AngII dependent sodium and water reabsorption [34]. Kim et al. found that GLP-1 receptor is present in the cardiac atria and stimulates the secretion of ANP through promoting translocation of the Rapgef4 to the membrane when GLP-1R is activated by agonist liraglutide [15]. The ANP deficiency can cause salt-sensitive hypertension [35]. In addition, high salt intake can reduce ANP level in salt-sensitive patients but not in salt-resistant patients during high salt intake [36]. However, the mechanism remains unknown. We found that high salt intake reduced the GLP-1 level in the circulation, which may partly explain the phenomenon that high salt intake decreases the concentration of ANP due to GLP-1R activation disorder. However, further studies especially population studies are required to validate those findings in suitable cohorts.

In our study, we found that 24h-urinary Na^+ was significantly less in SS subjects (156.2±13.9 mmol/day) compared with SR subjects (175.3±12.9 mmol/day) at the baseline.

Previous studies have shown that sodium excretion is delayed in SS individuals. The salt intake in residents in China is alarmingly higher than the current recommended amount (5 g/day) [37]. A large number of studies have shown that in response to high salt diets, SR individuals are acutely and chronically resistant to salt -induced hypertension because they rapidly excrete salt and retain little of it so that their blood volume, and therefore blood pressure, does not increase. Conversely, SS individuals develop salt-induced hypertension due to an impaired renal capacity to excrete salt that causes greater salt retention and blood volume expansion than that occurs in SR individuals [38]. In addition, excessive salt intake in susceptible characters induces inappropriate central and sympathetic nervous system responses [39]. Discomfort in SS subjects caused by adjust disorder may contribute to their slightly reduced salt intake compared with SR subjects. We infer that the relatively delayed urinary sodium excretion and slightly limited salt intake in SS subjects lead to the difference in the baseline 24h-urinary Na^+ compared with SR subjects. Certainly, it is undeniable that the number of recruited subjects in this study is limited, which may result in a deviation.

Our present study has the limitation that should be addressed. The recruited population in this study was restricted to Northern Chinese individuals and relatively small. Therefore, our observations will require validation in larger and more diverse cohorts to determine the generalizability in other ethnicities and populations with different backgrounds. In addition, the serological parameters we measured were limited because the blood sample collected from each subject was insufficient and the funding was limited. We expect to quantitatively measure serum total GLP-1, ANP, DPP-4 activity and other suitable parameters in the future.

Conclusions

Our human intervention study has demonstrated that the circulating GLP-1 level increases with the change from the baseline to a low-salt diet and decreases with the change from low-salt to high-salt diet in Chinese normotensive SS but not in SR subjects. These findings may contribute to a better understanding of the underlying mechanism of salt

sensitivity and may have potential clinical and public health implications.

Disclosure Statement

The authors declare that there is no conflict of interest.

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References

- 1 Ibrahim MM, Damasceno A: Hypertension in developing countries. *Lancet* 2012;380:611-619.
- 2 O'Donnell M, Mente A, Yusuf S: Sodium intake and cardiovascular health. *Circ Res* 2015;116:1046-1057.
- 3 Weinberger MH: Pathogenesis of salt sensitivity of blood pressure. *Curr Hypertens Rep* 2006;8:166-170.
- 4 Weinberger MH, Fineberg NS, Fineberg SE, Weinberger M: Salt sensitivity, pulse pressure, and death in normal and hypertensive humans. *Hypertension* 2001;37:429-432.
- 5 Appel LJ, Frohlich ED, Hall JE, Pearson TA, Sacco RL, Seals DR, Sacks FM, Smith SC, Vafiadis DK, Van Horn LV: The importance of population-wide sodium reduction as a means to prevent cardiovascular disease and stroke: A call to action from the American Heart Association. *Circulation* 2011;123:1138-1143.
- 6 He FJ, MacGregor GA: Salt reduction lowers cardiovascular risk: meta-analysis of outcome trials. *Lancet* 2011;378:380-382.
- 7 Stocker SD, Madden CJ, Sved AF: Excess dietary salt intake alters the excitability of central sympathetic networks. *Physiol Behav* 2010;100:519-524.
- 8 Baggio LL, Drucker DJ: Biology of incretins: GLP-1 and GIP. *Gastroenterology* 2007;132:2131-2157.
- 9 Ravassa S, Zudaire A, Díez J: GLP-1 and cardioprotection: from bench to bedside. *Cardiovasc Res* 2012;94:316-323.
- 10 Yu M, Moreno C, Hoagland K, Dahly A, Ditter K, Mistry M, Roman RJ: Antihypertensive effect of glucagon-like peptide 1 in Dahl salt-sensitive rats. *J Hypertens* 2003;21:1125-1135.
- 11 Hocher B, Reichetzedder C, Alter ML: Renal and Cardiac Effects of DPP 4 Inhibitors – from Preclinical Development to Clinical Research. *Kidney Blood Press Res* 2012;36:65-84.
- 12 von Websky K, Reichetzedder C, Hocher B: Physiology and pathophysiology of incretins in the kidney. *Curr Opin Nephrol Hypertens* 2014;23:54-60.
- 13 Crajoinas RO, Oricchio FT, Pessoa TD, Pacheco BP, Lessa LM, Malnic G, Girardi AC: Mechanisms mediating the diuretic and natriuretic actions of the incretin hormone glucagon-like peptide-1. *Am J Physiol Renal Physiol* 2011;301:F355-363.
- 14 Kim M, Platt MJ, Shibasaki T, Quaggin SE, Backx PH, Seino S, Simpson JA, Drucker DJ: GLP-1 receptor activation and Epac2 link atrial natriuretic peptide secretion to control of blood pressure. *Nat Med* 2013;19:567-575.
- 15 Hirata K, Kume S, Araki S, Sakaguchi M, Chin-Kanasaki M, Isshiki K, Sugimoto T, Nishiyama A, Koya D, Haneda A, Kashiwagi A, Uzu T: Exendin-4 has an anti-hypertensive effect in salt-sensitive mice model. *Biochem Biophys Res Commun* 2009;380:44-49.
- 16 Eljovich F, Weinberger MH, Anderson CA, Appel LJ, Bursztyn M, Cook NR, Dart RA, Newton-Cheh CH, Sacks FM, Laffer CL: Salt Sensitivity of Blood Pressure: A Scientific Statement From the American Heart Association. *Hypertension* 2016;68:e7-e46.

- 17 Drucker DJ: The role of gut hormones in glucose homeostasis. *J Clin Invest* 2007;117:24-32.
- 18 Goud A, Zhong J, Peters M, Brook RD, Rajagopalan S: GLP-1 Agonists and Blood Pressure: A Review of the Evidence. *Curr Hypertens Rep* 2016;18:16.
- 19 Skov J, Dejgaard A, Frøkiær J, Holst JJ, Jonassen T, Rittig S, Christiansen JS: Glucagon-like peptide-1 (GLP-1): effect on kidney hemodynamics and renin-angiotensinaldosterone system in healthy men. *J Clin Endocrinol Metab* 2013;98:E664-671.
- 20 Asmar A, Simonsen L, Asmar M, Madsbad S, Holst JJ, Frandsen E, Moro C, Jonassen T, Bülow: Renal extraction and acute effects of glucagon-like peptide-1 on central and renal hemodynamics in healthy men. *Am J Physiol Endocrinol Metab* 2015;308:E641-E649.
- 21 Skov J, Holst JJ, Gøtze JP, Frøkiær J, Christiansen JS: Glucagonlike peptide-1: effect on pro-atrial natriuretic peptide in healthy males. *Endocr Connect* 2014;3:11-16.
- 22 Green BD, Hand KV, Dougan JE, McDonnell BM, Cassidy RS, Grieve DJ: GLP-1 and related peptides cause concentration-dependent relaxation of rat aorta through a pathway involving KATP and cAMP. *Arch Biochem Biophys* 2008;478:136-142.
- 23 Liu Q, Adams L, Broyde A, Fernandez R, Baron AD, Parkes DG: The exenatide analogue AC3174 attenuates hypertension, insulin resistance, and renal dysfunction in Dahl salt-sensitive rats. *Cardiovasc Diabetol* 2010;9:1.
- 24 Sufiun A, Rafiq K, Fujisawa Y, Rahman A, Mori H, Nakano D, Kobori H, Ohmori K, Masaki T, Kohno M, Nishiyama A: Effect of dipeptidyl peptidase-4 inhibition on circadian blood pressure during the development of salt-dependent hypertension in rats. *Hypertens Res* 2015;38:237-243.
- 25 Gutzwiller JP, Hruz P, Huber AR, Hamel C, Zehnder C, Drewe J, Gutmann H, Stanga Z, Vogel D, Beglinger C: Glucagon-like peptide-1 is involved in sodium and water homeostasis in humans. *Digestion* 2006;73:142-150.
- 26 Song P, Onishi A, Koepsell H, Vallon V: Sodium glucose cotransporter SGLT1 as a therapeutic target in diabetes mellitus. *Expert Opin Ther Targets* 2016;20:1109-1125.
- 27 Lehmann A, Hornby PJ: Intestinal SGLT1 in metabolic health and disease. *Am J Physiol Gastrointest Liver Physiol* 2016;310:G887-G898.
- 28 Ikari A, Tachihara Y, Kawano K, Suketa Y: Differential regulation of Na⁺,K⁺-ATPase and the Na⁺-coupled glucose transporter in hypertensive rat kidney. *Biochim Biophys Acta* 2001;1510:118-124.
- 29 Vahl TP, Drazen DL, Seeley RJ, D'Alessio DA, Woods SC: Meal-anticipatory glucagon-like peptide-1 secretion in rats. *Endocrinology* 2010;151:569-575.
- 30 Nijima A: Effects of taste stimulation on the efferent activity of the autonomic nerves in the rat. *Brain Res Bull* 1991;26:165-167.
- 31 Reimann F, Gribble FM: Glucose-sensing in glucagon-like peptide-1-secreting cells. *Diabetes* 2002;51:2757-2763.
- 32 Phaner MJ, Galligan JJ, Swain GM: Increased catecholamine secretion from single adrenal chromaffin cells in DOCA- salt hypertension is associated with potassium channel dysfunction. *ACS Chem Neurosci* 2013;4:1404-1413.
- 33 Coffman TM: Under pressure: the search for the essential mechanisms of hypertension. *Nat Med* 2011;17:1402-1409.
- 34 Oliveira-Souza M, Malnic G, Mello-Aires M: Atrial natriuretic peptide impairs the stimulatory effect of angiotensin II on H⁺-ATPase. *Kidney Int* 2002;62:1693-1699.
- 35 Lynch AI, Claas SA, Arnett DK: A review of the role of atrial natriuretic peptide gene polymorphisms in hypertension and its sequelae. *Curr Hypertens Rep* 2009;11:35-42.
- 36 Campese VM, Tawadrous M, Bigazzi R, Bianchi S, Mann AS, Oparil S, Raij L: Salt intake and plasma atrial natriuretic peptide and nitric oxide in hypertension. *Hypertension* 1996;28:335-340.
- 37 Hipgrave DB, Chan SY, Li XY, Wu Y: Salt and Sodium Intake in China. *JAMA* 2016;315:703-705.
- 38 Coffman TM: The inextricable role of the kidney in hypertension. *J Clin Invest* 2014;124:2341-2347.
- 39 Majid DS, Prieto MC, Navar LG: Salt-Sensitive Hypertension: Perspectives on Intrarenal Mechanisms. *Curr Hypertens Rev* 2015;11:38-48.