Effects of Salt Loading on Plasma Osteoprotegerin Levels and Protective Role of Potassium Supplement in Normotensive Subjects

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Background: Excess dietary salt is strongly correlated with cardiovascular disease, morbidity, and mortality. Conversely, potassium likely elicits favorable effects on cardiovascular disorders. In epidemiological studies, increased plasma osteoprotegerin (OPG) concentrations are associated with atherosclerosis and vascular deaths. Our study was designed to examine the effects of salt intake and potassium supplementation on plasma OPG levels in normotensive subjects.

Methods and Results: The 18 normotensive subjects were selected from a rural community in China. They were sequentially maintained on low-salt diet for 7 days (3 g/day, NaCl), high-salt diet for 7 days (18 g/day), and high-salt diet with potassium supplementation for 7 days (18 g/day of NaCl+4.5 g/day of KCl). High-salt intake enhanced plasma OPG levels (252.7 ± 13.9 vs. 293.4 ± 16.1 pg/mL). This phenomenon was abolished through potassium supplementation (293.4 ± 16.1 vs. 235.1 ± 11.3 pg/mL). Further analyses revealed that the OPG concentration positively correlated with 24-h urinary sodium excretion (r=0.497, P<0.01). By contrast, OPG concentration negatively correlated with 24-h urinary potassium excretion (r=-0.594, P<0.01).

Conclusions: Salt loading can enhance the production of circulating OPG. Potassium supplementation can reverse the effects of excessive OPG. Our study results may improve our understanding of the roles of salt and potassium in the risk of cardiovascular disorders.

Key Words: Cardiovascular disorders; Dietary interventions; Osteoprotegerin; Potassium; Salt
mentation on plasma OPG levels in non-obese, normoten-
sive subjects.

**Methods**

**Subjects**

A total of 18 male subjects with similar dietary preferences were recruited from a rural community in northern China. A brief medical questionnaire was administered. Subjects with a history of hypertension, obesity, liver or renal disease, or diabetes mellitus were excluded. Hypertension was defined as the mean systolic BP ≥140 mmHg and/or mean diastolic BP ≥90 mmHg. Obesity was defined as body mass index (BMI) ≥28 kg/m². All subjects were nonsmokers. The study protocol was approved by the Ethics Committee of Xi’an Jiaotong University Medical School. Written informed consent was given by each subject. The procedures were performed in accordance with institutional guidelines.

**Protocol**

The protocol consisted of a series of investigations, including a 3-day baseline period during which clinical history and physical examination data (height, weight, and BP) were obtained. The subjects were given a normal salt diet, followed by a low-salt diet (51.3 mmol or 3 g of NaCl/day) for 7 days, high-salt diet (307.7 mmol or 18 g of NaCl/day) for 7 days, and high-salt diet with potassium supplementation (60 mmol or 4.5 g of KCl/day) for 7 days. In the base-

**Results**

**Profiles of the Study Subjects**

All the enrolled subjects completed this interventional study. Their average age was 50.7±6.6 years, and systolic BP and diastolic BP were 117.2±9.2 and 72.4±6.2 mmHg, respectively (Table 1). The average 24-h urinary sodium excretion was 173.2±12.1 mmol/day, which corresponded to an intake of 8 g/day of salt. The average daily urinary potassium excretion was 45.3±5.3 mmol/day.

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**Table 1. Baseline Demographic and Clinical Characteristics of the Normotensive Study Subjects**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (years)</td>
<td>50.7±6.6</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>9/9</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>21.8±0.6</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>117.2±9.2</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>72.4±6.2</td>
</tr>
<tr>
<td>Fasting glucose (mmol/L)</td>
<td>3.92±0.91</td>
</tr>
<tr>
<td>Hemoglobin (g/L)</td>
<td>134.5±2.7</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>1.36±0.89</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>4.05±1.0</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/L)</td>
<td>2.24±0.83</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/L)</td>
<td>1.17±0.25</td>
</tr>
<tr>
<td>Serum creatinine (µmol/L)</td>
<td>56.98±9.21</td>
</tr>
<tr>
<td>Serum sodium (mmol/L)</td>
<td>142.0±1.09</td>
</tr>
<tr>
<td>Serum potassium (mmol/L)</td>
<td>3.94±0.33</td>
</tr>
<tr>
<td>Plasma OPG level (pg/mL)</td>
<td>265.1±11.3</td>
</tr>
</tbody>
</table>

BMI, body mass index; BP, blood pressure; DBP, diastolic BP; HDL, high-density lipoprotein; LDL, low-density lipoprotein; OPG, osteoprotegerin; SBP, systolic BP.

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

<table>
<thead>
<tr>
<th>SBP</th>
<th>DBP</th>
<th>24-h Urinary Na⁺ (mmol/day)</th>
<th>24-h Urinary K⁺ (mmol/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>117.2±9.2</td>
<td>72.4±6.2</td>
<td>173.2±12.1</td>
</tr>
<tr>
<td>Low-salt diet</td>
<td>119.3±9.5</td>
<td>73.1±4.6</td>
<td>99.6±3.7</td>
</tr>
<tr>
<td>High-salt diet</td>
<td>120.6±11.1</td>
<td>74.5±6.6</td>
<td>244.5±13.8</td>
</tr>
<tr>
<td>High-salt diet+potassium supplement</td>
<td>117.4±10.9</td>
<td>73.2±6.5</td>
<td>259.1±4.7</td>
</tr>
</tbody>
</table>

*P<0.05 vs. low-salt diet; *P<0.05 vs. high-salt diet. Abbreviations as in Table 1.
Effects of Dietary Intervention on BP and 24-h Urinary Sodium and Potassium Excretion

Table 2 shows the BP responses to the dietary interventions. The BP responses to the low-salt diet, high-salt diet, and high-salt diet with potassium supplement intervention did not change significantly. The 24-h sodium and potassium excretion in urine was calculated at the end of the intervention period to ensure compliance with the study protocol. Urinary sodium excretion was significantly decreased as the baseline was changed to the low-salt diet, but increased as the low-salt diet was changed to the high-salt diet (P<0.05; Table 2). Potassium supplementation increased urinary potassium excretion and slightly increased urinary sodium excretion. These results confirmed that the subjects complied with the dietary intervention protocol.

Effects of High Salt Intake and Potassium Supplementation on Fasting OPG Levels

There was no difference in plasma OPG levels between the baseline and the low-salt diet period (265.1±11.3 vs. 252.7±13.9 pg/mL; Figure 1). High-salt intake enhanced the plasma OPG levels (252.7±13.9 pg/mL vs. 293.4±16.1 pg/mL). The high-salt-diet-induced increase in plasma OPG was prevented by potassium supplementation (293.4±16.1 vs. 235.1±11.3 pg/mL). Further analyses revealed that the OPG level correlated with the 24-h urinary sodium excretion (r=0.497, P<0.01; Figure 2). The plasma OPG level negatively correlated with the 24-h urinary potassium excretion (r=0.594, P<0.01; Figure 3). Moreover, the OPG level positively correlated with the 24-h urinary sodium excretion Na/K ratio (r=0.311, P=0.008; Figure 4).

Figure 1. Effect of salt intake and potassium supplement on fasting osteoprotegerin levels in all study subjects.

Figure 2. Correlation between plasma osteoprotegerin levels and 24-h urinary sodium excretion (mmol/day) in all study subjects on a low-salt diet and on a high-salt diet.

Figure 3. Correlation between plasma osteoprotegerin levels and 24-h urinary potassium excretion (mmol/day) in all study subjects on a high-salt diet and on a high-salt diet with potassium supplement.

Figure 4. Correlation between plasma osteoprotegerin level and 24-h urinary Na/K ratio in all study subjects at baseline and in the diet intervention period.
This study found that high-salt intake can stimulate OPG production. Conversely, potassium supplementation can prevent the effects of high-salt diet on plasma OPG levels. Moreover, the plasma OPG level positively correlated with the 24-h urinary Na/K ratio.

OPG is associated with the onset and progression of cardiovascular disease (CVD); this substance has been considered as a potential biomarker of cardiometabolic disorders. Although OPG was initially identified as a bone reabsorption regulator, it is synthesized by mononuclear leukocytes and endothelial cells; OPG is also expressed in the central nervous system, thyroid, heart, liver, lung, testes, ovaries, pancreas, adrenals, and kidneys. OPG regulates several important organ systems by inhibiting the interaction between RANKL and its receptor RANK. The OPG/RANKL/RANK system is involved in the pathogenesis of endothelial dysfunction, vascular inflammation, and vascular calcification. In 2001, the first study reporting a positive association of OPG with all-cause and CVD death and incident diabetes was published. In the prospective, population-based Bruneck Study, Kiechl and colleagues observed a significant association of OPG with the 10-year incidence of CVD defined as myocardial infarction, stroke, transient ischemic attack, or peripheral artery disease and vascular death associated with myocardial infarction, stroke, ruptured aneurysm, or sudden cardiac death; OPG is also related to the initiation and progression of carotid atherosclerosis. Increased OPG levels have been observed in patients with unstable angina, acute myocardial infarction, or significant coronary artery stenosis. Lewis et al conducted a prospective study of elderly women and demonstrated that increased circulating OPG levels and renal dysfunction can predict 15-year CVD and all-cause mortality rates. Furthermore, OPG is associated with atherosclerosis and diabetic complications, such as neuropathy, nephropathy, or retinopathy. However, studies have yet to determine whether OPG is causally related to atherosclerotic disease progression or is implicated as a modulatory response to subclinical CVD. In endothelial cells, OPG acts as a survival and antiapoptotic factor. It protects endothelial cells from apoptosis in vitro and promotes neovascularization in vivo. OPG enhances endothelial cell proliferation in microvessels. Therefore, high circulating levels of OPG may indicate a compensatory mechanism by which injuries in blood vessel walls induce the release of OPG from the inner blood vessel wall to the circulation. OPG also plays a pathogenic role in atherogenesis, inducing the expression of ICAM-1, VCAM-1, and E-selectin on endothelial cells and consequently promoting leukocyte adhesion, which is an early step in endothelial cell dysfunction. At high concentrations, OPG can stimulate metalloprotease activity in vascular smooth muscle cells. Therefore, further in vitro and in vivo studies should be performed to establish the interaction between OPG and endothelial dysfunction or inflammation.

Although the mechanisms underlying the association of high-salt intake and cardiovascular events remain unresolved, studies have confirmed that endothelial dysfunction and inflammation play crucial roles in this phenomenon. Li et al demonstrated that a 5 mmol/L increase in salt concentration causes a 25% decrease in nitric oxide (NO) synthase activity in vivo. High-salt diet impairs endothelium-dependent relaxation by decreasing NO levels and increasing superoxide production in rats. Our previous dietary intervention studies also showed that a high-salt diet can impair endothelial function and facilitate inflammation in normotensive subjects, especially in salt-sensitive patients. OPG present study demonstrated that salt loading significantly enhanced the plasma OPG level and that the 24-h urinary sodium excretion positively associated with the OPG level in humans. This study provided a novel finding that explains the link between sodium intake and CVD incidence. However, further studies should be performed to confirm the role of OPG in adverse salt-induced outcomes.

Our work also provides novel insights into the effects of potassium supplementation on the influence of high-salt diet on OPG levels. In particular, potassium supplementation can reverse the effects of high-salt diet on OPG levels. However, the molecular mechanisms modulating the serum OPG level in response to potassium supplementation remain elusive. Our previous studies demonstrated that potassium supplementation in normotensive subjects may influence NO synthesis by inhibiting asymmetrical dimethylarginine production. Wyss et al showed that potassium directly restores the vasorelaxation of resistance arterioles in non-hypertensive DOCA/salt-fed mice, possibly via its antioxidant effect. Overall, potassium supplementation may counteract the salt-induced OPG increase by inhibiting oxidative stress and protecting endothelial functions. Thus, this phenomenon contributes to a reduction in CVD risk.

Study Limitations
Our observation cannot be considered as a general finding to be applied to other populations, because of the small sample size. Further studies should be conducted to validate our findings in a larger and more diverse sample. The mechanisms by which salt loading affects plasma OPG and the protective effect of potassium supplementation need further investigation.

In conclusion, our human intervention study found that salt loading could increase the amount of circulating OPG. Potassium supplementation could reverse the effects of excessive OPG. These findings may enhance our understanding of the roles of salt and potassium in CVD morbidity and mortality. Our study may also have potential clinical and public health implications.

Grants
This study was supported by grant 81200512 (to F.-Q.L.) from the Natural Science Foundation of China.

References
Salt, Potassium and Osteoprotegerin


