Potassium supplementation blunts the effects of high salt intake on serum retinol-binding protein 4 levels in healthy individuals

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ABSTRACT

Aims/Introduction: Excessive dietary salt or low potassium intakes are strongly correlated with insulin resistance (IR) and type 2 diabetes mellitus. In epidemiological and experimental studies, increased serum retinol-binding protein 4 (RBP4) contributes to the pathogenesis of type 2 diabetes mellitus. Herein, we hypothesized that RBP4 might be an adipocyte-derived "signal" that plays the crucial role in salt-related insulin resistance or type 2 diabetes mellitus. This study aimed to assess whether salt consumption and potassium supplementation influence serum RBP4 levels in healthy individuals.

Materials and Methods: A total of 42 participants (aged 25–50 years) in a rural area of Northern China were successively provided normal (3 days at baseline), low-salt (7 days; 3 g/day NaCl) and high-salt (7 days; 18 g/day) diets, and a high-salt diet with potassium additive (7 days; 18 g/day NaCl and 4.5 g/day KCl). Urinary sodium and potassium were measured to ensure compliance to dietary intervention. Then, RBP4 levels were evaluated by enzyme-linked immunosorbent assay.

Results: High salt intake significantly raised serum RBP4 levels in healthy participants (17.5 ± 0.68 vs 28.6 ± 1.02 μg/mL). This phenomenon was abrogated by potassium supplementation (28.6 ± 1.02 vs 17.6 ± 0.88 μg/mL). In addition, RBP4 levels presented positive ($r = 0.528$, $P < 0.01$) and negative ($r = -0.506$, $P < 0.01$) associations with 24-h urinary sodium- and potassium excretion levels.

Conclusions: RBP4 synthesis is motivated by high salt intake and revoked by potassium supplementation. Our pioneer work has contributed to the present understanding of salt-induced insulin resistance or type 2 diabetes mellitus.

INTRODUCTION

Individuals consuming high amounts of salt are at elevated risk of cardiovascular events, and the related health outcomes constitute a public health concern worldwide[1]. Multiple reports have established associations of high sodium consumption with insulin resistance (IR) or type 2 diabetes mellitus[2,3]. Potassium is considered to exert beneficial effects on cardiovascular diseases and associated mortality. Recently, a population-based study showed that reduced dietary potassium correlates with new-onset diabetes[4]. Meanwhile, it was shown that both dietary and serum potassium levels constitute a potential risk factor for thiazide-induced diabetes[5–7].

Recent studies have focused on adipocytes as the source of "adipokines," because they are involved in the pathogenesis of obesity-associated ailments, including IR, type 2 diabetes mellitus and cardiovascular disease. In addition to adipokines specifically produced in the fat tissue, an increasing number of well-known molecules are secreted by adipocytes. Animal and human experiments have shown that high-sodium diets increase fat tissue mass, as well as adipocyte size[3,8]. Our previous study also showed that elevated salt consumption stimulates

the synthesis of several adipokines, including adiponectin and tumor necrosis factor-α[9].

Retinol-binding protein 4 (RBP4), a 21-kDa member of the "lipocalin superfamily," was originally considered to be mostly found in the liver; it constitutes the only retinol (vitamin A) transporter[10]. More recently, Yang et al.[11] found that significant amounts of circulating RBP4 are released from adipocytes. Transgenic overexpression of human RBP4 and administration of recombinant RBP4 in healthy mice reduce insulin sensitivity, which is ameliorated by RBP4 gene deletion or RBP4 level normalization in obese rodents[11]. Indeed, previous reports have shown that high serum RBP4 levels are associated with IR in humans. A study by Sun et al.[12] included 2,091 Chinese adult participants (aged 50–70 years), and found plasma RBP4 to independently correlate with 6-year odds of developing type 2 diabetes mellitus. Hence, RBP4 is considered a novel adipokine in humans, possibly relating to obesity, IR, type 2 diabetes and metabolic syndrome.

Little attention has been focused on the link between salt intake and RBP4. To assess the influence of salt or potassium on IR or type 2 diabetes mellitus, prospective trials that minimize bias are required. We tested the hypothesis that RBP4 synthesis contributes to the progression of IR in excessive salt intake and potassium supplementation. Therefore, the effects of salt consumption and added potassium on serum RBP4 levels were assessed in healthy individuals.

METHODS

Participants

A total of 42 participants with comparable eating habits were recruited from a rural community of Northern China and briefly queried regarding their medical history. Participants with a history of obesity, hypertension, liver or renal diseases, IR, or type 2 diabetes mellitus were excluded. Hypertension was indicated by systolic blood pressure (BP) ≥140 mmHg and/or diastolic BP ≥90 mmHg. According to the criteria recommended by the Working Group on Obesity in China[13], obese individuals had body mass index (BMI) values ≥28 kg/m^2. The participants had no history of smoking. The study obtained approval from the institutional ethics committee of Xi'an Jiaotong University Medical School, and all participants provided written informed consent.

Protocol

First, the participants were assessed for 3 days in the baseline period, recording their clinical and physical (height, weight and BP) data. During this initial experimental phase, they were provided a regular salt diet. Then, the participants received low-salt (3 g of NaCl daily; 7 days) and high-salt (18 g of NaCl daily; 7 days) diets successively, followed by a high-salt diet and potassium supplementation (4.5 g of KCl daily) for 7 days (Figure 1). The participants received intensive dietary intervention, when the participants arrived at the study kitchen for their breakfast, lunch and dinner during the entire intervention period. All foods were prepared without salt, and prepacked salt was added to meal when it was served by the study staff. The participants were instructed to avoid table or cooking salt, high-sodium foods and nitrite-/nitrate-rich foods throughout the study. The study protocol was consistent with Genetic Epidemiology Network of Salt Sensitivity (GenSalt)[14].

Biochemical analysis

Blood glucose levels were assessed by the glucose oxidase method. Serum total cholesterol, triglyceride and high-density lipoprotein cholesterol levels were also evaluated. Blood specimens for fasting serum RBP4 levels were cooled on ice immediately after collection. The serum was kept at −80°C until analysis. RBP4 levels were assessed by sandwich enzyme-linked immunosorbent assay with anti-RBP4 antibodies (Wuhan USCN Sciences Corporation, Wuhan, China). Five serum specimens were used for intra- and interassay variations, and coefficients ranging between 3.4% and 4.8% (mean 4.1%), and from 4.7% to 6.2% (mean 5.4%), respectively, were obtained.

Twenty-four hour urinary sodium and potassium level assessment

Urine specimens (24 h) were obtained at baseline and on the final days of various intervention periods, and frozen at −40°C until use. Sodium and potassium levels in the urine were measured with ion-selective electrodes (Hitachi Ltd., Tokyo, Japan).

Statistical analysis

Data are shown as the mean ± standard deviation. Repeated measures ANOVA was used to assess biochemical parameters. Differences between biochemical markers obtained during the various dietary intervention periods were calculated by analysis of variance with the repeated measures design. Age, sex and BMI were adjusted in multivariable analysis. SPSS 16.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analyses, with two-tailed $P < 0.05$ showing statistical significance.

RESULTS

Characteristics of the participants

All enrolled participants completed the present interventional study. They were aged 50.9 ± 1.29 years, with systolic and diastolic BPs of 110.7 ± 2.2 and 72.6 ± 1.3 mmHg, respectively (Table 1). The mean 24-h sodium level in urine was 183.2 ± 10.1 mmol/day, corresponding to 8 g of salt consumed per day. The mean potassium level in urine was 45.3 ± 2.8 mmol/day.

Effects of dietary intervention on BP, and urinary sodium and potassium excretion

As shown in Table 2, BP remained relatively stable after all three interventions. Sodium levels in urine were markedly reduced after a change from baseline (normal) to a low-salt diet, and showed an increase after low-salt diet replacement by a high-salt diet ($P < 0.05$; Table 2). Potassium supplementation

3 days	7 days	7 days	7 days
Baseline : regular diet	Low salt diet : 3g/day 51 mmol Na	Low salt diet : 18g/day 308 mmol Na	Potassium Supplement : 60 mmol K and 308 mmol Na

Figure 1 | Overview of dietary intervention.

resulted in elevated potassium levels and slightly higher sodium levels in urine.

Effects of high salt consumption and potassium supplementation on RBP4 synthesis

The present results showed that serum RBP4 amounts were significantly decreased after low salt intake compared with baseline values (17.5 ± 0.68 vs 26.8 ± 1.22 µg/mL, $P < 0.01$). A high-salt diet resulted in elevated serum RBP4 levels compared with a low-salt diet (28.6 ± 1.02 vs 17.5 ± 0.68 µg/mL; Figure 2). Interestingly, high-salt diet-associated elevation of serum RBP4 was prevented by potassium supplementation (28.6 ± 1.02 vs 17.6 ± 0.88 µg/mL). Meanwhile, RBP4 concentration was associated with 24-h urinary sodium amounts in both low- and high-salt intervention phases ($r = 0.528$, $P < 0.01$). Finally, serum RBP4 concentration was negatively correlated with 24 h urinary potassium levels in both high-salt

and high-salt/potassium supplementation intervention phases ($r = -0.506$, $P < 0.01$).

DISCUSSION

The present study showed that excessive salt consumption stimulates RBP4 synthesis, which might contribute to IR and diabetes mellitus. Meanwhile, potassium supplementation blunts this phenomenon.

Accumulating evidence suggests that high serum RBP4 levels correlate with IR in humans[12,14–19], as well as cardiovascular disease, including hypertension, stroke and atherosclerosis[20–23]. RBP4 downregulates the glucose transporter, GLUT4, constituting the rate-limiting step in insulin-induced glucose transport across the muscle and adipocyte membrane[11,24]. Furthermore, RBP4 can also exacerbate endothelial function and

Table 1 | Baseline demographic and clinical characteristics

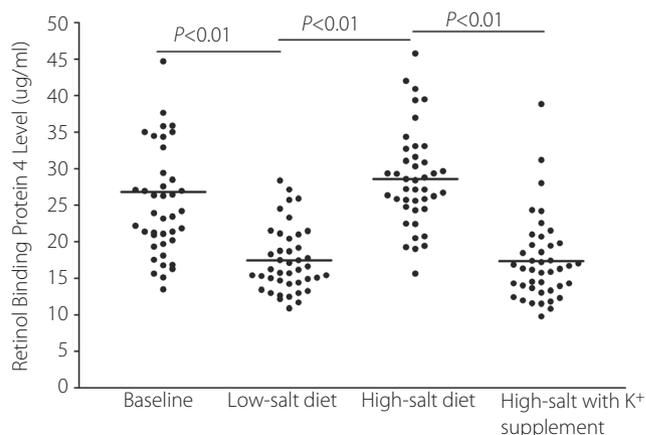| Parameter	Values
Mean age (years)	50.9 ± 1.29
Sex (male/female)	21/21
BMI (kg/m^2)	23.5 ± 0.42
Systolic BP (mm Hg)	110.7 ± 2.2
Diastolic BP (mm Hg)	72.6 ± 1.3
Fasting glucose (mmol/L)	3.9 ± 0.1
Total cholesterol (mmol/L)	4.18 ± 0.13
Triglycerides (mmol/L)	1.31 ± 0.11
LDL cholesterol (mmol/L)	2.35 ± 0.11
HDL cholesterol (mmol/L)	1.21 ± 0.04
Serum creatinine (µmol/L)	57.3 ± 1.35

Figure 2 | Effect of salt intake and potassium supplementation on serum retinol-binding protein 4 levels.

Table 2 | Blood pressure levels (mm Hg) and 24-h urinary sodium and potassium excretions (mmol/day) at baseline and during dietary interventions

	SBP	DBP	24-h Urinary Na$^+$ (mmol/day)	24-h Urinary K$^+$ (mmol/day)
Baseline	110.7 ± 2.2	72.6 ± 1.3	183.2 ± 10.1	45.3 ± 2.8
Low-salt diet	108.7 ± 1.8	73.5 ± 1.1	98.1 ± 5.3	36.9 ± 2.3
High-salt diet	117.3 ± 2.6	77.7 ± 1.3	228.3 ± 10.9	42.9 ± 3.6
High-salt diet with potassium supplement	107.5 ± 1.9	72.2 ± 1.3	273.4 ± 9.6	79.2 ± 3.1

*$P < 0.05$ vs low-salt diet. DBP, diastolic blood pressure; SBP, systolic blood pressure.

programmed cell death by increasing oxidative stress in the mitochondria through modulation of mitochondrial fusion and fission[19,25–27], which might also promote hypertension and atherosclerosis occurrence.

Human and experimental studies have shown that in addition to promoting hypertension occurrence, high sodium consumption results in IR[2,3,28,29]. Insulin sensitizers (for example, pioglitazone), which ameliorate insulin sensitivity in fat-fed and obese animals, do not prevent high-salt-associated IR[30–32], showing that high-salt-associated IR differs from other IR models. High-sodium consumption results in IR, mainly because of vascular dysfunction in muscles, in the case of no myocyte-associated or liver IR[28]. This finding directly contrasts the notion that IR originates from high fat/obesity, in which insulin signaling responsible for glucose uptake is repressed. Interestingly, quinapril prevents the development of high-sodium-derived muscle IR by preserving microvascular insulin responsiveness[28]. The present study is the first to show that salt loading significantly enhanced serum RBP4 levels. We previously reported that a high-salt diet impairs endothelial function in normotensive individuals[33], especially in salt-sensitive patients. We believe that sodium-derived IR might be associated with elevated RBP4, which leads to vascular dysfunction. The current findings provide new insights into the association of sodium intake with IR.

We also found that potassium supplementation reversed the influence of a high-salt diet on RBP4 levels; however, the underlying mechanisms are unknown. Recent reports have assessed the associations of potassium with obesity and metabolic syndrome, and found that high potassium levels alleviate obesity and metabolic syndrome risk[7,33–36]. We recently reported potassium supplementation in normotensive individuals affects nitrogen oxide production by reducing asymmetric dimethylarginine levels during high salt loading[37]. Furthermore, potassium supplementation counteracts salt-associated osteoprotegerin elevation through oxidative stress reduction and endothelial function protection[38]. Overall, potassium supplementation might prevent salt-associated IR by inhibiting the expression of RBP4, which can stimulate the oxygen-reactive free radical generation and endothelial dysfunction.

The present research had several remarkable advantages. First, the participants were recruited from rural communities where lifestyle and environmental risk factors were homogeneous. In addition, participants with a BMI >28 kg/m^2 were
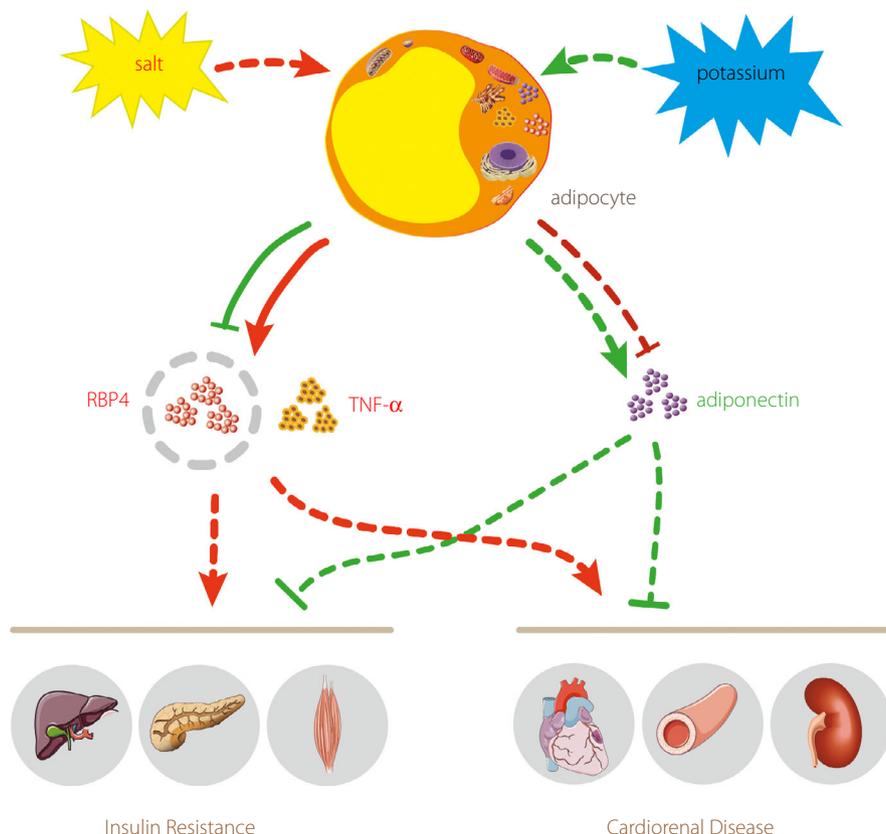

Figure 3 | The potential mechanism of diet-induced insulin resistance and cardiovascular disease. RBP4, retinol-binding protein 4; TNF-α, tumor necrosis factor-α.

excluded; hence, the hybrid effect of BMI might have been minimized. The compliance with the diet intervention was guaranteed through the urinary sodium excretion assessment.

The limitations of the present study should be mentioned. First, the sample size was relatively small, and these findings should be confirmed in more extensive studies. In addition, the causal relationship between RBP4 and IR was not estimated. Finally, RBP4 amounts were determined by enzyme-linked immunosorbent assay, whereas immunoblot standardized to RBP4 is the method of choice for such assessment.

The present study might give a tantalizing clue to the mechanisms by which high sodium intake contributes to IR. Potassium supplementation can reverse the effect of elevated RBP4 levels (Figure 3). These findings also shed some new light on a therapeutic target for the treatment of IR.

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DISCLOSURE

The authors declare no conflict of interest.

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