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Low-salt diet increases insulin resistance in healthy subjects

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ABSTRACT

Low-salt (LS) diet activates the renin-angiotensin-aldosterone and sympathetic nervous systems, both of which can increase insulin resistance (IR). We investigated the hypothesis that LS diet is associated with an increase in IR in healthy subjects. Healthy individuals were studied after 7 days of LS diet (urine sodium <20 mmol/d) and 7 days of high-salt (HS) diet (urine sodium >150 mmol/d) in a random order. Insulin resistance was measured after each diet and compared statistically, unadjusted and adjusted for important covariates. One hundred fifty-two healthy men and women, aged 39.1 ± 12.5 years (range, 18-65) and with body mass index of 25.3 ± 4.0 kg/m^2, were included in this study. Mean (SD) homeostasis model assessment index was significantly higher on LS compared with HS diet (2.8 ± 1.6 vs 2.4 ± 1.7, $P < .01$). Serum aldosterone (21.0 ± 14.3 vs 3.4 ± 1.5 ng/dL, $P < .001$), 24-hour urine aldosterone (63.0 ± 34.0 vs 9.5 ± 6.5 μg/d, $P < .001$), and 24-hour urine norepinephrine excretion (78.0 ± 36.7 vs 67.9 ± 39.8 μg/d, $P < .05$) were higher on LS diet compared with HS diet. Low-salt diet was significantly associated with higher homeostasis model assessment index independent of age, sex, blood pressure, body mass index, serum sodium and potassium, serum angiotensin II, plasma renin activity, serum and urine aldosterone, and urine epinephrine and norepinephrine. Low-salt diet is associated with an increase in IR. The impact of our findings on the pathogenesis of diabetes and cardiovascular disease needs further investigation.

1. Introduction

Low dietary salt intake is recommended as one of the public health measures to decrease the risk of cardiovascular disease [1]. However, low-salt intake stimulates aldosterone production through activation of the renin-angiotensin-aldosterone system (RAAS) [2]. We recently demonstrated an association between aldosterone and insulin resistance (IR) in healthy subjects [3]. Moreover, sympathetic nervous system is activated by low-salt diet as shown by an increase in urine norepinephrine levels [4,5]. Activation of the sympathetic nervous system may also increase IR [6]. Therefore, low

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dietary salt intake may be associated with an increase in IR. Previous studies on the effect of salt intake on IR in healthy subjects have shown contradictory results. Some studies showed an increase in IR [7,8], whereas others found no such effect or a decrease in IR [9-11]. To investigate this further, we analyzed data from a large cohort of healthy subjects studied under carefully controlled conditions. Furthermore, we investigated whether the effect of salt intake on IR could be explained by known risk factors for IR.

2. Methods

This is an analysis of data collected during the studies conducted as part of the International HyperPath (Hypertensive Pathotype) cohort [12] and includes subjects from 3 sites: Boston, Salt Lake City, and Nashville. Study subjects included men and women aged 18 to 65 years who were generally free of any significant medical or psychiatric problems. The studies were approved by the Human Research Committee at each site, and informed consent was obtained. All participants were studied after 7 days of an isocaloric, high-salt (HS) diet and low-salt (LS) diet in a random order. Compliance with salt intake was monitored by measuring 24-hour urine sodium. Those with urine sodium less than 150 mmol/d on HS diet or greater than 20 mmol/d on LS diet were excluded.

After 7 days of respective diet, participants collected 24-hour urine for sodium, creatinine, aldosterone, cortisol, epinephrine, and norepinephrine. They were then kept fasting and supine overnight for 8 to 10 hours. Next day, baseline blood pressure was measured. Mean arterial blood pressure (MAP) was calculated as MAP = diastolic blood pressure + (systolic − diastolic)/3. Fasting supine blood samples were obtained for plasma renin activity (PRA), aldosterone, angiotensin II, cortisol, sodium, potassium, glucose, and insulin. Angiotensin II (Bachem, Weil am Rhein, Germany) was infused intravenously at 3 ng/(kg min) for 45 minutes following which serum aldosterone was measured again. Blood pressure and heart rate were monitored every 2 minutes during the angiotensin II infusion.

All laboratory assays were performed at a central laboratory as previously described [13]. Urine and serum aldosterone were measured by solid-phase radioimmunoassay by the Coat-A-Count method (Diagnostic Products, Los Angeles, CA). Urine and serum cortisol was measured by Access Cortisol assay (Beckman Coulter, Chaska, MN). Urine epinephrine and norepinephrine were measured by radioimmunoassay by first converting them enzymatically to *N*-acylmetaneprine and *N*-acylnormetaneprine (LDN 2 CAT RIA; Immuno Biological Laboratories, Minneapolis, MN). Urine and serum sodium and potassium were assayed by flame photometry (Nova Biomedical, Waltham, MA). Urine creatinine was measured with the ACE Creatinine Reagent (Alfa Wasserman, West Caldwell, NJ). Insulin was assayed by chemiluminescence immunoassay using the Access Immunoassay System (Beckman Coulter).

The homeostasis model assessment (HOMA) was used as the measure of IR and was calculated as HOMA = [plasma glucose (in millimoles per liter) × plasma insulin (in micro-units per milliliter)]/22.5 [14]. The data were summarized using means and standard deviations. Measurements obtained on LS diet were compared with measurements obatined on HS diet by paired *t* test. Mixed-model analyses were performed to assess the impact of diet on the natural log of HOMA while controlling for single and multiple covariates (SAS version 9.1, Cary, NC).

3. Results

We identified 227 subjects studied on both LS and HS diets. Seventy-five subjects were excluded from analysis because of urine sodium greater than 20 mmol/d on LS diet or less than 150 mmol/d on HS diet. One hundred fifty-two subjects were included in this analysis. The subjects were 39.1 ± 12.5 years old and included 55% women. Eighty percent of subjects were white. Subject characteristics and laboratory values obtained on LS and HS diets are shown in Table 1. Body mass index (BMI) and blood pressure were lower and measures of RAAS were higher on LS compared with HS diet. Urine norepinephrine levels were also higher on LS diet, whereas the urine cortisol levels were low. The HOMA index was significantly higher on LS compared with HS diet (2.82 ± 1.6 vs 2.45 ± 1.69, $P < .01$).

Change in HOMA from LS to HS diet (ΔHOMA) did not correlate with changes in serum aldosterone, PRA, angiotensin II, stimulated serum aldosterone, urine aldosterone, and urine norepinephrine (all *P*s = not significant). On mixed-model analyses, the effect of diet on HOMA remained significant after controlling individually for each of the covariates in Table 1 (all *P*s < .05). A multiple-variable model was developed to assess the impact of diet on HOMA while controlling for multiple potentially important variables simultaneously. Potential predictors besides diet were age, sex, BMI, serum potassium, serum sodium, MAP, serum aldosterone, serum angiotensin II, serum and urine cortisol, urine aldosterone, and urine norepinephrine. The final parsimonious

Table 1 – Comparison of subject characteristics and laboratory parameters on HS vs LS diet

	LS diet	HS diet	*P*
BMI (kg/m^2)	24.6 ± 4.1	25.3 ± 4.1	<.0001
Mean arterial blood pressure (mm Hg)	78.1 ± 8.2	82.6 ± 9.2	<.0001
Serum Na (mmol/L)	139.0 ± 5.4	140.0 ± 5.3	NS
Serum K (mmol/L)	4.1 ± 0.4	4.1 ± 0.4	NS
Serum cortisol (μg/dL)	12.3 ± 4.4	11.9 ± 4.1	NS
Serum aldosterone (ng/dL)	21.0 ± 13.5	3.4 ± 1.4	<.0001
PRA (ng/[mL h])	3.1 ± 2.1	0.39 ± 0.40	<.0001
Angiotensin II (pg/mL)	43.9 ± 22.6	28.1 ± 15.9	<.0001
Serum aldosterone after angiotensin II infusion (ng/dL)	45.2 ± 21.8	11.2 ± 6.6	<.0001
Urine Na (mmol/d)	7.5 ± 4.9	237.8 ± 70.9	<.0001
Urine aldosterone (μg/d)	63.0 ± 34.0	9.6 ± 6.5	<.0001
Urine cortisol (μg/d)	37.4 ± 24.4	52.7 ± 25.9	<.0001
Urine epinephrine (μg/d)	11.5 ± 6.3	12.2 ± 6.5	NS
Urine norepinephrine (μg/d)	78.0 ± 36.7	67.9 ± 39.8	<.05
Urine creatinine (mg/d)	1367 ± 408	1401 ± 397	NS
HOMA	2.82 ± 1.6	2.45 ± 1.69	.002

model contained only diet, age, and BMI (all Ps < .001). The HOMA index remained significantly higher on LS diet in the multiple-variable model that controlled for age and BMI.

4. Discussion

Our study shows that low salt intake is associated with higher IR. The effect of dietary salt intake on insulin sensitivity has been controversial. Some previous studies have shown an increase in IR with low-salt diet [7,8], whereas others found no such effect or even a decrease in IR [9-11]. These discrepancies may be due to differences in study populations, levels of salt restriction, or study methods or to the relatively small sample size in previous studies of healthy individuals. Two studies that showed a decrease or no change in IR on LS diet included only 7 to 8 subjects and excluded women [9,10]. Another study that showed no change in IR included 34 men, but the subjects were not provided a standardized diet and were studied after a moderate salt restriction (urine sodium 70 ± 45.1 mmol/d on LS diet and 175 ± 72.1 mmol/d on HS diet) [11]. Our results are consistent with those of Townsend et al [8] who studied 20 healthy subjects, both men and women, and compared IR after standardized diets containing 20 vs 200 mmol/d sodium for 6 days. Our study is the largest study in healthy subjects, includes both men and women, was performed under carefully controlled conditions, and used a sodium content for the HS diet that is comparable to the average sodium intake in the US population [15].

We found an increase in aldosterone and norepinephrine, both of which may contribute to an increase in IR on LS diet. Angiotensin II levels were also higher on LS diet, and angiotensin II has been shown to interfere with insulin signaling pathways [16]. However, none of these factors could individually explain the increase in IR; salt intake remained an independent predictor of HOMA after including them individually in the analysis. Moreover, change in HOMA on LS vs HS diet did not correlate with corresponding changes in components of the RAAS. This lack of correlation may indicate a different mechanism for LS-diet–induced IR or may be due to the large variability in RAAS components with dietary salt restriction. Consistent with previous studies, urine cortisol was lower on LS diet [17,18], thus ruling out the possibility of high cortisol production as a mediator of IR. The possibility of other unidentified factors involved in LS-diet–induced IR cannot be ruled out.

High salt intake is considered a public health problem in the United States. High dietary salt intake is associated with higher blood pressure, a known cardiovascular disease risk factor. In a recent study using computerized models, it was estimated that reduction in salt intake could reduce the number of deaths by 44 000 to 92 000 [19]. However, this study and other similar studies were based on the estimated cardiovascular disease risk reduction associated with a fall in blood pressure. Activation of RAAS and changes in IR were not included in these models. Available data do not support universal recommendation for any particular level of dietary salt intake [20].

In conclusion, in healthy subjects, LS diet is associated with an increase in IR. The impact of our findings on the pathogenesis of diabetes and cardiovascular disease warrants further investigation.

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