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Effects of Aging and Alterations in Dietary Sodium Intake on Total Nitric Oxide Production

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Abstract

Animal studies suggest that nitric oxide (NO) deficiency is linked to salt-sensitive hypertension and that NO activity decreases during normal aging. This study investigates the impact of increasing age and manipulations in dietary salt intake on biochemical indices of the NO system in healthy humans. We measured $\text{NO}_2 + \text{NO}_3$ (NO_x ; stable oxidation products of NO) and cyclic guanosine monophosphate (cGMP; major second messenger) in plasma and urine of 30 healthy subjects aged 22 to 77 years. Subjects were maintained on controlled low NO_x and low-, normal-, or high-salt diets for 3 days. Salt sensitivity of blood pressure was seen only in the oldest subjects. Plasma renin activity was suppressed by a high salt intake in all age groups, and baseline values declined with advancing age. Neither age nor salt intake correlated with indices of NO activity over the third 24-hour period of controlled salt intake. In a subgroup of subjects aged 33 ± 4 years challenged with ultrahigh sodium intake (400 mEq/24 h), again there was no increase in $\text{NO}_2 + \text{NO}_3$ or cGMP measures. In contrast to animal studies, there is no correlation in humans between either salt intake or age and total NO production and activity, indicated by $\text{NO}_2 + \text{NO}_3$ and cGMP measures. This does not preclude undetected alterations occurring in NO production and/or activity in strategic locations in the kidney and cardiovascular system. Limitations of blood and urine measurements of $\text{NO}_2 + \text{NO}_3$ and cGMP as indices of NO activity are discussed.

Index Words

Sodium excretion; blood pressure (BP); salt-sensitive hypertension; cyclic guanosine monophosphate (cGMP); $\text{NO}_2 + \text{NO}_3$ (NO_x); nitric oxide (NO)

With Advancing Age, the kidney is less able to either conserve sodium in response to dietary restriction or remove sodium after excess intake.^{1,2} Both aging rats and humans have a blunted ability to excrete an acutely administered sodium load,^{1,3,4} and pressure natriuresis is attenuated in the old rat.^{5,6} According to Guyton et al,⁷ impaired pressure natriuresis leads to salt-sensitive hypertension, and the incidence of salt-sensitive hypertension increases substantially in humans with increasing age,⁸ reflected by an increased frequency of low-renin essential hypertension.⁹

Increased nitric oxide (NO) production occurs in response to high dietary salt intake in normal rats, and chronic NO synthase (NOS) inhibition combined with high dietary salt intake can lead to volume-dependent hypertension.¹⁰⁻¹³ In addition, the genetically salt-sensitive hypertension in the Dahl rat is associated with defective NO production in the face of high sodium intake.^{14,15} NO directly inhibits sodium transport in several parts of the tubule. NO is

also a powerful renal vasodilator, and in NO deficiency experimentally induced by NOS inhibition, pressure natriuresis is attenuated.^{16,17} It therefore is widely held that an appropriate increase in NO production in the kidney and peripheral vasculature is essential for maintenance of normal blood pressure (BP) and sodium balance during increased sodium intake.

In aging rats, vascular (indicated from in vitro vascular reactivity studies)¹⁸ and total NO production (indicated from decreases in 24-hour excretion of the oxidation products of NO [NO_x])^{19,20} decline. These observations raise the possibility that age-dependent decrements in the NO system contribute to increased salt-dependent hypertension in older humans. These clinical studies were designed to investigate this possibility and specifically to test the following hypotheses: (1) in healthy humans, NO production increases in response to increased dietary sodium, detectable as an increase in 24-hour urinary NO_x excretion (24-hour U_{NO_xV}); (2) this increased NO production is necessary for appropriate natriuretic and hemodynamic adaptations to high sodium intake; and (3) the increase in NO production (and U_{NO_xV}) in response to high salt intake is blunted in old subjects, contributing to the development of salt-sensitive hypertension.

Methods

Experiments were conducted on 30 healthy subjects aged 22 to 77 years recruited from faculty, staff, students, and spouses of patients in the Nephrology Clinic. Subjects were divided into age groups: group 1 was young (age, 20 to 39 years), group 2 was middle-aged (age, 40 to 59 years), and group 3 was old (age, ≥65 years). Inclusion criteria were no history of renal or cardiovascular disease and no medications that could influence cardiovascular and/or renal function. Informed consent was obtained from each subject, and all procedures were approved by the West Virginia University Institutional Review Board. All subjects had shown compliance with the diet (discussed next) and complete collection of 24-hour urine specimens, evidenced by 24-hour urinary sodium excretions (U_{NaV}) within 10% of dietary sodium intake.

At recruitment, body weight, height, and baseline BP were measured. Subjects were given a nutritionally complete 3-day diet (~2,000 calories/24 h) that contained a low NO_x content (~330 μmol of NO_x per 24 hours; ~20% of normal NO_x intake) and a low total-sodium content of 60 mEq/24 h. For studies with higher sodium intakes, we used the basic diet supplemented with salt packets and/or bouillon according to individual taste. Normal sodium intake was set at 130 mEq/24 h, and high sodium intake, at 240 mEq/24 h. People of all ages followed these three diets, although only six young, six middle-aged, and four old subjects completed all three diets. In addition, seven young and middle-aged subjects followed an ultrahigh-sodium diet of 400 mEq/24 h of sodium to separately test whether an extreme sodium challenge could evoke increased U_{NO_xV}. Additional calories were provided when necessary in the form of NO_x-free and sodium-free foods. All subjects kept a record of all food ingested and reported food not consumed.

Subjects followed the diet for 3 days, and during the third 24-hour period, all urine was collected. A blood sample was obtained in the morning, coincident with the end of the 24-hour urine collection and after a fast of at least 12 hours. BP was measured (triplicate determinations, 5 minutes apart) while subjects were quietly seated. All blood samples were collected with EDTA, and after centrifugation, plasma for cyclic guanosine monophosphate (cGMP) analysis was stored with a phosphodiesterase inhibitor.

The following analyses were conducted. NO_x concentration of plasma and urine was measured using the Griess assay after conversion of nitrate to nitrite, described previously by us.²¹ Plasma and urine cGMP were measured by Cayman Chemicals (Ann Arbor, MI) ELISA Kit. Plasma and urine sodium were measured by flame photometer. In some studies, plasma renin activity

(PRA) was measured by radioimmunoassay of generated angiotensin I using the Gamma-Coat method (Incstar, Stillwater, MN), adapted from the original method by Haber et al.²² All data are reported as mean $\pm$ SE. Statistics were by paired or unpaired *t*-test, one-way analysis of variance, analysis of variance, and regression analysis. Statistical significance is defined as *P* less than 0.05.

Results

Tables 1 and 2 list data for all subjects on this study. As shown in Table 1, the three age groups were well matched for body weight and body surface area.

All subjects participated in the low-sodium intake experiments. As shown in Table 1, by day 3, subjects in each age group were in sodium balance because $U_{Na}V$ was approximately equal to sodium intake (sodium, ~ 60 mEq/24 h). The reduction from normal dietary sodium intake had no impact on systolic or diastolic BPs in groups 1 and 2, whereas in group 3, systolic BP decreased with low salt intake (Table 1). Not all subjects participated in all other experiments with normal or high sodium intakes (see individual group numbers, Tables 1 and 2). Normal sodium intake (130 mEq/24 h) did not alter BP from the prediet value in any age group. All subjects were in balance by day 3 on both normal and high sodium intakes. Although BP trended upward in older subjects with high salt intakes, this was not significantly different from the prediet value (Table 1). Systolic BP data are shown in Fig 1. Average baseline BP (gray squares) increased with age ($P < 0.052$ for young versus middle-aged; $P < 0.002$ for old versus young). Despite the low numbers of subjects, the salt dependence of BP in the old subjects is striking, with a decrease in systolic BP on low-sodium versus control diets, as well as a difference in systolic BP on the high sodium versus the low sodium intake ($P < 0.05$) seen only in group 3.

Table 2 lists indices of activity of the NO system. In general, there was little impact of either age or salt intake on NO_x or cGMP. There was no indication of increases in plasma NO_x concentrations as dietary salt intake increased, and there were no systematic differences in $U_{NO_x}V$ in response to either salt intake or age. Regression analysis shows no relationship between age and $U_{NO_x}V$ on any dietary salt intake (Fig 2), and there was no correlation between sodium excretion (which balanced salt intake) and $U_{NO_x}V$ for any age group or the entire data set combined (Fig 3). Neither salt intake nor age had a consistent effect on plasma cGMP levels, although there were some minor differences (Table 2). Urinary excretion of cGMP ($U_{cGMP}V$) showed no trend in response to variations in either salt intake or age (Table 2), and there was no correlation between $U_{NO_x}V$ and $U_{cGMP}V$ by regression analysis at any age or for all subjects ($r^2 = 0.1031$, data not shown). In addition, there was no relationship between mean BP and $U_{NO_x}V$ when all available data were subjected to regression analysis (not shown) or when the paired change in BP versus change in $U_{NO_x}V$ was plotted from low to high or low to ultrahigh sodium intakes (Fig 4).

Table 3 lists the subset of data from subjects who completed the low-, normal-, and high-sodium intake studies, and these data allow a paired analysis of the effect of salt. As with the larger group listed in Tables 1 and 2, there was no systematic relationship between either salt intake or age on any indices of activity of NO. Systolic BP was higher on the high- versus low-salt diet for group 3, old subjects, in accordance with the expected salt sensitivity of BP seen in the aging population (Fig 1).

In seven subjects selected from groups 1 and 2 (age, 33 ± 4 years), an ultrahigh-sodium load (400 mEq/24 h) was given to establish whether increased $U_{NO_x}V$ could be elicited in response to an extreme sodium challenge. Paired data are listed in Table 3. Subjects were in sodium balance at day 3, and there was no evidence of salt sensitivity in this age group. There was no increase in $U_{NO_x}V$ and $U_{cGMP}V$ with ultrahigh sodium intake, and there was no relationship

between $U_{NOX}V$ and BP on these extremes of sodium intake. Plasma NO_X levels decreased slightly, and there was no effect on plasma cGMP.

As shown in Fig 5, PRA declined with increasing age and was lower in groups 2 and 3 versus group 1 for any given salt intake. PRA showed the expected response to alterations in sodium intake, and PRA was lower on both normal- and high-sodium versus low-sodium diets in groups 1 and 3. In middle-aged subjects, PRA was significantly lower on high- versus low-sodium diets (Fig 5).

Discussion

The major findings in the present study are: (1) in healthy humans, there is no evidence that total NO production decreases with advancing age when using the indices of plasma and urinary NO_X or cGMP; (2) there is no response in these indices to 3 days of dietary salt manipulation, suggesting that in healthy humans, total NO production is not changed by alterations in sodium intake over this time frame; and (3) although old subjects tended to have increased BP and characteristics of salt sensitivity of BP, there was no obvious relationship between BP and the indices of total NO production.

Animal studies have strongly implicated increased NO activity in cardiovascular and/or renal responses to high salt intake. For example, in the normal rat, dietary sodium loading evokes a large increase in total NO production, detectable as increased $U_{NOX}V$.^{10,11,23,24} There is evidence that dietary sodium loading upregulates the neuronal NOS protein in the inner medullary collecting duct,²⁴ as well as increasing glomerular endothelial nitric oxide synthase (eNOS) messenger RNA and NO production²⁵ after 3 to 4 days of high salt intake. Functional studies indicate that total renal blood flow and blood flow to the papilla are under enhanced NO-dependent vasodilatory tone when salt intake is high.^{23,26,27} In vivo and in vitro data suggest that NO has an important natriuretic role in the renal handling of sodium in a number of species.^{16,17,28,29} In addition, chronic NO deficiency is associated with both experimentally induced and genetically determined hypertension in animals during high salt intake.^{12-15,28} The consensus is that increased NO production must occur in the kidney and vasculature in response to increased sodium intake for appropriate natriuretic and vascular adaptations to occur. An important assumption made by many workers is that increased $U_{NOX}V$ reflects this increased renal and/or cardiovascular NO production.

We conducted the present experiments to investigate the relationship between NO production and sodium intake in healthy humans. We found that a large increase in sodium intake and excretion (from 60 to 400 mEq/24 h) was not associated with an increase in 24-hour $U_{NOX}V$. Clearly, the seven individuals challenged with the ultrahigh-sodium diet underwent appropriate vascular and natriuretic responses to the salt load because after 3 days of 400 mEq/24 h of sodium intake, sodium excretion increased to equal intake, with no change in BP (Table 4). In addition, considering all data from the low-, normal-, and high-salt intake studies regardless of age, we found no correlation between $U_{NOX}V$ and either sodium intake or $U_{Na}V$ (Fig 3) or BP (Fig 4). Thus, our observations in humans indicate that unlike the rat, total NO production measured from 24-hour $U_{NOX}V$ does not increase with increased sodium intake, at least over a 3-day period. In accordance with the present findings, Campese et al³⁰ reported that plasma NO_X concentrations did not increase in healthy subjects given a high-salt diet. Conversely, Facchini et al³¹ reported an inverse relationship between change in BP and change in $U_{NOX}V$ in healthy subjects in response to low versus high sodium intakes. We did not observe such a relationship in our study, in which subjects were studied days 2 through 3 of the diet rather than days 3 through 5 of the controlled salt intake in the study by Facchini et al.³¹ We have no explanation for this discrepancy, although it is important to note that salt balance and

unchanged BP was achieved by days 2 and 3 in our young and middle-aged subjects in the absence of a change in $U_{NOX} V$.

What is the significance of our findings? Do they imply that in humans, NO is unimportant in the normal response to high sodium intake, or alternatively, are the methods used to assess NO activity in the kidney and/or cardiovascular system giving misleading information? To focus on NO_X measurements, under optimal measurement conditions (eg, dietary control of NO_X intake, regulated activity), 24-hour $U_{NOX} V$ probably gives a qualitative measure of total NO production.³² The crucial question is whether total NO production reflects and always changes in parallel with renal and vascular NO production. Probably not, based on our earlier animal studies, because the large increases in 24-hour $U_{NOX} V$ seen in the normal rat in response to high salt intake or pregnancy can be dissociated from sodium excretion or BP.³² In vitro NOS-activity studies suggest that in normal rats, total renal and vascular NO generated contributes only a small fraction to the total-body NO_X pool (B. Santmyre and C. Baylis, unpublished data). Thus, biologically significant variations in endothelial and/or renal NO bioactivity will be associated with undetectable changes in total-body NO_X production; with large changes in total NO_X , this may reflect sources other than those involved in cardiovascular and/or renal control.

We also found no correlation between $U_{cGMP} V$ and either sodium intake or $U_{Na} V$. This finding was not anticipated, particularly because atrial natriuretic peptide, which is stimulated by high salt intake, also signals through cGMP. However, cGMP is cleared by a combination of excretion and metabolism by phosphodiesterases; thus, plasma and/or urinary cGMP may not provide a quantitative index of NO production in any given state because the contribution by metabolism may vary. It has been reported that tissue cGMP, but not plasma concentration of cGMP or 24-hour cGMP excretion, correlates with NOS activity in the chronically NOS-inhibited rat.³³

Because of the limitations of the NO_X and cGMP measurements in extracellular fluid discussed previously, the present findings do not necessarily imply that NO does not have a role in the renal and/or vascular response to salt in healthy humans. A recent intervention study suggested a role for increased NO in the renal and/or cardiovascular response to increased sodium intake. A high-salt diet produced an impressive amplification of the pressor, renal vasoconstrictor, and antinatriuretic effects of acute NOS inhibition in healthy humans, suggesting that NO activity increases in the kidney and peripheral vasculature under conditions of sodium loading.³⁴ Unfortunately, the present study supports the notion that even measured under optimal conditions, simple plasma and urine analyses of NO_X and cGMP are not reliable indices of renal or vascular NO activity.

The primary goal of our experiments is to investigate the activity of the NO system in healthy aging humans. Based on animal studies,¹⁸⁻²⁰ we had anticipated that baseline total NO production would decrease with advancing age, and in addition, the ability of a high salt intake to increase NO production would be attenuated with aging. However, there was clearly no decline in total NO production with advancing age in our subjects, and variations in dietary salt intake had no differential effects on NO-cGMP levels in individuals of differing ages. However, given the measures used in our study, we cannot determine whether the hemodynamically active component of the NO system is altered by age in healthy humans or contributes to the increased incidence of salt-sensitive hypertension seen in the elderly. To date, there have been no other studies of the NO system in aging humans. However, there have been observations in blacks, who also have a high incidence of salt-sensitive hypertension. Patients with salt-sensitive and salt-resistant hypertension show similar declines in plasma NO_X levels with a high sodium intake,³⁰ similar to our findings in aging humans. Infusion of L-arginine, the substrate for NOS, evokes a blunted renal vasodilatory response in patients with

salt-sensitive versus salt-resistant hypertension and controls.³⁵ This implies the existence of a specific renal NO defect in salt sensitivity. Although the issue of salt sensitivity was not specifically addressed, Cardillo et al³⁶ observed reduced NO-dependent vasodilator activity in the forearm circulation during mental stress in blacks. In general, as with the aging literature, there is little direct clinical information on the relationship between NO deficiency and salt sensitivity, although animal studies certainly support this relationship.³⁷

In contrast to our ambiguous findings on the NO system in healthy humans, PRA behaved exactly as predicted, with suppression by high salt intake in all age groups, as well as an age-dependent decline in PRA.^{38,39} This, together with the finding of salt sensitivity of BP in the oldest subjects, is reassuring that we are looking at a healthy human population over a range of ages.

In conclusion, using the available biochemical indices of NO_x and cGMP, we have no evidence that NO production (1) increases in response to increased sodium intake, (2) decreases with advancing age, or (3) is associated with salt sensitivity of BP in humans. Differences in responses between humans and rats in terms of increases in total NO production (from increased total NO_x output) suggest that rats may have a large pool of readily stimulated NO that originates from sources not involved in cardiovascular and/or renal regulation.³² Overall, the present study increases our skepticism about the value of NO_x and cGMP measurements as noninvasive indices of activity of NO in the cardiovascular system and kidney in humans.

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Fig 1.

Prediet and postdiet systolic BP (SBP) in young, middle-aged, and old subjects before and at the end of 3 days of low (x; 60 mEq/24 h), normal (◇; 130 mEq/24 h), and high sodium intake (● 240 mEq/24 h). *Reduction in SBP with low sodium intake versus control, $P < 0.05$.

Φ Difference in SBP on low versus high sodium intake, $P < 0.05$. Large gray squares to the left of the prediet data points give overall baseline SBP for each of the three age groups.

Fig 2. Relationship between age and 24-hour U_{NOx}V for subjects on low (×; 60 mEq/24 h; $r^2 = 0.00$), medium (◊; 130 mEq/24 h; $r^2 = 0.12$), and high sodium (●; Na; 240 mEq/24 h; $r^2 = 0.03$) intakes. The solid line gives overall relationship for age versus U_{NOx}V regardless of sodium intake ($r^2 = 0.016$).

Fig 3. Relationship between 24-hour $U_{Na}V$ and 24-hour $U_{NOx}V$ for young ($\circ$; age, 22 to 39 years; $r^2 = 0.02$), middle-aged ($\square$; age, 40 to 59 years; $r^2 = 0.08$), and old (Δ ; age, 65+ years; $r^2 = 0.14$) subjects. The solid line gives the relationship between $U_{Na}V$ and $U_{NOx}V$ for all subjects regardless of age ($r^2 = 0.06$).

Fig 4.

Relationship between the change in mean arterial BP (delta MAP) and delta 24-hour U_{NOXV} in subjects at 2 to 3 days of high sodium intake (240 mEq/24 h) and the same subjects at 2 to 3 days of low sodium intake (60 mEq/24 h). Data given by the open symbols. (○) Young; (□) middle aged; (△) old. (◆) Separate delta U_{NOXV} versus delta MAP for the seven individuals with both low and ultrahigh sodium (400 mEq/24 h) intakes. $r = 0.03$; $P =$ not significant.

Fig 5.

PRA (in nanograms of angiotensin I per milliliter per hour) for young (group 1), middle-aged (group 2), and old (group 3) individuals on the low-salt (LS; 60 mEq/24 h), normal-salt (NS; 130 mEq/24 h), and high-salt (HS; 240 mEq/24 h) diets. * $P < 0.05$ versus group 1 (young subjects). # $P < 0.05$ versus low salt intake.

Age, Body Weight and Surface Area, BP, and 24-Hour Sodium Excretion in Subjects of Different Ages and Sodium Intakes

Table 1

Group	No. of Patients	Sex (M/F)	Age (y)	BW (kg)	BSA (units)	Predict BP (mm Hg)	Postdiet BP (mm Hg)	U _{Na} V (mEq/24 h)
Low sodium intake (60 mEq/24 h)								
1	10	6/4	28 ± 2	69 ± 5	1.80 ± 0.06	118 ± 4/74 ± 2	117 ± 4/77 ± 3	63 ± 3
2	12	3/9	49 ± 2*	69 ± 4	1.77 ± 0.06	124 ± 5/83 ± 2	121 ± 4/83 ± 3	63 ± 3
3	8	3/5	71 ± 1*	71 ± 3	1.75 ± 0.05	130 ± 9/74 ± 4	117 ± 5/71 ± 3 [†]	56 ± 7
Normal sodium intake (130 mEq/24 h)								
1	9	5/4	29 ± 2	67 ± 5	1.77 ± 0.07	110 ± 3/74 ± 2	111 ± 3/75 ± 1	128 ± 6
2	11	3/8	50 ± 2*	68 ± 3	1.75 ± 0.04	120 ± 4/79 ± 3	121 ± 3/79 ± 2	133 ± 5
3	6	2/4	72 ± 2*	72 ± 4	1.77 ± 0.06	129 ± 7/75 ± 4	126 ± 6/75 ± 4	125 ± 7
High sodium intake (240 mEq/24 h)								
1	6	3/3	29 ± 2	70 ± 8	1.78 ± 0.08	116 ± 3/78 ± 2	116 ± 4/77 ± 2	241 ± 16
2	7	2/5	52 ± 2*	66 ± 4	1.72 ± 0.06	123 ± 6/81 ± 3	126 ± 6/83 ± 3	234 ± 10
3	4	1/3	72 ± 3*	69 ± 5	1.72 ± 0.08	128 ± 6/80 ± 4	139 ± 14/79 ± 6	238 ± 13

NOTE. Subjects are divided into three age groups: group 1, 20 to 39 years; group 2, 40 to 59 years; and group 3, 65+ years.

Abbreviations: BW, body weight; BSA, body surface area.

* $P < 0.05$ versus group 1, 1-way analysis of variance.

[†] $P < 0.05$ versus prediet BP, paired t -test.

Table 2
Effect of Alterations in Sodium Intake on Subjects of Different Ages on Various Indices of NO System Activity

Group	No. of Patients	P _{NOX} (μmol/L)	P _{cGMP} (pmol/mL)	U _{NOX} V (μmol/24 h)	U _{cGMP} V (pmol/24 h)
Low sodium intake (60 mEq/24 h)					
1	10	26 ± 3	3.66 ± 0.87	722 ± 129	250 ± 73
2	12	33 ± 3	4.26 ± 0.80	684 ± 57	237 ± 37
3	8	42 ± 6*	6.78 ± 1.35*	785 ± 160	164 ± 84
Normal sodium intake (130 mEq/24 h)					
1	9	31 ± 7	4.88 ± 1.90 (8)	849 ± 81	186 ± 39
2	11	30 ± 4	6.23 ± 1.23	583 ± 96*	292 ± 59
3	6	47 ± 9	7.05 ± 1.29	535 ± 37 (5)	189 ± 46
High sodium intake (240 mEq/24 h)					
1	6	19 ± 3	4.23 ± 1.36	610 ± 115	193 ± 29
2	7	20 ± 5 [†]	3.32 ± 0.41 [‡]	535 ± 37	155 ± 59
3	4	32 ± 5	8.02 ± 3.06	567 ± 132	126 ± 30

NOTE. (n) indicates the n for a specific value, where different from the group n.

Abbreviations: P_{NOX}, plasma NO_x concentration; P_{cGMP}, plasma cGMP concentration.

* Different versus group 1.

[†] Different versus low salt.

[‡] Different versus normal sodium.

Table 3
Impact of Altering Sodium Intake in Subjects of Different Ages on Several Variables

Group	No. of Patients	U _{Na⁺vi} (mEq/24 h)	BP(mm Hg)		P _{eGMP} (pmol/mL)	P _{NOX} (μmol/L)	U _{NOX} V (μmol/24 h)	U _{eGMP} V (pmol/24 h)
			Pre	Post				
Low sodium intake (60 mEq/ 24 h)	1	6	116 ± 6/74 ± 4	115 ± 6/80 ± 5	2.74 ± 0.45	26 ± 5	823 ± 194	185 ± 53
	2	6	126 ± 9/81 ± 3	124 ± 7/82 ± 3	3.04 ± 0.47	30 ± 3	763 ± 67	249 ± 51
	3	4	128 ± 12/72 ± 4	119 ± 7/71 ± 3	5.76 ± 1.59	45 ± 10	624 ± 144	53 ± 27
Normal sodium intake (130 mEq/24 h)	1	6	110 ± 4/75 ± 2	110 ± 4/75 ± 1	3.11 ± 0.99	31 ± 10	849 ± 101	181 ± 50
	2	6	120 ± 5/80 ± 3	121 ± 5/81 ± 2	3.31 ± 0.84	28 ± 6	716 ± 110	240 ± 58
	3	4	129 ± 8/75 ± 4	124 ± 5/75 ± 3	4.96 ± 1.58	35 ± 4	563 ± 160	183 ± 50
High sodium intake (240 mEq/24 h)	1	6	116 ± 3/78 ± 2	116 ± 4/77 ± 2	4.27 ± 1.36	19 ± 3	610 ± 115	193 ± 29

Group	No. of Patients	U _{Na⁺ Vⁱ} (mEq/24 h)	P _{NOX} (μmol/L)	P _{cGMP} (pmol/mL)	BP(mm Hg)		U _{NOX} V (μmol/24 h)	U _{cGMP} V (pmol/24 h)
					Pre	Post		
2	6	235 ± 12 [*]	22 ± 5	3.47 ± 0.46	121 ± 7/81 ± 4	124 ± 7/83 ± 3	551 ± 40	171 ± 68
3	4	238 ± 13 [*]	32 ± 5	2.79 ± 0.42	128 ± 7/80 ± 4	139 ± 14/79 ± 6 [†]	567 ± 132	126 ± 30

NOTE. Only subjects completing all three diets are included.
Abbreviations: P_{NOX}, plasma NO_x concentration; P_{cGMP}, plasma cGMP concentration.

^{*} Different versus group 1.

[†] Different versus low sodium intake by repeated-measures analysis of variance.

Table 4
Seven Healthy Subjects on Low and Ultrahigh Dietary Sodium Intake

Na Intake (mEq)	U _{Na^v} (μmol/min)	BW (kg)	BP(mm Hg)		U _{NOX^v} (μmol/24 h)	P _{NOX} (μmol/L)	U _{cGMP^v} (pmol/24 h)	PcGMP (pmol/mL)
			Pre	Post				
60	60 ± 4	70 ± 5	117 ± 4/80 ± 4	117 ± 4/81 ± 4	794 ± 162	31 ± 5	318 ± 116	3.4 ± 0.4
400	397 ± 13 [*]	72 ± 7	119 ± 4/73 ± 3	117 ± 3/75 ± 3	735 ± 33	17 ± 2 [*]	391 ± 127	2.7 ± 0.3

NOTE. Subjects aged 33 ± 4 years.
Abbreviations: BW, body weight; PNOX, plasma NO_x concentration; PcGMP, plasma cGMP concentration.
^{*} Low salt versus ultrahigh salt by paired *t*-test.