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Serum nitrate and nitrite levels in patients with hypertension and ischemic stroke depend on diet: A multicenter study

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ABSTRACT

Objectives: Nitric oxide, NO, is the key vasorelaxing agent produced by vascular endothelium. Stable end products of NO oxidation, nitrate and nitrite (NOx), are considered as markers of endothelial dysfunction associated with hypertension. Our goal was to compare NOx levels in patients with hypertension on controlled low nitrate diet with NOx levels in general population without dietary restrictions.

Patients and methods: The study included 1005 participants split into 8 groups: healthy subjects ($n = 28$) and patients with moderate ($n = 37$) and malignant hypertension ($n = 24$) on low nitrate diet; healthy subjects ($n = 9$) and patients with stroke ($n = 44$) on low nitrate diet; normotensive subjects ($n = 354$) and patients with elevated blood pressure ($n = 430$) and severe hypertension ($n = 79$) without dietary restrictions.

Results: In groups with controlled low NOx diet, patients with moderate and malignant hypertension had significantly ($p < 0.01$) higher serum NOx versus healthy subject (66.9 ± 18.8 μM and 75.3 ± 22.7 μM versus 52.8 ± 28.8 μM, respectively). Patients with stroke had significantly ($p = 0.02$) higher serum NOx levels versus corresponding group of healthy subjects (106.3 ± 58.4 μM versus 71.2 ± 18.1 μM, respectively). In the absence of dietary control, differences in serum NOx levels between normotensive subjects and patients with elevated blood pressure and severe hypertension were not significant ($p > 0.1$).

Conclusion: Differences in serum NOx concentrations are associated with severity of hypertension only in patients on low nitrate diet. The highest concentrations of NOx were observed in patients with stroke. In the absence of dietary control, NOx levels were not associated with presence or severity of hypertension.

Introduction

Hypertension is an important health problem affecting over 40% of adult population throughout the world. Hypertension is a risk factor for myocardial infarction and stroke. Elevated blood pressure can be caused by endothelial dysfunction manifested as various lesions of regulation of vascular tone and altered balance of vasoconstrictors and vasorelaxants [1].

Nitric oxide, NO, is one of the key vasorelaxing agents produced by vascular endothelium [2]. Under aerobic conditions in the body, nitric oxide is oxidized to its stable metabolites, nitrate and nitrite ions (NOx). NOx can be metabolized back to NO through a series of reduction steps catalyzed by various enzymes [3,4]. NOx can be exogenously consumed with nutrients, including water, fruits and vegetables, cured processed meats, and fish preserves. Thus, steady state level of NOx in the blood or serum is determined by consumption of NOx and oxidation of endogenously synthesized NO as well as by metabolism of NOx and urinary excretion of NOx [5].

NOx levels in the serum of patients with various diseases were measured and documented in hundreds of published studies. However, specificity and diagnostic or prognostic values of this parameter are poorly understood [6]. In theory, NOx can be used as a marker of endothelial dysfunction or hypertension provided that its excretion and consumption can be controlled carefully. To our knowledge, direct comparison of NOx levels in hypertensive patients with and without dietary control of nitrate consumption has not been described in the literature.

The goal of the present study was to measure NOx levels in patients with hypertension who had dietary control in a hospital setting with low consumption of NOx and to compare their values to NOx levels in general population. The data indicate that limiting NOx consumption by dietary control in patients with hypertension or stroke results in significantly elevated serum NOx versus matched health subjects. In general population without dietary control, these differences were not significant.

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Materials and methods

The study was approved by the ethics committees of all participating institutions and was performed according to local and international guidelines for clinical studies. All patients gave their informed consent. In addition to this, participants of groups 1 through 5 agreed to strictly follow diet recommendations for 5–7 days prior to withdrawal of blood and gave their consent for other procedures of the study.

Patients were treated with common antihypertensive medications including inhibitors of angiotensin-converting enzyme, beta-blockers, diuretics, and other medications as needed (statins, calcium antagonists, aspirin, etc).

Patients and diet

The study was conducted in three institutions in Russia including Pirogov Medical University in Moscow (groups 1, 2, and 3), Orenburg State Medical Academy in Orenburg (groups 4 and 5), and National Research Center for Preventive Medicine in Moscow (groups 6, 7, and 8). For basic description of the groups, see Table 1.

General exclusion criteria for all groups except group 4 (patients with stroke) included acute cardiac or cerebral events within 6 months prior to blood sampling; acute inflammation; chronic kidney failure stages III, IV, or V with glomerular filtration less than 60 ml/min/1.73 m^2; decompensated type 1 or type 2 diabetes with levels of hemoglobin glycation over 7.5%; left ventricular ejection fraction less than 40%; any oncological disease; any hematological disease affecting platelet count or blood coagulation; and any immune or autoimmune disease. In group 4, two patients had stage 1 kidney failure apparently caused by chronic pyelonephritis.

Office blood pressure was measured with an aneroid manometer. Subjects were required to stay in a sitting position for 5 min prior to the measurement. In 10 min, measurement was repeated and average value was used to diagnose hypertension as blood pressure exceeding 139/89 mm Hg according to WHO guidelines.

Groups on low NOx diet

Control group 1 in Moscow included 28 healthy volunteers (14 male and 14 female aged 30–85 years; mean 58 ± 8 years) with normal blood pressure. They were asked to specifically pay abstain from consumption of high NOx products for 5–7 days prior to withdrawal of blood for NOx assay. Their low NOx diet was low on fruits and vegetables (especially green leaf salads) and rich in grains, meat, and dairy. All kinds of processed meat (hotdogs, bacon, sausage, and similar products) were strictly forbidden.

Another control group 5 in Orenburg included 9 healthy subjects (5 male and 4 female aged 39–67 years; mean 53 ± 9 years). They were asked to follow similar nutritional guidelines but as evident from Table 1, local food habits can account for significant differences between group 1 and group 5. Thus, this group 5 was needed as a control for the stroke group 4 because group 4 was recruited in Orenburg. Orenburg is about 1500 kilometers to the southeast of Moscow and local produce there might have higher NOx contents.

Groups 2 and 3 in Moscow included 37 patients with moderate hypertension (16 male and 21 female aged 39–87 years; mean 66 ± 12 years) and 24 patients with malignant hypertension and concomitant ischemic heart disease, heart failure, or previous myocardial infarction (14 male and 10 female aged 39–87 years; mean 58 ± 11 years), respectively. The patients were maintained on the low NOx diet in the hospital setting.

Group 4 in Orenburg comprised 44 patients with ischemic stroke aged 39–83 years (mean 62 ± 11 years, 25 male, 19 female). Diagnosis was verified with brain CT or MRT, and cerebral blood flow lesions were assessed with extracranial and transcranial ultrasound. All patients had neurological and ophthalmological symptoms characteristic for ischemic stroke. These patients were maintained on the low NOx diet in the hospital setting.

Groups without dietary restrictions

Groups 6 through 8 were a part of the ongoing Stress Aging and Health in Russia (SAHR) study recruited within the prospective population cohort survey. The protocol of the study was described elsewhere and some findings have been already published [7]. In brief, the cohort was randomly selected from the pool of subjects previously enrolled in general epidemiological studies. Anamnesis was collected using a questionnaire, and diagnostic procedures including blood pressure measurement and withdrawal of a blood sample for NOx assay were conducted during two visits to the outpatient hospital of Center for Preventive Medicine in Moscow.

Group 6 comprised 354 subjects with normal blood pressure 120–139/80–89 mm Hg (55–87 years of age). Group 7 comprised 430 subjects with moderate hypertension 140–180/90–110 mm Hg (55–87 years of age). Group 8 comprised 79 subjects with severe hypertension >180/110 mm Hg (55–87 years of age). In these groups, exclusion criteria included patients with major cardiac events, diseases of the kidney, liver and endocrine system, cancer, and recent infections. Subjects in groups 6 through 8 had no dietary restrictions.

NOx assay

Blood was withdrawn from the ulnar vein after overnight fasting of at least 12 h. Serum was prepared and stored frozen at −26 °C until the assay. Concentrations of NOx were measured in deproteinized serum using Griess reaction after reduction with vanadium (III) as described originally [8] and modified by us previously [9]. All reagents for the

Table 1
Serum NOx levels in groups of subjects.

Group	Condition	*n*	Blood pressure, mm Hg systolic/diastolic	Serum NOx, mean ± SD (median), μM	Use of diuretics/nitrates, % of all patients in group[a]
Dietary control of NOx consumption					
1	Healthy	28	Normal	52.77 ± 28.77 (48.3)	None
2	Moderate hypertension	37	140–160/90–100	66.91 ± 18.82 (65.1)	15 (41%)/3 (8%)
3	Malignant hypertension	24	220–260/120–140	75.28 ± 22.66 (76.6)	10 (41%)/2 (8%)
4	Stroke	44	110–170/80–100	106.33 ± 58.44 (97.9)	8 (18%)/1 (2%)
5	Stroke control, healthy	9	normal	71.22 ± 16.13 (73.3)	None
No dietary control					
6	Normal blood pressure	354	120–139/80–89	61.76 ± 37.54 (53.6)	None
7	Elevated blood pressure	430	140–180/90–110	58.74 ± 28.31 (54.9)	94 (48%)/unknown
8	Severe hypertension	79	>180/100	54.22 ± 23.65 (51.8)	113 (59%)/unknown

[a]Diuretics included furosemide, spironolactone, and hypochlorothiazide; organic nitrates included nitroglycerine and isosorbide mononitrate.