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Original article

The Effect of Beetroot Powder (BETA-NO•-BOOST), a Natural Source of Nitrate and Nitrite Ions, on Serum Lipids and Cardiovascular and Inflammatory Markers: A Pilot Study

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Abstract: *Aim* — Beetroot is a natural source of nitrate and nitrite ions and is known to provide various benefits in healthy volunteers. The present pilot clinical study was aimed to evaluate the effects of beetroot powder (BETA-NO•-BOOST) on serum lipids and inflammatory and metabolic markers in patients with early-stage arterial hypertension to assess feasibility of a subsequent expanded randomized crossover-controlled trial.
Materials and Methods — The present study enrolled 22 male patients with early-stage AH treated for 7 days with an oral daily dose of 20 g BETA-NO•-BOOST equivalent to 650-700 mg of nitrate and nitrite ions (NOx). Blood samples were collected at baseline and at endpoint and used to assay serum levels of lipids and inflammatory and metabolic markers.
Results — Total levels of cholesterol and alanine aminotransferase were decreased by 10% and 18.5% ($p<0.05$), respectively, in all patients after 7-day use of beetroot powder. Hemoglobin concentration was decreased by 3.2% in 71% of the participants ($p=0.001$). The levels of C-reactive protein were decreased by 1.7-fold and leukocyte counts were decreased by $1.2x10^9$/L in 57% and 62% of the participants, respectively ($p<0.05$). Levels of blood glucose, creatinine, aspartate aminotransferase, triglycerides, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and platelets were decreased in more than 50% of the participants.
Conclusion — The results support feasibility of an expanded randomized crossover controlled study of BETA-NO•-BOOST, suggesting that consumption of dietary nitrate from natural plant sources may be associated with nitric oxide production to account for potential beneficial use of BETA-NO•-BOOST in various inflammatory and cardiovascular diseases.

Keywords: Pilot study, beetroot powder, inflammatory markers, arterial hypertension, BETA-NO•-BOOST, nitrate, nitrite.

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Introduction

Arterial hypertension (AH) is one of clinical manifestations of advanced stages of endothelial dysfunction attributed to dysregulation of the vascular tone and resulting in a sustained elevation of blood pressure [1]. According to World Health Organization, AH affects approximately 20% of the global population [2]. In Russian Federation, the prevalence of AH was 53.9% in 2023 [3]. Appropriate management of AH is needed to prevent high mortality due to chronic and acute cardiovascular and cerebrovascular disorders. Standard cardiovascular risk factors include hyperlipidemia, diabetes mellitus, AH, and smoking, which are associated with altered nitric oxide (NO) production, endothelial dysfunction, and eventual development of atherosclerotic lesions.

NO is one of the key paracrine and endocrine mediators responsible for normal regulations of the vascular tone, and NO deficiency may be due to reduced synthesis of NO from L-arginine or NO inactivation by reactive oxygen species produced in endothelial dysfunction or inflammation [4]. Endothelial dysfunction is one of the early stages of various cardiovascular diseases and is manifested as AH, metabolic syndrome, inflammation, or obesity [4].

The results of a number of clinical studies indicate that decreased levels of NO can be replenished by various dietary supplements providing exogenous sources of nitrate and nitrite ions (NOx) that can be directly reduced to NO through various metabolic pathways or indirectly enhance endogenous NO production or stability [5-8]. Regular consumption of exogenous NOx normalizes circulating and tissue levels of NO [6-8]. Raw beetroot is known to have a very high NOx content naturally [9]. Thus, beetroot was used to develop dry powder "BETA-NO•-BOOST" (*Figure 1*) that was used as a natural source of NOx in the present pilot clinical trial. Clinical trials of beetroot-derived nutritional supplements in Russia have not been reported previously. Thus, the present study aimed to evaluate the effect of beetroot powder on serum lipids and inflammatory and metabolic markers.

Material and Methods

Patients

The present pilot study enrolled 22 male patients based on the following inclusion criteria: male; 18-60 years of age; diagnosed with early-stage AH or compensated AH, with blood pressure levels determined by 24-h blood pressure monitoring while on a stable regimen of antihypertensive therapy; and signed informed consent for participation in the trial. Participants consumed a daily oral dose of 20 g of beetroot powder equivalent to 650-700 mg NOx for 7 days. All participants were instructed to abstain from consumption of certain vegetables (beetroot, green leafy vegetables and salads, and cabbage), preserved foods, and processed meat products for at least 12 h prior to blood sampling. The study was approved by Local Independent Ethic Committee of National Medical Research Center for Therapy and Preventive Medicine compliant with the ethical principles of the 1964 Helsinki Declaration; Approval No: 01-07124; date of registration: 28.10.2024.

The following exclusion criteria were applied: intolerance to beetroot powder; diabetes mellitus; acute or exacerbated chronic diseases; and voluntary withdrawal from the study.

Study Protocol

Patients ingested a daily oral dose of 20 g of dry beetroot powder, which was equivalent to 650-700 mg of NOx as determined previously [9], over 7 days. The participants continued their regular previously prescribed antihypertensive therapy. Blood samples were collected from the cubital vain at baseline and endpoint for analysis.

Beetroot Powder

Finely dispersed beetroot powder (BETA-NO•-BOOST) was produced by infrared dehydration and subsequent milling and provided by the Scientific and Production Center for Healthy Nutrition Technologies of Razumovsky Saratov State Medical University commissioned by National Medical Research Center for Therapy and Preventive Medicine. NOx concentrations in were quantified as described previously [9] to assess NOx equivalent of the daily dose [6].

Biochemical Assays

Blood samples were collected after fasting from the cubital vein into Vacutainer tubes (Becton Dickinson, Crawley, UK). Serum was obtained by centrifugation at 1,000 g for 20 min. at room temperature, aliquoted, and stored at -24°C until analysis.

Erythrocyte and platelet counts were determined in whole blood using an automated MEK-8222K hematology analyzer (Nihon Kohden, Japan) and Isotonac-3, Hemolynac-3N, and Hemolynac-5 reagents supplied by Nihon Kohden.

Figure 1. The Label of beetroot powder named "BETA-NO-BOOST" (in Russian).

Table 1. Hematological and biochemical parameters of the participants (N=22) at baseline and endpoint after 7-day consumption of beetroot powder at a dose of 20 g/day equivalent to 650-700 mg NOx/day

Parameters	*Reference*	*Baseline, Median (IQR 25%; 75%)*	*Endpoint, Median (IQR 25%; 75%)*	*P**
Age (years)		46.5 (40.0; 52.0)		
NOx (µM)		28.92 (22.2; 47.4)	69.76 (46.23; 105.82)	0.008
Total cholesterol (mmol/L)	<5.0	5.91 (4.95; 7.13)	5.33 (4.62; 6.21)	0.07
HDL-cholesterol (mmol/L)	>1.0	1.51 (1.30; 1.68)	1.36 (1.21; 1.53)	0.18
LDL-cholesterol (mmol/L)	See note[1]	3.92 (3.24; 4.53)	3.57 (2.97; 4.51)	0.06
Triglycerides (mmol/L)	<1.7	1.14 (1.04; 1.38)	1.09 (0.79; 1.37)	0.37
C-reactive protein (mg/L)	See note[2]	1.28 (0.86; 1.78)	0.95 (0.51; 1.79)	0.97
Creatinine (µmol/L)	65-110	90.00 (82.70; 101.30)	89.90 (83.35; 95.00)	0.06

HDL, high-density lipoprotein; IQR, interquartile range; LDL, low-density lipoprotein. *P-values were calculated using Wilcoxon matched pairs test for two dependent variables. [1] Normal LDL cholesterol levels are risk-dependent: <3.0 mmol/L for low risk; <2.6 mmol/L for moderate risk; <1.8 mmol/L for high risk; <1.4 mmol/L for very high risk; and <1.0 mmol/L for extreme risk. [2] For cardiovascular risk assessment, C-reactive protein level <1.0 mg/L is considered low risk; 1.0-3.0 mg/L indicates average risk; and >3.0 mg/L indicates high risk. A level up to 5 mg/L is considered normal in the absence of other clinical manifestations.

Table 2. Statistically significant changes in various parameters after 7-day supplementation with beetroot powder

Parameter (unit)	*Patients, %*	*Baseline, Median (IQR 25%; 75%)*	*Endpoint, Median (IQR 25%; 75%)*	*P**
HDL-cholesterol (mmol/L)	62	1.56 (1.32; 1.67)	1.29 (1.16; 1.40)	0.0008
LDL-cholesterol (mmol/L)	57	4.23 (3.40; 5.07)	3.09 (2.93; 3.97)	0.002
Triglycerides (mmol/L)	62	1.18 (1.08; 1.53)	0.89 (0.79; 1.12)	0.001
C-reactive protein (mg/L)	57	1.26 (0.97; 2.09)	0.75 (0.44; 1.17)	0.002
Creatinine (µmol/L)	62	100.8 (86.2; 104.1)	85.8 (81.4; 93.6)	0.003
Glucose (mmol/L)	62	6.0 (5.4; 6.5)	5.7 (5.1; 5.8)	0.002
AST (U/L)	66,6	26.50 (23.00; 46.00)	23.50 (21.00; 40.00)	0.002
Leukocytes (× 10^9/L)	62	7.20 (6.7; 8.00)	6.00 (5.40; 6.30)	0.002
Platelets (cells/µL)	52	250 (191; 288)	210 (174; 239)	0.003
Erythrocytes (× 10^6/µL)	52	5.10 (4.81; 5.38)	4.73 (4.7; 5.11)	0.003
Hemoglobin (g/L)	71	154.0 (149.0; 162.0)	149.0 (139.0; 155.0)	0.001

AST, aspartate aminotransferase; HDL, high-density lipoprotein; IQR, interquartile range; LDL, low-density lipoprotein. *P-values were calculated using Wilcoxon matched pairs test for two dependent variables.

Levels of total cholesterol, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were determined enzymatically using an Architect C650-7000 analyzer (Abbott, USA) and the corresponding reagent kits. Triglycerides were measured by the glycerophosphate oxidase method. High-density lipoprotein (HDL) cholesterol was quantified using the detergent-accelerated homogeneous enzymatic method. Creatinine was assayed by the Jaffe method. C-reactive protein (CRP) was measured by immunoturbidimetry. Glucose was determined by the hexokinase method. All assay kits were provided by Abbott (USA). Low-density lipoprotein (LDL) cholesterol was estimated using the Friedewald equation.

Serum NOx levels were assayed as described previously [9].

Statistical Analysis

The data were analyzed using SPSS IBM Statistics version 23 (USA) and Statistica version 7 (USA). Paired values at baseline and endpoint for continuous variables were not normally distributed, and the differences were thus evaluated by non-parametric Wilcoxon signed rank test. Data are presented as the median and 25-75percentile range. A p-value of <0.05 was considered statistically significant.

Results

The present study enrolled 22 male patients 46.5 (40.0-52.0) years of age, with a low (2.23%) cardiovascular risk according to SCORE (Systematic COronary Risk Evaluation). Patients (N=8) were prescribed antihypertensive therapy, including angiotensin II receptor blockers (36.3%; N=8), and diuretics (9%; N=2), and lipid-lowering medications, including statins (13.6%; N=3) and ezetimibe (4.5%; N=1). None of the participants were treated with anticoagulants.

Almost all biochemical parameters in the serum were within normal reference ranges at baseline, with the exception of slightly elevated total and LDL cholesterol levels (*Table 1*). Consumption of beetroot powder for 7 days resulted in a 2.4-fold increase in serum NOx (*Table 1*). The levels of total cholesterol were decreased by 0.58 mmol/L (by 10%; P<0.05), and the levels of ALT were decreased by 6.7 U/L (by 18.5%; P<0.05) in all participants. The changes in the total cholesterol and NOx levels were positively correlated with each other (Spearman rank correlation coefficient r=0.5; P=0.04). However, no other correlations between various parameters were observed at baseline or endpoint. The levels of HDL cholesterol and triglycerides were somewhat decreased at endpoint but this trend did not reach significance (P>0.05).

More than 50% of the participants demonstrated a significant decrease in inflammatory markers (*Table 2*), including CRP and leukocyte counts, which were reduced by 1.7- and 1.2-fold in 57% and 62% of the participants, respectively (*Table 2*). A significant decrease in hemoglobin levels by 5 g/L was observed in 71% of the participants, although the endpoint values remained well within the normal reference range. A decreased in the levels of glucose was observed in 62% of patients.

Discussion

This pilot study revealed a 10% decrease in the baseline levels of total cholesterol after 7 days of beetroot powder supplementation in patients with early-stage arterial hypertension.

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A positive correlation between changes in NOx and cholesterol levels was observed. However, NOx levels are known to be negatively correlated with total and LDL cholesterol levels (r=-0.40; p<0.01; and r=-0.47; p<0.003; respectively) in patients with hypercholesterolemia [10]. Another study demonstrated that prolonged supplementation with dietary nitrate did not influence plasma cholesterol levels [11]. Thus, these associations should be further assessed in an expanded crossover study.

Only certain plant-based nutritional supplements induce a decreased in cholesterol levels. For example, a study that used *Lycium barbarum* as natural plant-based source of dietary nitrate did not demonstrate an association between consumption of the supplement and cholesterol [12]. Moreover, organic juice from beet leaf and stalk was shown to have a positive effect on lipid profile parameters [13].

Supplementation of obese mice with inorganic nitrate is known to moderately influence carbohydrate and lipid metabolism [14]. In the present study, beetroot powder consumption was associated with a reduction in glucose levels by 0.3 mmol/L in 62% of patients. This finding is consistent with a study of Henstridge et al., which demonstrated that nitrate supplementation is beneficial for glucose metabolism independent of plasma insulin concentration [15].

A decrease in hemoglobin levels in 71% of patients observed in the present study is consistent with a 15% decrease shown in a study in rats treated with a nitrate supplement [16]. Other investigations reported that a similar dose of nitrate modulates tissue oxygen transport in rodents and humans [17, 18]. The mechanism of these changes may involve NO-mediated vasodilation that leads to reduced systemic oxygen demand to eventually attenuate the production of erythropoietin, which controls hemoglobin levels, resulting in downregulation of hemoglobin concentrations in the blood.

Beneficial influence of BETA-NO•-BOOST in the present study included a decrease in inflammatory markers (CRP and leukocyte counts) in more than 50% of the participants. The results of other authors support these findings indicating that nitrate supplementation significantly decreases inflammation and adverse consequences of oxidative stress [19]. This effect is partially mediated by commensal microflora that covert nitrate to NO to reduce the levels of leukocyte and platelet adhesion molecules, including ICAM-1 [19]. Additionally, NOx supplementation modulates intestinal microbiome and enhances antimicrobial activity against certain pathogens, including *Helicobacter pylori*, *Yersinia enterocolitica*, and *Salmonella enteritidis* [19].

Conclusion

Moderate consumption of plant-derived exogenous NOx induced a range of beneficial effects that may contribute to a reduction in cardiovascular risk factors. The findings of the present study demonstrated feasibility of an expanded controlled randomized crossover study. Thus, intake of nitrate-containing plant-based nutritional supplements may be beneficial for prevention and treatment of chronic cardiovascular and inflammatory diseases.

Limitations

The conclusions of the present pilot study are based on relatively small number of participants, and the results require confirmation in an expanded randomized crossover-controlled trial of beetroot powder (BETA-NO•-BOOST).

Ethical approval

The study was approved by Local Independent Ethic Committee of National Medical Research Center for Therapy and Preventive Medicine compliant with the ethical principles of the 1964 Helsinki Declaration; Approval No: 01-07124; date of registration: 28.10.2024.

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Author Contributions

Material collection and processing: N.L. Bogdanova; statistical analysis, manuscript writing, study concept and design: N.G. Gumanova; pilot testing execution, data collection, manuscript editing: K.S. Samatova; pilot testing execution, data collection: A.I. Korolev and V.S. Ososkov; administration: A.Y. Gorshkov and A.R. Kiselev; project supervision: O.M. Drapkina.

Conflict of Interest

The authors declare no conflicts of interest.

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