

Original article

## Pharmacokinetic study and phytochemical analysis of beetroot powder as an initial stage of the development of an NO-boosting formulation as a food supplement with cardioprotective properties and potential donor of nitric oxide

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**Abstract:** *Background* — Production of nitric oxide (NO) can be modulated endogenously by enzymatic synthesis of NO from L-arginine within the body or exogenously via diet. Bioactive NO levels can be influenced by adding various foods rich in nitrite and nitrate (NOx).

*Objective* — The aim of the present study was to investigate pharmacokinetics and perform phytochemical analysis of NOx as an active component in finely dispersed powder of a dried beetroot formulation to assess the shelf life that corresponds to minimal losses in the NOx contents while preserving optimal pharmacokinetic characteristics.

*Results* — The beetroot powder was manufactured by infrared dehydration of beetroot. The NOx content per dry weight of the beetroot powder was 25.2 g/kg (25,200 mg/kg), which was 10-fold higher than NOx content per kg of untreated beetroot. The data indicated that powdered beetroot can be stored at room temperature for at least a year without detectable loss in nitrate content. Tmax for NOx was 30 min after loading, and Cmax for NOx was 209 μM or 52% of the loading dose of 400 mg contained in 11 g of dry beetroot powder. The half-life of NOx delivered to the blood plasma (T1/2) was 10 h.

*Conclusion* — The recommended daily dose of the beetroot powder is 16 g, which is equivalent to 400 mg of NOx. Considering pharmacokinetic characteristics, the data indicated that the beetroot formulation is able to boost NO levels after being added to the diet and may be used to normalize the level of NOx in the blood and tissues to compensate for pathological changes in synthesis or bioavailability of endogenous NO.

**Keywords:** NO boosting, stable metabolites of nitric oxide, beetroot, exogenous sources of nitrate and nitrite ions, arterial hypertension.

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### Introduction

Discovery of endogenous nitric oxide (NO) synthesis in the body in 1980s and subsequent identification of various signaling pathways linked to NO represented one of the main achievements in cardiovascular medicine [1]. NO regulates important functions of the cardiovascular, immune, and nervous systems and is involved in blood coagulation and inflammation. Imbalance in NO levels is associated with multiple diseases linked to excessive or insufficient synthesis or bioavailability of NO.

Human body has a functional NO cycle starting from NO synthesis and followed by oxidation of NO by oxygen to form nitrite (NO<sub>2</sub><sup>-</sup>) and nitrate (NO<sub>3</sub><sup>-</sup>). Then, NO<sub>3</sub><sup>-</sup> may be reduced to NO<sub>2</sub><sup>-</sup> by various enzyme, and NO<sub>2</sub><sup>-</sup> may be further reduced to form bioactive NO [1]. These molecules have specific biological and physiological activities, some of which are associated with the regulation of the cardiovascular system [1, 2].

Biomedical studies demonstrated that NO production and levels are modulated by changes in endogenous synthesis of NO by NO-synthases from L-arginine and by exogenous consumption of

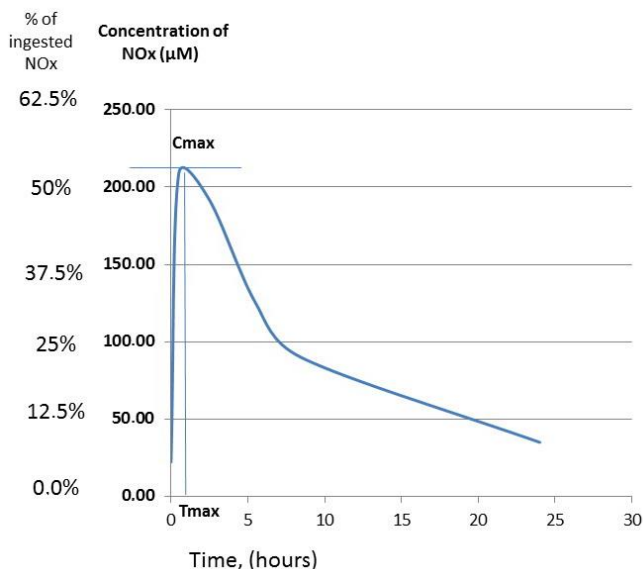
NO metabolites or precursors [1, 2]. The levels of bioactive NO are influenced by diet containing various plant-based products that have high levels of nitrate or nitrite ([Table 1](#)). Regular consumption of NOx has been shown to normalize the levels of NO in the blood and tissues and thus compensate for alterations in endogenous synthesis of NO [1, 2].

Healthy nutrition that compensates for pathological changes in NO synthesis is important for prophylaxis of cardiovascular diseases. The data shown in [Table 1](#) indicate that beetroot has the highest level of NO<sub>3</sub><sup>-</sup> [3] and is considered a suitable source for exogenous nutritional boosting of NO levels.

The goal of the present study was to facilitate the development of an NO-boosting nutritional supplement formulated from beetroot. Thus, we aimed to assay the contents of NOx in finely dispersed dried beetroot powder to determine NOx shelf life as a potential NO precursor and to determine pharmacokinetic properties of NOx in the blood within 24 h.

**Table 1. Classification of vegetables based on the NO<sub>3</sub>- ion contents [3]**

Contents of NO <sub>3</sub> <sup>-</sup> ion (mg/kg wet weight)	Vegetables
Very low (<200)	Asparagus, artichoke, beans, eggplant, garlic, onion, green beans, mushrooms, peas, pepper, potato, summer squash, sweet potato, tomato, watermelon
Low (200-500)	Broccoli, carrot, cauliflower, cucumber, squash, chicory
Medium (500-1000)	Cabbage, dill, turnip, savoy cabbage
High (1000-2500)	Celery, celery cabbage, fennel, endive, leeks, parsley
Very high (>2400)	Celery, watercress, chervil, beets, spinach, arugula



**Figure 1.** Dynamics of NO<sub>x</sub> concentrations in the serum of healthy volunteers after a single ingestion of beetroot powder solution (N=10).

## Material and Methods

### Pharmacokinetic study

The study enrolled 10 healthy volunteers who signed an informed consent and agreed to use the nutritional supplement at the dose of 11 g dissolved in water orally. The Local Ethics Committee of Saratov State Medical University, Saratov, Russia approved the protocol of the study according to Helsinki Declaration. All participants were warned to avoid consumption of vegetables (beetroot, green salad, and cabbage) and processed foods within 12 h prior to the consumption of the beetroot powder solution. Prior to the ingestion of the solution, a catheter was placed in the cubital vein, and the blood was collected at six time points: before ingestion of the solution and in 0.5, 2.5, 5.5, 8.5, and 24 h after the ingestion. The pharmacokinetic curve was constructed by plotting the mean NO<sub>x</sub> concentration in the serum for all 10 volunteers versus time.

### Serum preparation

The serum was prepared by centrifugation of the blood at 1,000 g for 20 min at room temperature and was aliquoted and stored at -24C until NO<sub>x</sub> assay.

### Beetroot powder formulation used for NO<sub>x</sub> assay

Finely dispersed beetroot powder was prepared by infrared dehydration using an infrared drying chamber IKS-70, Russia. The chamber was supplied by the Research Center of Healthy Nutrition Technology of Razumovsky Medical University, Saratov, Russia. The powder was dark-pink colored and had characteristic beetroot

odor. A total of 100 mg of the powder was dissolved in 10 ml of deionized water to prepare 10 g/l solution that was filtered through a paper filter and used to prepare dilutions for the NO<sub>x</sub> assay.

### Assay of NO<sub>x</sub> in biological media and fluids.

The method is based on diazotization reaction after reduction of nitrate to nitrite by vanadium chloride (III) in deproteinized medium. The method has been developed by us previously and registered by the Ministry of Health Care of Russian Federation in 2008 under the name "Express method for the assay of nitric oxide metabolites in biological media as markers of vascular endothelial dysfunction" (certificate AA 0001634 for the use of novel medical technology number 2008/229; October 23, 2008).

NO<sub>x</sub> was assayed in the beetroot samples twice, including immediately after receipt of the packaging from the manufacturing facility and in 12 months after storage at room temperature in a dry place.

NO<sub>x</sub> was measured by the Griess reaction after reduction with VCl<sub>3</sub> solution in 1 M HCl as described previously [4, 5]. Optical density was measured at 540 nm using a plate reader (Tecan Infinite 200 Pro, Switzerland). NO<sub>x</sub> concentrations were calculated using Magellan software (Tecan) based on a calibration curve constructed using variable nitrate concentrations from 5 to 320 μM. Serum samples were deproteinized by ultrafiltration through SPIN-X UF columns (Corning, UK)

## Results

### Assay of NO<sub>x</sub> in beetroot powder solutions

NO<sub>x</sub> concentration in 10 g/l aqueous beetroot powder solution was 2.43±0.25 mM. Assuming molecular weight of KNO<sub>3</sub> of 101.1 g/mol, the mean concentration of NO<sub>x</sub> in the solution was 0.25 g/l. Thus, the mean content of NO<sub>x</sub> was estimated to be 25.2 g/kg dry weight of the powder. The second assay was performed after 12 months of storage. In this case, the concentration of NO<sub>x</sub> was 2.40±0.32 mM. The two values were compared using Mann-Whitney test, and the P value was >0.05, indicating that the storage of the powder for 12 months did not result in significant changes in the content of NO<sub>x</sub>. Thus, the dry powder can be stored for at least 12 months.

Based on previously published studies [7-9], we have selected the dose of 400 mg of NO<sub>x</sub> for pharmacokinetic experiments in volunteers, and this dose corresponded to 16 g of the beetroot powder.

### Pharmacokinetics of the beetroot powder

The pharmacokinetic curve after the ingestion of a single dose of the beetroot powder corresponding to 400 mg of NO<sub>x</sub> was based on the mean concentrations of NO<sub>x</sub> in the serum of 10 healthy volunteers (Figure 1).

The data indicated that T<sub>max</sub> was 30 min after the ingestion of the formulation. This T<sub>max</sub> corresponded to C<sub>max</sub> of 209 μM (approximately 52% of the total ingested dose of 400 mg). Half-life of the active ingredient NO<sub>x</sub> (T<sub>1/2</sub>) was estimated to be 10 h. The rate of absorption appeared to be relatively fast and was approximately equal to 7 μM NO<sub>x</sub>/min, reaching the C<sub>max</sub> after 30 min.

### Discussion

The data of [Table 1](#) [3] and our measurements indicated that the content of NO<sub>3</sub><sup>-</sup> was 2.4 g/kg of wet beetroot weight, corresponding to a very high level. These results are in agreement with the data of another study [6] that used ionometry to measure content NO<sub>3</sub><sup>-</sup> to be 1.90 g/kg in beetroot according to the State Standard of Russian Federation number 4329-77.4. Thus, infrared dehydration increased the content of NO<sub>x</sub> in the product by an order of magnitude. Use of dry powder simplifies storage and consumption of NO<sub>x</sub> as NO precursor and is expected to facilitate further development of beetroot-based nutritional supplements.

Previous studies estimate effective doses of nitrate based on tolerance to physical load, effect on blood pressure, and other cardiovascular parameters. On average, the dose of nitrate in clinical studies was 400 mg [6-10]. This dose corresponded to approximately 16-20 g of beetroot powder used in the present study.

Beetroot-based formulations have been shown to be beneficial for physical fitness in athletes [11-13]. Positive effects of beetroot nitrate on high-load exercise tolerance were confirmed in numerous studies [14-17].

Recent DASH study (Dietary Approaches to Stop Hypertension) demonstrated that consumption of large volumes of fruits and vegetables while limiting the consumption of total fat and saturated fat lowered blood pressure compared to that in subjects fed a diet with low volume of vegetables and fruits and excessive consumption of saturated fat [18-22]. Moreover, Mediterranean diet is linked to low cardiovascular and cancer mortality compared to standard Western diet, which contains only 6% of NO<sub>x</sub> content of a typical Mediterranean diet [23-30]. These findings suggest that beneficial effects of Mediterranean diet may be due to high levels of NO<sub>x</sub> and polyphenols [25-30]. Thus, NO<sub>x</sub> supplementation in the form of a beetroot powder formulation is expected provide considerable cardiovascular health benefits.

### Conclusion

The results of the present study indicated that the content of NO<sub>x</sub> was 25.2 g/kg of dried beetroot powder. The powder used in the present study was stored for 12 months without a detectable decrease in the content of NO<sub>x</sub>. The estimated oral dose of the powder was 16-20 g corresponding to 400 mg of nitrate. The data confirm feasibility of the development of a nutritional supplement using formulation used in the present study to boost the levels of NO in the body and benefit the cardiovascular system.

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### Conflicts of interest

The authors declare no conflicts of interest.

### Author participation

Generation and collection of the data: N.L. Bogdanova; statistical analysis, conceptualization, design, editing, and writing: N. G. Gumanova; administration: A. R. Kiselev, O.M. Drapkina.

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