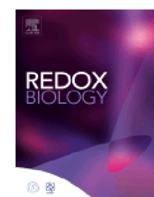
Redox Biology 5 (2015) 234–242

Contents lists available at ScienceDirect

Redox Biology

journal homepage: www.elsevier.com/locate/redox



Research Paper

Effects of long-term dietary nitrate supplementation in mice

Michael P. Hezel [a,*], Ming Liu [a], Tomas A. Schiffer [a], Filip J. Larsen [a], Antonio Checa [b], Craig E. Wheelock [b], Mattias Carlström [a], Jon O. Lundberg [a], Eddie Weitzberg [a,*]

[a] Department of Physiology and Pharmacology, Karolinska Institutet, Nanna Svartz väg 2, Stockholm 171 77, Sweden
[b] Division of Physiological Chemistry 2, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden

ARTICLE INFO

Article history:
Received 30 April 2015
Received in revised form
26 May 2015
Accepted 26 May 2015
Available online 29 May 2015

Keywords:
Nitrate
Toxicity
Long-term
Supplementation
Treatment
Survival

ABSTRACT

Background: Inorganic nitrate (NO_3^-) is a precursor of nitric oxide (NO) in the body and a large number of short-term studies with dietary nitrate supplementation in animals and humans show beneficial effects on cardiovascular health, exercise efficiency, host defense and ischemia reperfusion injury. In contrast, there is a long withstanding concern regarding the putative adverse effects of chronic nitrate exposure related to cancer and adverse hormonal effects. To address these concerns we performed in mice, a physiological and biochemical multi-analysis on the effects of long-term dietary nitrate supplementation.
Design: 7 week-old C57BL/6 mice were put on a low-nitrate chow and at 20 weeks-old were treated with $NaNO_3$ (1 mmol/L) or NaCl (1 mmol/L, control) in the drinking water. The groups were monitored for weight gain, food and water consumption, blood pressure, glucose metabolism, body composition and oxygen consumption until one group was reduced to eight animals due to death or illness. At that point remaining animals were sacrificed and blood and tissues were analyzed with respect to metabolism, cardiovascular function, inflammation, and oxidative stress.
Results: Animals were supplemented for 17 months before final sacrifice. Body composition, oxygen consumption, blood pressure, glucose tolerance were measured during the experiment, and vascular reactivity and muscle mitochondrial efficiency measured at the end of the experiment with no differences identified between groups. Nitrate supplementation was associated with improved insulin response, decreased plasma IL-10 and a trend towards improved survival.
Conclusions: Long term dietary nitrate in mice, at levels similar to the upper intake range in the western society, is not detrimental.
© 2015 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Introduction

Dietary intake of inorganic nitrate has for more than half a century been considered potentially harmful due to its bioconversion to nitrite and carcinogenic nitrosamines [1,2]. After the discovery of nitric oxide (NO) as a biological signaling molecule nitrate and nitrite have been used as stable surrogate markers of NO synthase-derived NO [3]. More recently an alternative pathway of NO generation was described in which serial reduction of nitrate and nitrite to NO and other bioactive nitrogen intermediates occurs [4]. This NO synthase (NOS) independent pathway relies on retention of nitrate anions through salivary concentration from the plasma. Nitrate is reduced to nitrite by oral commensal bacteria, swallowed, absorbed and further reduced to NO and other bioactive nitrogen oxides in blood and tissues [5,6]. A number of studies have demonstrated robust NO-like bioactivity after ingestion of nitrate. These include a reduction in blood pressure [7,8], inhibition of platelet aggregation [8,9], improved vascular function [10] and increased mitochondrial efficiency [11]. The crucial involvement of oral bacteria in nitrate bioactivation is evident from experiments using an antibacterial mouthwash, or if the test persons avoid swallowing of saliva after nitrate ingestion. Under these conditions the nitrate effects are greatly reduced or lost [8,12–14]. A paradox regarding the proposed harmful effects of dietary nitrate is the fact that vegetables are the greatest source of this anion. Long term longitudinal studies have specifically identified

Abbreviations: ACh, acetylcholine; ADMA, asymmetric dimethylarginine; AU, arbitrary units; AUC, area under the curve; BP, blood pressure; cGMP, cyclic guanidine monophosphate; DeXA, Dual energy X-ray absorptiometry; eNOS, endothelial nitric oxide synthase; HOMA-IR, homeostatic model assessment of insulin resistance; IL-6, interleukin-6; IL-10, interleukin-10; iNOS, inducible nitric oxide synthase; IPGTT, intraperitoneal glucose tolerance test; IPITT, intraperitoneal insulin tolerance test; MAP, Mean arterial pressure; NADPH, nicotinamide adenine dinucleotide phosphate; NO, nitric oxide; NOS, nitric oxide synthase; NaCl, sodium chloride; $NaNO_3$, sodium nitrate; NO_3^-, nitrate; PE, phenylephrine; SDMA, symmetric dimethylarginine; SEM, standard error of measure; SNP, sodium nitroprusside; T3, triiodothyronine; T4, thyroxine

* Corresponding authors.
E-mail addresses: Michael.Hezel@ki.se (M.P. Hezel), Eddie.Weitzberg@ki.se (E. Weitzberg).

http://dx.doi.org/10.1016/j.redox.2015.05.004
2213-2317/© 2015 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

green leafy vegetable intake, which is the dominant nitrate source, as inversely proportional to the risk of diabetes in aging females and cardiovascular disease in males [15,16].

The recent studies showing beneficial cardiovascular and metabolic effects of dietary nitrate in animal models and humans have been short term or acute. Other long term dietary studies have been performed investigating the putative toxicity of nitrate and nitrite using high doses of these anions [17–21]. This study aimed at determining the long term effects of dietary nitrate intake in mice at doses similar to what can be achieved in humans by a diet rich in vegetables. During nitrate or matched sodium chloride supplementation of approximately 18 months, we looked at the parameters for metabolic, cardiovascular and inflammatory differences.

Materials and methods

Animals

All animal procedures in this study were in strict adherence to the *Guide for the Care and Use of Laboratory Animals* as adopted by the U.S. National Institutes of Health and were approved by the Regional Animal Care and Use Committee of Stockholm, Sweden. Six week-old male C57Bl/6 mice ($n=27$) were obtained from Charles River (Sulzfeld, Germany). After 1 week acclimatization the animals were fed low nitrate chow (0.06 μg/g NO_3, D06041501DW, Research Diets, New Brunswick, NJ, USA) and housed in rooms at 22 °C with a 12/12 day-night cycle. After 3 months, the animals were separated into similar weight groups of 14 and 13 with 1 mM sodium chloride (NaCl) or 1 mM sodium nitrate ($NaNO_3$) respectively in the drinking water. Over this period, weight and food and water uptake were monitored (Fig. 1). The health of the animals was monitored using an institutional physiological checklist and if behavior resulted in a combined score of 0.4 the animal was sacrificed and blood and organs collected. Seven animals were sacrificed due to poor health and four animals died of natural causes during the experiment.

Materials

All chemicals not specified were obtained from Sigma-Aldrich at the highest grade available.

Body composition

Body composition by dual energy X-ray absorptiometry (DeXA) was measured under isoflurane anesthesia using the PIXImus imager (General Electric, Niskayuna, NJ, USA) according to manufacturer's instructions [22].

Intraperitoneal glucose tolerance test

Animals were starved for 6 h, separated individually, fasting blood glucose recorded (Freestyle Lite, Abbott Diabetes Care, Alameda, CA, USA). Following an intraperitoneal injection of D-glucose (2 g/kg BW) blood glucose was measured at 15, 30, 60 and 120 min [22].

Intraperitoneal insulin tolerance test

Under non-fasting conditions the mice were separated and initial blood glucose measurement was taken from the tail, an intraperitoneal insulin load (0.75 IE kg^{-1}) was administered and blood glucose was measured at 15, 30, 45, 60, 75, 90, and 120 min [22].

Blood pressure

Blood pressure was monitored using the Coda High Throughput Noninvasive Tail Monitoring System (Kent Scientific, Torrington, CT, USA) following the manufacturer's protocol. Systolic, diastolic and mean arterial pressure measurements were collected over 5 consecutive days and averaged [22].

Indirect calorimetry

Individual mouse oxygen consumption was measured using an INCA indirect calorimeter (Somedic, Horby, Sweden). Animals were placed in the equipment between 5 pm and 6 pm and underwent 36 h at 24 °C and 24 h at the thermoneutral 34 °C maintaining a 12/12 light dark cycle consistent with animal room timing.

Mitochondrial isolation

After euthanizing the mouse, the soleus muscle was extracted, weighed and immediately added to 1 mL of ice-cold isolation medium: 100 mM sucrose, 100 mM KCl, 50 mM Trizma–HCl, 1 mM KH_2PO_4, 0.1 mM EGTA and 0.2% BSA (weight). The sample was homogenized with a pair of scissors ($\approx$5 min) and left for sedimentation. The supernatant was removed and the sample was washed by adding new isolation medium again followed by sedimentation. This procedure was repeated twice whereupon isolation medium containing bacterial protease (0.2 mg/mL) was added and the sample was left on ice for 30 s followed by low intensity

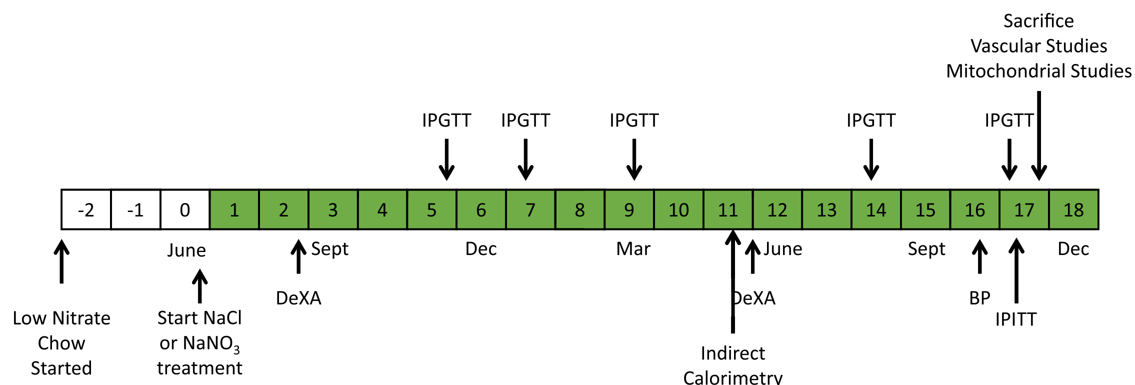


Fig. 1. Experimental timeline. Diagram of the experimental procedures on the mice over the course of the experiment. The green squares indicate period of supplementation. NaCl, sodium chloride; $NaNO_3$, sodium nitrate; DeXA, dual energy X-ray absorptiometry; IPGTT, intraperitoneal glucose tolerance test; BP, blood pressure; IPITT, intraperitoneal insulin tolerance test.

Download English Version:

https://daneshyari.com/en/article/1923159

Download Persian Version:

https://daneshyari.com/article/1923159

Daneshyari.com