

Effects of dietary nitrate on oxygen cost during exercise

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Abstract

Aim: Nitric oxide (NO), synthesized from L-arginine by NO synthases, plays a role in adaptation to physical exercise by modulating blood flow, muscular contraction and glucose uptake and in the control of cellular respiration. Recent studies show that NO can be formed *in vivo* also from the reduction of inorganic nitrate (NO_3^-) and nitrite (NO_2^-). The diet constitutes a major source of nitrate, and vegetables are particularly rich in this anion. The aim of this study was to investigate if dietary nitrate had any effect on metabolic and circulatory parameters during exercise.

Method: In a randomized double-blind placebo-controlled crossover study, we tested the effect of dietary nitrate on physiological and metabolic parameters during exercise. Nine healthy young well-trained men performed submaximal and maximal work tests on a cycle ergometer after two separate 3-day periods of dietary supplementation with sodium nitrate ($0.1 \text{ mmol kg}^{-1} \text{ day}^{-1}$) or an equal amount of sodium chloride (placebo).

Results: The oxygen cost at submaximal exercise was reduced after nitrate supplementation compared with placebo. On an average VO_2 decreased from 2.98 ± 0.57 during CON to $2.82 \pm 0.58 \text{ L min}^{-1}$ during NIT ($P < 0.02$) over the four lowest submaximal work rates. Gross efficiency increased from 19.7 ± 1.6 during CON to $21.1 \pm 1.3\%$ during NIT ($P < 0.01$) over the four lowest work rates. There was no difference in heart rate, lactate [Hla], ventilation (VE), VE/VO_2 or respiratory exchange ratio between nitrate and placebo during any of the submaximal work rates.

Conclusion: We conclude that dietary nitrate supplementation, in an amount achievable through a diet rich in vegetables, results in a lower oxygen demand during submaximal work. This highly surprising effect occurred without an accompanying increase in lactate concentration, indicating that the energy production had become more efficient. The mechanism of action needs to be clarified but a likely first step is the *in vivo* reduction of dietary nitrate into bioactive nitrogen oxides including nitrite and NO.

Keywords calorie, electron transport, energy expenditure, glycolysis, metabolism, mitochondria, nitric oxide, nitrite, uncoupling protein, VO_2 .

Physiological adaptation to exercise involves major cardiovascular and metabolic changes. Oxygen consumption increases dramatically in the active muscles with a parallel increase in the muscle blood flow. In these processes, the endogenous gas nitric oxide (NO) plays an important regulatory role. NO increases blood flow to the muscles and modulates muscular contraction

and glucose uptake (for review see Stamler & Meissner 2001). In addition, it is involved in the control of cellular respiration through interaction with enzymes of the mitochondrial respiratory chain (for review, see Moncada & Erusalimsky 2002).

In vitro studies published in the 1990s showed that NO was a modulator of mitochondrial respiration via

the pedal system the subjects were familiar with from training. The bicycle ergometer was computer-controlled, permitting a constant work rate regardless of the cadence the subject chose to pedal with. The pedalling cadence was individually chosen in the range of 70–90 rpm but each individual held exactly the same preferred cadence during the NIT and the CON trials. This was to minimize differences in work output due to changes in muscle recruitment patterns.

Pulmonary ventilation (V_E), oxygen uptake (VO_2), CO_2 output (V_{CO_2}) and respiratory exchange ratio (RER) were measured at 10 s intervals by a computerized gas analyser (AMIS 2001, Odense, Denmark) connected to a flow meter which the subjects breathed through via a mouthpiece and a plastic tube. Heart rate (HR) was continuously recorded during the tests with a portable heart rate monitor (Polar S610; Polar, Kempele, Finland). Capillary blood samples (20 μ L) were collected from the fingertip and were analysed for lactate ([Hla]) using a Biosen C-Line Sport Analyser (EKF Diagnostics, Magdeburg, Germany). Haemoglobin concentration ([Hb]) at rest was determined with the capillary blood taken from the fingertip and analysed with an Hb-measuring device (Hemocue, Ångelholm, Sweden). Haematocrit (Hct) was determined by centrifuging the capillary blood at 15133g for 3 min.

Pre-tests

Each subject attended the laboratory twice within a 2-week period before the first main tests. The first pre-test was carried out to familiarize the subject with the bicycle ergometer and the testing procedure. The subjects did a preliminary test at five submaximal levels with every level lasting for 5 min. There was no rest between the different submaximal levels. VO_2 was continuously measured with the AMIS 2001. At the end of each submaximal level capillary blood was taken from the fingertip and analysed for [Hla]. At every work rate the subjects rated their perceived exertion on the Borg's RPE-scale (Borg 1970), and both the central and muscular exertion were rated. After 8 min of recovery, the subject was instructed to cycle for as long as possible at a work rate corresponding to his calculated maximal oxygen uptake (Åstrand & Rodahl 1970). During this test the subjects actual VO_{2peak} was measured and if the subject was able to cycle for longer than 7 min extra power of 20–30 W was added every minute until exhaustion. One and three minutes after the maximal test capillary blood was sampled from the fingertip for analysis of [Hla].

Before the second pre-test, the submaximal levels were adjusted so that they corresponded to 45, 60, 70, 80 and 85% of VO_{2peak} . The maximal work rate was

also adjusted, if necessary, so that the time to exhaustion was kept between 4 and 7 min.

The main tests

The subjects refrained from heavy exercise 3 days prior to the main tests and avoided all exercise the day before the tests. They were also told to eat their last light meal at least 3 h before the start of the tests. When the subjects came to the laboratory they received their last dose of either placebo or nitrate and were allowed to rest in the supine position for 60 min before the test commenced.

All subjects used a standardized warm up procedure of 5 min of cycling at 100 W followed by 5 min of rest. The submaximal and maximal tests were performed in the same way as the second pre-test with five submaximal work rates lasting 5 min each, without rest between the different levels. Identical work rates were used during the two main tests. Venous blood (9 mL) was drawn at rest 45 min after the last nitrate/placebo-dose was ingested and again immediately after the VO_{2peak} test. The blood was placed in an ice bath and centrifuged within 5 min at 1300 rpm and 4°C. The plasma was separated and kept at –80°C until it was analysed for its nitrate and nitrite concentrations by a chemiluminescence assay as described previously (Lundberg & Govoni 2004).

Statistics and calculations

Results are expressed as mean \pm standard deviation (mean \pm SD). Submaximal work parameters at multiple work rates were analysed by ANOVA with two-way repeated measures. Paired *t*-tests were used to evaluate the difference between the nitrate and the placebo trials when appropriate. The significance level was set as $P < 0.05$.

Gross efficiency (GE) was defined as the work rate divided by the actual energy expenditure (EE). The EE was in turn calculated with the Brouwer equation (Brouwer 1957).

Delta efficiency (DE) was defined as the increase in work rate, divided by the increase in EE (Gaesser & Brooks 1975). The DE was based on the four lowest work rates which were analysed with linear regression. The oxygen pulse is defined as VO_2/HR .

Results

Blood pressure at rest

The average resting systolic blood pressure was lower after nitrate supplementation (112 ± 8 mmHg) compared with placebo (120 ± 5.9 , $P < 0.01$). The diastolic blood pressure was also lower after nitrate (68 ± 5.5

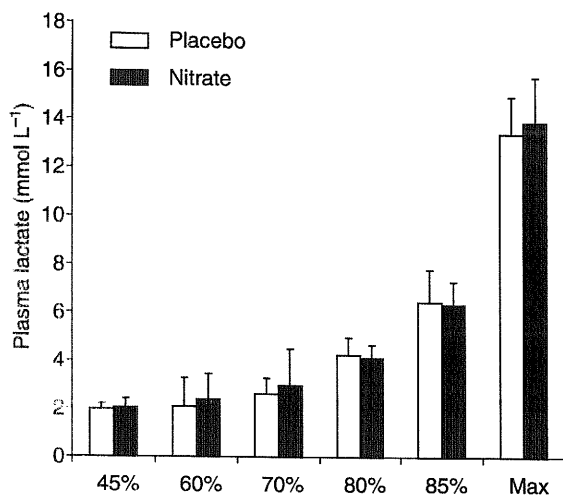


Figure 3 Plasma lactate concentration measured at six different work rates (% of VO_{2peak}) after dietary supplementation with sodium nitrate ($0.1 \text{ mmol kg}^{-1} \text{ day}^{-1}$ for 3 days, filled bars) or an equal amount of sodium chloride (placebo, empty bars).

The chosen cadence varied slightly within the group (mean 82 ± 6 rpm at all submaximal workloads), but was identical for every individual on the two occasions (NIT and CON). Mean work rate was 140 ± 13 W at 45% VO_{2peak} , 190 ± 17 W at 60% VO_{2peak} , 229 ± 21 W at 70% VO_{2peak} , 261 ± 26 W at 80% VO_{2peak} , and 288 ± 26 W at 85% VO_{2peak} .

Maximal work capacity

There was no significant difference in the VO_{2peak} between the NIT and CON trials (4.49 ± 0.44 and $4.61 \pm 0.28 \text{ L min}^{-1}$, respectively, $P = 0.29$, t -test). These values were also not significantly different from the VO_{2peak} achieved during the pre-test ($4.54 \pm 0.32 \text{ L min}^{-1}$). Likewise, no significant differences were noted either in $V_{E_{max}}$ (NIT 182 ± 21.4 vs. CON $186 \pm 21.7 \text{ L min}^{-1}$, $P = 0.5$, t -test), HR_{max} (NIT 189.8 ± 7.0 vs. CON $190.3 \pm 7.5 \text{ beats min}^{-1}$, $P = 0.94$, t -test) or maximal work rate (NIT 360.6 ± 32.8 vs. CON $358.9 \pm 32.3 \text{ W}$, $P = 0.35$, t -test). There was no difference between NIT and CON in the rating of perceived exertion (Borg RPE-scale) at any workload (submaximal or max).

Cadence at VO_{2peak} was identical in CON and NIT (86 ± 6 rpm). The workload at VO_{2peak} was 361 ± 33 W for NIT and 359 ± 32 W for CON.

Comment to the results

In the present study a significantly reduced oxygen demand at the four lowest submaximal work rates was noted after nitrate administration. The fifth work rate,

at approx. 85% VO_{2peak} , was well above the lactate threshold in several subjects and thus the anaerobic energy production became more pronounced. This led to the involvement of accessory muscle groups and a noticeable change in motion pattern. At this work rate the VO_2 did not reach a stable steady-state level (in the majority of the subjects the VO_2 increased more than 150 mL min^{-1} during the last 3 min of the work rate) and is therefore unsuitable for the calculation of muscular efficiency. During the four lowest work rates the VO_2 reached a stable steady-state in all subjects. The reason for including the fifth work rate in the protocol was to receive a lactate value above the lactate threshold and thereby get an indication of changes in the upper part of the lactate curve.

Discussion

One of the fundamentals of exercise physiology is the remarkably tight coupling between oxygen uptake and workload during submaximal cycle ergometer exercise (Åstrand & Rodahl 1970). The consumption of oxygen at a given workload is near identical between different individuals or when measured in the same individual at different occasions. If the findings of the present study hold true, it is possible that this textbook knowledge will have to be revised. We show that dietary supplementation with inorganic nitrate, in an amount achievable through a diet rich in vegetables, results in a reduced VO_2 during submaximal work and a significant increase in muscular efficiency. These highly surprising effects occurred without any changes in the maximal attainable work rate. We are not aware of any previously described dietary or pharmacological approaches to decrease oxygen consumption during exercise.

At this early stage the mechanism behind the effects of dietary nitrate can only be speculated upon. Nevertheless, there is reason to believe that the observed effects involve initial reduction of nitrate to nitrite. Nitrate itself is believed to be biologically inert and cannot be metabolized by mammalian cells. However, after ingestion nitrate re-enters the mouth via the salivary glands and is effectively reduced by commensal bacteria thereby forming nitrite. In contrast to nitrate, the nitrite ion was recently shown to possess a wide range of bioactivities (Lundberg *et al.* 2004, Gladwin *et al.* 2005, Lundberg & Weitzberg 2005). In this study, we did indeed note an increase in plasma nitrite after the nitrate treatment period thereby confirming *in vivo* reduction of nitrate as described previously (Lundberg & Govoni 2004, Larsen *et al.* 2006). Another finding in support of nitrite being bioactive was its effective consumption during exercise in contrast to the unchanged levels of plasma nitrate. Ultimately the bioactivity of nitrite is likely related to its

ence of training and competing. It is improbable that a few visits to the laboratory would change their efficiency during cycling to any noteworthy extent. The fact that the subjects used the same cycling shoes, clip-on pedals and the same seat position as they were used to during training makes this even more unlikely. Most importantly, the randomization procedure used in this study rules out any such differences. Marechal & Gailly (1999) demonstrated a faster relaxing velocity of muscle fibres in *in situ* experiments during administration of an NO-donor, thereby implicating a neuromuscular modulatory effect of NO. It remains to be proven if this can improve the muscular efficiency during cycling.

The finding that the oxygen pulse at a given work rate decreases by nitrate supplementation is a direct effect of the lower oxygen demand at that work rate. However, there is no difference in oxygen pulse at a given absolute oxygen uptake. The lack of effect of nitrate on VE/VO₂ or oxygen pulse indicates that the improved efficiency originates from muscular or mitochondrial adaptations rather than from central adaptations in the heart or the lungs.

In contrast to submaximal work we found no significant effects of nitrate supplementation on the metabolic variables during maximal work. This may be explained by changes in physiological functions at a maximum level of effort compared with a submaximal level. During maximal or near maximal work, the modulatory physiological effects by nitrate are likely to be overridden by emergency reactions. However, we cannot exclude that the lack of effect during maximal work is due to a depletion of the systemic nitrite stores. In addition, we cannot exclude that there is a true difference also at the highest workloads that would have been revealed in a larger group of study subjects.

There are a number of remaining questions that need to be addressed in future studies besides revealing the exact mechanism of action for the effects of dietary nitrate. First, the importance of gender, age, physical fitness and disease is unclear as we only included young healthy well-trained men. Second, we do not know if the effects are limited to physical exercise or if nitrate also modulates resting energy expenditure, and we also need to explore if the effect is specific for a certain tissue or a population of mitochondria. Third, it is possible that an 'NO-like' bioactivity such as that of nitrate, also could effect substrate utilization. Fourth, nitrate intake varies greatly in the population and between different cultures and it will be important to establish the dose-effect ranges and the duration of effects.

In summary, our findings show a lower oxygen cost during submaximal work after dietary supplementation with nitrate, in amounts achievable through a diet rich in vegetables. This remarkable effect occurred without an accompanying increase in plasma lactate, indicating

that the energy production had become more efficient. The mechanism of action and main targets need to be clarified but the process likely involves *in vivo* reduction of nitrate into bioactive nitrogen oxides including nitrite and NO.

Conflict of interest

The authors declare no conflict of interest.

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