Effect of dietary nitrate supplementation on metabolic rate during rest and exercise in human: A systematic review and a meta-analysis

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ABSTRACT

Background: Recent randomized controlled trials have suggested that dietary nitrate (NO3⁻), found in beetroot and other vegetables, and inorganic NO3⁻ salts decrease metabolic rate under resting and exercise conditions.

Objective: Our aim was therefore to determine from a systematic review and meta-analysis whether dietary NO3⁻ supplementation significantly reduces metabolic rate, expressed as oxygen uptake (VO2), under resting and exercise conditions in healthy humans and those with cardiorespiratory diseases.

Design: A systematic article search was performed on electronic databases (PubMed, Scopus and Web of Science) from February to March 2015. The inclusion criteria included 1) randomized controlled trials; 2) studies reporting the effect of NO3⁻ on VO2 under resting and/or exercise conditions; 3) comparison between dietary NO3⁻ supplementation and placebo. Random-effects models were used to calculate the pooled effect size.

Results: Twenty nine randomized placebo-controlled trials were included in the systematic review, and 26 of which were included in the meta-analysis. Dietary NO3⁻ supplementation significantly decreases VO2 during submaximal intensity exercise [0.26 (95% IC: 0.38, 0.15), p < 0.01], but not in the sub-analysis of subjects with chronic diseases [0.09 (95% IC: 0.50, 0.32), p = 0.67]. When data were separately analyzed by submaximal intensity domains, NO3⁻ supplementation reduces VO2 during moderate [0.29 (95% IC: −0.48, −0.10), p < 0.01] and heavy [−0.33 (95% IC: −0.54, −0.12), p < 0.01] intensity exercise. When the studies with the largest effects were excluded from the meta-analysis, there is a trend for a VO2 decrease under resting condition in dietary NO3⁻ supplementation [−0.28 (95% IC: −0.62, 0.05), p = 0.10].

Conclusion: Dietary NO3⁻ supplementation decreases VO2 during exercise performed in the moderate and heavy intensity domains in healthy subjects. The present meta-analysis did not show any significant effect of dietary NO3⁻ supplementation on metabolic rate in subjects with chronic diseases, despite enhanced exercise tolerance.

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1. Introduction

Low oxygen availability limits the sustainable metabolic rate in a wide range of conditions, such as in chronic cardiorespiratory diseases, environmental hypoxia or in athletes when oxygen carrying capacity reaches its maximal during intense exercise [1–3]. Metabolic rate is minimal under resting, thermoneutral, and post-absorptive conditions, comprising the energy expenditure required to sustain vital body functions [4]. Brain, liver, heart, and kidneys account for 60–70% of resting energy expenditure, whereas skeletal muscles account for 20–30% in humans [5]. During exercise, metabolic rate can be increased up to 20-fold during maximal intensity exercise in athletes to meet the energy needs of working muscles [4]. Metabolic rate is commonly expressed as the rate of oxygen uptake (VO2), which reflects whole body oxidative metabolism and is measured by indirect calorimetry [6]. There is however little possibility to reduce resting metabolic rate, or metabolic rate for a given work rate during exercise [7]. Nitric oxide (NO) is an ubiquitous signaling molecule produced through the NO synthase pathway that possesses the ability to lower metabolic rate through an increase in mitochondrial efficiency caused by factors such as the inhibition of enzyme cytochrome c oxidase activity by NO, or the inhibition of uncoupled respiration [8]. NO synthesis through the nitric oxide synthase (NOS) pathway is however dependent on L-arginine and oxygen availability, and becomes limited in hypoxia [9]. However, dietary nitrate (NO3) found in abundance in green leafy and root vegetables have been shown to represent an important alternative source of NO [10]. Dietary NO3 are absorbed from the small intestine, and after an entero-salivary recirculation and concentration into the saliva, are converted into nitrite (NO2) by oral NO3 reductase bacteria [10]. The swallowed salivary NO2 are either reduced to NO and other nitrogen species in the acidic stomach or absorbed from the intestine [10].

In humans, increased NO bioavailability was first shown to result in decreased VO2 during exercise [7,11,12]. Recently, Larsen et al. (2014) also showed in a randomized controlled trial that resting metabolic rate is reduced by 4.2% in healthy subjects following a 3-day dietary NO3 supplementation [13]. The decrease in resting metabolic rate with dietary NO3 supplementation was proposed to mimic the beneficial effect of calorie restriction or resveratrol supplementation, which were previously shown to improve metabolic health parameters [14,15]. A number of studies have also been conducted in patients with chronic disease conditions that severely impair oxygen delivery and/or utilization, such as chronic obstructive pulmonary disease, heart failure, or peripheral arterial disease [16–20].

Our purpose was therefore to conduct a systematic review and meta-analysis of the randomized controlled trials exploring the effect of inorganic NO3 supplementation on the metabolic rate, expressed as VO2, under resting and/or exercise conditions. A secondary purpose of the meta-analytical procedures was to determine factors, such as population characteristics, supplementation duration, and dietary NO3 dose that could be responsible of the hypothesized changes in metabolic rate.

2. Methods

The systematic review and the meta-analysis were conducted according to the established guidelines in Cochrane Handbook for Systematic Reviews of Interventions, and were reported according to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines [21]. The criteria of PICOS (Participants, Interventions, Comparators, Outcomes, Study design) approach are presented in Table 1.

2.1. Type of studies

Randomized clinical trials that investigated the effect of dietary NO3 under the form of inorganic NO3 salts or present in beetroot...
The studies included achieved increased dietary NO3 intake by consumption of beetroot juice rich in inorganic NO3 or nitrate salts dissolved in aqueous solutions (potassium nitrate or sodium nitrate). Supplementation duration, dietary NO3 dosage, adherence to protocols, dropout rate, duration of the washout period between dietary NO3 and placebo supplementation, and adverse events. Participant characteristics were also extracted with the following information whenever available: age, number of male and female subjects, health status (healthy, chronic obstructive pulmonary disease, heart failure, and peripheral arterial disease), anthropometric characteristics, physical activity or exercise training level and maximal oxygen uptake (VO2max) to reflect aerobic fitness.

2.2. Type of interventions

The studies included achieved increased dietary NO3 intake by consumption of beetroot juice rich in inorganic NO3 or nitrate salts dissolved in aqueous solutions (potassium nitrate or sodium nitrate). Supplementation duration, dietary NO3 dose, type of supplementation (beetroot juice or salts) were parameters used as potential sources of heterogeneity in random-effects models. For studies where metabolic rate was assessed during exercise, we defined the exercise intensity domain as moderate, heavy, or severe based on the existing literature [22,23] in order to determine whether the effect of dietary NO3 supplementation is affected by exercise intensity. Studies where exercise was performed in the moderate, heavy and severe intensity domains were also pooled together for a separate analysis of metabolic rate during submaximal intensity exercise (<VO2max). Metabolic rate measured during maximal exercise tests were included in a specific random-effects model. The criteria used to determine the exercise intensity are presented in Table 2.

Inorganic NO3 salts and beetroot juice supplementation were included in the same meta-analysis to evaluate the pooled effect size, because they provide similar amount of bioavailable NO3. However, because beetroot is rich in antioxidants and polyphenols that affect NO metabolism, and inhibit nitrosative stress [10], inorganic NO3 salts and beetroot juice supplementation were subsequently separated for subgroups meta-analyses to assess potential differences between the two forms of supplementation.

### Table 1

Description of PICOS approach used in the systematic review and meta-analysis.

<table>
<thead>
<tr>
<th>Components</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Participants</td>
<td>Adult (&gt;18 years with or without chronic disease conditions that severely impair oxygen delivery and/or utilization in particular.</td>
</tr>
<tr>
<td>Interventions</td>
<td>Increased dietary NO3 intake by consumption of vegetables rich in NO3 such as beetroot, or nitrate salt solutions without any restriction regarding supplementation characteristics.</td>
</tr>
<tr>
<td>Comparators</td>
<td>Placebo with negligible nitrate content</td>
</tr>
<tr>
<td>Outcomes</td>
<td>Metabolic rate expressed as VO2</td>
</tr>
<tr>
<td>Study design</td>
<td>Randomized controlled trials</td>
</tr>
<tr>
<td></td>
<td>Cross-over design</td>
</tr>
<tr>
<td></td>
<td>Parallel design</td>
</tr>
<tr>
<td></td>
<td>Double- or single-blind, or otherwise</td>
</tr>
</tbody>
</table>

NO3: nitrate; VO2: oxygen uptake.

### Table 2

Criteria for the determination of exercise intensity domains.

<table>
<thead>
<tr>
<th>Intensity domains</th>
<th>Physiological demarcation</th>
<th>Corresponding % VO2max or aerobic peak power</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moderate</td>
<td>Below the gas exchange threshold</td>
<td>&lt;60%</td>
</tr>
<tr>
<td>Heavy</td>
<td>Between the gas exchange threshold and the critical power</td>
<td>From 60 to 80%</td>
</tr>
<tr>
<td>Severe</td>
<td>Above critical power</td>
<td>&gt;80%</td>
</tr>
<tr>
<td>Maximal</td>
<td>Exercise performed up to subject’s exhaustion with the attainment of VO2max or VO2peak</td>
<td></td>
</tr>
</tbody>
</table>

The critical power is defined as the intensity above which the attainment of VO2max is elicited, which is maintained up to exhaustion.

#### 2.3. Type of outcome measures

The primary outcome of the meta-analysis was the change in metabolic rate expressed as absolute or relative VO2 (mL/min⁻¹, L/min⁻¹, mL/min⁻¹ kg⁻¹) under resting and exercise conditions in response to dietary NO3 supplementation. Secondary outcome were the changes in NO3 and NO2 plasma concentrations after dietary NO3 supplementation compared to placebo condition.

#### 2.4. Search strategy

Studies were identified by searching electronic databases and screening reference lists of articles. The systematic review was limited to the articles published in English, and no limits were applied for publication date. The main search was conducted to PubMed, Scopus and Web of Science and was undertaken from February 2015 to March 2015. The following search terms were used in all electronic databases: dietary, exogenous, infusion, inorganic, nitrate, nitrite, beetroot, beet root, oxygen uptake or consumption, metabolic rate and energy expenditure.

#### 2.5. Study selection and data extraction

Titles, abstracts and articles screening was carried out separately by two reviewers (M.P.C., J.A). Firstly, the viewing stage consisted in analysis of titles and abstracts. Reference lists of the articles that met the eligibility criteria were also searched for additional eligible articles. Uncertainties about the eligibility of a study were resolved by consensus.

Data extraction was realized by two authors using data collection sheet, with one author in charge of data extraction, and the second author checked the collected data. Corresponding authors were contacted for missing data when necessary.

#### 2.6. Data analysis

The complete meta-analysis of outcome measures was run through Review Manager Software (Review Manager (RevMan) [Windows 7, Microsoft]. Version 5.3. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014.). DerSimonian and Laird random-effects models were used to estimate the pooled effect size.
effect size for VO2 under resting and exercise conditions. Data are reported as standardized mean differences for VO2 at rest and during exercise with 95% Confidence Interval (CI), and presented in forest plots. The threshold for statistical significance was set at \( p = 0.05 \). Heterogeneity among studies was evaluated using the Cochrane’s Q test \((p < 0.10)\) and \(I^2\) values (low \(\geq 25\%), moderate \(\geq 50\%), high \(\geq 75\%)\), and between-studies variance was calculated \((\text{Tau}^2, \chi^2 > 1)\). Subgroup analyses were performed to determine the effect of the following factors on random-effects models: supplementation form (inorganic NO3 salts or beetroot juice), experimental condition (normoxia or hypoxia), dietary NO3 dose, supplementation duration, plasma NO3/NO2-response (high or low NO3 and/or NO2 response) and health status (healthy or pathological subjects). The median value for each parameter included in subgroup analyses was used to separate into two subgroups: dietary NO3 dose (high \(> 7.5 \text{ mmol} vs. \text{ low }\leq 7.5 \text{ mmol})\), supplementation duration (short \(< 3 \text{ days} vs. \text{ long }\geq 3 \text{ days})\), and change in plasma NO3/NO2 levels (high NO3 \(> +574\% vs. \text{ low }\leq +574\%); high NO2 \(> +115.5\% vs. \text{ low }\leq +115.5\%)\). Sensitivity analysis was performed to determine the influence of the studies with the largest effects on the pooled effect size of resting studies. The risk assessment of bias was performed separately by two authors (M.P.-C. and J.A.) in accordance with Cochrane Risk of Bias Tool’s items. Identification of publication bias was performed by funnel plot analysis [24].

3. Results

3.1. Study selection

Based on selected search terms, the initial search in electronic database retrieved 3314 articles, of which 53 articles were potentially eligible for inclusion after title and abstract reading. Thereafter, 29 articles were identified as eligible, and included in the systematic review for qualitative analysis. Twenty-six articles were included in meta-analysis to perform quantitative analysis. The remaining studies were excluded because: data were unavailable after request to the corresponding author, a heterogeneity in work rate between the NO3 and placebo conditions in exercise studies, and no assessment of the change in NO3 and NO2 plasma levels. A flow chart of the literature search procedure is presented in Fig. 1.

3.2. Study characteristics

Most included studies were randomized, double- or single-blind, placebo controlled, parallel or crossover trials, and conducted between 2007 and 2015. Twenty-three studies were conducted in active or trained, young and healthy subjects. One study was conducted in the elderly \((63.5 \pm 3.0 \text{ years})[25]\). One study was conducted in overweight, but otherwise healthy subjects[26]. Four studies were performed in populations with chronic disease conditions. Two studies were conducted in patients with chronic obstructive pulmonary disease [16,19], one study in patients with peripheral arterial disease [17], and one study in patients with heart failure with preserved ejection fraction [20]. The meta-analysis included a total of 264 participants with 7–17 participants per study. The main characteristics of the 29 studies included are presented in Supplemental Table 1.

NO3 supplementation was achieved by intake of inorganic NO3 salts (nitrate sodium or nitrate potassium solutions) in 6 studies and beetroot juice in 23 studies. The following placebo were used: equimolar sodium chloride solutions in 5 studies, isovolumetric...
NO3-free maltodextrin solution in 1 study, NO3-depleted beetroot juice in 12 studies, apple-blackcurrant juice in 4 studies, blackcurrant juice in 3 studies, orange juice in 2 studies, prune juice in 1 study and tomato juice in 1 study. There was a single ingestion of dietary NO3 in 13 studies and a supplementation duration ranged from 3 to 15 days in 15 studies with washout period between 2 and 14 days, with the exception of one study with parallel design, where the placebo and beetroot juice supplementation were initiated 4 days before baseline testing and continued during 6 weeks of exercise training [27]. The last ingestion most often occurred 2.5–3 h before VO2 measurement, which corresponds to peak plasma NO2 concentrations after dietary NO3 ingestion [28,29], with the exception of one study where the ingestion was 1 h before assessment [13]. The daily amount of NO3 ingested ranged from 5.1 to 19.5 mmol.

Regarding additional dietary NO3 intake, participants were asked to refrain from consuming NO3-rich food items in 12 studies, three of which applied nutritional guidelines [30] or standardized dinner and breakfast [27,31]. Seventeen studies reported no dietary restriction, but 10 of these studies indicated that the participants were instructed to replicate their food intake in the two conditions (nitrate vs placebo), and 4 studies used standardized meals before experimental tests following supplementation periods [32–35]. The subjects were instructed to refrain from caffeine and alcohol 6 and 24 h before tests in studies investigating the effect of supplementation on exercise performance. The study participants were asked also to refrain from strenuous activity within the 24–48 h preceding testing sessions. In all studies, the subjects were instructed not to use antibacterial mouthwashes that inhibit the NO3 to NO2 conversion by oral anaerobic bacteria [28].

### 3.3. Risk of bias within studies

Most studies included were considered as having low risk of bias according to Cochrane Risk of Bias Tool’s items [36]. Two studies were rated as having high risk of bias because of the lack of blinding for experimental beverages of participants and research personnel. A number of studies were rated as having an unclear risk of bias in one or more items as follows: random sequence generation in 1 study, blinding of participants and personnel in 9 studies, blinding of experimental outcome assessments in 3 studies, and incomplete outcome data in 3 studies. The allocation of interventions was exclusively randomized, and washout period and dropout rate were reported in all trials. The risk of bias assessment is reported in Supplemental Fig. 1. Eligible trials were mostly double- (n = 16) or single-blind (n = 9) when placebo with different taste, smell and appearance compared to dietary NO3 supplementation were used. Seven studies reported that the participants were concealed to experimental hypotheses, whereas the purpose of trials was communicated as the comparison of physiologic responses after two beverage intakes or treatment solutions. Otherwise, the authors notified that the study participants were not informed about the potential physiologic effects of inorganic NO3 salts and beetroot juice supplementation. Eighteen studies did not report whether the study participants were aware of the properties of inorganic NO3 and of the true aim of study. In two trials, the study participants were informed that the purpose of the study was to test the effect of a NO3-rich beverage on exercise performance [16,33]. Eighteen studies reported funding sources and 8 studies disclosed potential conflict of interest.

### 3.4. Results of individual studies

Dietary NO3 supplementation was well tolerated, and no side effects were reported. Some participants reported beeturia (red urine) and red stools after beetroot juice supplementation, but these events were considered benign.

The summary of main results in each included study is displayed in Supplemental Table 1. Two studies reported a reduced VO2 under resting condition after dietary NO3 supplementation [13,31], one of which was performed under hypoxic condition [31]. Only one study was designed to specifically investigate the effect of dietary NO3 on resting metabolic rate and reported a 4.2% decrease [13]. Four studies did not report any effect of dietary NO3 supplementation on baseline VO2 measurements, which were performed before the beginning of exercise tolerance tests [12,26,30,37]. VO2 was significantly decreased following dietary NO3 supplementation in 12 studies during moderate intensity steady-state exercise [7,11,12,26,31,32,35,38–40], heavy intensity exercise [7,26,32,41,42], and severe intensity exercise [7,12,26,38]. The decreased VO2 was associated with enhanced exercise tolerance during severe intensity exercise [11,12,35,38]. Six studies reported no change in VO2 during exercise with dietary NO3 supplementation [25,33,34,37,43,44]. Several studies investigated the effect of dietary NO3 on time trial performance and reported VO2 measurements. One study showed that VO2 was significantly decreased during a 4-min all-out maximal effort [45], but 2 studies did not report an effect of dietary NO3 supplementation on VO2 during 4·km and 16.1-km time trials and during 50 mile time trials, respectively [46,47]. However, the power output to VO2 ratio during exercise significantly increased in the two latter studies, indicative of a decrease in the O2 cost of exercise. Two studies showed that VO2max was decreased during maximal intensity exercise [30,48] without change in time to exhaustion after inorganic NO3 supplementation, and one study reported no effects on VO2max [7]. Finally, one study investigated the combined effect of beetroot juice supplementation and hypoxic endurance training on aerobic fitness and reported a similar increase in VO2max between NO3 supplementation and placebo [27].

Regarding patients with chronic disease conditions, 2 studies in chronic obstructive pulmonary disease did not report any change in VO2 after beetroot juice supplementation [16,19], despite increased exercise capacity [16]. In patients with peripheral arterial disease, exercise tolerance was enhanced and the onset of claudication pain was delayed, but VO2 was significantly reduced at the first stage of the incremental exercise test only [17]. In patients with heart failure with preserved ejection fraction, beetroot juice supplementation resulted in higher VO2peak and increased total work achieved during a supine-cycle maximal exercise test [20]. Regarding metabolic rate under non-exercise conditions, baseline VO2 measured before exercise tolerance tests was unchanged following beetroot juice ingestion in 2 studies in patients with chronic obstructive pulmonary disease [16,19]. However, a study conducted in patients with peripheral arterial disease showed a trend toward a lower resting VO2 after beetroot juice ingestion [17].

### 3.5. Synthesis of results

#### 3.5.1. Pooled effect size

Dietary NO3 supplementation resulted in a significant VO2 decrease during submaximal intensity exercise [−0.26 (95% IC: −0.38, −0.15), p < 0.01] (Fig. 2). When data from the three submaximal intensity domains were analyzed separately, dietary NO3 supplementation resulted in significant VO2 decrease during moderate intensity exercise [−0.29 (95% IC: −0.48, −0.10), p < 0.01] (Fig. 3) and heavy intensity exercise [−0.33 (95% IC: −0.54, −0.12), p < 0.01] (Fig. 4). However, there was no significant effect of dietary NO3 relative to placebo under resting condition [0.01 (95% IC: −0.47, 0.50), p = 0.96] (Fig. 5), during severe intensity exercise [−0.14 (95% IC: −0.38, 0.09), p = 0.24] (Fig. 6) and during maximal...
3.5.2. Subgroup analyses

Subgroup analyses showed no effect of any of the selected parameters on VO2 during submaximal intensity exercise, except for health status, where dietary NO3 supplementation significantly decreased VO2 in healthy subjects \([0.28 (95\% \text{ IC}: 0.40, 0.16), \ p < 0.01]\), but not in patients with chronic diseases \([-0.09 (95\% \text{ IC}: -0.50, 0.32), \ p = 0.67]\).

Subgroup analysis for each exercise intensity domain showed that treatment form did not change the effect of NO3 supplementation on VO2, with significant VO2 decrease during moderate [beetroot juice: \(0.27 (95\% \text{ IC}: 0.50, 0.04), \ p = 0.02\); inorganic NO3 salts: \(0.38 (95\% \text{ IC}: 0.76, 0.00), \ p = 0.05\)] and heavy intensity exercise [beetroot juice: \(0.30 (95\% \text{ IC}: 0.69, -0.12), \ p < 0.01\), but not high NO3 dose \([-0.17 (95\% \text{ IC}: -0.50, 0.32), \ p = 0.67]\).

**Fig. 2.** Forest plot of the effect size for the change in VO2 during submaximal intensity exercise with dietary NO3 supplementation.
Fig. 3. Forest plot of the effect size for the change in VO$_2$ during moderate intensity exercise with dietary NO$_3$ supplementation.

Fig. 4. Forest plot of the effect size for the change in VO$_2$ during heavy intensity exercise with dietary NO$_3$ supplementation.
supplementation form, dietary NO3maximal intensity exercise following the subgroup analyses by of the small number of studies performed under hypoxia[27,31,42].

\[0.48\], \(p = 0.08\), 0.51), \(p = 0.08\), 0.74, 0.11), \(p = 0.15\), the effects on VO2 was no longer significant during heavy intensity exercise. When the analysis was limited to healthy subjects, there were trends towards significant VO2 decrease under resting condition \(\pm 0.37\) (95% IC: \(-0.80, 0.05\)), \(p = 0.08\) and during maximal intensity exercise \(\pm 0.29\) (95% IC: \(-0.63, 0.06\)), \(p = 0.10\).

\[0.38\] (95% IC: \(-0.80, 0.05\)), \(p = 0.08\) and during maximal intensity exercise \(\pm 0.29\) (95% IC: \(-0.63, 0.06\)), \(p = 0.10\).

\[0.17\] (95% CI: \(-1.15, 0.82\)), \(p = 0.20\). High NO3 dose significantly decreases VO2 during heavy intensity exercise \(-0.53\) (95% IC: \(-1.16, -0.01\)), \(p = 0.05\), and low NO3 dose was associated with a trend toward significant effect size \(-0.24\) (95% IC: \(-0.50, 0.02\)), \(p = 0.07\).

The supplementation duration affects the change in VO2, with the longer supplementation duration resulting in a significant VO2 decrease during moderate intensity exercise \(-0.62\) (95% IC: \(-1.05, -0.19\)), \(p < 0.01\), that is not observed with short duration supplementation \(-0.14\) (95% IC: \(-0.35, 0.07\)), \(p = 0.19\). Surprisingly, short duration supplementation was associated with a significant decrease in VO2 during heavy intensity exercise \(-0.30\) (95% IC: \(-0.57, -0.04\)), \(p = 0.02\), but not with long duration supplementation \(-0.38\) (95% IC: \(-0.87, 0.10\)), \(p = 0.12\).

No change was found under resting condition and in severe and maximal intensity exercise following the subgroup analyses by supplementation form, dietary NO3 dose and supplementation duration.

NO3 supplementation has no effect on VO2 in patients with chronic disease conditions [resting condition: \(-0.04\) (95% IC: \(-0.57, 0.48\)), \(p = 0.87\); moderate intensity exercise: 0.01 (95% IC: \(-0.50, 0.51\)), \(p = 0.98\)]. When the patients with chronic disease conditions were excluded from the analysis, healthy subjects had a decreased VO2 during moderate intensity exercise \(-0.33\) (95% IC: \(-0.54, -0.13\)), \(p < 0.01\).

Subgroup analysis for hypoxic condition is not reported because of the small number of studies performed under hypoxia[27,31,42]. Subgroup analysis based on plasma NO3/NO2 response is not reported because of the different analytical approaches used to assess NO3/NO2 that results in extremely large differences of concentrations between studies.

### 3.5.3. Influence of studies with the largest effects

When the exclusion of studies with the largest effects was performed, the random-effects models were affected under resting condition with a trend toward a VO2 decrease \(-0.28\) (95% IC: \(-0.62, 0.05\)), \(p = 0.10\). The sensitivity analysis also showed that the exclusion of studies with the largest effects did not change the effects of NO3 supplementation on VO2 during submaximal intensity exercise \(-0.23\) (95% IC: \(-0.35, -0.01\)), \(p < 0.01\), and when the three submaximal intensity domains were analyzed separately (moderate intensity exercise \(-0.26\) (95% IC: \(-0.43, -0.09\)), \(p < 0.01\)), heavy intensity exercise \(-0.26\) (95% IC: \(-0.48, -0.04\)), \(p = 0.02\), and severe intensity exercise \(-0.11\) (95% IC: \(-0.35, 0.14\)), \(p = 0.38\). The exclusion of studies with the largest effects did not change the effect of NO3 supplementation during exercise performed at maximal intensity \(-0.17\) (95% IC: \(-0.47, 0.13\)), \(p = 0.27\).

The results of the subgroup analyses for the factors potentially affecting metabolic rate were unchanged for moderate and severe intensity exercise when the studies with the largest effects were excluded. When the analysis was limited to beetroot juice supplementation \(-0.19\) (95% IC: \(-0.46, 0.07\)), \(p = 0.15\) and high NO3 dose \(-0.31\) (95% IC: \(-0.74, 0.11\)), \(p = 0.15\), the effects on VO2 was no longer significant during heavy intensity exercise. When the analysis was limited to healthy subjects, there were trends towards significant VO2 decrease under resting condition \(-0.37\) (95% IC: \(-0.80, 0.05\)), \(p = 0.08\) and during maximal intensity exercise \(-0.29\) (95% IC: \(-0.63, 0.06\)), \(p = 0.10\).

### 3.5.4. Publication bias

The analysis of funnel plots indicates an overall symmetric distribution of the studies around the pooled effect size for

\[\begin{align*}
\text{Fig. 6.} & \quad \text{Forest plot of the effect size for the change in VO2 during severe intensity exercise with dietary NO3} \\
\text{Fig. 7.} & \quad \text{Forest plot of the effect size for the change in VO2 during maximal intensity exercise with dietary NO3} \\
\end{align*}\]
moderate, heavy and severe intensities of exercise, suggesting no publication bias. Under resting condition as well as for pooled submaximal intensity exercises and maximal intensity exercise, the funnel plots showed an overall asymmetric distribution of effect sizes of the individual studies (See Supplemental Fig. 2A, B and C) that may be related to the significant heterogeneity in these models (see thereafter).

3.5.5. Heterogeneity and inconsistency

We observed no significant heterogeneity and inconsistency for submaximal intensity exercise \([Q = 45.12 (p = 0.80), \tau^2 = 0, I^2 = 0\%]\). However, there was significant heterogeneity for long duration supplementation \([Q = 28.37 (p = 0.10), \tau^2 = 0.10\) and low inconsistency \([I^2 = 30\%]\), which disappeared when we excluded the studies with the largest effects \([Q = 15.57 (p = 0.62), \tau^2 = 0, I^2 = 0\%]\).

There was high heterogeneity in random-effects models for resting condition \([Q = 39.77 (p < 0.01), \tau^2 = 0.51]\) and maximal intensity exercise \([Q = 17.99 (p = 0.02), \tau^2 = 0.25]\), with high \([I^2 = 72\%]\) and moderate \([I^2 = 56\%]\) inconsistency, respectively. Under resting condition, beetroot juice supplementation \([Q = 36.70 (p < 0.01), \tau^2 = 1.08]\), low NO3 dose \([Q = 36.86 (p < 0.01), \tau^2 = 0.91]\), long duration supplementation \([Q = 34.24 (p < 0.01), \tau^2 = 0.64]\) and healthy subjects \([Q = 37.13 (p < 0.01), \tau^2 = 0.74]\) had large heterogeneity, and high inconsistency \([I^2 = 84\%, 81\%, 94\%, and 78\%\), respectively]. Regarding maximal intensity exercise, the subgroup analyses for beetroot juice supplementation \([Q = 15.03 (p = 0.01), \tau^2 = 0.40]\), low NO3 dose \([Q = 16.82 (p = 0.01), \tau^2 = 0.39]\), long duration supplementation \([Q = 14.83 (p < 0.01), \tau^2 = 0.87]\) and healthy subjects \([Q = 17.09 (p = 0.02), \tau^2 = 0.31]\) resulted in increased heterogeneity and inconsistency \([I^2 = 67\%, 64\%, 80\%, and 59\%\), respectively].

The exclusion of the studies with the largest effects altered substantially the random-effects models, with a reduced heterogeneity and inconsistency under resting condition \([Q = 15.35 (p = 0.088), \tau^2 = 0.12, I^2 = 41\%]\) and during maximal intensity exercise \([Q = 5.13 (p = 0.64), \tau^2 = 0, I^2 = 0\%]\). Regarding sources of heterogeneity, the sensitivity analyses were associated with reduced inconsistency with beetroot juice supplementation \([I^2 = 69\%]\), low NO3 dose \([I^2 = 57\%]\) and healthy subjects only \([I^2 = 48\%]\) under resting condition. The heterogeneity remained significant in the beetroot juice supplementation subgroup \([Q = 12.93 (p = 0.01), \tau^2 = 0.36]\) and low NO3 dose subgroup \([Q = 11.50 (p = 0.04), \tau^2 = 0.22]\). The exclusion of the studies with the largest effects in maximal intensity exercise model changed both the heterogeneity and inconsistency as follows: beetroot juice supplementation \([Q = 4.04 (p = 0.40), \tau^2 = 0, I^2 = 1\%]\), low NO3 dose \([Q = 2.94 (p = 0.71), \tau^2 = 0, I^2 = 0\%]\), long duration supplementation \([Q = 2.18 (p = 0.34), \tau^2 = 0.02, I^2 = 8\%]\) and healthy subjects \([Q = 2.95 (p = 0.82), \tau^2 = 0, I^2 = 0\%]\).

4. Discussion

4.1. Summary of evidence

The main findings from the present meta-analysis are that dietary NO3 supplementation is associated with i) a significantly decreased metabolic rate when exercise is performed in the moderate and heavy intensity domains, and ii) that inorganic NO3 salts and beetroot juice supplementation induced similar effect on VO2 in these two exercise intensity domains. The results are however inconclusive regarding the effects of dietary NO3 dose and supplementation duration. However, there was no significant effect on VO2 in severe and maximal intensity exercise, suggesting that the effects of NO on the O2 cost of exercise are alleviated as the intensity of exercise approaches the maximum. The exercise intensity domains used in the present study were based on thresholds expressed as percentage \(VO2_{\text{peak}}\), that may be debatable. The main effects of dietary NO3 supplementation on VO2 under resting and exercise are summarized and displayed in Table 3.

In contrast to moderate intensity exercise, we found that dietary NO3 supplementation does not significantly alter metabolic rate under resting condition, although a trend toward a significantly reduced metabolic rate appeared when the studies with the largest effects were excluded. The lack of effects of NO3 supplementation under resting condition may be related to physicochemical conditions in high metabolic rate organs and muscle that weakly contribute to the activation of the nitrate-nitrite-NO pathway. In contrast, NO2 bioactivity is enhanced in conditions of low oxygen pressure and pH by enhanced NO2 conversion into NO species [10]. This nitrite reductase activity could thus be hypothesized to be strongly activated during conditions such as exercise and/or chronic cardiac and respiratory disease. However, only one study reported a trend towards a lower VO2 under resting condition in peripheral arterial disease [17], and results of the meta-analysis suggest that the effects of dietary nitrate supplementation on VO2 is decreased as the intensity of exercise increases, which does not support the hypotheses. Otherwise, most eligible studies included in the meta-analysis for metabolic rate under resting condition provided less reliable measurement of metabolic rate under resting condition. Indeed, the primary purpose of these studies was to investigate the effect of dietary NO3 on the O2 cost of exercise in subjects who did not fast overnight, and VO2 was measured at rest before the beginning of exercise tests, with indirect calorimetry devices used for VO2 measurement during exercise, which may be less adequate for measurement under resting condition. Thus further studies are required to determine whether dietary NO3 supplementation significantly affects resting metabolic rate. In addition, only one study was specifically designed to investigate the effect of dietary NO3 on resting metabolic rate, and used the most appropriate methodology with the VO2 measurement performed with a ventilated hood and subjects tested after an overnight fast, and showed a significant decline in resting metabolic rate [13]. Larsen et al. also showed a NO2 dose-dependent increase of mitochondrial p50 for oxygen in vitro, which was also observed after NO3 supplementation [39], and is strongly \((R^2 = 0.66)\) and negatively associated with basal metabolic rate [49]. Hence, an increase in NO2 bioavailability through dietary NO3 supplementation can reasonably be expected to result in decreased basal metabolic rate. Only a limited number of treatments have previously been reported to decrease resting metabolic rate. For example, basal metabolic rate was shown to decrease by 4% in healthy obese subjects supplemented during 30 days with resveratrol, a natural phenol present in various dietary vegetable components [15]. Resting metabolic rate has also been shown to decrease by 6% in healthy overweight subject who had followed a 6-month caloric restriction [50,51].

Regarding the effect of dietary NO3 in populations with cardiovascular and respiratory diseases, we did not observe a significant decrease in metabolic rate that could counteract the impaired oxygen carrying capacity. It is important to note that the studies included or not in the meta-analysis reported enhanced exercise tolerance in patients with chronic obstructive pulmonary disease [16,18], peripheral arterial disease [17], and heart failure with preserved ejection fraction [20]. In addition and in contrast to our hypothesis, beetroot juice supplementation in patients with heart failure with preserved ejection fraction resulted in increased \(VO2_{\text{peak}}\), which was associated with enhanced vasodilatory reserve and cardiac output at peak exercise [20]. Of note, another study reported an increase in \(VO2_{\text{max}}\) after 15 days of beetroot juice
supplementation in healthy subjects, in which an increased local muscle perfusion and cardiac output were hypothesized to be factors contributing to this change [40]. We were also unable to determine the effect of either aerobic fitness level (VO$_{2\max}$) or activity/training status, as many studies included subjects with large range of VO$_{2\max}$ or did not report physical activity and training history, which did not allow categorizing the populations according to the aerobic fitness and/or activity/training status. It was recently found that the decrease in the O$_2$ cost of exercise was negatively correlated with VO$_{2\max}$ and that the increase in NO$_3$ plasma levels with dietary NO$_3$ supplementation was lower in subjects with high VO$_{2\max}$ [52]. These results confirm those of previous studies where the NO$_3$ supplementation did not affect VO$_2$, exercise tolerance or exercise performances in highly endurance trained subjects [33,34,43,47].

4.2. Limitations

There are limitations inherent to meta-analysis that should be considered in the interpretation of the results due to the small number of studies in several models and the reduced sample size in most studies. A significant heterogeneity also appeared under resting condition and during maximal intensity exercise, which was reduced when the studies with the largest effects were excluded in sensitivity analysis.

The meta-analysis included a narrow range of subjects in terms of age, gender, health status and physical activity level. The over-representation of active or highly endurance trained, young, and healthy male participants is a limitation, making the applicability of the findings to other populations debatable. Subgroup analysis by gender was for example not feasible due to the low number of female participants (28 females/236 males) in the whole population of the meta-analysis and the lack of study specifically investigating women. Two previous studies reported a lower decrease in blood pressure [53] and no decrease in platelet reactivity [54] in women supplemented with dietary NO$_3$ compared to men, which suggest that women may have different metabolic responses to increased NO$_3$ intake, especially when NO synthesis is limited by hypoxia. This is supported by the findings from 2 studies performed in hypoxic conditions that reported VO$_2$ decrease at rest and during moderate intensity exercise following a 6-day beetroot juice supplementation [31], and during heavy intensity exercise after a single ingestion of beetroot juice [42]. These results warrant further works, though NO$_3$ supplementation failed to facilitate the adaptations to hypoxic environment during 6-week of hypoxic training program [27].

5. Conclusion and perspectives

Previous meta-analyses already showed that dietary NO$_3$ significantly decreases systolic blood pressure [56] and may have a therapeutic potential in patients with cardiovascular diseases and significantly improves exercise tolerance in healthy subjects [57]. Mechanistically, there were clear evidences from in vitro studies that NO increases mitochondrial oxidative efficiency [39,58]. The present meta-analysis supports that increasing NO bioavailability through dietary NO$_3$ translates into a significantly decreased O$_2$ cost during exercise performed in the heavy and moderate intensity domains, but not at exercise intensities close to the maximum. These effects of dietary NO$_3$ supplementation may be of interest in patients with cardiovascular or respiratory diseases that severely limits exercise capacity, with potential improvement of exercise tolerance that may positively affect their quality of live. On the opposite, there is also a need to determine the factors responsible for the lack of effect on VO$_2$ in subjects with high VO$_{2\max}$, as short term beetroot juice supplementation is used as an easy, available and healthy ergogenic aid by athletes. Directions for future studies should also include research into the dose and duration of supplementation in order to provide recommendation for NO$_3$ supplementation specific to each population.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.niox.2016.01.001.

References


