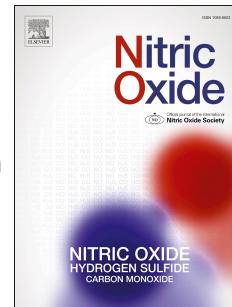


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Effect of dietary nitrate supplementation on metabolic rate during rest and exercise in human: a systematic review and a meta-analysis

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Short running head: Dietary nitrate intake on metabolic rate

¹Abbreviations: I²: inconsistency value; NO₃⁻: nitrate; NO: nitric oxide; NOS: nitric oxide synthase; NO₂⁻: nitrite; PICOS: Participants, Interventions, Comparators, Outcomes, Study design; PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses; τ²: between-studies variance; VO₂: oxygen uptake, VO_{2max}: maximal oxygen uptake.

1 **ABSTRACT**

2 **Background:** Recent randomized controlled trials have suggested that dietary nitrate (NO_3^-),
3 found in beetroot and other vegetables, and inorganic NO_3^- salts decrease metabolic rate under
4 resting and exercise conditions.

5 **Objective:** Our aim was therefore to determine from a systematic review and meta-analysis
6 whether dietary NO_3^- supplementation significantly reduces metabolic rate, expressed as
7 oxygen uptake (VO_2), under resting and exercise conditions in healthy humans and those with
8 cardiorespiratory diseases.

9 **Design:** A systematic article search was performed on electronic databases (PubMed, Scopus
10 and Web of Science) from February to March 2015. The inclusion criteria included 1)
11 randomized controlled trials; 2) studies reporting the effect of NO_3^- on VO_2 under resting
12 and/or exercise conditions; 3) comparison between dietary NO_3^- supplementation and
13 placebo. Random-effects models were used to calculate the pooled effect size.

14 **Results:** Twenty nine randomized placebo-controlled trials were included in the systematic
15 review, and 26 of which were included in the meta-analysis. Dietary NO_3^- supplementation
16 significantly decreases VO_2 during submaximal intensity exercise [-0.26 (95% IC: -0.38, -
17 0.15), $p < 0.01$], but not in the sub-analysis of subjects with chronic diseases [-0.09 (95% IC: -
18 0.50, 0.32), $p = 0.67$]. When data were separately analyzed by submaximal intensity domains,
19 NO_3^- supplementation reduces VO_2 during moderate [-0.29 (95% IC: -0.48,-0.10), $p < 0.01$]
20 and heavy [-0.33 (95% IC: -0.54,-0.12), $p < 0.01$] intensity exercise. When the studies with
21 the largest effects were excluded from the meta-analysis, there is a trend for a VO_2 decrease
22 under resting condition in dietary NO_3^- supplementation [-0.28 (95% IC: -0.62, 0.05), $p =$
23 0.10].

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

28

29 **Keywords:** Dietary nitrate, metabolic rate, rest, exercise, chronic diseases.

30 INTRODUCTION

31 Low oxygen availability limits the sustainable metabolic rate in a wide range of conditions,
32 such as in chronic cardiorespiratory diseases, environmental hypoxia or in athletes when
33 oxygen carrying capacity reaches its maximal during intense exercise [1–3]. Metabolic rate is
34 minimal under resting, thermoneutral, and postabsorptive conditions, comprising the energy
35 expenditure required to sustain vital body functions [4]. Brain, liver, heart, and kidneys
36 account for 60-70 % of resting energy expenditure, whereas skeletal muscles account for 20-
37 30% in humans [5]. During exercise, metabolic rate can be increased up to 20-fold during
38 maximal intensity exercise in athletes to meet to the energy needs of working muscles [4].
39 Metabolic rate is commonly expressed as the rate of oxygen uptake (VO_2), which reflects
40 whole body oxidative metabolism and is measured by indirect calorimetry [6]. There is
41 however little possibility to reduce resting metabolic rate, or metabolic rate for a given work
42 rate during exercise [7]. Nitric oxide (NO)¹ is an ubiquitous signaling molecule produced
43 through the NO synthase pathway that possesses the ability to lower metabolic rate through an
44 increase in mitochondrial efficiency caused by factors such as the inhibition of enzyme
45 cytochrome c oxidase activity by NO, or inhibition of uncoupled respiration [8]. NO synthesis
46 through the nitric oxide synthase (NOS) pathway is however dependent on L-arginine and
47 oxygen availability, and becomes limited in hypoxia [9]. However, dietary nitrate (NO_3^-)
48 found in abundance in green leafy and root vegetables have been shown to represent an
49 important alternative source of NO [10]. Dietary NO_3^- are absorbed from the small intestine,
50 and after an entero-salivary recirculation and concentration into the saliva, are converted into
51 nitrite (NO_2^-) by oral NO_3^- reductase bacteria [10]. The swallowed salivary NO_2^- are either
52 reduced to NO and other nitrogen species in the acidic stomach or absorbed from the intestine
53 [10].

54 In humans, increased NO bioavailability was first shown to result in decreased VO₂ during
55 exercise [7, 11, 12]. Recently, Larsen et al. (2014) also showed in a randomized controlled
56 trial that resting metabolic rate is reduced by 4.2 % in healthy subjects following a 3-day
57 dietary NO₃⁻ supplementation [13]. The decrease in resting metabolic rate with dietary NO₃⁻
58 supplementation was proposed to mimic the beneficial effect of calorie restriction or
59 resveratrol supplementation, which were previously shown to improve metabolic health
60 parameters [14, 15]. A number of studies have also been conducted in patients with chronic
61 disease conditions that severely impair oxygen delivery and/or utilization, such as chronic
62 obstructive pulmonary disease, heart failure, or peripheral arterial disease [16–20].
63 Our purpose was therefore to conduct a systematic review and meta-analysis of the
64 randomized controlled trials exploring the effect of inorganic NO₃⁻ supplementation on the
65 metabolic rate, expressed as VO₂, under resting and/or exercise conditions. A secondary
66 purpose of the meta-analytical procedures was to determine factors, such as population
67 characteristics, supplementation duration, and dietary NO₃⁻ dose that could be responsible of
68 the hypothesized changes in metabolic rate.

69

70 **METHODS**

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

76

77 *Type of studies*

78 Randomized clinical trials that investigated the effect of dietary NO_3^- under the form of
79 inorganic NO_3^- salts or present in beetroot juice on metabolic rate, expressed as VO_2 , in
80 human were included. The following experimental variables were extracted from each study:
81 resting or exercise conditions, supplementation duration, dosage, adherence to protocols,
82 dropout rate, duration of the washout period between dietary NO_3^- and placebo
83 supplementations, and adverse events. Participant characteristics were also extracted with the
84 following information whenever available: age, number of male and female subjects, health
85 status (healthy, chronic obstructive pulmonary disease, heart failure, and peripheral arterial
86 disease), anthropometric characteristics, physical activity or exercise training level and
87 maximal oxygen uptake ($\text{VO}_{2\text{max}}$) to reflect aerobic fitness.

88

89 *Type of interventions*

90 The studies included achieved increased dietary NO_3^- intake by consumption of beetroot juice
91 rich in inorganic NO_3^- or nitrate salts dissolved in aqueous solutions (potassium nitrate or
92 sodium nitrate). Supplementation duration, dietary NO_3^- dose, type of supplementation
93 (beetroot juice or salts) were parameters used as potential sources of heterogeneity in random-

94 effects models. For studies where metabolic rate was assessed during exercise, we defined the
95 exercise intensity domain as moderate, heavy, or severe based on the existing literature [22,
96 23] in order to determine whether the effect of dietary NO₃⁻ supplementation is affected by
97 exercise intensity. Studies where exercise was performed in the moderate, heavy and severe
98 intensity domains were also pooled together for a separate analysis of metabolic rate during
99 submaximal intensity exercise (<VO_{2max}). Metabolic rate measured during maximal exercise
100 tests were included in a specific random-effects model. The criteria used to determine the
101 exercise intensity are presented in **Table 2**.

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

108

109 *Type of outcome measures*

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

115

116 *Search Strategy*

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

124

125 *Study selection and data extraction*

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

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

133

134 *Data analysis*

135 The complete meta-analysis of outcome measures was run through Review Manager Software
136 (Review Manager (RevMan) [Windows 7, Microsoft]. Version 5.3. Copenhagen: The Nordic
137 Cochrane Centre, The Cochrane Collaboration, 2014.). DerSimonian and Laird random-
138 effects models were used to estimate the pooled effect size for VO_2 under resting and exercise
139 conditions. Data are reported as standardized mean differences for VO_2 at rest and during
140 exercise with 95% Confidence Interval (CI), and presented in forest plots. The threshold for

141 statistical significance was set at $p = 0.05$. Heterogeneity among studies was evaluated using
142 the Cochrane's Q test ($p < 0.10$) and I² values (low $\geq 25\%$, moderate $\geq 50\%$, high $\geq 75\%$), and
143 between-studies variance was calculated (Tau^2 , $\tau^2 > 1$). Subgroup analyses were performed to
144 determine the effect of the following factors on random-effects models: supplementation form
145 (inorganic NO₃⁻ salts or beetroot juice), experimental condition (normoxia or hypoxia),
146 dietary NO₃⁻ dose, supplementation duration, plasma NO₃⁻/NO₂⁻ response (high or low NO₃⁻
147 and/or NO₂⁻ response) and health status (healthy or pathological subjects). The median value
148 for each parameter included in subgroup analyses was used to separate into two subgroups:
149 dietary NO₃⁻ dose (high > 7.5 mmol vs. low ≤ 7.5 mmol), supplementation duration (short ≤ 3
150 days vs. long > 3 days), and change in plasma NO₃⁻/NO₂⁻ levels (high NO₃⁻ $> +574\%$ vs. low
151 NO₃⁻ $\leq +574\%$; high NO₂⁻ $> +115.5\%$ vs. low NO₂⁻ $\leq +115.5\%$). Sensitivity analysis was
152 performed to determine the influence of the studies with the largest effects on the pooled
153 effect size of resting studies. The risk assessment of bias was performed separately by two
154 authors (M.P.-C. and J.A.) in accordance with Cochrane Risk of Bias Tool's items.
155 Identification of publication bias was performed by funnel plot analysis [24].

156 **RESULTS**157 *Study selection*

158 Based on selected search terms, the initial search in electronic database retrieved 3314
159 articles, of which 53 articles were potentially eligible for inclusion after title and abstract
160 reading. Thereafter, 29 articles were identified as eligible, and included in the systematic
161 review for qualitative analysis. Twenty-six articles were included in meta-analysis to perform
162 quantitative analysis. The remaining studies were excluded because: data were unavailable
163 after request to the corresponding author, a heterogeneity in work rate between the NO_3^- and
164 placebo conditions in exercise studies, and no assessment of the change in NO_3^- and NO_2^-
165 plasma levels. A flow chart of the literature search procedure is presented in **Figure 1**.

166

167 *Study characteristics*

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

178 NO_3^- supplementation was achieved by intake of inorganic NO_3^- salts (nitrate sodium or
179 nitrate potassium solutions) in 6 studies and beetroot juice in 23 studies. The following

180 placebo were used: equimolar sodium chloride solutions in 5 studies, isovolumetric NO_3^- -free
181 maltodextrin solution in 1 study, NO_3^- -depleted beetroot juice in 12 studies, apple-
182 blackcurrant juice in 4 studies, blackcurrant juice in 3 studies, orange juice in 2 studies, prune
183 juice in 1 study and tomato juice in 1 study. There was a single ingestion of dietary NO_3^- in 13
184 studies and a supplementation duration ranged from 3 to 15 days in 15 studies with washout
185 period between 2 and 14 days, with the exception of one study with parallel design, where the
186 placebo and beetroot juice supplementation were initiated 4 days before baseline testing and
187 continued during 6 weeks of exercise training [27]. The last ingestion most often occurred 2.5
188 to 3 hours before VO_2 measurement, which corresponds to peak plasma NO_2^- concentrations
189 after dietary NO_3^- ingestion [28, 29], with the exception of one study where the ingestion was
190 1 hour before assessment [13]. The daily amount of NO_3^- ingested ranged from 5.1 to 19.5
191 mmol.

192 Regarding additional dietary NO_3^- intake, participants were asked to refrain from consuming
193 NO_3^- -rich food items in 12 studies, three of which applied nutritional guidelines [30] or
194 standardized dinner and breakfast [27, 31]. Seventeen studies reported no dietary restriction,
195 but 10 of these studies indicated that the participants were instructed to replicate their food
196 intake in the two conditions (nitrate vs placebo), and 4 studies used standardized meals before
197 experimental tests following supplementation periods [32–35]. The subjects were instructed
198 to refrain from caffeine and alcohol 6 and 24 hours before tests in studies investigating the
199 effect of supplementation on exercise performance. The study participants were asked also to
200 restrain from strenuous activity within the 24–48 hours preceding testing sessions. In all
201 studies, the subjects were instructed not to use antibacterial mouthwashes that inhibit the NO_3^-
202 to NO_2^- conversion by oral anaerobic bacteria [28].

203

204 *Risk of bias within studies*

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

225

226 *Results of individual studies*

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

230 The summary of main results in each included study is displayed in Supplemental Table 1.

231 Two studies reported a reduced VO_2 under resting condition after dietary NO_3^-

232 supplementation [13, 31], one of which was performed under hypoxic condition [31]. Only

233 one study was designed to specifically investigate the effect of dietary NO_3^- on resting

234 metabolic rate and reported a 4.2% decrease [13]. Four studies did not report any effect of

235 dietary NO_3^- supplementation on baseline VO_2 measurements, which were performed before

236 the beginning of exercise tolerance tests [12, 26, 30, 37]. VO_2 was significantly decreased

237 following dietary NO_3^- supplementation in 12 studies during moderate intensity steady-state

238 exercise [7, 11, 12, 26, 31, 32, 35, 38–40], heavy intensity exercise [7, 26, 32, 41, 42], and

239 severe intensity exercise [7, 12, 26, 38]. The decreased VO_2 was associated with enhanced

240 exercise tolerance during severe intensity exercise [11, 12, 35, 38]. Six studies reported no

241 change in VO_2 during exercise with dietary NO_3^- supplementation [25, 33, 34, 37, 43, 44].

242 Several studies investigated the effect of dietary NO_3^- on time trial performance and reported

243 VO_2 measurements. One study showed that VO_2 was significantly decreased during a 4-min

244 all-out maximal effort [45], but 2 studies did not report any effect of dietary NO_3^-

245 supplementation on VO_2 during 4-km and 16.1-km time trials and during 50 mile time trials,

246 respectively [46, 47]. However, the power output to VO_2 ratio during exercise significantly

247 increased in the two latter studies, indicative of a decrease in the O_2 cost of exercise. Two

248 studies showed that $\text{VO}_{2\text{max}}$ was decreased during maximal intensity exercise [30, 48] without

249 change in time to exhaustion after inorganic NO_3^- supplementation, and one study reported no

250 effects on $\text{VO}_{2\text{max}}$ [7]. Finally, one study investigated the combined effect of beetroot juice

251 supplementation and hypoxic endurance training on aerobic fitness and reported a similar

252 increase in $\text{VO}_{2\text{max}}$ between NO_3^- supplementation and placebo [27].

253 Regarding patients with chronic disease conditions, 2 studies in chronic obstructive

254 pulmonary disease did not report any change in VO_2 after beetroot juice supplementation [16,

255 19], despite increased exercise capacity [16]. In patients with peripheral arterial disease,
256 exercise tolerance was enhanced and the onset of claudication pain was delayed, but VO_2 was
257 significantly reduced at the first stage of the incremental exercise test only [17]. In patients
258 with heart failure with preserved ejection fraction, beetroot juice supplementation resulted in
259 higher $\text{VO}_{2\text{peak}}$ and increased total work achieved during a supine-cycle maximal exercise test
260 [20]. Regarding metabolic rate under non-exercise conditions, baseline VO_2 measured before
261 exercise tolerance tests was unchanged following beetroot juice ingestion in 2 studies in
262 patients with chronic obstructive pulmonary disease [16, 19]. However, a study conducted in
263 patients with peripheral arterial disease showed a trend toward a lower resting VO_2 after
264 beetroot juice ingestion [17].

265

266 *Synthesis of results*

267 Pooled effect size

268 Dietary NO_3^- supplementation resulted in a significant VO_2 decrease during submaximal
269 intensity exercise [-0.26 (95% IC: -0.38, -0.15), $p < 0.01$] (**Figure 2**). When data from the
270 three submaximal intensity domains were analyzed separately, dietary NO_3^- supplementation
271 resulted in significant VO_2 decrease during moderate intensity exercise [-0.29 (95% IC: -0.48,
272 -0.10), $p < 0.01$] (**Figure 3**) and heavy intensity exercise [-0.33 (95% IC: -0.54,-0.12), $p <$
273 0.01] (**Figure 4**). However, there was no significant effect of dietary NO_3^- relative to placebo
274 under resting condition [0.01 (95% IC: -0.47, 0.50), $p = 0.96$] (**Figure 5**), during severe
275 intensity exercise [-0.14 (95% IC: -0.38, 0.09), $p = 0.24$] (**Figure 6**) and during maximal
276 intensity exercise [0.02 (95% IC: -0.42, 0.46), $p = 0.93$] (**Figure 7**).

277

278 Subgroup analyses

279 Subgroup analyses showed no effect of any of the selected parameters on VO_2 during
280 submaximal intensity exercise, except from health status, where dietary NO_3^- supplementation
281 significantly decreased VO_2 in healthy subjects [-0.28 (95% IC: -0.40, -0.16), $p < 0.01$], but
282 not in patients with chronic diseases [-0.09 (95% IC: -0.50, 0.32), $p = 0.67$].

283 Subgroup analysis for each exercise intensity domain showed that treatment form did not
284 change the effect of NO_3^- supplementation on VO_2 , with significant VO_2 decrease during
285 moderate [beetroot juice: -0.27 (95% IC: -0.50, -0.04), $p = 0.02$; inorganic NO_3^- salts: -0.38
286 (95% IC: -0.76, 0.00), $p = 0.05$] and heavy intensity exercise [beetroot juice: -0.30 (95% IC: -
287 0.55, -0.04), $p = 0.02$; inorganic NO_3^- salts: -0.41 (95% IC: -0.81, -0.01), $p = 0.04$].

288 Subgroup analysis by dose showed that low NO_3^- dose had a significant effect during
289 moderate intensity exercise [-0.41 (95% IC: -0.69, -0.12), $p < 0.01$], but not high NO_3^- dose [-
290 0.17 (95% IC: -0.43, 0.09), $p = 0.20$]. High NO_3^- dose significantly decreases VO_2 during
291 heavy intensity exercise [-0.53 (95% IC: -1.06, -0.01), $p = 0.05$], and low NO_3^- dose was
292 associated with a trend toward significant effect size [-0.24 (95% IC: -0.50, 0.02), $p = 0.07$].

293 The supplementation duration affects the change in VO_2 , with the longer supplementation
294 duration resulting in a significant VO_2 decrease during moderate intensity exercise [-0.62
295 (95% IC: -1.05, -0.19), $p < 0.01$], that is not observed with short duration supplementation [-
296 0.14 (95% IC: -0.35, 0.07), $p = 0.19$]. Surprisingly, short duration supplementation was
297 associated with a significant decrease in VO_2 during heavy intensity exercise [-0.30 (95% IC:
298 -0.57, -0.04), $p = 0.02$], but not with long duration supplementation [-0.38 (95% IC: -0.87,
299 0.10), $p = 0.12$].

300 No change was found under resting condition and in severe and maximal intensity exercise
301 following the subgroup analyses by supplementation form, dietary NO_3^- dose and
302 supplementation duration.

303 NO₃⁻ supplementation has no effect on VO₂ in patients with chronic disease conditions
304 [resting condition: -0.04 (95% IC: -0.57, 0.48), p = 0.87; moderate intensity exercise: 0.01
305 (95% IC: -0.50, 0.51), p = 0.98]. When the patients with chronic disease conditions were
306 excluded from the analysis, healthy subjects had a decreased VO₂ during moderate intensity
307 exercise [-0.33 (95% IC: -0.54, -0.13), p < 0.01].

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

312

313 Influence of studies with the largest effects

314 When the exclusion of studies with the largest effects was performed, the random-effects
315 models were affected under resting condition with a trend toward a VO₂ decrease [-0.28 (95%
316 IC: -0.62, 0.05), p = 0.10]. The sensitivity analysis also showed that the exclusion of studies
317 with the largest effects did not change the effects of NO₃⁻ supplementation on VO₂ during
318 submaximal intensity exercise [-0.23 (95% IC: -0.35, -0.11), p < 0.01], and when the three
319 submaximal intensity domains were analyzed separately (moderate intensity exercise [-0.26
320 (95% IC: -0.43, -0.08), p < 0.01], heavy intensity exercise [-0.26 (95% IC: -0.48, -0.04), p =
321 0.02], and severe intensity exercise [-0.11 (95% IC: -0.35, 0.14), p = 0.38]). The exclusion of
322 studies with the largest effects did not change the effect of NO₃⁻ supplementation during
323 exercise performed at maximal intensity [-0.17 (95% IC: -0.47, 0.13), p = 0.27].

324 The results of the subgroup analyses for the factors potentially affecting metabolic rate were
325 unchanged for moderate and severe intensity exercise when the studies with the largest effects
326 were excluded. When the analysis was limited to beetroot juice supplementation [-0.19 (95%

327 IC: -0.46, 0.07), p = 0.15] and high NO₃⁻ dose [-0.31 (95% IC: -0.74, 0.11), p = 0.15], the
328 effects on VO₂ was no longer significant during heavy intensity exercise. When the analysis
329 was limited to healthy subjects, there were trends towards significant VO₂ decrease under
330 resting condition [-0.37 (95% IC: -0.80, 0.05), p = 0.08] and during maximal intensity
331 exercise [-0.29 (95% IC: -0.63, 0.06), p = 0.10].

332

333 *Publication bias*

334 The analysis of funnel plots indicates an overall symmetric distribution of the studies around
335 the pooled effect size for moderate, heavy and severe intensities of exercise, suggesting no
336 publication bias. Under resting condition as well as for pooled submaximal intensity exercises
337 and maximal intensity exercise, the funnel plots showed an overall asymmetric distribution of
338 effect sizes of the individual studies (See Supplemental Figure 2A, B and C) that may be
339 related to the significant heterogeneity in these models (see thereafter).

340

341 *Heterogeneity and Inconsistency*

342 We observed no significant heterogeneity and inconsistency for submaximal intensity
343 exercise [Q = 45.12 (p = 0.80), τ² = 0, I² = 0 %]. However, there was significant heterogeneity
344 for long duration supplementation [Q = 28.37 (p = 0.10), τ² = 0.10] and low inconsistency [I²
345 = 30 %], which disappeared when we excluded the studies with the largest effects [Q = 15.57
346 (p = 0.62), τ² = 0, I² = 0 %].

347 There was high heterogeneity in random-effects models for resting condition [Q = 39.77 (p <
348 0.01), τ² = 0.51] and maximal intensity exercise [Q = 17.99 (p = 0.02), τ² = 0.25], with high
349 [I² = 72 %] and moderate [I² = 56%] inconsistency, respectively. Under resting condition,
350 beetroot juice supplementation [Q = 36.70 (p < 0.01), τ² = 1.08], low NO₃⁻ dose [Q = 36.86 (p

351 < 0.01), $\tau^2 = 0.91$], long duration supplementation [$Q = 34.24$ ($p < 0.01$), $\tau^2 = 6.74$] and
352 healthy subjects [$Q = 37.13$ ($p < 0.01$), $\tau^2 = 0.74$] had large heterogeneity, and high
353 inconsistency [$I^2 = 84\%$, 81 %, 94 %, and 78 %, respectively]. Regarding maximal intensity
354 exercise, the subgroup analyses for beetroot juice supplementation [$Q = 15.03$ ($p = 0.01$), τ^2
355 = 0.40], low NO_3^- dose [$Q = 16.82$ ($p = 0.01$), $\tau^2 = 0.39$], long duration supplementation [$Q =$
356 14.83 ($p < 0.01$), $\tau^2 = 0.87$] and healthy subjects [$Q = 17.09$ ($p = 0.02$), $\tau^2 = 0.31$] resulted in
357 increased heterogeneity and inconsistency [$I^2 = 67\%$, 64 %, 80 %, and 59 %, respectively].

358 The exclusion of the studies with the largest effects altered substantially the random-effects
359 models, with a reduced heterogeneity and inconsistency under resting condition [$Q = 15.35$ (p
360 = 0.08), $\tau^2 = 0.12$, $I^2 = 41\%$] and during maximal intensity exercise [$Q = 5.13$ ($p = 0.64$), $\tau^2 =$
361 0, $I^2 = 0\%$]. Regarding sources of heterogeneity, the sensitivity analyses were associated with
362 reduced inconsistency with beetroot juice supplementation [$I^2 = 69\%$], low NO_3^- dose [$I^2 = 57$
363 %] and healthy subjects only [$I^2 = 48\%$] under resting condition. The heterogeneity remained
364 significant in the beetroot juice supplementation subgroup [$Q = 12.93$ ($p = 0.01$), $\tau^2 = 0.36$]
365 and low NO_3^- dose subgroup [$Q = 11.50$ ($p = 0.04$), $\tau^2 = 0.22$]. The exclusion of the studies
366 with the largest effects in maximal intensity exercise model changed both the heterogeneity
367 and inconsistency as follows: beetroot juice supplementation [$Q = 4.04$ ($p = 0.40$), $\tau^2 = 0$, $I^2 =$
368 1 %], low NO_3^- dose [$Q = 2.94$ ($p = 0.71$), $\tau^2 = 0$, $I^2 = 0\%$], long duration supplementation [Q
369 = 2.18 ($p = 0.34$), $\tau^2 = 0.02$, $I^2 = 8\%$] and healthy subjects [$Q = 2.95$ ($p = 0.82$), $\tau^2 = 0$, $I^2 = 0$
370 %].

371

372 **DISCUSSION**373 *Summary of evidence*

374 The main findings from the present meta-analysis are that dietary NO_3^- supplementation is
375 associated with i) a significantly decreased metabolic rate when exercise is performed in the
376 moderate and heavy intensity domains, and ii) that inorganic NO_3^- salts and beetroot juice
377 supplementation induced similar effect on VO_2 in these two exercise intensity domains. The
378 results are however inconclusive regarding the effects of dietary NO_3^- dose and
379 supplementation duration. However, there was no significant effect on VO_2 in severe and
380 maximal intensity exercise, suggesting that the effects of NO on the O_2 cost of exercise are
381 alleviated as the intensity of exercise approaches the maximum. The exercise intensity
382 domains used in the present study were based on thresholds expressed as percentage $\text{VO}_{2\text{peak}}$
383 that may be debatable. The main effects of dietary NO_3^- supplementation on VO_2 under
384 resting and exercise are summarized and displayed in **Table 3**.

385 In contrast to moderate intensity exercise, we found that dietary NO_3^- supplementation does
386 not significantly alter metabolic rate under resting condition, although a trend toward a
387 significantly reduced metabolic rate appeared when the studies with the largest effects were
388 excluded. The lack of effects of NO_3^- supplementation under resting condition may be related
389 to physicochemical conditions in high metabolic rate organs and muscle that weakly
390 contribute to the activation of the nitrate-nitrite-NO pathway. In contrast, NO_2^- bioactivity is
391 enhanced in conditions of low oxygen pressure and pH by enhanced NO_2^- conversion into NO
392 species [10]. This nitrite reductase activity could thus be hypothesized to be strongly activated
393 during conditions such as exercise and/or chronic cardiac and respiratory disease. However,
394 only one study reported a trend towards a lower VO_2 under resting condition in peripheral
395 arterial disease [17], and results of the meta-analysis suggest that the effects of dietary nitrate
396 supplementation on VO_2 is decreased as the intensity of exercise increases, which does not

397 support the hypotheses. Otherwise, most eligible studies included in the meta-analysis for
398 metabolic rate under resting condition provided less reliable measurement of metabolic rate
399 under resting condition. Indeed, the primary purpose of these studies was to investigate the
400 effect of dietary NO₃⁻ on the O₂ cost of exercise in subjects who did not fast overnight, and
401 VO₂ was measured at rest before the beginning of exercise tests, with indirect calorimetry
402 devices used for VO₂ measurement during exercise, which may be less adequate for
403 measurement under resting condition. Thus further studies are required to determine whether
404 dietary NO₃⁻ supplementation significantly affects resting metabolic rate. In addition, only
405 one study was specifically designed to investigate the effect of dietary NO₃⁻ on resting
406 metabolic rate, and used the most appropriate methodology with the VO₂ measurement
407 performed with a ventilated hood and subjects tested after an overnight fast, and showed a
408 significant decline in resting metabolic rate [13]. Larsen et al. also showed a NO₂⁻ dose-
409 dependent increase of mitochondrial p50 for oxygen in vitro, which was also observed after
410 NO₃⁻ supplementation [39], and is strongly ($R^2=0.66$) and negatively associated with basal
411 metabolic rate [49]. Hence, an increase in NO₂⁻ bioavailability through dietary NO₃⁻
412 supplementation can reasonably be expected to result in decreased basal metabolic rate. Only
413 a limited number of treatments have previously been reported to decrease resting metabolic
414 rate. For example, basal metabolic rate was shown to decrease by 4 % in healthy obese
415 subjects supplemented during 30 days with resveratrol, a natural phenol present in various
416 dietary vegetable components [15]. Resting metabolic rate has also been shown to decrease by
417 6% in healthy overweight subject who had followed a 6-month caloric restriction [50, 51].
418 Regarding the effect of dietary NO₃⁻ in populations with cardiovascular and respiratory
419 diseases, we did not observe a significant decrease in metabolic rate that could counteract the
420 impaired oxygen carrying capacity. It is important to note that the studies included or not in
421 the meta-analysis reported enhanced exercise tolerance in patients with chronic obstructive

422 pulmonary disease [16, 18], peripheral arterial disease [17], and heart failure with preserved
423 ejection fraction [20]. In addition and in contrast to our hypothesis, beetroot juice
424 supplementation in patients with heart failure with preserved ejection fraction resulted in
425 increased $\text{VO}_{2\text{peak}}$, which was associated with enhanced vasodilatory reserve and cardiac
426 output at peak exercise [20]. Of note, another study reported an increase in $\text{VO}_{2\text{max}}$ after 15
427 days of beetroot juice supplementation in healthy subjects, in which an increased local muscle
428 perfusion and cardiac output were hypothesized to be factors contributing to this change [40].
429 We were also unable to determine the effect of either aerobic fitness level ($\text{VO}_{2\text{max}}$) or
430 activity/training status, as many studies included subjects with large range of $\text{VO}_{2\text{max}}$ or did
431 not report physical activity and training history, which did not allow categorizing the
432 populations according to the aerobic fitness and/or activity/training status. It was recently
433 found that the decrease in the O_2 cost of exercise was negatively correlated with $\text{VO}_{2\text{max}}$, and
434 that the increase in NO_2^- plasma levels with dietary NO_3^- supplementation was lower in
435 subjects with high $\text{VO}_{2\text{max}}$ [52]. These results confirm those of previous studies where the
436 NO_3^- supplementation did not affect VO_2 , exercise tolerance or exercise performances in
437 highly endurance trained subjects [33, 34, 43, 47].

438

439 *Limitations*

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

445 The meta-analysis included a narrow range of subjects in terms of age, gender, health status
446 and physical activity level. The over-representation of active or highly endurance trained,

447 young, and healthy male participants is a limitation, making the applicability of the findings
448 to other populations debatable. Subgroup analysis by gender was for example not feasible due
449 to the low number of female participants (28 females/236 males) in the whole population of
450 the meta-analysis and the lack of study specifically investigating women. Two previous
451 studies reported a lower decrease in blood pressure [53] and no decrease in platelet reactivity
452 [54] in women supplemented with dietary NO₃⁻ compared to men, which suggest that women
453 may have different metabolic responses to increased NO₃⁻-NO₂⁻-NO bioavailability.

454 Due to the limited number of studies that investigated the effect of dietary NO₃⁻
455 supplementation on exercise tolerance and metabolic rate under hypoxic conditions [27, 31,
456 42], the meta-analysis could not confirm that dietary NO₃⁻ affects metabolic rate in these
457 conditions. The rationale for investigating the effect of dietary NO₃⁻ supplementation in
458 hypoxic condition is that native highlanders exhibit high circulating plasma NO₃⁻ and NO₂⁻
459 levels due to an up-regulation of NOS activity that may contribute to their acclimatization to
460 environmental hypoxia [55]. Hence, it has been hypothesized that subjects not acclimatized to
461 hypoxia may benefit from enhanced NO bioavailability provided by increased dietary NO₃⁻
462 intake, especially when NO synthesis is limited by hypoxia. This is supported by the findings
463 from 2 studies performed in hypoxic conditions that reported VO₂ decrease at rest and during
464 moderate intensity exercise following a 6-day beetroot juice supplementation [31], and during
465 heavy intensity exercise after a single ingestion of beetroot juice [42]. These results warrant
466 further works, though NO₃⁻ supplementation failed to facilitate the adaptations to hypoxic
467 environment during 6-week of hypoxic training program [27].

468

469 *Conclusion and Perspectives*

470 Previous meta-analyses already showed that dietary NO₃⁻ significantly decreases systolic
471 blood pressure [56] and may have a therapeutic potential in patients with cardiovascular

472 diseases and significantly improves exercise tolerance in healthy subjects [57].
473 Mechanistically, there were clear evidences from in vitro studies that NO increases
474 mitochondrial oxidative efficiency [39, 58]. The present meta-analysis supports that
475 increasing NO bioavailability through dietary NO_3^- translates into a significantly decreased O_2
476 cost during exercise performed in the heavy and moderate intensity domains, but not at
477 exercise intensities close to the maximum. These effects of dietary NO_3^- supplementation may
478 be of interest in patients with cardiovascular or respiratory diseases that severely limits
479 exercise capacity, with potential improvement of exercise tolerance that may positively affect
480 their quality of live. On the opposite, there is also a need to determine the factors responsible
481 for the lack of effect on VO_2 in subjects with high $\text{VO}_{2\text{max}}$, as short term beetroot juice
482 supplementation is used as an easy, available and healthy ergogenic aid by athletes.
483 Directions for future studies should also include research into the dose and duration of
484 supplementation in order to provide recommendation for NO_3^- supplementation specific to
485 each population.

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487

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493

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TABLES**TABLE 1.** Description of PICOS approach used in the systematic review and meta-analysis

Components	Criteria
Participants	Adult > 18 years with or without chronic disease conditions that severely impair oxygen delivery and/or utilization in particular.
Interventions	Increased dietary NO ₃ ⁻ intake by consumption of vegetables rich in NO ₃ ⁻ such as beetroot, or nitrate salt solutions without any restriction regarding supplementation characteristics.
Comparators	Placebo with negligible nitrate content
Outcomes	Metabolic rate expressed as VO ₂
Study design	Randomized controlled trials Crossover design Parallel design Double- or single-blind, or otherwise

NO₃⁻: nitrate; VO₂: oxygen uptake.

TABLE 2. Criteria for the determination of exercise intensity domains

Intensity domains	Physiological demarcation	Corresponding % VO_{2max} or aerobic peak power
Moderate	Below the gas exchange threshold	< 60%
Heavy	Between the gas exchange threshold and the critical power	From 60 to 80%
Severe	Above critical power	> 80 %
Maximal	Exercise performed up to subject's exhaustion with the attainment of VO _{2max} or VO _{2peak}	

The critical power is defined as the intensity above which the attainment of VO_{2max} is elicited, which is maintained up to exhaustion.

TABLE 3. Summary of dietary nitrate supplementation effects on VO₂

Random-effects models	Effect size	Significance (p < 0.05)
Resting condition	0.01 (95% IC: -0.47, 0.50)	p = 0.96
Submaximal intensity exercise	-0.26 (95% IC: -0.38, -0.15)	p < 0.01
Moderate intensity exercise	-0.29 (95% IC: -0.48, -0.10)	p < 0.01
Heavy intensity exercise	-0.33 (95% IC: -0.54,-0.12)	p < 0.01
Severe intensity exercise	-0.14 (95% IC: -0.38, 0.09)	p = 0.24
Maximal intensity exercise	0.02 (95% IC: -0.42, 0.46)	p = 0.93

FIGURES

FIGURE 1. Flow chart of the selection process in literature search

FIGURE 2. Forest plot of the effect size for the change in VO₂ during submaximal intensity exercise with dietary NO₃⁻ supplementation

FIGURE 3. Forest plot of the effect size for the change in VO₂ during moderate intensity exercise with dietary NO₃⁻ supplementation

FIGURE 4. Forest plot of the effect size for the change in VO₂ during heavy intensity exercise with dietary NO₃⁻ supplementation

FIGURE 5. Forest plot of the effect size for the change in VO₂ under resting condition with dietary NO₃⁻ supplementation

FIGURE 6. Forest plot of the effect size for the change in VO₂ during severe intensity exercise with dietary NO₃⁻ supplementation

FIGURE 7. Forest plot of the effect size for the change in VO₂ during maximal intensity exercise with dietary NO₃⁻ supplementation

FIGURE 8. Summary of dietary nitrate supplementation effects on VO₂

* Indicates a significant effect size ($p < 0.05$).

ONLINE SUPPLEMENTAL MATERIAL

SUPPLEMENTAL TABLE 1. Main characteristics of the studies included in the systematic review and meta-analysis

SUPPLEMENTAL FIGURE 1. Summary of risk of bias assessment by bias domains

SUPPLEMENTAL FIGURE 2. Funnel plots of the effect size for the change in VO₂

A. Pooled submaximal intensity exercise

B. Resting condition

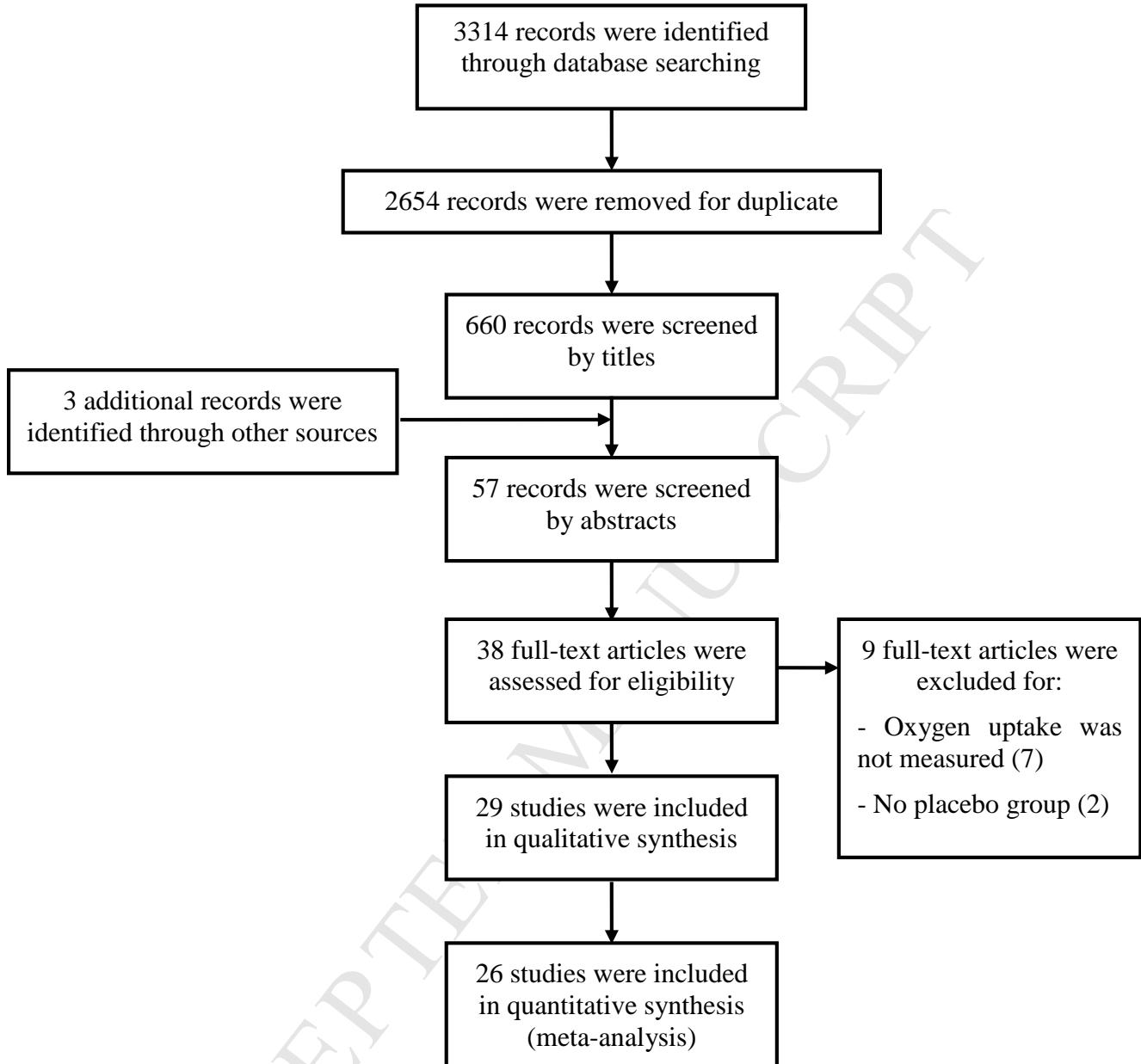
C. Maximal intensity exercise

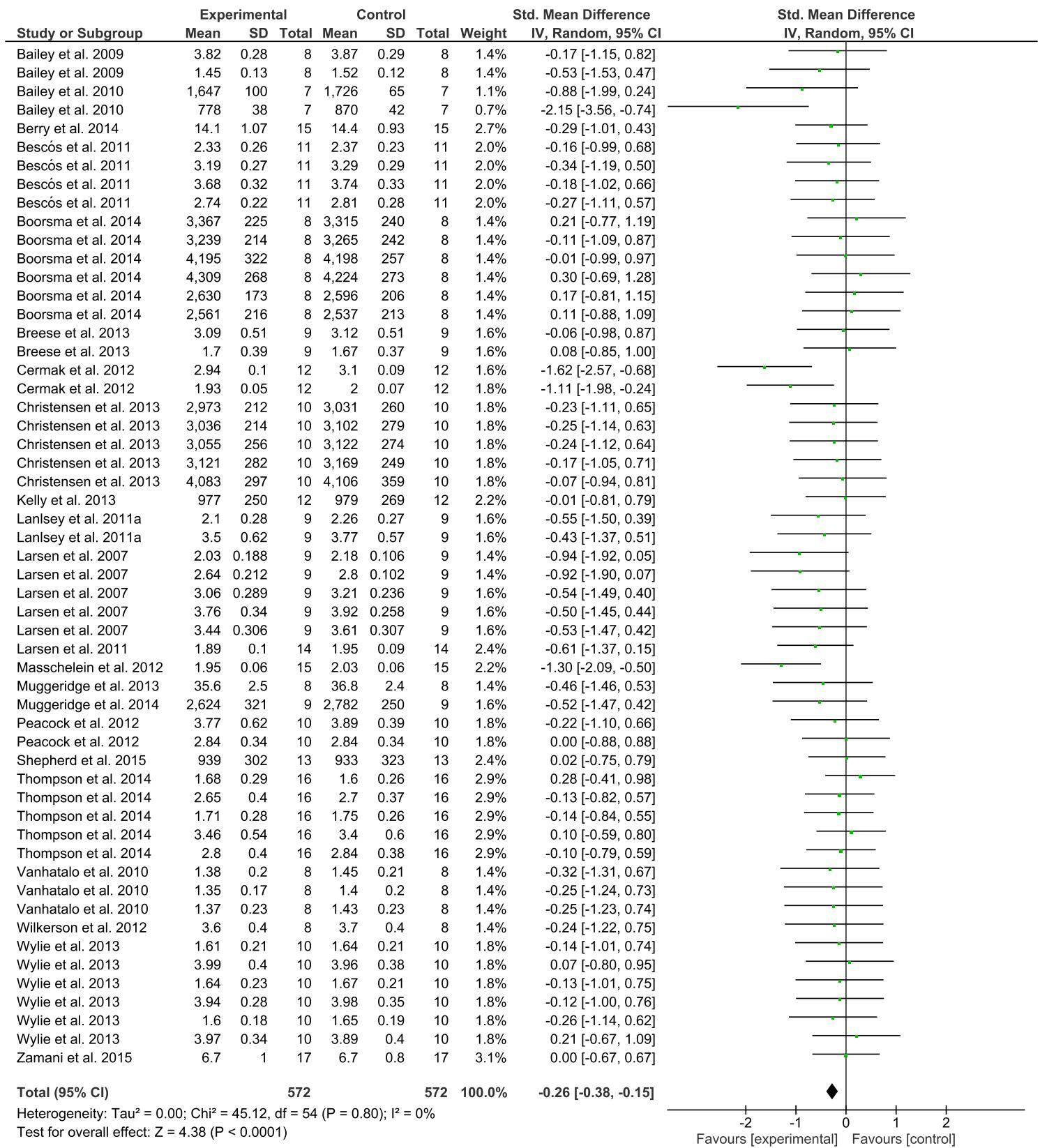
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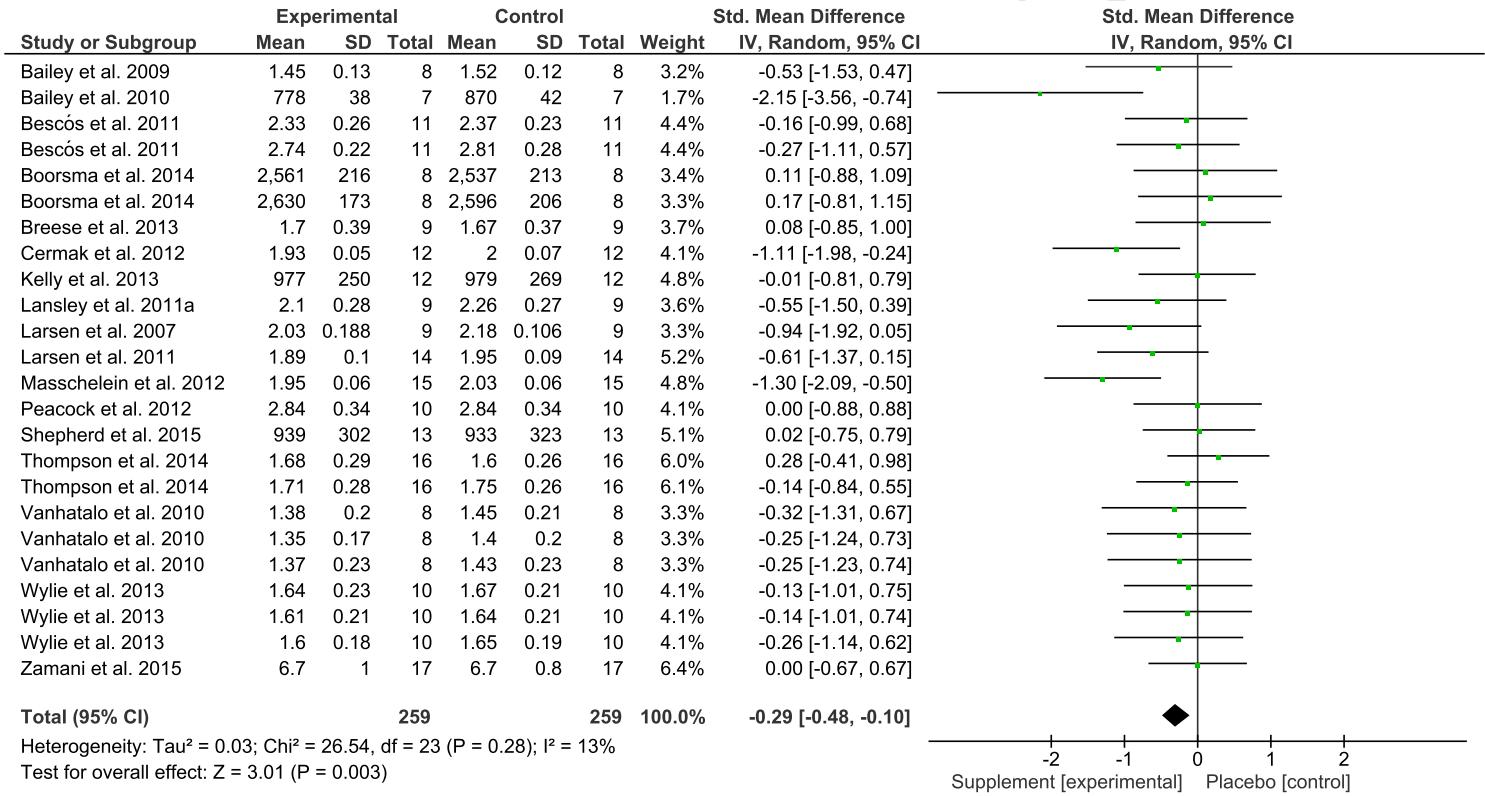
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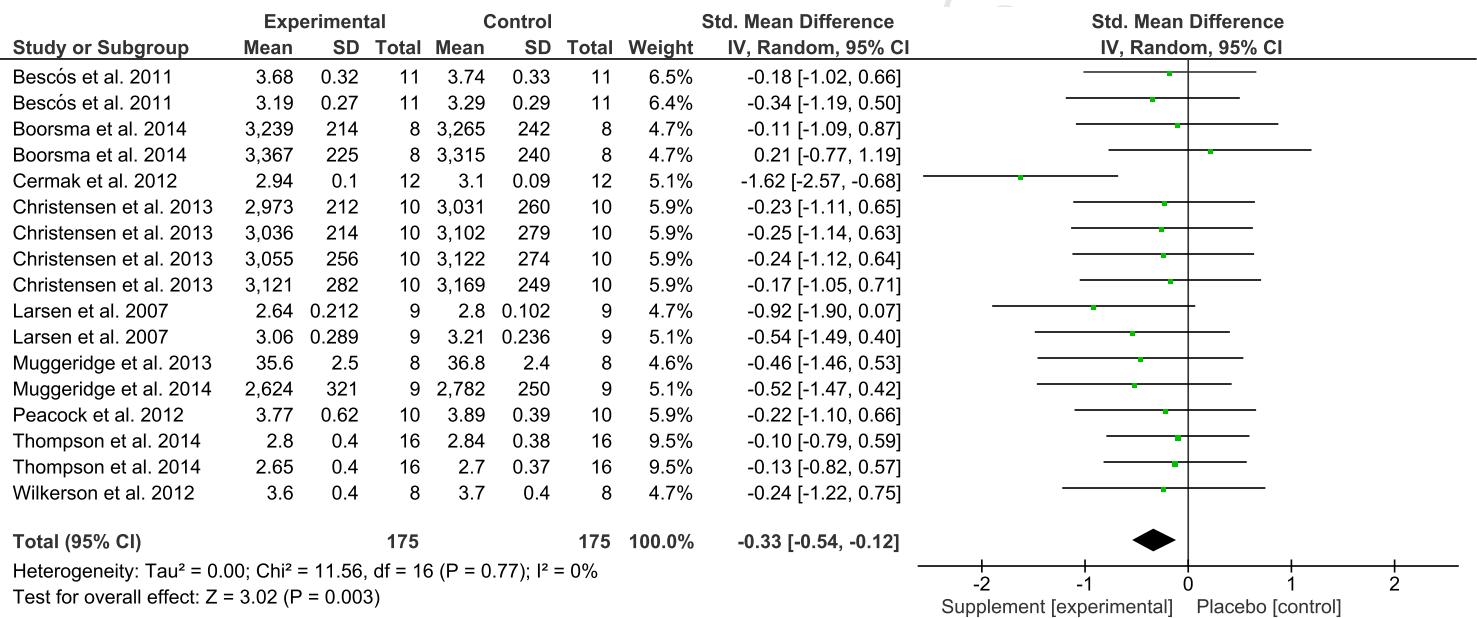
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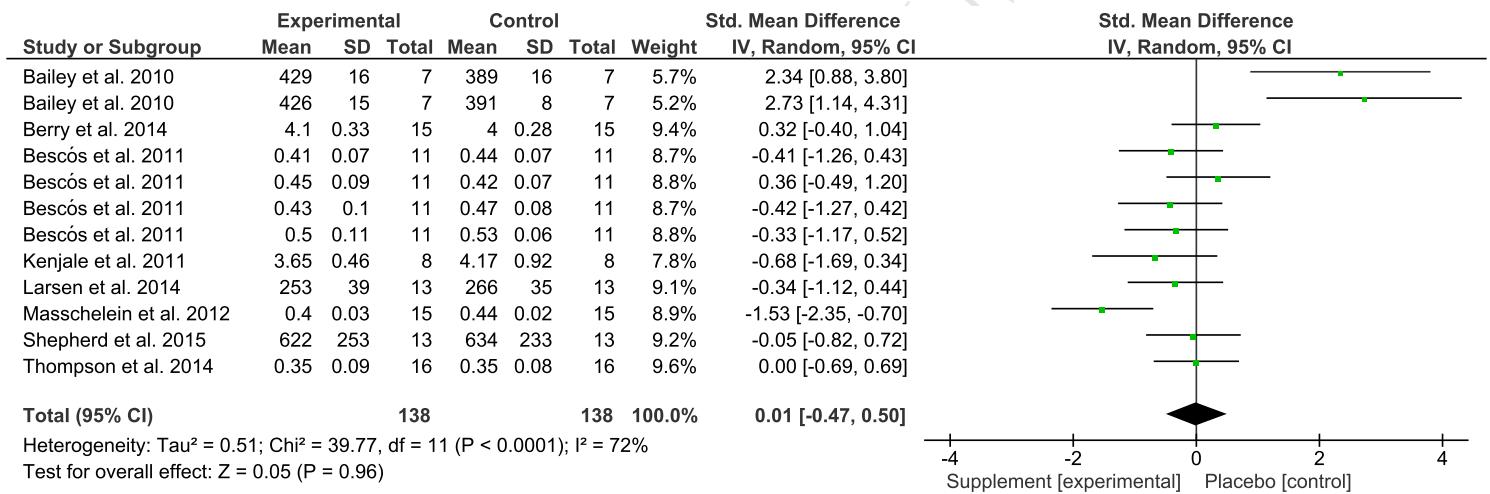
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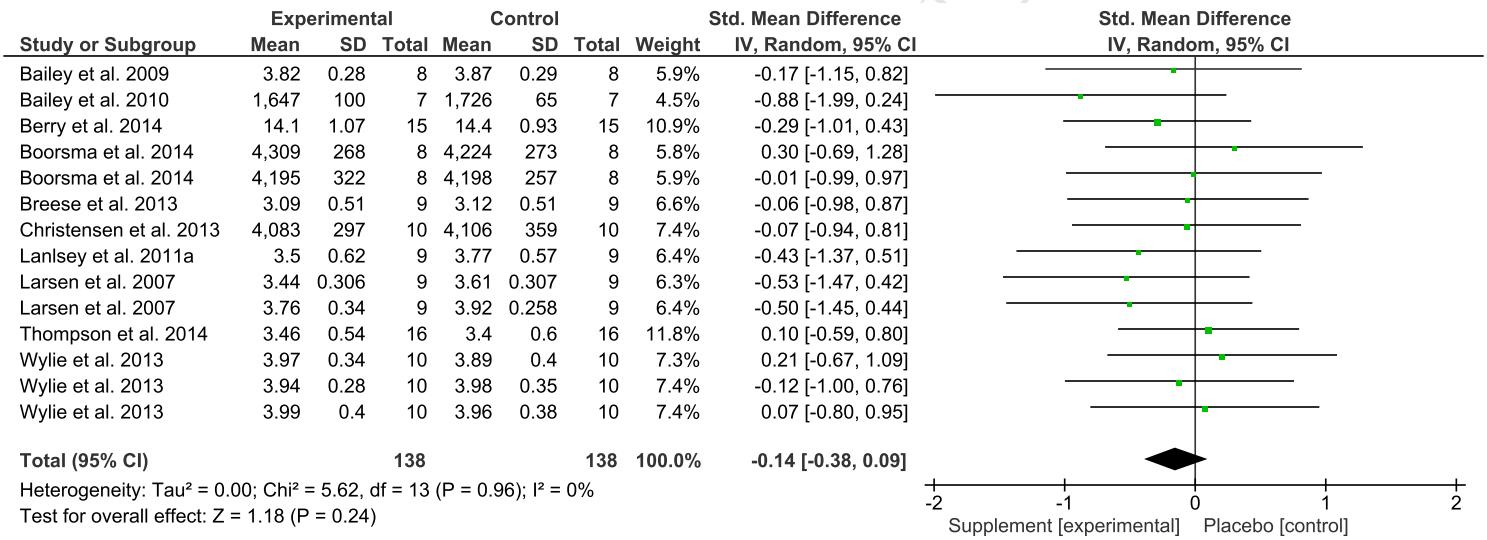


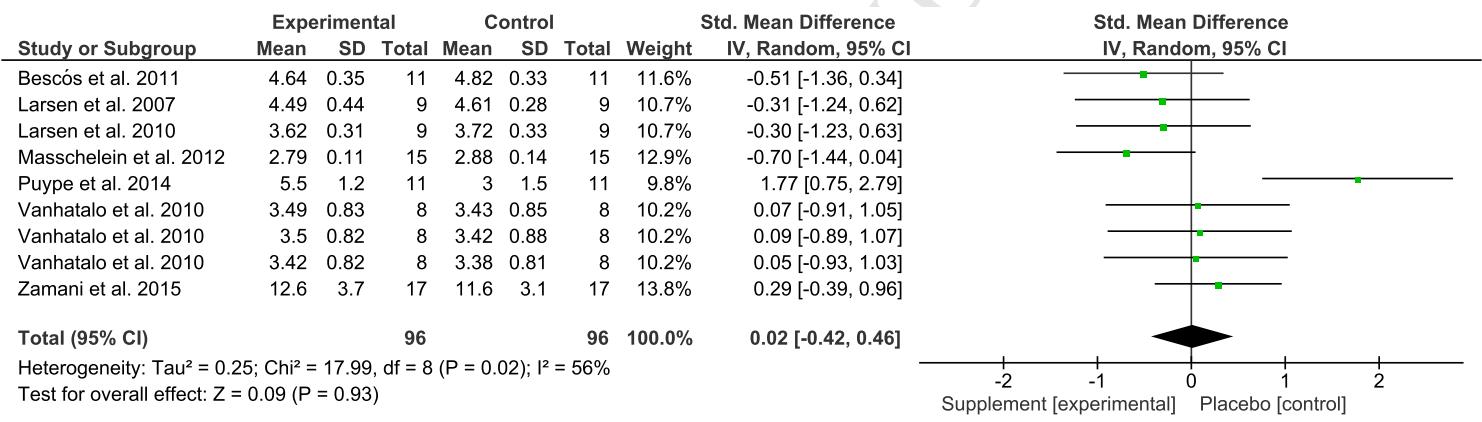


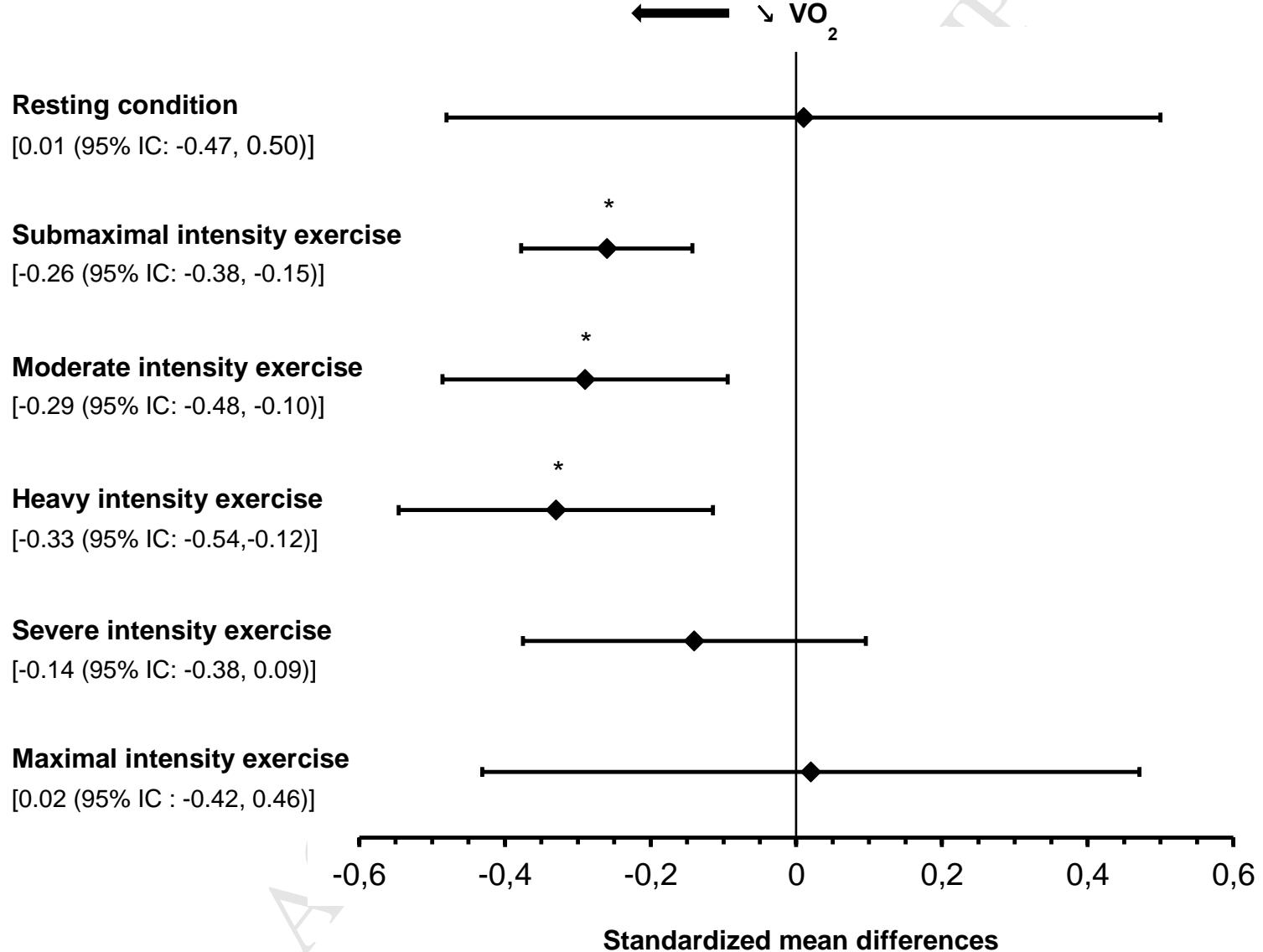












HIGHLIGHTS

- Dietary nitrate (NO_3^-) intake does not affect resting metabolic rate.
- NO_3^- intake does not affect oxygen uptake (VO_2) during maximal intensity exercise.
- NO_3^- supplementation decreases VO_2 during moderate and heavy intensity exercise.
- Exercise tolerance may be increased in disease, despite no effect on VO_2 .