Nitrate (NO$_3^-$) and nitrite (NO$_2^-$) have been known predominantly as undesired molecules in the food chain with potentially harmful effects or as inert oxidative end products of endogenous nitric oxide (NO) metabolism (21). However, research carried out during the last decade has shown that nitrate and nitrite are physiologically recycled in blood and tissues to form NO and other bioactive nitrogen oxides (20). When inorganic nitrate is ingested, it is rapidly absorbed in the upper gastrointestinal tract and its bioavailability is almost 100%. Most absorbed inorganic nitrate is ultimately excreted in the urine, but up to 25% of plasma nitrate is actively taken up by the salivary glands and excreted in the saliva (32). In the mouth, facultative anaerobic bacteria on the surface of the tongue reduce nitrate to nitrite (30). Salivary nitrite then can be further converted to NO in the stomach (24), but it is also clear that a substantial part of swallowed nitrite is absorbed intact to increase circulating plasma nitrite (20). This nitrite can be converted to NO and other bioactive nitrogen oxides in blood and tissues under appropriate physiological conditions (22). This pathway complements the classic l-arginine NO synthase pathway and is especially enhanced during tissue acidosis and hypoxia, when NO formation by NO synthases may be compromised (20). A recent study showed that when this circuit was interrupted by not swallowing saliva for 3 h after ingestion of nitrate-rich beverages, the rise in plasma nitrite, but not nitrate, was blocked (36). Hence, this pathway is required to increase circulating nitrite concentration after nitrate load. A picture is now emerging of the important functions of the nitrate–nitrite–NO pathway in the regulation of blood pressure and blood flow (16), gastric integrity (22), and tissue protection against ischemic injury (28). The nutritional aspect of these findings is intriguing because diet constitutes the main source of nitrate in humans, with vegetables accounting for 60%–80% of our daily intake.

Tissue acidosis and low oxygen tension are present during physical exercise. In this metabolic state, the reduction of nitrite is probably greatly enhanced. Recent studies have reported that dietary nitrate supplementation decreases whole-body oxygen consumption (VO$_2$) at low and moderate intensities of exercise in healthy subjects (1,2,15). In addition,
two recent studies showed that VO₂ values decreased significantly at higher intensities of exercise after several days of dietary nitrate supplementation (14,18). Although several hypotheses have attempted to explain how nitrate administration reduces the O₂ cost of exercise, the exact mechanism is currently unclear. The first research group to report the effects of nitrate supplementation on cardiorespiratory adaptation to exercise suggested that much of the O₂ reduction is due to the improvement in mitochondrial respiration with an increase in the P/O ratio (17). A recent study by Bailey et al. (1) suggested that this response could be derived from a reduction in phosphocreatine degradation, which diminishes the ATP cost of muscle force production.

Currently, it is known that the exercise response is different in highly trained athletes and the untrained population. Chronic exercise training induces improvements in vascular structures, muscle tissues, and the metabolism of NO (23,31). To date, studies have failed to report an improvement in the cardiorespiratory response in an athletic population using the classic precursor of NO (L-arginine) (4,19). However, in elderly populations with endothelial dysfunction, L-arginine supplements effectively enhance exercise capacity (9). In addition, a recent study by Koppo et al. (13) reported that L-arginine supplementation speeds VO₂ kinetics in healthy males. Other studies using supplementation with L-citrulline (an alternative precursor of NO) showed a significant increase in plasma L-arginine concentration, but no effects on performance, in well-trained cyclists (29,30). Moreover, previous studies of nitrate supplementation (1,2,14,15,18) assessed the effect of prolonged supplementation (between 3 and 6 d) in an attempt to increase the systemic levels of nitrate and nitrite. However, there is evidence of acute effects of nitrate on the cardiovascular system because it lowers blood pressure 3 h after ingestion in healthy subjects (36). One very recent study assessed the effect of acute ingestion of nitrate on physically active people, but these subjects were not highly trained (34).

Accordingly, in this study, we aimed to assess the effect of a single dose of nitrate given before cycling exercise on the cardiorespiratory and metabolic response in endurance athletes at different intensities. Moreover, we investigated the influence of nitrate supplementation on plasma levels of nitrate and nitrite over time. We hypothesized that dietary nitrate may not be effective in improving the cardiorespiratory adaptation to exercise at low to moderate intensities who are highly adapted to cycling. However, at higher intensities, at which acidosis and low oxygen tension occur, the nitrate–nitrite–NO pathway could be activated and increase tolerance to high-intensity cycling, which is measured as the time to task failure.

**METHODS**

**Subjects.** Eleven male cyclists and triathletes (age = 34.3 ± 4.8 yr, body weight = 73.3 ± 5.6 kg, body mass index = 23.7 ± 1.5 kg·m⁻², VO₂peak = 65.1 ± 6.2 mL·kg⁻¹·min⁻¹, sum of six skinfolds [triceps, subscapular, supraspinial, abdominal, medial calf, and front thigh] = 55.5 ± 13.8 mm) volunteered to participate in this study. Athletes were members of competitive cycling or triathlon squads, and none of them reported any medical conditions at the time of the study. None of the subjects smoked tobacco. The procedures used in this study were approved by the Ethics Committee of the Catalonian Sports Council. All subjects gave their written informed consent after an explanation of the experimental procedures and before the commencement of the study.

**Nitrate supplementation.** Subjects were randomly assigned in a double-blind crossover design to receive a single dose of either sodium nitrate (10 mg·kg⁻¹·kg⁻¹ of body mass; code 18211 [Acofarma, Madrid, Spain]) or the placebo (sodium chloride) dissolved in 250 mL of water. The two drinks could not be distinguished by taste or appearance. The beverage was ingested 3 h before the test because this period is consistent with the pharmacokinetics of nitrate and the peak of circulating nitrite indicated in previous studies (36). During this period, the subjects remained under resting conditions in the laboratory and did not ingest food and fluids, apart from water, to guarantee hydration status. A diet with low levels of moderate- or high-nitrate content foods (green vegetables, beetroot, strawberries, grapes, and tea) was followed for 3 d before the tests. During this time, athletes received nutritional guidelines and were encouraged to follow a high-CHO diet to optimize glycogen deposition. In addition, they were told to avoid alcohol, caffeine products, and dietary supplements 48 h before the exercise test. A 7-d washout separated the supplementation periods.

**Ergometry test.** The subjects were required to report to the laboratory on three occasions. The first test was carried out to familiarize the subject with the bicycle ergometer, gas analyzer, and the testing procedure. The next two tests were performed under identical conditions and used to assess the effect of the dietary nitrate and placebo. Tests were carried out during the cycling off-season in November and December to ensure that training or competitions would not affect the results of the study. All tests were performed at the same time of day (+1 h) on an electronically braked cycle ergometer (Lode Excalibur Sport, Groningen, The Netherlands) under controlled conditions (22°C ± 1°C, 40%–60% relative humidity, P₀ = 760–770 mm Hg). Before and after the study, the cycle ergometer was calibrated for power outputs of 25–1000 W at different cadences and was found to be within 1% of a true value. The participants cycled at a self-selected pedal rate of between 70 and 100 rpm. This pedal rate, along with saddle and handle bar height and configuration, was recorded and reproduced in subsequent tests. The protocol of the test was divided into two parts: submaximal and maximal exercise intensity. Initially, the subjects completed four submaximal workloads corresponding to 2.0, 2.5, 3.0, and 3.5 W·kg⁻¹ of body mass with every load lasting for 6 min, interspersed with 3 min of passive recovery. Five minutes after completion of the submaximal workloads, subjects performed
a continuous incremental exercise test to volitional exhaustion. Starting at 3.0 W·kg⁻¹, the work rate increased by 0.5 W·kg⁻¹ every minute until task failure as a measure of exercise tolerance. The maximal power output (W_max) was calculated using the formula:

\[ W_{max} = W_E + (W_I/t) t_E \]  

where \( W_{max} \) = maximal power output (W), \( W_E \) = power output of the last stage completed (W), \( W_I \) = work rate increment (W), \( t \) = workload duration (s), \( t_E \) = duration of the final stage (s).

Gas analysis. During all the tests, oxygen uptake (\( V\text{O}_2 \)), minute ventilation (\( V_E \)), carbon dioxide production (\( V\text{CO}_2 \)), and the RER were measured breath-by-breath by a computerized gas analyzer (Cosmed Quark PFT-Ergo, Rome, Italy). Before each test, ambient conditions were measured, and the gas analyzers and respiratory flowmeter were calibrated with high-precision calibration gases (16.00% ± 0.01% O₂ and 5.00% ± 0.01% CO₂; Scott Medical Products, Plumsteadville, PA) and a 3-L calibration syringe (Hans Rudolph, Shawnee, KS), respectively, following the manufacturer’s instructions.

Data analysis procedures. Breath-by-breath \( V\text{O}_2 \) data from submaximal bouts of exercise were initially examined to exclude errant breaths caused by coughing, swallowing, sighing, and others. Values greater than 4 SD from the local mean were removed. The first 20 s of data after the onset of exercise (i.e., the phase 1, cardiodynamic component) was deleted, and a nonlinear least squares algorithm was used to fit the data thereafter (SigmaPlot 8.0; SPSS, Inc., Chicago, IL). A single-exponential model was used to analyze the oxygen uptake kinetics of four submaximal rates of exercise, as described in the following equation:

\[ \dot{V}\text{O}_2(t) = \dot{V}\text{O}_2\text{baseline} + A_r [1 - e^{-(t - t_{DP})/\tau_P}] \]  

where \( \dot{V}\text{O}_2(t) \) represents the absolute \( \dot{V}\text{O}_2 \) at a given time; \( \dot{V}\text{O}_2\text{baseline} \) represents the mean \( \dot{V}\text{O}_2 \) in the baseline period; \( A_r \), \( T_{DP} \), and \( \tau_P \) represent the amplitude, time delay, and time constant, respectively, describing the phase 2 (i.e., primary component) increase in \( \dot{V}\text{O}_2 \) above baseline. \( \dot{V}\text{O}_2\text{baseline} \), and end-exercise \( \dot{V}\text{O}_2 \) were defined as the mean \( \dot{V}\text{O}_2 \) measured during the final 30 s before starting each submaximal workload and during the final 30 s of each submaximal workload, respectively. In addition, the gross efficiency (GE) was calculated as the mean of the data collected in the last 180 s of every submaximal workload in the steady state with RER < 1.0 using the formula:

\[ \text{GE} (%) = \frac{\text{work rate (W)/energy expended (J·s⁻¹)}}{100} \]  

The energy expenditure was in turn calculated with the Brouwer equation (7):

\[ \text{energy expenditure (J·s⁻¹)} = \left[ \frac{(3.869 \dot{V}\text{O}_2) + (1.195 V\text{CO}_2)}{4.186/60} \right] \times 1000 \]  

The \( \dot{V}\text{O}_2\text{peak} \) during the incremental test was determined as the mean \( \dot{V}\text{O}_2 \) measured over the final 60 s of exercise. To determine the ventilatory threshold (VT) and the respiratory compensation point (RCP), data were averaged at 30-s intervals and analyzed by two independent reviewers, according to methods described by Wasserman et al. (35). HR was continuously recorded during the test with a portable HR monitor and HR_max was defined as the HR at the point of exhaustion (RS800 SD; Polar, Kempele, Finland).

Blood sampling. A small catheter was inserted into an antecubital vein for venous blood sampling. Four blood samples were collected to analyze nitrate and nitrite: 1) during resting conditions, 2) 3 h after supplement or placebo ingestion, 3) in the first minute after the fourth submaximal load, and 4) in the first minute after the maximal test. Venous blood was drawn with a 5-mL syringe EDTA and was immediately centrifuged at 1000g for 20 min to separate plasma from blood cells. Plasma samples were then centrifuged for 30 min at 14,000g in 10K filters (Amicon Ultra; Millipore, Billerica, MA) to remove proteins. The supernatant was recovered and used to measure nitrite and nitrate levels by detecting the liberated NO in a gas-phase chemiluminescence reaction with ozone using a nitric oxide analyzer (NOA 280i; Sievers, Boulder, CO).

Nitrate levels were determined following an adaptation of the method described by Braman and Hendrix (6). Briefly, the purge vessel was loaded with a saturated VCl₃ solution in 1 M HCl and heated to 90°C with a current of hot water. To prevent damage to the NOA from the hydrochloric acid vapor, a gas bubbler filled with 1 M NaOH was installed between the purge vessel and the NOA. A nitrate standard (5–200 μM) was used to calculate the nitrate concentration. Ten microliters of the filtered sample or standard was injected into the purge vessel, and the area under the curve of NO peaks was recorded and processed using NOAnalysis™ Liquid software v. 3.2 (IONICS, Boulder, CO).

Nitrite levels were determined following an adaptation of the method described by Castegnaro et al. (8). Briefly, the purge vessel was loaded with 50 mM KI in glacial acetic acid and 400 μL of antifoam. A nitrite standard (0.5–10 μM) was used to calculate the nitrite concentration. One hundred microliters of the filtered sample or standard were injected into the purge vessel and the area under the curve of NO peaks was recorded and processed using NOAnalysis™ Liquid software v. 3.2 (IONICS).

In addition, seven samples of capillary blood (10 μL) were collected from the ear lobe to analyze lactate ([HLa]) using a Lactate Photometer plus DP100 (Diaglobal GmbH, Berlin, Germany): 1) during resting conditions, 2) in the first minute after each submaximal load, and 3) at 3 and 5 min after the maximal test.

Statistics. Results are expressed as means ± SEM. A paired t-test was used to evaluate the differences between the placebo and the nitrate groups, where appropriate. To investigate the influence of time and treatment, the data were treated with two-way ANOVA with repeated measures on both time and treatment. The data were assessed to determine the normal distribution, and post hoc analyses
were performed via Tukey HSD. The significance level was set at $P < 0.05$, whereas a trend was noted when $P < 0.10$.

**RESULTS**

**Plasma nitrate and nitrite kinetics.** The concentrations of nitrate were similar (nitrate = $30 \pm 12$ $\mu$M, placebo = $28 \pm 10$ $\mu$M) before intake. Three hours after ingestion, the plasma levels of nitrate had increased significantly in the nitrate group ($250 \pm 80$ $\mu$M, $P < 0.001$) but remained unchanged in the placebo group ($29 \pm 8$ $\mu$M). The nitrate concentrations in plasma were not affected at any sample point after placebo treatment (Fig. 1). After nitrate supplementation, the plasma levels were significantly lower after submaximal ($234 \pm 82$ $\mu$M, $P = 0.027$) and maximal ($237 \pm 85$ $\mu$M, $P = 0.045$) exercise compared with the peak value reached 3 h after supplementation ($250 \pm 80$ $\mu$M) (Fig. 1).

There were no differences between treatments in the levels of nitrite under fasting conditions (nitrate = $2005 \pm 158$, placebo = $2053 \pm 278$ nM). Conversion of nitrate to nitrite was evident from the increased plasma nitrite levels 3 h after nitrate supplementation ($2313 \pm 157$ nM, $P = 0.017$) compared with the placebo ($1998 \pm 206$ nM). During nitrate treatment, nitrite levels were significantly lower after maximal exercise ($2126 \pm 251$ nM, $P = 0.044$) than the peak value reached 3 h after supplementation ($2313 \pm 157$ nM) (Fig. 1). Nitrite also tended to be lower after the placebo treatment and maximal exercise ($1916 \pm 168$ nM) than under fasting conditions ($2053 \pm 278$ nM, $P = 0.056$) (Fig. 1).

**Submaximal work parameters.** The cardiorespiratory values during the four bouts of exercise after nitrate supplementation and the placebo are shown in Table 1. There were no significant differences between the nitrate and placebo in VO$_2$, VCO$_2$, $V_{E}$, RER, HR, and GE. In addition, we did not find changes in the time constant and primary amplitude of VO$_2$ at any submaximal load (Table 1). The mean work rate was $147 \pm 11$ W at $2$ W·kg$^{-1}$, $183 \pm 14$ W at $2.5$ W·kg$^{-1}$, $220 \pm 17$ W at $3$ W·kg$^{-1}$, and $257 \pm 20$ W at $3.5$ W·kg$^{-1}$. The chosen cadence was $87 \pm 8$ rpm on the two occasions (nitrate and placebo).

**Maximal work parameters.** After nitrate supplementation, VO$_{2peak}$ dropped from $4.82 \pm 0.33$ to $4.64 \pm 0.35$ L·min$^{-1}$ ($P = 0.010$) (Table 2). In addition, VO$_2$ tended to be lower at the respiratory compensation point after nitrate supplementation ($4.31 \pm 0.28$ L·min$^{-1}$) than after the placebo ($4.44 \pm 0.23$ L·min$^{-1}$, $P = 0.068$) (Table 2). The ratio between oxygen consumption and power was significantly decreased at the VO$_{2peak}$ level after nitrate ingestion ($P = 0.031$) (Fig. 2). Other cardiorespiratory parameters such as HR, pulmonary ventilation, and carbon dioxide production were unaffected by nitrate supplementation. There was no significant difference in time to exhaustion between treatments (nitrate = $416 \pm 32$ s, placebo = $409 \pm 27$ s, $P = 0.169$) at the maximal intensity of exercise. The workload at VO$_{2peak}$ was $416 \pm 29$ W for nitrate and $410 \pm 28$ W for the placebo ($P = 0.318$).

**Blood lactate concentration.** No differences were found in blood lactate accumulation between conditions at any point of submaximal or maximal exercise intensity (Fig. 3).

**DISCUSSION**

In agreement with our first hypothesis, this research showed that cardiorespiratory adaptation at low to moderate intensities of exercise was not modified by a single administration of nitrate (10 mg·kg$^{-1}$) in well-trained cyclists. Although our second hypothesis of nitrate-induced enhancement of tolerance to high-intensity cycling was not confirmed, we found that the VO$_{2peak}$ was significantly reduced without affecting the maximal attainable work, blood lactate, or other cardiorespiratory parameters. This was coupled with consumption of plasma nitrite mainly in the nitrate group, which probably indicates a reduction of this anion to NO and other bioactive nitrogen species.
The levels of plasma nitrate had increased by 86.9% ± 8.4% (P < 0.05) 3 h after supplementation compared with the placebo, which is consistent with previous studies (1,2,15,18,36). In addition, we found that plasma nitrate was significantly lower after submaximal (234 ± 82 μM; P = 0.027) and maximal (237 ± 85 μM; P = 0.045) exercise than its values at 3 h after supplementation (250 ± 80 μM). This fact is difficult to attribute to the effect of exercise alone because the level of nitrate was no different after the incremental than after submaximal exercise. Previous research showed that nitrate remained stable after exercise (15). One likely explanation for this finding is related to the pharmacokinetics of nitrate after dietary ingestion. There is evidence that the plasma levels of nitrate increased rapidly within the 30 min after nitrate supplementation to peak at 1.5 h (18,36). The half-life of plasma nitrate in humans is approximately 5 h, and there is a substantial decrease after 4 h of ingestion (36). In this study, the timing was at the borderline of the nitrate half-life because athletes completed submaximal and maximal workloads at 3 h 45 min (±10 min) and 4 h 5 min (±14 min), respectively.

Effects of an acute dose of nitrate on blood levels of nitrate and nitrite. The levels of plasma nitrate had increased by 86.9% ± 8.4% (P < 0.05) 3 h after supplementation compared with the placebo, which is consistent with previous studies (1,2,15,18,36). In addition, we found that plasma nitrate was significantly lower after submaximal (234 ± 82 μM; P = 0.027) and maximal (237 ± 85 μM; P = 0.045) exercise than its values at 3 h after supplementation (250 ± 80 μM). This fact is difficult to attribute to the effect of exercise alone because the level of nitrate was no different after the incremental than after submaximal exercise. Previous research showed that nitrate remained stable after exercise (15). One likely explanation for this finding is related to the pharmacokinetics of nitrate after dietary ingestion. There is evidence that the plasma levels of nitrate increased rapidly within the 30 min after nitrate supplementation to peak at 1.5 h (18,36). The half-life of plasma nitrate in humans is approximately 5 h, and there is a substantial decrease after 4 h of ingestion (36). In this study, the timing was at the borderline of the nitrate half-life because athletes completed submaximal and maximal workloads at 3 h 45 min (±10 min) and 4 h 5 min (±14 min), respectively.

Additional studies are needed to pinpoint the exact mechanisms behind this finding. Nitrate takes longer to appear in the circulation than nitrate, peaking between 2.5 and 3 h (36). This delay is due to the enterosalivary circulation of these compounds. Most of the absorbed nitrate is ultimately excreted in the urine, but up to 25% of plasma is also excreted in the saliva (22). In the oral cavity, commensal facultative anaerobic bacteria reduce nitrate to nitrite by the action of nitrate reductase enzymes. The nitrite is swallowed, and in the acidic environment of the stomach, it is reduced to NO or reenters the circulation as nitrite (21). Because inorganic nitrite is the main precursor of NO and other bioactive nitrogen oxides, we decided on a 3-h period between supplement ingestion and the start of exercise to ensure that the nitrite in plasma had peaked. Curiously, basal levels of nitrite in this study were higher than in previous studies of healthy populations (1,15,18,34).

**TABLE 1. Cardiorespiratory dynamics during low- to moderate-intensity exercise after supplementation with nitrate or placebo (n = 11).**

<table>
<thead>
<tr>
<th>Load</th>
<th>2.0 W kg⁻¹</th>
<th>2.5 W kg⁻¹</th>
<th>3.0 W kg⁻¹</th>
<th>3.5 W kg⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Placebo</td>
<td>Nitrate</td>
<td>Placebo</td>
<td>Nitrate</td>
</tr>
<tr>
<td>VO₂max (L min⁻¹)</td>
<td>4.02 ± 0.07</td>
<td>4.04 ± 0.07</td>
<td>4.01 ± 0.07</td>
<td>4.07 ± 0.08</td>
</tr>
<tr>
<td>VCO₂max (L min⁻¹)</td>
<td>2.37 ± 0.23</td>
<td>2.28 ± 0.28</td>
<td>2.30 ± 0.22</td>
<td>2.32 ± 0.27</td>
</tr>
<tr>
<td>Time constant, t (s)</td>
<td>13.4 ± 5.4</td>
<td>14.5 ± 5.3</td>
<td>14.3 ± 4.3</td>
<td>16.6 ± 6.2</td>
</tr>
<tr>
<td>VO₂ at VT (L min⁻¹)</td>
<td>1.98 ± 0.21</td>
<td>2.38 ± 0.23</td>
<td>2.30 ± 0.22</td>
<td>2.80 ± 0.27</td>
</tr>
<tr>
<td>VCO₂ at VT (L min⁻¹)</td>
<td>0.31 ± 0.06</td>
<td>0.41 ± 0.08</td>
<td>0.41 ± 0.07</td>
<td>0.44 ± 0.08</td>
</tr>
<tr>
<td>HR at VT</td>
<td>10.8 ± 1.9</td>
<td>12.1 ± 2.7</td>
<td>14.2 ± 10.6</td>
<td>14.4 ± 2.8</td>
</tr>
<tr>
<td>HR at RCP</td>
<td>48.9 ± 6.4</td>
<td>51.0 ± 6.3</td>
<td>59.8 ± 7.0</td>
<td>59.6 ± 6.6</td>
</tr>
<tr>
<td>RER</td>
<td>0.73 ± 0.08</td>
<td>0.77 ± 0.10</td>
<td>0.82 ± 0.09</td>
<td>0.86 ± 0.10</td>
</tr>
<tr>
<td>VO₂ at RCP (L min⁻¹)</td>
<td>0.23 ± 0.07</td>
<td>0.37 ± 0.08</td>
<td>0.52 ± 0.08</td>
<td>0.74 ± 0.10</td>
</tr>
<tr>
<td>VO₂ at VT (L min⁻¹)</td>
<td>111 ± 8</td>
<td>126 ± 10</td>
<td>124 ± 11</td>
<td>142 ± 12</td>
</tr>
<tr>
<td>VO₂max (L min⁻¹)</td>
<td>156.4 ± 16.5</td>
<td>151.0 ± 22.6</td>
<td>2.02 ± 2.71</td>
<td>2.30 ± 3.17</td>
</tr>
<tr>
<td>RER</td>
<td>0.27 ± 2.75</td>
<td>0.23 ± 2.30</td>
<td>0.09 ± 0.44</td>
<td>0.27 ± 3.74</td>
</tr>
<tr>
<td>HR at VT</td>
<td>182 ± 14</td>
<td>182 ± 14</td>
<td>182 ± 14</td>
<td>182 ± 14</td>
</tr>
</tbody>
</table>

Values are means ± SD.
RER, respiratory exchange ratio; HR, heart rate; VCO₂, expired carbon dioxide; VO₂, oxygen uptake.

**TABLE 2. Metabolic and circulatory response to maximal exercise after dietary supplementation with nitrate or placebo (n = 11).**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Placebo</th>
<th>Nitrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>VO₂peak (L min⁻¹)</td>
<td>4.62 ± 0.33</td>
<td>4.64 ± 0.35*</td>
</tr>
<tr>
<td>VO₂ max (L min⁻¹)</td>
<td>4.44 ± 0.23</td>
<td>4.31 ± 0.28**</td>
</tr>
<tr>
<td>VO₂ at VT (L min⁻¹)</td>
<td>3.52 ± 0.32</td>
<td>3.45 ± 0.23</td>
</tr>
<tr>
<td>VCO₂ peak (L min⁻¹)</td>
<td>5.25 ± 0.45</td>
<td>5.18 ± 0.50</td>
</tr>
<tr>
<td>VO₂max (L min⁻¹)</td>
<td>156.4 ± 16.5</td>
<td>151.0 ± 22.6</td>
</tr>
<tr>
<td>RER</td>
<td>1.09 ± 0.08</td>
<td>1.12 ± 0.07</td>
</tr>
<tr>
<td>HRpeak (beats min⁻¹)</td>
<td>182 ± 14</td>
<td>182 ± 14</td>
</tr>
</tbody>
</table>

Values are means ± SD.
* Statistical significance between nitrate and placebo (P < 0.05).
** Statistical tendency between nitrate and placebo (P < 0.10).
VO₂peak, peak of oxygen consumption at ventilatory threshold; VO₂max, maximum minute ventilation; VO₂ at RCP, oxygen consumption at respiratory compensation point; VO₂ at VT, oxygen consumption at ventilatory threshold; VO₂peak, peak of oxygen consumption.

**FIGURE 2—Rate between oxygen consumption and power at ventilatory threshold (VT), at respiratory compensation point (RCP), and at peak of oxygen consumption (VO₂peak) (n = 11).** *Statistical significance between nitrate and placebo (P < 0.05).
These differences may be due to methodological issues or the present subjects’ high level of training. Accordingly, Rassaf et al. (25) showed that plasma nitrite is directly proportional to exercise capacity. Another recent study showed higher levels of nitrite in the Tibetan population because of adaptation to altitude (11). Interestingly, inhabitants of high altitudes had higher maximal work rates than inhabitants of lower altitudes (11). To sum up, more studies are needed to establish the normal levels of plasma nitrite in highly trained athletes.

Effects of an acute dose of nitrate on the physiological response to low to moderate exercise. We found no statistical differences in cardiorespiratory adaptation to exercise at low to moderate intensities between the nitrate and placebo groups (Table 1). In contrast, previous studies showed significant improvements in exercise efficiency at low to moderate intensities after dietary nitrate supplementation (1,2,15). The present study design differed from these studies in two main aspects. The first is the duration of the treatment. Although previous studies followed several days (between 3 and 6 d) of nitrate supplementation, we assessed the effect of only one dose before exercise. Interestingly, we showed that an acute dose of sodium nitrate equivalent to 10 mg kg⁻¹ produced a similar increase in plasma nitrate (218 ± 68 μM) as a 3-d supplement of sodium nitrate (212 ± 28 μM), in which 8.5 mg kg⁻¹ d⁻¹ were ingested (18). Thus, the difference in VO₂ response to submaximal exercise between studies is probably not due to the availability of plasma nitrate. The second difference was the characteristics of the subjects analyzed in each study. All previous studies have been carried out in healthy volunteers with a VO₂peak between 45 and 58 mL kg⁻¹ min⁻¹ (1,2,14,15,18,34). In our study, all subjects were endurance athletes with high VO₂peak (65.1 ± 6.2 mL kg⁻¹ min⁻¹). In this regard, training may alter the physiological response to exercise. Mitochondrial volume and aerobic capacity in type II fibers increase greatly in endurance athletes (12). Decreases in submaximal oxygen uptake after endurance training may be due to changes in the working muscle’s oxidative capacity and metabolic processes, represented by an increase in the activity of the mitochondrial enzymes (33). Evidence to support this argument is that NO production seems to be a temporary response to chronic exercise that progresses to structural vascular and muscle adaptations (23). Nevertheless, other anatomical, biochemical, and biomechanical (pedaling technique) factors, among others, should not be excluded because they may contribute to the improvement of movement efficiency when normal and athletic populations are compared (12). Collectively, the data obtained at this moment suggest that the effects of acute nitrate supplementation at low to moderate intensities of exercise might be more limited in endurance-trained athletes than in moderately trained subjects.

Effects of an acute dose of nitrate on the physiological response to maximal exercise. Nitrate supplementation showed a trend toward reducing O₂ cost of exercise when athletes exceeded the RCP point. Differences between nitrate and placebo conditions became significant at maximal intensity of exercise (VO₂peak). In addition, we found that the mean ratio between VO₂peak and W' peak fell significantly after dietary nitrate ingestion. These findings confirm results reported by two recent studies when moderately trained subjects were supplemented for 3 and 6 d with inorganic nitrate and nitrate-rich beetroot juice, respectively (14,18). However, these surprising reductions in VO₂peak and in the ratio of VO₂peak and W' peak were not linked with impairment of performance. We found that tolerance to exercise measured as time to exhaustion was maintained after nitrate supplementation (nitrate = 416 ± 32 s, placebo = 409 ± 27 s). This physiological change occurred without any effect on other cardiorespiratory parameters (HR, V̇e, VCO₂ and RER), as well as lactate concentrations, which suggests that the reduction in VO₂peak could not be originated from alterations in the energetic cost of cardiorespiratory support processes.

However, the mechanistic bases for the reduced VO₂peak after nitrate ingestion have not been described in full. It is known that the nitrate–nitrite–NO pathway is gradually activated as the oxygen supply is limited and nitrite is converted to NO under hypoxic and acidic conditions (21). Therefore, as maximal intensity of exercise reproduces these physiological conditions, synthesis of NO could be derived by nitrite oxidation. Interestingly, in the current study, when athletes were supplemented with nitrate before exercise, it was found that plasma nitrite levels decreased significantly just after finishing maximal workload, suggesting activation of nitrate–nitrite–NO pathway (Fig. 1). There is evidence that NO donors, which evoke a small increase in NO, improve muscle metabolism, preventing an excess of calcium release and subsequently modulating the ATP cost of force production (26). In addition, it is known that one of the most energetically costly processes during skeletal muscle contraction

![Graph showing plasma lactate concentration at rest conditions, after every submaximal workloads equivalents to 2.0, 2.5, 3.0, and 3.5 W kg⁻¹, and at 3 and 5 min after maximal exercise in both conditions (nitrate and placebo).](http://www.acsm-msse.org)
is sarcoplasmic reticulum calcium pumping, which may account for up to 50% of the total ATP turnover (3). From this viewpoint, a recent study by Bailey et al. (1) found that a decrease in O2 cost of exercise after dietary nitrate supplementation was related to a reduction in ATP cost of muscle force production. On the other hand, it is widely accepted that NO is involved in the regulation of mitochondrial O2 consumption. In mitochondria, a reduction in the O2 cost of ATP resynthesis would require either more protons to be pumped per O2 molecule reduced or the use of an alternative terminal electron acceptor. Recent studies have shown that demands of mitochondrial oxygen consumption increase in vitro when NO donors are added (5) and decrease in vivo when the NOS inhibitor l-NAME is added (27). In relation to these findings, an interesting study by Larsen et al. (17) indicates that mitochondrial respiration, measured in vitro as the amount of oxygen reduced per ATP produced (P/O ratio), is significantly improved after dietary nitrate supplementation in humans. However, all these findings, including the reduction of ATP cost of force production reported by Bailey et al. (1) as well as the improvement in mitochondrial function indicated by Larsen et al. (17) after nitrate supplementation, have been reported only when subjects performed exercise at low to moderate intensity. Currently, it is unclear whether the fall in the VO2peak found in the current study could be explained by these metabolic mechanisms or whether there are other pathways linked to this intriguing physiological response. Further research is needed to elucidate the mechanistic bases of VO2peak reduction in well-trained athletes after dietary nitrate consumption.

In conclusion, acute dietary nitrate administration 3 h before an exercise test increases plasma levels of nitrate and nitrite. In contrast with previous studies carried out in moderately trained subjects, we did not find that nitrate supplementation enhances cardiorespiratory adaptation to exercise at low to moderate exercise intensity. However, we found that the VO2peak was significantly reduced when athletes ingested nitrate. These in vivo data were found without any changes in cardiorespiratory and performance parameters, which suggests that nitrate and its reaction products could play an important role in oxygen consumption at maximal intensity of exercise in well-trained athletes.

The present study was funded by the National Institute of Physical Education, Laboratory of Physical Activity Science of University of Balearic Islands, by the Spanish Ministry of Health (DPS2008-07033-C03-03), and by FEDER funds. Raúl Bescós is a Ph.D. candidate supported by the University Department, Research and Information Society (AGAUR) of the Generalitat of Catalonia. The authors thank the athletes for participating in this study. The authors thank the personnel of the Sports Physiology Department and the Research Group, GIRSANE, of the High Performance Center (CAI) of Barcelona, for their technical and experienced support during the study. The authors also thank Xavier Àbalos, Jairo Vázquez, and Lara Rodríguez for their support in data collection during the study. The results of the present study do not constitute endorsement by the American College of Sports Medicine.

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