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The effects of dietary nitrate (beetroot juice) supplementation on exercise performance: A review

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Abstract: Nitric oxide (NO) is a potent vasodilator that increases blood flow and induces various intracellular actions such as increased mitochondrial and contractile efficiency. NO bioavailability may be increased by direct consumption of dietary nitrate and its sequential reduction to nitrite, a regulator of NO-induced hypoxic signaling. Dietary nitrate consumption reduces blood pressure, protects from ischemia-reperfusion injury, and improves endothelial dysfunction. Recently, the popularity of dietary nitrate as an ergogenic aid has been increased dramatically. Most exercise studies have administered dietary nitrate in the form of beetroot juice containing 5-8 mmol of nitrate and a few studies have used sodium and potassium nitrate (8-10 mg/kg). The most prominent and consistent effects of dietary nitrate supplementation is a reduction in the oxygen cost of exercise and an increase in exhaustion time at submaximal workloads. This effect was observed after either a single bolus (2-3 h prior to exercise) or a long-term (2-15 days) supplementation. The ergogenic effects of beetroot supplementation appear to be dose-dependent and are most often observed after long-term ingestion (approximately 6 days), at high exercise intensities and in less fit individuals. The ergogenic value of beetroot supplementation in endurance athletes is not clear; many studies have documented no improvements and a few studies an enhanced performance (0.4% to 3%) in time- and distance-trials. Clearly, more research is needed to document (i) the optimal dosage of beetroot ingestion for enhancing exercise performance in athletes, (ii) the effects of dietary nitrate consumption on training adaptations, (iii) the efficacy of beetroot supplementation in increasing exercise tolerance in individuals with chronic disease, and (iv) the safety of long-term beetroot consumption.

Keywords: Dietary Nitrate, Nitric Oxide, Nitrite, Beetroot, Endurance, Exercise, VO2, Performance, Supplements

1. Introduction

Nitric oxide (NO) is a molecule that has been implicated in numerous vascular, contractile, and metabolic physiological processes (1-3). NO is biosynthesized by endogenous and exogenous processes. Endogenously, NO is produced by enzymatic and non-enzymatic pathways. The first pathway includes the oxidation of L-arginine to NO, a reaction catalyzed by the enzyme nitric oxide synthase (NOS); the second pathway involves a non-enzymatic (NOS-independent) reduction of inorganic nitrate to NO via the "nitrate-nitrite-NO" pathway. It should be noted that the L-arginine-NOS system contributes to recycling of nitrate and nitrite for the operation of the endogenous non-enzymatic "nitrate-nitrite-NO" pathway (4).

The NOS-independent processes occur also exogenously, by the direct consumption of dietary nitrate and its sequential reduction to nitrite and then to NO, enabling the "nitrate-nitrite-NO" pathway. In fact, it is believed that the biological effects of dietary nitrate are mediated by its conversion to NO. After ingestion, the nitrate is rapidly absorbed from the upper part of the small intestine into the plasma reaching the peak concentration in about 1 to 2h (5). About 25% of the ingested dietary nitrate is absorbed from the plasma by the salivary glands and undergoes metabolic conversion to nitrite in the oral cavity. Portion of the swallowed salivary nitrite is reduced to NO in the stomach and the remaining enters the systemic circulation and is converted to NO in the blood and body tissues (6, 7). This pathway is of great importance for production of NO under hypoxic conditions (mismatch of O_2 delivery and utilization).

Green leafy vegetables are the major dietary sources of nitrate. Particularly, plants grown in low exposure to sunlight, such as roots, have higher nitrate content compared to nitrate present in leaves and stems. Among leafy vegetables and roots with high nitrate concentration (>1000 mg/kg) is arugula (rocket), spinach, lettuce, radish, and beetroot that contain 140-260 mg of nitrate per 100 g (62 mg of nitrate equal 1 mmol) (8, 9). Numerous studies have pointed out the beneficial effects of dietary nitrate consumption on reducing blood pressure, on inhibiting platelet aggregation, on protecting from ischaemia-reperfusion injury, and on improving endothelial dysfunction (6, 7, 9).

Over the last five years, however, the popularity of dietary nitrate as an ergogenic aid has been increased dramatically. The rationale for the possible ergogenic effects of dietary nitrate is based on several facts *collectively*. First, NO and nitrite, the products of nitrate, are potent vasodilators that increase blood flow (1, 10, 11) and hence the O_2 delivery and oxygenation in working muscles (2, 12). Second, the ingestion of dietary nitrate increases the plasma nitrite bioavailability which acts as a reservoir for the "nitrate-nitrite-NO" pathway and as a regulator of hypoxic signaling for NO-induced vasodilatation (6, 10, 13). Third, the hypoxic and acidic conditions, as those observed during exercise, facilitate the reduction of nitrite to NO and potentiate the physiological effects of exogenously administered nitrite (11). Finally, the beneficial effects of nitrate/nitrite/NO on muscle blood flow and on mitochondrial and contractile efficiency (2, 14-17) may alter the muscle's hemodynamic and metabolic responses to exercise (18-20). In fact, there is evidence that pre-exercise plasma nitrite concentrations are independent predictors of exercise capacity and performance (12, 21-24).

In this review we include studies that have examined the effects of dietary nitrate on physiological responses and exercise performance in relation to exercise intensity (submaximal, maximal), training status (trained, untrained) and type of nitrate administration (acute, long-term). Furthermore, we discuss the possible mechanistic approaches for the improved exercise performance after dietary nitrate supplementation.

2. The Larsen's et al. Study (2007)

The pioneering study showing that dietary nitrate consumption may reduce energy cost during exercise was performed by Larsen et al. (2007)(25). Nine healthy, well-trained men performed cycling exercise at exercise intensities ranging from 45% to 100% of VO_2max after a 3-day sodium nitrate supplementation (0.1 mmol/kg/d). Dietary nitrate reduced VO_2 and increased gross efficiency at work rates corresponding from 45% to 80% of VO_2max compared with placebo. In contrast, dietary nitrate supplementation had no effect on VO_2 at maximal or near-maximal work rates (85% and 100% of VO_2max);

neither effect was evident on heart rate, blood lactate, cycling cadence, ventilation, and respiratory exchange ratio (RER) at sub-maximal and maximal work rates. Interestingly, Larsen et al. showed that the increase in metabolic efficiency was elicited after dietary nitrate ingestion that is attainable through a consumption of leafy vegetables and roots.

The observation by Larsen et al. (2007) was followed by a bulk of studies investigating the effects of acute (single dose) and long-term (repeated doses) dietary nitrate supplementations on metabolic and circulatory responses and on exercise capacity and performance in healthy, non-athletic. population and in athletes. It should be noted that in the vast majority of studies dietary nitrate was administered in the form of beetroot juice.

3. Acute (Single dose) Nitrate Supplementation

For the purposes of this review, acute administration is considered the ingestion of dietary nitrate within 24h prior to exercise. Of the 19 studies that examined the effects of an acute (single-dose) of dietary nitrate supplementation, 8 were conducted in untrained to moderately trained individuals (Table 1), 10 in well-trained or elite athletes (Table 2) and only 1 in pathological population (Table 1). The dose of nitrate and the time of supplementation prior to exercise in these studies ranged from 5 to 19.5 mmol and from 75 to 180 min, respectively.

3.1. Supplementation in Untrained to Moderately Trained (Non-Athletic) Population

In the first study examining the ergogenic effects of a *single dose* of beetroot juice, Vohhatalo et al. (2010) administered in two occasions beetroot juice (5.2 mmol NO_3^-) and blackcurrant juice (negligible NO_3^-), 2.5h prior to exercise (26). Beetroot juice supplementation significantly reduced the O_2 cost at the completion of moderate exercise and improved metabolic efficiency (less increase in VO_2 relative to the increase in external work). In the same study, the participants performed a maximal ramp incremental test until exhaustion. The results showed that a single-dose of beetroot juice during maximal effort had no effects on physiological, metabolic, and performance parameters. In line, recent studies reported that VO_2 was significantly reduced at the completion of 5 min of cycling at 40%, 60%, and 80% of VO2max (27, 28); whereas, VO_2max did not change (29) after a single dose of nitrate supplementation compared to placebo. The above studies also examined the effects of acute nitrate supplementation on systemic and cerebrovascular hemodynamics (27, 28) and on cerebral and muscle de-oxygenation levels (29) during moderate-high intensity exercise. In Bond's et al. studies, a single bolus of nitrate (750 mg) administered 2 h before exercise significantly increased cerebral arterial blood flow velocity and reduced the cerebrovascular resistance (CVR), the

rate-pressure product (RPP, an index of myocardial oxygen demand) and the systolic blood pressure compared to placebo, during cycling at 40%, 60%, and 80% of VO$_2$max. The reduced RPP and CVR have important clinical implications for heart failure and hypertensive patients, respectively, in order to exercise with less cardiac strain and reduced risk for stroke. The responses for cardiac output, heart rate, and diastolic blood pressure were not affected by nitrate ingestion. Thompson et al. (2014) reported reduced cerebral and muscle HHb concentration during exercise at 50% and 70% of VO$_2$max. This finding implies a reduced O$_2$ extraction response supporting the evidence of a reduced O$_2$ cost (25) and increased mitochondrial efficiency (15) after nitrate supplementation.

A few studies have examined in non-athletic population the effects of an acute nitrate supplementation on exercise capacity and performance (24, 29, 30). Murphy et al. (2012) documented that moderately trained individuals improved the performance in an all-out 5-km treadmill run by 3% after a single consumption of 200 g baked beetroot ($\geq$500 mg of nitrate). More specifically, consumption of baked beetroot increased both the mean and the peak running velocities (by 3% and 5%, respectively); average rating of perceived exertion and heart rate were not different between the beetroot and the placebo conditions. In line, Thompson et al. (2014) showed that a single dose of beetroot juice supplementation (5.0 mmol NO$_3^-$) increased by 16% the time to exhaustion at 90% VO$_{2max}$ compared with placebo. Wylie et al. (2013) evaluated the effects of acute nitrate supplementation on performance in team sport-specific exercise (24). Consumption of large doses of nitrate (28.6 mmol) over 30 h, in form of concentrated beetroot juice, increased the distance covered in the Yo-Yo intermittent recovery test by 4.2%.

Two studies completed by the same group of scientists examined the effect of acute nitrate supplementation on skeletal muscle metabolism. More specifically, Vohhatalo et al. (2011) evaluated whether potentiating the nitrate-nitrite-NO cycle with a single bolus of beet root juice would attenuate the deleterious effect of hypoxia on muscle metabolism and performance (20). They administered 0.75 L of beetroot juice (9.3 mmol of nitrate) or nitrate-depleted beetroot juice, in three equal doses, to 9 healthy moderately fit individuals 24 h, 12 h and 2.5 h prior exercise. Twenty-four hours following the first supplementation the participants performed knee-extension exercise until exhaustion, in three occasions: in hypoxia after beetroot ingestion (BR), in hypoxia after placebo (PL) ingestion, and in normoxia after placebo (PL). In support to authors' hypothesis, beetroot administration increased the time to exhaustion in hypoxia and abolished the deleterious effects of hypoxia on exercise performance. The ergogenic effects of nitrate were attributed to the reduced muscle metabolic perturbation, as observed by the smaller and slower reduction during exercise for phosphocreatine (PCr), inorganic phosphate (Pi) and pH, as well as to faster PCr recovery in BR-hypoxia and PL-normoxia versus PL-

hypoxia. The authors suggested that the PCr-sparing effect of nitrate supplementation is possibly due to the improved matching of O$_2$ supply to demand. In their next study, the same group of scientists examined the effects of acute administration of nitrate-rich beetroot juice on muscle force production and muscle metabolism during repeated isometric maximum voluntary contractions (31). In two different occasions, the participants consumed 2.5 h prior exercise beetroot juice with (0.25 L with 10.2 mmol nitrate) and without nitrate (0.25 L with 0.17 mmol nitrate). The two exercise protocols consisted of (i) two bouts of continuous one-leg extension for 24 s, at 1 kg below the maximum, with 4 min recovery between bouts, and (ii) 50 isometric maximum voluntary contractions of 6.6 s intercepted by 2.2 s of rest. The force production (peak, mean, end, and fatigue index), as well as mean PCr changes (mean cost, mM; mean force/PCr depletion, N/mM) were not affected when a single dose of nitrate-rich beetroot juice was administered 2.5 h before exercise. The reason for the contrasting results regarding the PCr responses between the Vohhatalo et al. (2011) and Fulford et al. (2013) studies maybe attributed to the hypoxic conditions of exercise in beetroot supplemented group in the first study. It is possible that the hypoxic environment, in the Vohhatalo et al. (2011) study, favored the reduction of nitrite to NO. This in turn, could increase the NO-induced vasodilation, resulting to better matching of VO$_2$ supply to demand, and hence, increased oxidative function and PCr-sparing.

To the best of our knowledge, only one study to date, has investigated the effects of acute nitrate administration in a pathological population (32). The authors reasoned that the documented effects of nitrate supplementation on increasing muscle blood flow would improve muscle oxygenation in patients with peripheral arterial disease delaying the claudication pain and improving the exercise tolerance. Indeed, the participants increased their walking time (by 18%) before the onset of claudication pain and time to exhaustion (by 17%) during a maximal incremental test after the consumption of beetrout juice (750 mg of nitrate) compared with placebo treatment (orange juice). The enhanced exercise capacity, after nitrate ingestion, was accompanied by increased oxygenation (O$_2$Hb) and blood volume (tHb), and reduced O$_2$ extraction (HHb) during walking, suggesting an enhanced bulk muscle blood flow and an improved matching of O$_2$ delivery to utilization. In line with previous studies in healthy individuals, acute nitrate supplementation reduced diastolic blood pressure during a maximal incremental test.

3.2. Supplementation in Athletes

Several studies over the last four years have evaluated the ergogenic effects of acute nitrate supplementation in cyclists, skiers, runners, rowers, kayakers, and team-sport athletes. Surprisingly, 5 out of 10 studies were performed in cyclists. Lansley et al. (2011) was the first to examine the effects acute nitrate supplementation on exercise performance in athletes highlighting its potential use as an ergogenic aid

(33). Nine competitive cyclists (VO$_2$max: 56 ml/kg/min) significantly improved performance in a 4 km and a 16.1-km time-trials following ingestion of beetroot juice (6.2 mmol of nitrate) compared with placebo. Beetroot consumption increased also the mean power output and the power output to VO$_2$ ratio, supporting previous findings of increased contractile and mitochondrial efficiency following nitrate supplementation (15, 19). In contrast to results obtained in untrained individuals, no reduction in VO$_2$ was observed across both distances. Bescós et al. (2011) had competitive cyclists (VO$_2$max: 65 ml/kg/min) cycle for 4 bouts of 6 min at submaximal intensities (35%-65% of peak power) and then perform a maximal incremental test (34). Sodium nitrate ingestion, 3 h prior to trials, had no significant effect on submaximal O$_2$ cost, on time to exhaustion, on maximal power, and on blood lactate; VO$_2$max, however, was significantly reduced by 3.7%. It should be noted that despite the substantial increase in plasma nitrate (86%) in this study, nitrite, the regulator of NO-induced hypoxic signaling, was increased only by 16%. Cermak et al. (2012) also failed to document an ergogenic effect of a single bolus of nitrate administration (8.7 mmol of nitrate) in well-trained cyclists (VO$_2$max: 60 ml/kg/min) (35). Performance parameters (time, average power and cycling cadence), as well as body fuel selection and metabolites (glucose, free fatty acids, and lactate) were not different after ingestion of nitrate-rich and nitrate-depleted supplements.

Table 1. *Studies on the effects of acute (single–dose) dietary nitrate supplementation on physiological responses and indices of exercise tolerance and exercise performance in non-athletic population*

Study	Participants	Dose of Nitrate (NO$_3^-$)	Change in NO$_2^-$ or NO	Time of ingestion	Study Design	Effects of BR ingestion
Vanhatalo (2010)	8 healthy, moderately fit men and women	BR juice (5.2 mmol NO$_3^-$)	↑36% NO$_2^-$	2.5h pre-exercise	Cycling: 2×5-min at 90% VT and a maximal test	At 90% VT: ↓VO$_2$ (~4%); ↓ VO$_2$/ work At Maximal: ↔VO$_2$max, peak and VT power, La$_b$, RERmax, VEmax, HRmax
Vanhatalo (2011)	9 healthy, moderately fit men and women	BR juice (9.3 mmol NO$_3^-$) in 3 equal doses	↑50% NO$_2^-$	24h, 12 h and 2.5h pre-exercise	Knee-extension in hypoxia and normoxia	In hypoxia: ↑ time to exhaustion, ↓ PCr breakdown, ↓ (faster) PCr recovery time
Fulford (2013)	8 healthy, moderately fit men	BR juice (10.2 mmol NO$_3^-$)	↑86% NO$_2^-$ (from placebo)	2.5h pre-exercise	Knee-extension and Isometric	↔ peak force, mean force, ↔end-exercise force; ↔PCr responses
Murphy (2011)	11 healthy, moderately fit men and women	Baked BR (≥500mg NO$_3^-$)	Not reported	75 min pre-exercise	5-km run trial	↑ mean and peak velocity; ↔ average RPE, HR; ↓ RPE at 1.8 km
Bond (2013)	12 healthy, active women	BR juice (750 mg NO$_3^-$)	↑300% NO	2h pre-exercise	Cycling at 40%, 60%, and 80% of VO$_{2max}$	↓ VO$_2$, RPP, SBP, CVRI; ↑ CBFV; ↔ CO, DBP, HR
Bond (2014)	12 healthy, active women	BR (750mg NO$_3^-$)	↑300% NO	2h pre-exercise	Cycling at 40%, 60%, and 80% of VO$_{2max}$	↓ VO$_2$, RPP, SBP; ↔ HR, DBP, VE, RER
Thompson (2014)	16 healthy, moderately fit men	BR juice (5.0 mmol NO$_3^-$)	↑79% NO$_2^-$	2h pre-exercise	Cycling at 50% and 70% VO$_{2max}$; All-out trial at 90% VO$_{2max}$	↔ VO$_{2max}$ At 50% and 70%: ↔ VO$_2$; ↔ RPE, HR; ↓ HHb All-out trial (90%): ↑ time to exhaustion
Wylie (2013)	14 recreational team-sport players	Multiple doses of BR juice (total of 28.6 mmol NO$_3^-$)	↑400% NO$_2^-$	Over 30h pre-exercise	Yo-Yo recovery 1 test	↑ Distance (by 4.2%) ↔ La$_b$
Kenjale (2011)	8 males and females with peripheral arterial disease	BR juice (~9 mmol NO$_3^-$)	↑186% NO$_2^-$	3h pre-exercise	Maximal incremental test	↑ Walking distance (by 17%); ↓ VO$_2$; ↓ HHb (reduced O$_2$ extraction)

↑ = increase; ↓ = decrease; ↔ = no change; NO$_2^-$ = nitrite; NO = nitric oxide; BR = beetroot; VT = ventilatory threshold; VO$_2$ = oxygen consumption; La$_b$ = blood lactate concentration; PCr = phosphocreatine; RPE = rate of perceived exertion; HR = heart rate; HHb = deoxygenated hemoglobin (index of O$_2$ extraction); RPP = rate-pressure product (index of myocardial work); SBP = systolic blood pressure; DBP = diastolic blood pressure; CVRI = cerebrovascular resistance index; CBFV = cerebral blood flow velocity; CO = cardiac output; VE = ventilation; RER = respiratory exchange ratio

Wilkerson et al. (2012) evaluated the efficacy of beetroot juice on improving performance in a longer event (23). Endurance cyclists (VO$_2$max: 63 ml/kg/min) executed a 50-mile time-trial following administration of nitrate-rich (6.2 mmol of nitrate) and nitrate-depleted beetroot juice. In line with the two previous studies, they did not observe a significant effect of beetroot juice on exercise performance and on average power, VO$_2$, HR, and lactate, but did document a 3% increase in mechanical efficiency (more power per VO$_2$). Interestingly, the increase in plasma nitrite

and improvement in performance time were significantly correlated (r = -0.83). This observation encouraged the authors to separate the participants in "responders" and "non-responders", based on the % increase in plasma nitrite following nitrate supplementation. They observed that analyzing the results only for "responders" to beetroot supplementation (>30% increase in nitrite), there was a significant improvement in the time (-2%) of completion of the 50-mile trial after beetroot ingestion. In contrast, in a study by Bescos et al. (2012) repeated doses of sodium nitrate administered for 3 days, failed to discriminate between responders (>50% increase in nitrite) and non-responders (<30% increase in nitrite) on the effects of nitrate supplementation on exercise performance (36). It should be also pointed out that several studies have reported no changes in exercise performance after a single-dose of dietary nitrate, even though almost all of them have evidenced increases of more than 30% in plasma nitrite.

The possible reason for the equivocal results of the ergogenic value of a single dose of beetroot juice ingestion between Lansley et al. (2011) and the subsequent studies, may be the dependency of the beetroot-effect on the intensity or the duration of exercise (23, 35). In the Lansley et al. (2011) study, the participants performed the trial at a higher intensity for a shorter time. It has been previously suggested that the ergogenic effects of beetroot juice supplementation on exercise performance would be more apparent during high-intensity exercise, when greater hypoxia is developed and a greater % of type II muscle fibers is activated (18). The hypoxic and acidic environment at higher intensity exercise may potentiate the conversion of nitrite to NO and thus, the physiological effects of nitrate supplementation. Furthermore, the effects of beetroot on the augmentation of blood flow and vascular conductance was evident mostly in muscles comprised of primarily type IIb fibers. The increase in blood flow and vascular conductance after beetroot supplementation was highly correlated with the percentage of type IIb muscle fibers (18). Thus, the beneficial effects of beetroot on exercise performance maybe more apparent during high-intensity exercise, when a greater % of type IIb muscle fibers is activated and greater hypoxia is developed. A recent study in trained cyclists partially supports the ergogenic value of beetroot juice in hypoxic conditions. Muggeridge et al. (2014) investigated the effects of a single-dose of beetroot ingestion on cycling performance in simulated altitude (2,500 m) (37). This is of significant practical value, since many cycling competitions are performed at altitude. Consumption of beetroot juice (5 mmol of nitrate) reduced O_2 cost and enhanced the 16.1-km performance at normobaric hypoxia.

Studies performed in elite skiers (VO_2max: 70 ml/kg/min), kayakers, runners (VO_2max: 80 ml/kg/min), and team-sport (VO_2max: 57 ml/kg/min) athletes agree that a single-dose of beetroot juice (5 and 19.5 mmol of nitrate) has no effect on exercise performance (time-trials) (38-40) and on all-out sprints (39, 41). The effect, however, on energy cost was unclear, with studies reporting either no change (38, 40) or

reduction (39). Hoon et al. (2014) examined the effects of two different doses of beetroot juice on simulated 2,000 m rowing performance. The authors reported that the moderate dose of nitrate (4.3 mmol) had no effect of 2,000 m rowing performance, whereas the high dose (8.4 mmol) improved performance (-1.8s) (42).

4. Long-Term Nitrate Supplementation

The ergogenic effects of nitrate supplementation have been partially attributed to the increased mitochondrial metabolic efficiency. This is in context with reports showing that continuous exposures of mammalian cells to NO for more than 6 days stimulate mitochondrial biogenesis (43, 44), which is unlikely to be observed with a single nitrate dose. Therefore, several studies examined the effects of long-term dietary nitrate (several doses over 2 to 15 days) supplementation on physiological responses and exercise performance in untrained to moderately trained individuals (Table 3) and in well-trained or elite athletes (Table 4).

4.1. Supplementation in Untrained to Moderately Trained (Non-Athletic) Population

Two years after Larsen's et al. (2007) study, Bailey et al. (2009) examined the effects of a 6-day beetroot supplementation (11.2 mmol/d of nitrate) on indices of muscle oxygenation and on VO_2 responses in healthy recreationally active men (45). Dietary beetroot reduced O_2 extraction (as evidenced by HHb response) and VO_2 during moderate intensity exercise (80% of ventilatory threshold), suggesting that the beneficial effects of repeated dietary nitrate consumption on reduced energy cost during exercise are attributed to mechanisms located within the muscle cell. The effects of beetroot supplements were different during severe exercise (70% between ventilatory threshold and VO_2max); that is, muscle oxygenation was unaffected after beetroot consumption, whereas, the exercise tolerance significantly increased (by 16%). In their follow up study, Bailey et al. (2010) attempted to study the mechanisms underlying the reduced energy cost after a short-term beetroot ingestion (5.1mmol/d of nitrate for 6 days). Muscle contractile efficiency was examined during knee-extension at low- and high-intensity work rates (19). There was a clear PCr-sparing effect and a reduced VO_2 response during low- and high-intensity exercise, after beetroot supplementation. Later studies, however, in elderly and young individuals failed to confirm the PCr-sparing effect of long-term (3-6 days) beetroot supplementation, during low (walking) and high (running) intensity exercise (46, 47). Furthermore, irrespective of the exercise intensity, the turnover rate for ATP_{total} was reduced after beetroot ingestion in Bailey et al. (2010) at expense of reduced ATP hydrolysis from the oxidative pathway and PCr breakdown. The reduced metabolic perturbation after beetroot ingestion tended to be related to the increased exercise tolerance. Interestingly, the reduction in PCr amplitude during the low-intensity exercise and in the PCr slow component during the high-intensity

exercise, were analogous to changes in the respective VO_2 parameters. The authors suggested that that the beneficial effects of dietary nitrate on the reduced O_2 cost during exercise, are elicited through improved coupling of skeletal muscle ATP hydrolysis and force production rather than by increased mitochondrial ATP production per O_2 consumed.

Table 2. *Studies on the effects of acute (single–dose) dietary nitrate supplementation on physiological responses and indices of exercise tolerance and exercise performance in athletes*

Study	Participants	Dose of Nitrate (NO_3^-)	Change in NO_2^- or NO	Time of ingestion	Study Design	Effects of beetroot ingestion
Lansley (2011)	9 competitive male cyclists	BR (6.2 mmol NO_3^-)	↑39% NO_2^-	2.5h pre-exercise	4km and 16.1km time-trials	↔ VO_2; ↑Power output (4.5%-6%); ↑ work/VO_2 (7-11%); ↑performance (~3.0%)
Bescós (2011)	11 competitive male cyclists	Sodium nitrate (10 mg/kg)	↑16% NO_2^-	3h pre-exercise	4×6-min at 35%-65% of PPO and a maximal test	At 35%-65% PPO: ↔ VO_2, VE, RER, La_b; Maximal: ↓VO_{2max} (3.7%); ↓VO_2/ work; ↔ time to exhaustion
Cermak (2012)	20 competitive male cyclists or triathletes	BR juice (8.7 mmol NO_3^-)	↑124% NO_2^-	2.5h pre-exercise	~1 h time trial (75% PPO)	↔ Performance, average power, average cadence; ↔HR, glucose, free fatty acids, La_b, insulin
Wilkerson (2012)	8 well-trained male cyclists	BR juice (6.2 mmol NO_3^-)	↑25% NO_2^-	2.5h pre-exercise	50-mile time-trial	↔ Performance time; ↔ average power, VO_2, HR, RPE, La_b; ↑ power/VO_2 (3.2%)
Muggeridge (2014)	9 trained male cyclists	BR juice (5 mmol NO_3^-)	↑134% NO_2^-	3h pre-exercise	Altitude: 15 min at 60% W_{max} 16.1 km time-trial	↓ VO_2; ↓ time (↑performance) by 2.2%
Peacock (2012)	10 junior elite cross-country skiers	Potassium nitrate (614mg NO_3^-)	↑127% NO_2^-	2.5h pre-exercise	55%VO_{2max} 75%VO_{2max} 5-km time-trial	↔ Performance time; ↔ VO_2, HR, RER, La_b
Muggeridge (2013)	8 trained kayakers	BR juice (5 mmol NO_3^-)	↑32% NO_2^-	3h pre-exercise	60%W_{max} all-out sprints 1-km time-trial	At 60%W_{max}: ↓ VO_2 Sprints: ↔ Peak Power 1-km: ↔ Performance time
Boorsma (2014)	8 elite 1,500-m runners	BR juice (19.5 mmol NO_3^-)	↑17-fold in *nitrate*	AWAD	55-80%VO_{2max} 1,500 m time-trial	↔ Performance time ↔ VO_2
Martin (2014)	16 team-sports males and females	BR juice (300 mg, ~5.0 mmol, NO_3^-)	AWAD	2h pre-exercise	all-out sprints on cycle ergometer	↓ Number of sprints ↓ Total work; ↔ Mean power
Hoon (2014)	10 highly trained rowers	BR juice (4.2 and 8.4 mmol NO_3^-)	AWAD	2h pre-exercise	Simulated 2,000-m rowing	At 4.2 mmol: ↔ Performance At 8.4 mmol: ↑ Performance

↑ = increase; ↓ = decrease; ↔ = no change; NO_2^- = nitrite; NO = nitric oxide; BR = beetroot; PPO = peak power output; VO_2 = oxygen consumption; La_b = blood lactate concentration; HR = heart rate; VE = ventilation; RER = respiratory exchange ratio; AWAD = information from abstracts without adequate data

In line, 7 days of beetroot juice supplementation increased the contractile properties of human skeletal muscle. Nitrate supplementation improved the evoked peak force at low frequencies and the explosive force at maximal twitch; there were no beetroot-effects on voluntary maximum or explosive force production (48). Subsequent studies showed that the ergogenic effects of long-term dietary nitrate supplementation are also evident during high-intensity exercise. More specifically, dietary nitrate ingestion (2 to 5 days) reduced O_2 cost during maximal exercise (49), and increased exercise tolerance across a range of severe-intensity exercise bouts performed above the ventilatory threshold (50). The critical power, however, which corresponds to the highest power output that can be supported mainly by aerobic pathways, was unaffected by long-term beetroot administration (50).

What was not clear from preceding studies (prior to 2011), is whether the beneficial effects of beetroot juice consumption on reducing the energy cost was attributed to dietary nitrate *per se,* and not to other bioactive compounds, such as bitaine and polyphenols, present in beetroot juice. Lansley et al. (2011) investigated this issue by using a nitrate-depleted beetroot juice as placebo (46). They confirmed previous findings of reduced O_2 cost during moderate- and high-intensity running, and of increased exercise tolerance (time to exhaustion) in nitrate-rich vs. nitrate-depleted beetroot juice. The novel important observations in that study were that 6 days of beetroot

administration (i) reduced the energy cost during very-low intensity exercise (walking), and (ii) did not change the mitochondrial oxidative capacity, suggesting that the improvement in aerobic exercise tolerance is not attributed to NO-induced mitochondrial biogenesis. Masschelein et al. (2012) examined the effects of a 6-day of beetroot supplementation on energy cost and oxygenation of muscle and brain in simulated severe hypoxia (51). Beetroot consumption for 6 days significantly ameliorated the deleterious effects of hypoxia increasing both muscle oxygenation during submaximal exercise (45% of VO2max), and time to exhaustion during maximal incremental testing; there were no effects of dietary nitrate on cerebral oxygenation. Resting energy expenditure and energy cost during submaximal exercise were significantly reduced (51).

VO_2 kinetics may alter the contribution of fuel utilization to energy production and affect the development of fatigue. More specifically, faster VO_2 kinetics during transition from rest to the first 2 min into exercise, may reduce the contribution of PCr and anaerobic glycolysis to energy production, whereas, a reduced VO_2 slow component delays the attainment of VO_2max during severe-intensity exercise increasing exercise tolerance (52, 53). Studies that evaluated the effect of long-term beetroot consumption on the primary amplitude of VO_2 kinetics revealed equivocal findings. Some studies reported that long-term administration of beetroot juice significantly reduced the primary amplitude of VO_2 during low-moderate exercise intensity (19, 45, 46), whereas other studies reported no effects (47, 54). Similarly, for high intensity exercise, there are reports showing increased primary amplitude of VO_2 after beetroot supplementation (45) and reports showing no changes (19, 46, 50, 54). The results for the phase II time constant are consistent for low-moderate intensity exercise, reporting no effects of beetroot juice (19, 45, 46, 54), and are contrasting for high-intensity, showing no effects (19, 46, 50), faster kinetics (54) or slower kinetics (45). Finally, the effect of beetroot juice on the VO_2 slow component also appears to divide the researchers into those observing (19, 45) and those not observing a beetroot-effect (46, 50, 54). Despite the divergent results on the effects of dietary intake on VO_2 slow component, all four studies reported an increased exercise time to fatigue. Collectively, long-term beetroot juice supplementation improved at least one parameter of VO_2 kinetics. That is, beetroot speeded VO_2 uptake in the first 1-2 min into exercise and/or reduced the magnitude of the VO_2 slow component or the mean response time (47, 54). The faster kinetics of VO_2 coincided with faster kinetics for HHb (faster O_2 extraction) (54). It should be also noted, that the effects of beetroot juice supplementation on VO_2 kinetics appear to be exercise-intensity dependent, with greater and more consistent effects observed during high intensity exercise.

4.2. Supplementation in Athletes

At the time of this review preparation, only seven studies examined the effects of long-term dietary nitrate supplementation on physiological responses and exercise performance in athletes. Three studies demonstrated an improved performance and four studies reported no effect of repeated beetroot supplementation of dietary nitrate (3-8d). The first two studies, performed in well trained athletes, showed that a 6-day beetroot ingestion (5.5 to 8 mmol of nitrate) improved the10-km cycling (55) and the 6×500 rowing (56) performance by 1.2% and 0.4% (by 1.7% in 4-6 reps), respectively. Finally, a recent study in master swimmers showed that beetroot juice (5.5 mmol of nitrate) consumed for 6 days, significantly increased the workload (by 6%) and reduced the energy cost (by 11%) at exercise intensity corresponding to the anaerobic threshold (57). Three points, however, should be considered before drawing conclusions from the above studies on the ergogenic value of long-term dietary nitrate supplementation in elite athletes. First, the within subject day-to-day variability in time trial performance was 1.0% that is very close to the reported effect of beetroot (55). Second, the worthwhile difference, index of practical significance, is very small for elite athletes (about 0.3% for rowers) (58). Third, the participants in Pinna's et al. study were master athletes with VO_2max values (43 ml/kg/min) comparable to those seen in young fit males; hence, it is not clear whether the documented effects in their study could be applied to athletes with higher aerobic fitness.

In contrast to the above results, more recent studies performed in endurance athletes and cyclists (VO_2max: 60-80 ml/kg/min) failed to observe an effect of repeated dietary nitrate supplementation in the form of either beetroot juice or sodium nitrate on exercise performance (36, 40, 59, 60). More specifically, Bescos et al. (2012) after a 3-day administration of sodium nitrate (10mg/kg/d) to endurance athletes reported no effect of dietary nitrate on mean distance covered and mean power exerted during the 40-min distance trial (36). Furthermore, no differences in respiratory parameters and blood lactate concentrations were observed between the nitrate and the placebo groups. In agreement, three subsequent studies also showed that beetroot administration for 4-8 days to elite 1500-m runners and cyclists had no effect on time-trial performance (40, 59, 60), on mean and peak power (59, 60), on submaximal VO_2 (40), on blood lactate (60), and on VO_2 kinetics and repeated sprinting ability (59). Furthermore, there was no effect of beetroot supplementation on adaptations to hypoxic training (60). In conclusion, the observations for the effects of repeated dietary nitrate supplementation on exercise performance in athletes remain largely divided.

Table 3. *Studies on the effects of long-term (several doses over 2-15 days) dietary nitrate supplementation on physiological responses and indices of exercise tolerance and exercise performance in non-athletic population*

Study	Participants	Dose of Nitrate (NO_3^-)	Change in NO_2^- or NO	Study Design	Effects of beetroot ingestion
Larsen (2007)	9 well-trained men	Sodium nitrate (0.1 mmol/kg /d for 2 d)	↑82% NO_2^-	Cycling maximal incremental test	At 45%-80% VO_2max: ↓ VO_2 cost and gross efficiency; ↔ HR, VE, RER At VO_2max: ↔ VO_2max, HR, VE, RPE, work
Bailey (2009)	8 healthy, recreationally active men	BR juice (5.5 mmol NO_3^-/d for 6d)	↑96% NO_2^- (6-d rise)	Cycling at moderate (<VT) and severe (>VT) work rates	At Moderate: ↓ HHb amplitude (reduced O_2 extraction); ↔ O_2Hb, tHb; ↓ VO_2 cost At Severe: ↔ HHb, O_2Hb, tHb; ↓VO_2 kinetics; ↓ VO_2 slow component; ↑ exercise tolerance
Bailey (2010)	7 healthy, recreationally active men	BR juice (5.1 mmol NO_3^-/d for 6d)	↑137% NO_2^- (4-6d rise)	Knee-extension at low (15% of MVC) and high (30% of MVC) intensity	At Low: ↓ VO_2 cost and ↑ work efficiency; ↓ PCr and Pi amplitudes and ↔pH; ↓ATP_{total}, ATP_{ox}, ATP_{PCr} and ↔ATP_{Gly} At High: ↓ VO_2 cost and ↑ work efficiency and exercise tolerance; ↓ PCr and VO_2 slow components and ↔pH; ↓ATP_{total}, ATP_{ox}, ATP_{PCr} and ↔ATP_{Gly}
Larsen (2010)	9 healthy men and women	Sodium nitrate (0.1 mmol NO_3^-/kg/d, 2d)	↑133% NO_2^-	Combined arm and leg maximal exercise	↓ VO_{2max} and O_2 pulse; ↔ VEmax, HRmax, RER, La_b, and time to exhaustion
Vanhatalo (2010)	8 moderately fit men and women	BR juice (5.2 mmol NO_3^-/day for 15 d)	↑46% NO_2^- at 15th d	Cycling: 2×5-min at 90% VT and a maximal test	At 90% VT: ↓VO_2 (~4-5%); ↓ VO_2/ work At Maximal: ↔ VO_{2max} and La_b; ↑ VT and peak power
Lansley (2011)	9 healthy active men	BR juice (6.2 mmol NO_3^-/d for 6d)	↑104% NO_2^-	Walking and running at moderate (<VT) and severe (>VT) exercise intensities	↓VO_2 at walking (-12%), at moderate-intensity (-7%) and at high-intensity running (-7%); ↑VO_2 primary amplitude at moderate intensity; ↑ time to exhaustion; ↔ mitochondrial oxidative capacity and ↔ PCr and pH changes
Masschelein (2012)	15 healthy active men	BR juice (0.07 mmol/kg/d NO_3^- for 6d)	↑40% NO_2^-	Cycling in hypoxia and normoxia at 45% VO_{2max} for 20 min and maximal test	In hypoxia: ↓ VO_2 at rest (-9%) and at 45% VO_{2max} (-4%); ↑ muscle oxygenation (+4-5%); ↑ exhaustion time at maximal test (+5%); ↔ brain oxygenation
Kelly (2013)	9 habitually active men	BR juice (8.2 mmol NO_3^-/d for 5d)	↑197% NO_2^-	Cycling above the ventilatory threshold	↑ exhaustion time; ↔ La_b; ↔ resting metabolic rate; ↔ critical power; ↔VO_2 kinetics
Kelly (2013)	12 older men and women	BR juice (9.6 mmol NO_3^-/d for 3d)	↑418% NO_2^-	6-min walking test	↑ VO_2 kinetics (faster); ↔ walking distance; ↔ VO_2 cost; ↔ PCr response;
Breese (2013)	9 healthy men and women	BR juice (8 mmol NO_3^-/d for 6d)	↑435% NO_2^-	Cycling at moderate (<VT) and severe (>VT) exercise intensities	Moderate: ↔ O_2 extraction (HHb) and tHb; ↔VO_2 kinetics Severe: ↑ HHb kinetics; ↓ HHb levels (reduced O_2 extraction); ↔ tHb; ↑VO_2 kinetics and ↔ VO_2 slow component; ↑ exercise tolerance
Fulford (2013)	8 moderately fit men	BR juice (10.2 mmol NO_3^- for 15d)	↑76% NO_2^- (vs. placebo)	Knee-extension and Isometric	↔ peak, mean, end-exercise force production; ↓PCr cost/mean force at end-exercise;
Haider (2014)	19 healthy men	BR juice (9.7 mmol NO_3^-/d for 7d)	AWAD	Voluntary and involuntary isometric contractions	↑ excitation-contraction coupling; ↑ evoked peak and explosive force; ↔ voluntary maximum and explosive force

↑ = increase; ↓ = decrease; ↔ = no change; NO_2^- = nitrite; NO = nitric oxide; BR = beetroot; VT = ventilatory threshold; VO_2 = oxygen consumption; La_b = blood lactate; PCr = phosphocreatine; Pi = inorganic phosphate; HHb = deoxygenated hemoglobin (index of O_2 extraction); O_2Hb = oxygenated hemoglobin; tHb = total hemoglobin (index of blood volume); VE = ventilation; RER = respiratory exchange ratio; AWAD = abstract without adequate data

Table 4. *Studies on the effects of long-term (several doses over 2-15 days) dietary nitrate supplementation on physiological responses and indices of exercise tolerance and exercise performance in athletes*

Study	Participants	Dose of Nitrate (NO_3^-)	Change in NO_2^- or NO	Study Design	Effects of beetroot ingestion
Cermak 2012	13 competitive male cyclists or triathletes	BR juice (8.0 mmol NO_3^- /day for 6 d)	↑ 20-fold in *nitrate*	2 × 30-min at 45% and 65% of PPO 10-km time-trial	At 45%-65% PPO: ↓ VO_2 cost; ↔ fuel selection, La_b and insulin: 10-km trial: ↑ Performance (1.2%) and average power output (2%);

Study	Participants	Dose of Nitrate (NO$_3^-$)	Change in NO$_2^-$ or NO	Study Design	Effects of beetroot ingestion
Bond (2012)	14 well-trained male rowers	BR juice (5.5 mmol NO$_3^-$ /day for 6 d)	Not reported	6 × 500-m with 90 s rest	↑ Performances in 4-6 repetitions (1.7%) and 1-6 (0.4%); ↔ HR, La$_b$, pH
Bescos (2012)	13 male cyclists and triathletes	Sodium nitrate (10 mg/kg) for 3 days	↑79% NO$_2^-$	40-min cycling distance-trial	↔ distance covered and mean power output; ↔ VO$_2$, VE, RER, and La$_b$
Christensen (2013)	10 elite cyclists	BR juice (500 ml, ~8 mmol NO$_3^-$ for 6 d)	↑298% of nitrate +NO$_2^-$	3 × 6-min Endurance trial (120 min) Repeated sprints (6×20 s)	↔ performance time and mean power output; ↔ repeated sprinting ability; ↔ VO$_2$, gross efficiency, HR, RER, and RPE; ↔ VO$_2$ kinetics
Puype (2014)	22 well fit men (VO2max: 60 ml/kg/min)	BR juice (0.07 mmol/kg NO$_3^-$/d for 4 d)	↑ 7.5-fold in nitrate	30-min cycling time-trial Incremental test	↔ time to exhaustion and VO$_2$max; ↔ peak and mean power output; ↔ submaximal and peak La$_b$; ↔ adaptations to hypoxic training
Pinna (2014)	14 master swimmers	BR juice (5.5 mmol NO$_3^-$/day for 6 d)	Not reported	Incremental test	↑ workload at AT; ↓ energy cost at AT
Boorsma (2014)	8 elite 1,500-m runners	BR juice (19.5 mmol NO$_3^-$ for 8d)	↑ 24-fold in nitrate	Runs at 55-80%VO$_{2max}$ 1,500 m time-trial	↔ Performance time ↔ VO$_2$

↑ = increase; ↓ = decrease; ↔ = no change; NO$_2^-$ = nitrite; NO = nitric oxide; BR = beetroot; AT = anaerobic threshold; VO$_2$ = oxygen consumption; La$_b$ = blood lactate concentration; RPE = rate of perceived exertion; HR = heart rate; VE = ventilation; RER = respiratory exchange ratio

There are several reasons for the less apparent ergogenic effects of nitrate supplementation in highly endurance trained individuals compared to their untrained or moderately trained counterparts. Athletes demonstrate increased NO-induced endothelial function and vascular control, higher muscle blood flow and are metabolically more efficient (consume less O$_2$ at a given workload) compared to untrained individuals. Since the ergogenic effects of dietary nitrate supplementation are primarily elicited by enhancing the metabolic efficiency, there is possibly less room for further improvements in exercise O$_2$ cost and performance in athletes. Furthermore, the effects of dietary nitrate supplementation on muscle contractile efficiency and blood flow were selectively documented in muscles with predominant type II fibers (> than 60%) (18). Thus, endurance trained athletes might not demonstrate the muscle fiber composition to obtain the effects of beetroot juice supplementation. Also, the mitochondrial content/activity and the aerobic potentials of type II muscle fibers are increased in endurance athletes (61). Furthermore, the increased capillarity in endurance athletes may reduce the extent and the likelihood of developing hypoxia at a given exercise intensity. Finally, endurance athletes have higher levels of basal nitrite (62, 63); hence they might require higher doses of nitrate to evidence an ergogenic effect (23, 35).

5. Potential Mechanisms for the Ergogenic Effects of Dietary Nitrate

One of the most prominent and consistently reported ergogenic effects of acute or long-term dietary nitrate supplementation is the reduced energy cost of exercise (increased exercise efficiency). The exact mechanisms for the ergogenic effect of nitrate administration have not been described in full. To the best of our knowledge, 7 studies (3

in humans and 4 in rodents) attempted to shed light into the mechanisms of nitrate-induced improvement in exercise efficiency. These mechanisms include: (i) increased muscle contractile efficiency, (ii) increased mitochondrial efficiency, and (iii) improved vascular control and blood flow.

Bailey et al. (2010) were the first to report an increased muscle contractile efficiency during exercise, after beetroot supplementation in healthy young males (19). They found that 6 days of nitrate supplementation (5.1 mmol/d of nitrate) resulted to analogous VO$_2$- and PCr-sparing effects and to reduced ATP turnover rate during low and high intensity exercise. The decreased total ATP turnover rate was attributed to reduced ATP hydrolysis derived from oxidative phosphorylation and PCr breakdown; the ATP turnover rate from the glycolytic pathway remained unchanged. It has been suggested that the possible mechanisms for the reduction in ATP cost after nitrate supplementation may be due to the effect of nitrate on contractile filaments (actomyosin-ATPase) or on Ca^{2+} handling (Ca^{2+}-ATPase) (3, 16, 19). To examine the above hypothesis, Hernandez et al. administered to rats inorganic nitrate (beetroot juice) for 7 days in a dose that is easily obtained by humans with high-nitrate diets and is similar to that eliciting an ergogenic effect (17). The authors observed a faster rate and greater magnitude of force production after nitrate, compared with placebo, only in fast-twitch (extensor digitorum longus) and not in slow-twitch (soleus) muscles. The enhanced muscular function was attributed to the improved Ca^{2+} handling, as documented by the increased myoplasmic free Ca^{2+} levels and overexpression of calsequestrin1 and dihydropyridine receptors proteins. Interestingly, the expression of these proteins did not change in the soleus muscle after the administration of beetroot, which was in line with the lack of changes in contractile force in the soleus muscle. The authors suggested that by lowering the threshold for fast-twitch muscle activation to achieve similar force production, the dietary nitrate supplementation may increase

the muscle contractile efficiency and reduce the effort and the number of motor units required for a given task without affecting the rate of force development. These results were recently confirmed in humans. In a double-blind randomized study, nitrate-rich beetroot juice enhanced the contractile properties of human skeletal muscle by improving the excitation-contraction coupling at low stimulation frequency (48).

There is evidence from a recent study that mitochondria constitute the main targets for the effects of nitrate on reduced O_2 consumption and increased exercise tolerance (15). The authors evaluated the mitochondrial efficiency after 3 days of dietary nitrate supplementation (0.1 mmol/kg/d of sodium nitrate) in healthy young males. They observed that the number of ATP molecules produced per O_2 consumed (P/O ratio) in skeletal muscle mitochondria increased by 19% after dietary nitrate ingestion compared with placebo. Importantly, the improved mitochondrial efficiency was correlated to the reduction in whole-body O_2 cost during exercise. The increased mitochondrial efficiency (P/O ratio) after nitrate supplementation was possibly attributed to the reduction in proton leak across the inner mitochondrial membrane, as suggested by the downregulation of adenine nucleotide transporter and UCP-3 proteins. In particular, the adenine nucleotide transporter catalyzes the exchange of ATP and ADP across the mitochondrial inner membrane and the cytoplasm; two-thirds of the basal proton conductance of mitochondria is catalyzed by this carrier (64). It is unlikely that improved metabolic efficiency after nitrate ingestion was due to changes in mitochondrial density or power, since no changes were observed in mitochondrial copy number, in expression of genes involved in mitochondrial biogenesis, and in the activity of citrate synthase and cytochrome c oxidase (16).

Recent studies propose that the beneficial effects of dietary nitrate supplementation on the metabolic control and exercise performance may be elicited through a non-intracellular mechanism. Ferguson at al. (2013) documented that dietary nitrate supplementation (1 mmol/kg/d) for 5 days enhanced vascular control and elevated skeletal muscle O_2 delivery during exercise in rats (18). The authors proposed this finding as the potential mechanism for the improved metabolic control after dietary nitrate supplementation. Interestingly, the augmenting effects of beetroot ingestion on blood flow and vascular conductance were most evident in muscles comprised of primarily type IIb fibers. The authors reasoned that the nitrate-induced increase in blood flow during exercise, would improve muscle O_2 perfusion and hence, increase the O_2 delivery/utilization matching, and would enhance the pressure gradient for blood-muscle O_2 flux. This would increase the contribution of oxidative pathways for energy production, would reduce the contribution of glycolytic pathway and the accumulation of lactate in muscles, and would delay fatigue (18). The above idea of NO availability (the end product of nitrate) rising the microvascular O_2 driving pressure during exercise, was previously documented by Ferreira et al. (65). Thus, in their subsequent two studies

Ferguson et al. administered beetroot juice to rats and examined the muscle hemodynamic responses during exercise. The authors reported that dietary nitrate supplementation improved skeletal muscle blood flow and O_2 delivery/utilization matching during exercise, and increased the microvascular O_2 pressure in a dose dependent manner (66, 67). More specifically, a low dose (0.3 mmol/kg/d; ~3.2mmol/d for humans) of beetroot juice, corresponding to a *typical* nitrate-rich diet consumed by humans, had no effects on muscle blood flow, vascular conductance, and microvascular (driving) O_2 pressure during exercise; whereas a high dose (1 mmol/kg/d; ~7.4 mmol/d for humans) significantly increased all parameters (67). The findings of Ferguson et al. are in line with an earlier report that the increased NO bioavalability after training is accompanied by a delayed fall in microvascular O_2 pressure during exercise (68). The improved microvascular O_2 pressure response during exercise, after nitrate supplementation may also well explain the reduced PCr degradation during high-intensity exercise (19, 20), and the improved exercise tolerance.

6. Conclusion

The vast majority of studies that examined the ergogenic value of dietary nitrate supplementation used beetroot juice and only a few administered sodium nitrate. The most prominent and consistent effects of dietary nitrate supplementation is a reduction in the oxygen cost of exercise, an increase in exercise tolerance at submaximal workloads, and an attenuation of the deleterious effects of hypoxic environment during exercise. The mechanistic bases for these effects are not clear yet, but appear to be of intracellular (increased contractile and mitochondrial efficiency) and hemodynamic (increased blood flow) origin. The ergogenic effects of beetroot ingestion were observed after a single bolus (2-3 h prior to exercise) and following long-term (several doses for 2-15 d) supplementation, and may be depended on the type of supplementation, the exercise intensity, and the fitness level. That is, the ergogenic beetroot-effects are more readily apparent after repeated ingestion (over approximately 6 days), during higher exercise intensities and in less fit individuals (69). Particularly for athletes, the ergogenic value of beetroot supplementation on exercise performance is not clear, with most studies documenting no significant improvements in time- and distance-trials. Given the paucity of data on the dose-response relationship between the effects of beetroot juice and exercise tolerance (70), studies should be conducted in various populations to establish the effectual dose for enhancing exercise performance. It is possible, for example, that athletes may require higher dosage to evidence an ergogenic effect. It should be noted, however, that most exercise studies exceeded the acceptable daily nitrate intake; yet, no adverse effects have been reported. Importantly, the ergogenic effects of beetroot supplementation should be examined in individuals with cardiovascular, respiratory, and metabolic diseases, since the potential of beetroot

ingestion to reduce energy cost and increase exercise tolerance is of practical importance for these individuals.

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