



DIETARY NITRIC OXIDE PRECURSORS AND EXERCISE PERFORMANCE

Andrew M. Jones, PhD | College of Life and Environmental Sciences | University of Exeter | United Kingdom

KEY POINTS

- Nitric oxide (NO) is a very important molecule in human physiology. It is involved in the regulation of blood flow, muscle contractility and mitochondrial respiration. Augmenting NO production may enhance exercise performance.
- NO can be synthesized by oxidation of the amino acid, L-arginine, or by reduction of nitrate and nitrite. Dietary supplements containing the NO precursors, L-arginine and nitrate, have been promoted as possible ergogenic aids.
- The efficacy of L-arginine supplementation is controversial. When combined with other compounds, there is some evidence that it may enhance exercise tolerance in sedentary or moderately trained, but not highly trained, subjects. L-arginine supplementation alone has a limited effect on NO production.
- L-citrulline supplementation may enable a higher level of extracellular L-arginine and enhanced NO availability. In some studies, L-citrulline with malate has improved muscle efficiency and in some others, L-citrulline has improved high-intensity aerobic exercise performance. Further studies are required to investigate the ergogenic potential of L-citrulline.
- Dietary nitrate supplementation, typically via beetroot juice ingestion, has been shown to reduce the oxygen cost of low-intensity exercise and to increase the time to exhaustion during high-intensity continuous and intermittent exercise. The available evidence indicates that these effects are more reliably observed in sub-elite athletes. It is unclear if elite athletes might benefit from longer periods, or higher doses, of nitrate supplementation.
- The efficacy of dietary supplementation with NO precursors is likely related to a range of factors including: the duration/intensity and type of exercise to be performed; the supplementation protocol, including the amount and duration of supplementation; and the training status of the subjects.

INTRODUCTION

Nitric oxide (NO) is a gaseous molecule that is synthesized at several locations in the body. NO has received significant attention in exercise physiology and sports nutrition, with many NO “supplements” being sold as potential ergogenic aids. This is based on the important role of NO in many physiological processes related to exercise and recovery, including the regulation of muscle contraction, mitochondrial respiration and blood flow (Stamler and Meissner, 2001).

In the “conventional” NO production pathway, specific nitric oxide synthase (NOS) enzymes catalyze a complex reaction leading to NO formation from the substrates, L-arginine and oxygen (O₂) (Moncada and Higgs, 1993). Nitrate and nitrite are the main oxidation products of NO. An alternative NOS-independent pathway of NO synthesis has also been discovered, based on the simple reduction of nitrate and nitrite to NO (Lundberg et al., 1994).

The use of NO precursors as dietary supplements for the enhancement of exercise performance is controversial (Álvares et al., 2011; Bescós et al., 2012a). These supplements typically contain L-arginine and/or L-citrulline, often alongside other ingredients. More recently, products containing nitrate have emerged, often in the form of a natural product such as beetroot juice. There is a lack of consensus in the scientific literature over the effectiveness of these supplements. This might

relate, in part, to methodological differences between studies, including the amount and duration of supplementation, the type of exercise performed, and the training status of the subjects.

The purpose of this Sports Science Exchange article is to review the effect of supplements designed to augment NO synthesis on exercise performance. Dietary NO precursors have been employed in a wide variety of exercise modalities and subject populations but the specific focus of this article is the efficacy of NO supplements on aerobic exercise performance in young healthy subjects.

RESERACH REVIEW

L-Arginine Supplementation

The semi-essential amino acid, L-arginine, is a natural constituent of dietary proteins. L-arginine is particularly abundant in watermelon juice, seafood, nuts and meat proteins. Dietary intake of L-arginine is ~4–5 g/d but L-arginine can also be synthesized in the kidney and liver, where it is formed from L-citrulline. L-arginine can also be taken up by endothelial cells and oxidized to produce NO (Moncada and Higgs, 1993).

The influence of L-arginine supplementation on exercise performance has been quite extensively investigated (Bescós et al., 2012a). Koppo

et al. (2009) reported that two weeks of L-arginine supplementation (6 g/d) resulted in a significant speeding of phase II $\dot{V}O_2$ kinetics at the onset of moderate-intensity cycle exercise. The reduced $\dot{V}O_2$ deficit might be expected to enhance exercise performance. Unfortunately, neither performance nor changes in indices of NO bioavailability, such as plasma concentrations of L-arginine or nitrite, were measured in this study. Olek et al. (2010) reported that acute L-arginine supplementation (2 g taken 60 min before exercise) did not alter Wingate 30-s cycle test performance or $\dot{V}O_2$. This might not be considered surprising, however, given that plasma values of nitrate/nitrite (biomarkers of NO production) were unchanged after L-arginine supplementation compared with placebo. In a comprehensive recent investigation, 15 recreationally active subjects completed moderate- and severe-intensity running bouts after acute supplementation with 6 g L-arginine or placebo (Vanhatalo et al., 2013). The plasma nitrite concentration was not different after L-arginine compared to placebo. Moreover, neither the $\dot{V}O_2$ cost of moderate-intensity exercise nor the time to exhaustion during severe-intensity exercise were different following L-arginine ingestion compared to placebo (Figure 1). In the same study, L-arginine with carbohydrate did not alter biomarkers of NO synthesis, exercise efficiency or time to exhaustion during cycle exercise compared to a carbohydrate-containing placebo beverage.

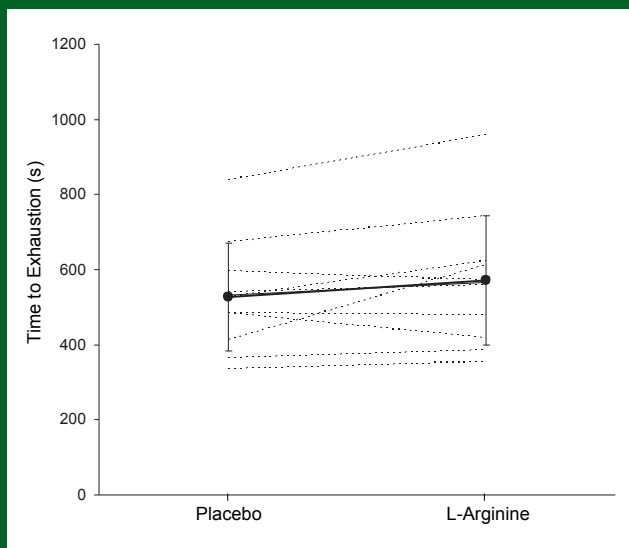


Figure 1: Time to exhaustion during severe-intensity treadmill running following acute L-arginine or placebo supplementation. Solid lines represent the group mean \pm SD responses and dashed lines show the responses of individual subjects. There was no significant effect of L-arginine supplementation. From Vanhatalo et al. (2013).

When well-trained subjects have been tested, L-arginine supplementation was not effective in altering the physiological responses to exercise or improving exercise performance (Bescós et al., 2009; Forbes et al., 2013). This may be because plasma concentrations of nitrate/nitrite appear to be generally insensitive to L-arginine supplementation despite the use of different durations (1-28 d) and doses (6-12 g) (Álvarez et al., 2011; Forbes et al., 2013). The bioavailability of dietary L-arginine is relatively low (~60%) and therefore some studies have

infused L-arginine intravenously (e.g., McConell et al., 2006). Even in these studies, however, no positive effect on exercise performance was found.

Interestingly, several studies reported an improved exercise performance with L-arginine supplementation when it is combined with other components, at least in untrained or moderately trained subjects. Bailey et al. (2010a) reported that L-arginine (6 g/d for 3 d) consumed in combination with other amino acids (including L-citrulline), antioxidants and vitamins, resulted in a lower $\dot{V}O_2$ during low-intensity cycle exercise and an increased time to exhaustion during high-intensity cycling. Also, Camic et al. (2010a) found an increased time to exhaustion during an incremental cycle test when L-arginine (3 g) plus grape seed extract was consumed for 28 d. Similarly, in older cyclists, Chen et al. (2010) found that supplementation of L-arginine (5 g/d for 21 d) with L-citrulline and antioxidants increased performance during an incremental cycle test. One suggested mechanism for the improved performance noted in these studies is that greater NO-mediated blood flow might enable more rapid clearance from the circulation of metabolites that have been related to the fatigue process such as potassium, ammonia and lactate (Camic et al., 2010b). While this explanation may be attractive, it is contradicted by evidence that L-arginine supplementation does not significantly increase blood flow in healthy humans (Adams et al., 1995).

In well-trained subjects, L-arginine supplementation appears to be less effective, even when taken in combination with other compounds. For example, Abel et al. (2005) found that neither endurance performance nor the physiological responses to exercise were altered when endurance cyclists took L-arginine (5.7 g) and aspartate for 28 d. As noted by Bescós et al. (2012a), this may be because training-induced improvements in cardiovascular and muscle metabolic function in athletes could negate any potential positive effects of dietary L-arginine supplementation.

The available evidence therefore suggests that L-arginine does not improve aerobic exercise performance either in recreationally active or well-trained athletes. It is possible that L-arginine in combination with certain other components may benefit exercise performance in untrained or moderately trained subjects. However, in these cases, it is not clear that L-arginine supplementation increased NO synthesis and it is possible that the improved exercise performance observed might result from the effects of other ingredients rather than L-arginine, *per se*.

L-Citrulline Supplementation

L-citrulline is a nonessential amino acid which is found mainly in watermelon but which can also be produced endogenously via synthesis from glutamine and via the conversion of L-arginine to NO. Interest in L-citrulline has increased recently due to its importance as a precursor of L-arginine. Unlike L-arginine, it bypasses hepatic metabolism and is not a substrate of the arginase enzymes. It is therefore possible that L-citrulline administration could be a more efficient way to elevate L-arginine in the human body.

Relatively few studies involving pure L-citrulline supplementation have been published. Hickner et al. (2006) assessed the effect of one dose of L-citrulline consumed either 3 h (3 g) or 24 h (9 g) before an incremental treadmill test in young healthy subjects. Surprisingly, L-citrulline lowered plasma insulin and nitrite concentrations and impaired exercise performance compared with placebo. More recently, Bailey et al. (2015) reported that, compared to both L-arginine and placebo supplementation, L-citrulline supplementation (6 g/d for 7 d) enhanced exercise tolerance and total work done during an aerobic performance trial. Consistent with this, Suzuki et al. (2016) found that 7 d of L-citrulline supplementation (2.4 g/d) led to a significant 1.5% reduction in time to complete a 4 km cycle time trial compared to placebo. On the other hand, acute L-citrulline supplementation (6 g taken 1 or 2 h before exercise) did not significantly alter performance during a series of aerobic and anaerobic exercise tests (Cutrufello et al., 2015).

In other studies, L-citrulline has been combined with malate, a tricarboxylic acid cycle intermediary. In one study, it was reported that 6 g/d of L-citrulline with malate for 16 d resulted in significant increases in the rate of oxidative ATP production during exercise and the rate of phosphocreatine recovery after exercise (Bendahan et al., 2002). L-citrulline with malate has also been reported to improve the efficiency of muscle contraction in rats (Giannesini et al., 2011). A single dose of L-citrulline with malate (8 g) was also found to increase the number of bench-press repetitions performed at 80% of 1-repetition maximum (RM) (Pérez -Guisado and Jakeman, 2010). In another study, however, L-citrulline with malate (12 g) did not improve multiple-sprint performance or time-to-exhaustion during high-intensity aerobic exercise compared to placebo (Cunniffe et al., 2016).

Despite the positive effects observed in some studies conducted to date, it has proven difficult to link the improved exercise performance with L-citrulline to an increased NO production. Additional research is necessary to determine the effects of L-citrulline supplementation on NO bioavailability, the physiological responses to exercise, and exercise performance. It appears that L-citrulline supplementation may be more effective if taken over several days rather than just acutely pre-exercise.

Nitrate Supplementation

The nitric oxide synthase (NOS)-independent pathway of NO production involves the reduction of nitrate and nitrite. Whereas the NOS pathway is O₂ dependent, the nitrate/nitrite-NO pathway is more active when tissue O₂ tension is low, suggesting that this pathway might be favoured during exercise (Lundberg and Weitzberg, 2009).

The main sources of nitrate in the diet are green leafy vegetables such as lettuce, spinach, rocket and beetroot, as well as drinking water. The NOS-dependent NO synthesis pathway also contributes to the overall production of nitrate and nitrite. The normal plasma nitrate and nitrite concentrations are 10-50 mM and 50-150 nM, respectively, although these values are highly sensitive to diet and training (Wylie et al., 2013a). Following ingestion, circulating nitrate is actively taken up by the salivary glands where it is concentrated before bacteria in the

mouth reduce it from nitrate to nitrite. Some of the swallowed nitrite is then absorbed and serves to increase circulating plasma nitrite which can be converted to NO in the blood and tissues under appropriate physiological conditions (Lundberg and Weitzberg, 2009).

The effect of dietary nitrate supplementation in the form of sodium nitrate has been investigated in several studies. In the first of these, Larsen et al. (2007) reported that the ingestion of sodium nitrate (0.1 mmol/kg for 3 d) reduced $\dot{V}O_2$ (~160 mL/min) during sub-maximal cycle exercise. This surprising effect occurred without changes in ventilation, heart rate, respiratory exchange ratio or blood lactate concentration, which suggests improved muscle efficiency after dietary nitrate consumption. A recent study with trained subjects, however, found that acute supplementation of sodium nitrate (10 mg/kg body mass (BM)) 3 h before exercise did not significantly reduce $\dot{V}O_2$ during moderate-intensity exercise nor improve exercise performance (Bescós et al., 2012b).

Several other studies have investigated the effect of dietary nitrate supplementation in the form of beetroot juice on exercise performance. Following supplementation with 500 mL/day of nitrate-rich beetroot juice for 6 d, Bailey et al. (2009) reported a significantly reduced O₂ cost of moderate-intensity exercise and improved time to exhaustion during high-intensity exercise compared with placebo. The same research group found similar effects during treadmill running (Lansley et al., 2011).

There are several mechanisms which may underpin a possible ergogenic effect of nitrate supplementation. Bailey et al. (2010b) used magnetic resonance spectroscopy to examine the muscle metabolic responses to exercise and reported that the reduced O₂ cost at moderate and high intensities after beetroot juice ingestion was accompanied by a sparing of muscle phosphocreatine. Consistent with this, there is evidence that NO may alter calcium handling and potentially reduce the ATP cost of force production (Hernández et al., 2012). In addition, Larsen et al. (2011) reported that human mitochondrial efficiency, measured as the amount of O₂ consumed per ATP produced (P/O ratio), was significantly improved after sodium nitrate ingestion compared with placebo. However, this improvement in mitochondrial efficiency was not confirmed in a recent study using beetroot juice (Whitfield et al., 2016). These improvements to intracellular energetics following nitrate supplementation may be complemented by increased blood flow to muscle, with greater distribution of O₂ to fast-twitch muscle (Ferguson et al., 2012).

Several studies have suggested that beetroot juice ingestion can improve exercise performance in sub-elite athletes (Figure 2). For example, both Lansley et al. (2011) and Cermak et al. (2012) found that completion time during cycle time trials (4, 10 and 16 km) was significantly reduced with beetroot juice supplementation compared with placebo. High-intensity intermittent exercise performance has also been reported to be enhanced both in recreational team sport players (Thompson et al., 2015; Wylie et al., 2013b) following beetroot juice supplementation. Attempts to reproduce these findings in highly trained athletes have, to date, with some exceptions (Bond et al., 2012;

Peeling et al., 2015), proved less successful (Boorsma et al., 2014; Christensen et al., 2013; Peacock et al., 2012). As was mentioned also for L-arginine supplementation, it is possible that the physiological adaptations associated with chronic training blunt the possible benefits to performance of dietary nitrate supplementation. It is interesting, however, that some well-trained athletes “respond” to nitrate supplementation while others do not (Boorsma et al., 2014; Wilkerson et al., 2012; Christensen et al., 2013). With regard to performance enhancement with nitrate, further research is necessary to determine the interactions of: 1) training status; 2) exercise type, intensity and modality; and 3) supplementation regimen including duration (acute vs. chronic) and appropriate dosing (Figure 2).

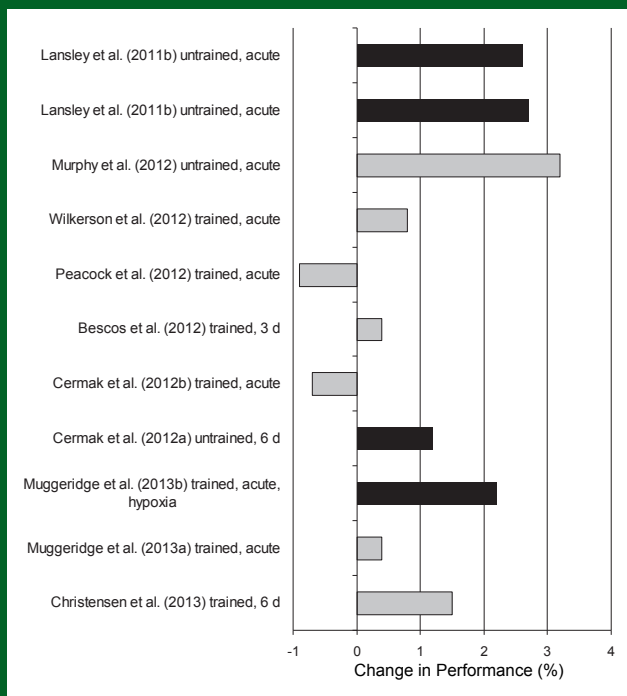


Figure 2: Effects of dietary nitrate supplementation vs. placebo on time trial performance. Black bars represent a significant improvement in performance and grey bars represent no significant change in performance.

Safety Considerations

L-arginine and L-citrulline supplementation is generally well tolerated. Similarly, other than the harmless purple coloring of urine and stools, nitrate supplementation with beetroot juice does not have significant side effects. The amount of inorganic nitrate in food and water has been strictly regulated because of its proposed role in the development of methemoglobinemia and cancer, and there is currently an acceptable daily intake (ADI) for humans of 3.7 mg nitrate per kg BM (although the US Environmental Protection Agency's reference level for nitrate in drinking water is higher than the current ADI at 7 mg/kg). However, the nitrate concentrations in food and water are unlikely to cause methemoglobinemia (Katan, 2009) and no causal link between dietary nitrate intake and gastric cancer in humans has been established (McKnight et al., 1999). A large green salad could have a nitrate

content that exceeds the current ADI and there is a growing view that nitrate is beneficial rather than harmful to human health and that the ADI should be revised.

PRACTICAL APPLICATIONS

- The scientific evidence supporting the use of L-arginine and L-citrulline supplements is limited. In general, there is insufficient evidence to show that these supplements increase NO production, improve exercise performance, or that performance improvements (where present) are related to increased NO synthesis.
- It remains possible that L-arginine supplementation, at least when combined with other compounds, may enhance exercise performance in sedentary or recreationally active subjects, but trained athletes do not appear to benefit.
- L-citrulline supplementation may present a more effective approach to elevating extracellular L-arginine concentration and, at least when consumed in combination with malate, L-citrulline holds some promise for the enhancement of exercise performance.
- The NOS-independent pathway of NO synthesis may be more amenable to dietary intervention. There is evidence that nitrate supplementation can reduce the O₂ cost of exercise, improve muscle efficiency and enhance exercise performance. However, the exercise intensity and duration along with the training status of the individual seem to be important in determining whether nitrate supplementation is ergogenic.
- Presently, it appears that highly trained subjects respond only minimally, if at all, to acute nitrate supplementation. It remains to be determined whether longer periods of supplementation, or higher doses of nitrate, are required to elicit performance benefits.

SUMMARY

Overall, there is a lack of consensus over whether dietary supplementation with the substrates required for NO synthesis improves exercise performance. L-arginine has not been convincingly shown to increase markers of NO synthesis or to alter the physiological responses to exercise. L-citrulline and nitrate appear to hold more promise and require further study. The effectiveness of dietary supplementation with NO precursors appears to be related to training status. It is well known that training can up-regulate NO metabolism and this may negate any possible benefits of supplementation. Nevertheless, there may be some circumstances, such as when muscle oxygenation is compromised, where supplementation with a dietary NO precursor may be beneficial. Also, because NO availability and vascular function are impaired with older age, it is possible that dietary NO precursors may enhance both cardiovascular health and exercise performance in middle-aged and older adults.

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