SSE #110

Sports Science Exchange (2013) Vol. 26, No. 110, 1-5

GATORADE SPORTS SCIENCE INSTITUTE

DIETARY NITRATE: THE NEW MAGIC BULLET?

Andrew M. Jones | Sport and Health Sciences | College of Life and Environmental Sciences | University of Exeter | United Kingdom

KEY POINTS

- Nitric oxide (NO) is vitally important in human physiology and it modulates many of the processes that are essential to exercise performance.
- Recent evidence indicates that NO availability can be enhanced by dietary supplementation with inorganic nitrate which is abundant in green leafy vegetables and beetroot.
- Dietary nitrate supplementation with 5-7 mmol nitrate (~0.1 mmol/kg body mass) reduces resting blood pressure, lowers the oxygen cost of sub-maximal exercise (i.e., enhances muscle efficiency) and may enhance exercise performance.
- These physiological effects can be observed as little as 3 h following nitrate consumption and can be maintained for at least 15 days if supplementation is continued.
- The optimal nitrate 'loading' regimen and the physical activities and populations in which nitrate supplementation might be most effective remain to be determined.
- Due to possible health risks associated with the consumption of nitrate salts, it is recommended that athletes wishing to explore the ergogenic potential of nitrate supplementation do so through increased consumption of nitrate-rich vegetable products such as beetroot juice.

INTRODUCTION

Nitric oxide (NO) is an important physiological signaling molecule that can modulate skeletal muscle function through its role in the regulation of blood flow, muscle contractility, glucose and calcium homeostasis, and mitochondrial respiration and biogenesis. Until quite recently, it was considered that NO was generated solely through the oxidation of the amino acid L-arginine in a reaction catalysed by nitric oxide synthase (NOS), and that nitrite (NO_2-) and nitrate (NO_3-) were inert by-products of this process. However, it is now clear that these metabolites can be recycled back into bioactive NO under certain physiological conditions. The reduction of NO_3- to NO_2- and subsequently of NO_2- to NO may be important as a means to increase NO production when NO synthesis by the NOS enzymes is impaired and in conditions of low O_2 availability, such as may occur in skeletal muscle during exercise.

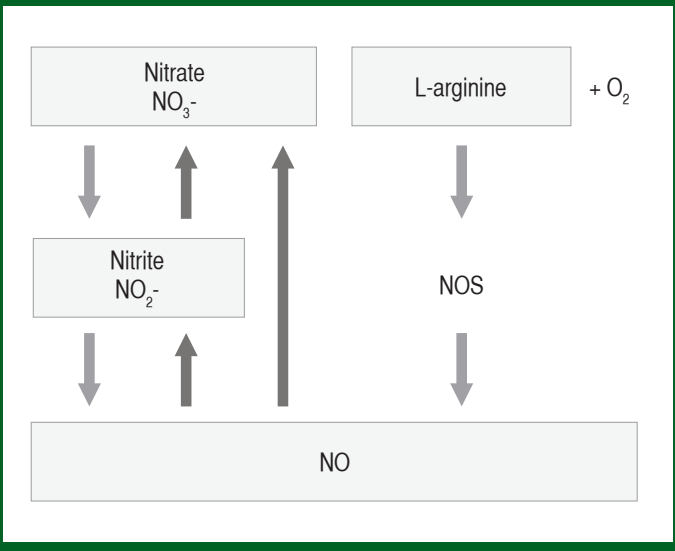

Figure 1: Relationship between nitric oxide (NO), nitrite (NO_2-) and nitrate (NO_3-). (NOS, nitric oxide synthase)

It is now known that tissue concentrations of nitrate and nitrite can be increased by dietary means. Green leafy vegetables such as lettuce, spinach, rocket, celery and beetroot are particularly rich in nitrate. Therefore, dietary nitrate supplementation represents a practical method to increase circulating plasma [NO_2-] and thus NO bioavailability. This has been demonstrated after ingestion of nitrate salts such as sodium nitrate (Larsen et al., 2007, 2010), as well as following nitrate-rich beetroot juice ingestion (Bailey et al., 2009, 2010; Vanhatalo et al., 2010; Webb et al., 2008). It is also possible to increase plasma [NO_2-] through increased consumption of whole nitrate-rich vegetables but nitrate content can vary according to soil conditions, time of year and storage. Given the importance of NO in vascular and metabolic control, there are sound theoretical reasons why augmenting NO bioavailability might be important in optimizing skeletal muscle function during exercise. Indeed, recent evidence indicates that elevating plasma [NO_2-] through dietary nitrate supplementation is associated with enhanced muscle efficiency, fatigue resistance and performance.

RESEARCH REVIEW

Nitrate and exercise. Larsen et al. (2007) reported that three days of sodium nitrate supplementation increased plasma [NO_2-] and reduced the O_2 cost of sub-maximal cycle exercise. These findings were surprising because it is well established that the O_2 cost of

exercising at a given sub-maximal power output is highly predictable. For example, during cycle ergometry, it is expected that pulmonary O_2 uptake ($\dot{V}o_2$) will increase by approximately 10 mL per minute for every additional Watt of external power output (i.e., the functional 'gain' is ~10 mL/min/W). The results of the Larsen et al. (2007) study were of considerable interest because they suggested that a short-term dietary intervention might improve exercise efficiency (i.e., reduce the energy required to exercise at the same intensity) and have the potential to enhance performance.

The findings of Larsen et al. (2007) were corroborated in the study of Bailey et al. (2009) in which nitrate was administered in the form of beetroot juice. Following three days of beetroot juice supplementation (0.5 L/day), the plasma [NO_2-] was doubled, the steady-state $\dot{V}O_2$ during moderate-intensity exercise was reduced (Figure 2) and the $\dot{V}O_2$ 'slow component' during severe-intensity exercise was attenuated. These results suggested that a short-term, natural dietary intervention improved the efficiency of muscular work.

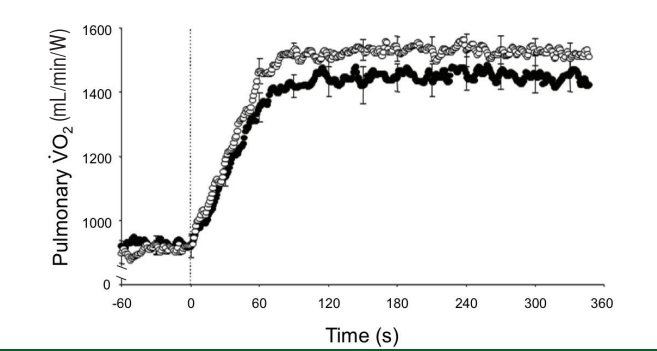

Figure 2: Reduction in O_2 uptake during 6 min of moderate-intensity cycle exercise following dietary nitrate supplementation (closed symbols) compared to placebo (open symbols).

The reduction in steady-state $\dot{V}O_2$ after nitrate supplementation was of the order of 5% in the studies of Larsen et al. (2007) and Bailey et al. (2009) in which supplementation was continued for 3-6 days. A similar reduction in steady-state $\dot{V}O_2$ during moderate-intensity cycle ergometry has been reported following acute nitrate supplementation. Vanhatalo et al. (2010) reported a significant reduction in steady-state $\dot{V}O_2$ just 2.5 h following beetroot juice ingestion, an effect that was maintained when supplementation was continued for 15 days (Figure 3). Importantly, habitual dietary nitrate intake was not restricted in this study, and yet resting blood pressure and steady-state $\dot{V}O_2$ were still significantly reduced. The reduction in $\dot{V}O_2$ following nitrate administration is not unique to cycling exercise, having also been observed during two-legged knee-extensor exercise (Bailey et al., 2010) and treadmill walking and running (Lansley et al., 2011a). Importantly, no reduction in $\dot{V}O_2$ was observed compared to a control condition when the subjects were supplemented with a placebo beetroot juice that had been depleted of nitrate using an ion-exchange resin (Lansley et al., 2011a). This confirmed that nitrate is the key 'active' ingredient responsible for the physiological changes observed following beetroot juice supplementation. It does not rule out, however, a synergistic role for other components of beetroot juice such as antioxidants, which may facilitate the reduction of nitrate to nitrite and NO. Collectively, these results indicate that the reduced $\dot{V}O_2$ following nitrate supplementation is reproducible and can be observed across a range of different supplementation regimens and exercise modalities.

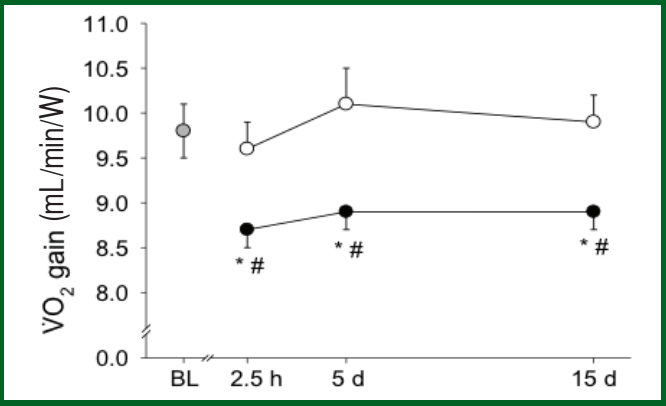

Figure 3: Reduction in the 'gain' of O_2 uptake following nitrate supplementation (closed symbols) compared to placebo (open symbols) and non-supplemented baseline (BL; gray symbol). Note that the gain is reduced from ~10 to ~9 mL/min/W following nitrate supplementation acutely (after 2.5 h) and that this effect persists if supplementation is continued for 15 days.

EXERCISE PERFORMANCE

Plasma [NO_2-] has recently been identified as an important correlate of exercise tolerance in healthy humans (Dreissigacker et al., 2010; Rassaf et al., 2007). Given that NO_3- supplementation increases plasma [NO_2-], this intervention may therefore have the potential to improve exercise tolerance. This hypothesis was tested in the study of Bailey et al. (2009). Plasma [NO_2-] was doubled and high-intensity exercise tolerance was enhanced by 16% following NO_3--rich beetroot juice supplementation. Subsequent experiments have reported improvements in exercise tolerance of 25% during two-legged knee-extensor exercise (Bailey et al., 2010), and 15% during treadmill running (Lansley et al., 2011a) following 6 days of beetroot juice supplementation. Improved incremental exercise performance has also been noted following 6 days of beetroot juice supplementation during single-legged knee extension exercise (Lansley et al., 2011a) and after 15 days of beetroot juice supplementation during cycle exercise (Vanhatalo et al., 2010).

It is well documented that exercise performance is compromised in a hypoxic environment relative to normoxia (21% O_2: sea level). In this regard, it is noteworthy that Vanhatalo et al. (2011) reported that nitrate supplementation with beetroot juice restored muscle performance in hypoxia (14% inspired O_2; equivalent to ~4000 meters or ~13,000 feet altitude) to that observed in the normoxic

control condition. Specifically, in hypoxia, nitrate supplementation resulted in a 20% extension of the time-to-exhaustion during high-intensity knee-extensor exercise. Vanhatalo et al. (2011) also reported that nitrate supplementation improved muscle oxidative function in hypoxia, suggesting that muscle oxygenation may have been enhanced. Consistent with this interpretation, Kenjale et al. (2011) reported that beetroot juice supplementation resulted in a 17-18% longer time to claudication pain and peak walking time during incremental exercise in patients with peripheral arterial disease. The authors attributed these effects to NO_2- -related improvement in peripheral tissue oxygenation. Collectively, these results have potential performance implications for athletes competing at altitude and for improving functional capacity in clinical conditions where tissue O_2 supply may be compromised.

As summarized above, during high-intensity constant-work-rate exercise, the improved exercise tolerance at a given power output following nitrate supplementation has been reported to be in the range of 16-25% (Bailey et al., 2009, 2010; Lansley et al., 2011a). However, the magnitude of improvement in 'actual' exercise performance would be expected to be far smaller; indeed, using the predictions of Hopkins et al. (1999), a ~20% improvement in time-to-exhaustion would be expected to correspond to an improvement in exercise performance (time taken to cover a set distance) of ~1-2%. This hypothesis was tested in the study of Lansley et al. (2011b) where competitive but sub-elite cyclists completed 4.0 and 16.1 km time trials on separate days, following acute beetroot juice ingestion. Consistent with the experimental hypothesis, nitrate administration improved 4.0 km and 16.1 km time trial performance by ~2.7 % compared to the placebo conditions (Lansley et al., 2011b). These improvements in exercise performance were consequent to the maintenance of a higher mean power output and an increase in the power output/$\dot{V}O_2$ ratio. Therefore, trained subjects were able to produce a higher power output for the same oxidative energy turnover (i.e., the inverse of a lower $\dot{V}O_2$ for the same power output; Bailey et al. 2009; Larsen et al., 2007), resulting in an improved exercise performance following nitrate supplementation. Improved cycle time trial performance following nitrate supplementation has also been reported by Cermak et al. (2012). These authors reported that six days of beetroot juice supplementation (8 mmol/day) significantly reduced $\dot{V}O_2$ at two sub-maximal work rates and improved mean power output and 10 km time trial performance (by 1.2%) in trained cyclists.

Despite these positive results with 'sub-élite' athletes, it remains unclear whether nitrate supplementation might enhance performance in athletes of the highest caliber. One study has reported that acute sodium nitrate administration did not significantly alter sub-maximal $\dot{V}O_2$ or incremental exercise performance in endurance athletes (Bescós et al., 2011). There may be several explanations for this apparent discrepancy. The resting plasma [NO_3-] and [NO_2-] is higher in athletes (Jungersten et al., 1997; Schena et al., 2002), which may reduce the scope for nitrate supplementation to improve exercise efficiency and performance in this population. Alternately, very highly trained individuals may require a larger nitrate dose to elicit similar changes in plasma [NO_2-] and exercise efficiency to those observed in recreationally active participants. Wilkerson et al. (2012) reported that acute nitrate supplementation did not enhance 50 km time trial performance in a group of well-trained cyclists, but also found a significant correlation (r = -0.83) between the increase in plasma [NO_2-] and the improvement in time trial performance. In this regard, the nitrate dosing regimen (i.e., amount and timing of ingestion) may be critical. It should also be considered that highly trained subjects are likely to have: 1) higher NOS activity such that the nitrate-nitrite-NO pathway may be relatively less important for the generation of NO; and 2) greater mitochondrial and capillary density which might limit the development of hypoxia and acidosis in skeletal muscle during exercise, preserving NOS function and reducing the requirement for nitrite reduction to NO. It should also be considered that it may be more difficult to discern possible performance improvements in elite athletes for methodological reasons. The likely performance effect might be ≤ 1% which, while still potentially highly meaningful during competition, may be difficult to measure reproducibly due to experimental noise and day-to-day variability. Further research is needed to elucidate the influence of NO_3- supplementation on exercise efficiency in athletes.

MECHANISMS

The reduced O_2 cost of exercise following nitrate supplementation is not associated with an elevated blood [lactate] (Bailey et al., 2009; Larsen et al., 2007), suggesting that there is no compensatory increase in anaerobic energy production as might be expected if oxidative metabolism were somehow inhibited. This indicates that nitrate supplementation results in a 'real' improvement in muscle efficiency. Theoretically, a lower O_2 cost of exercise for the same power output could result from: 1) a lower ATP cost of muscle contraction for the same force production (i.e., improved muscle contractile efficiency); and/or 2) a lower O_2 consumption for the same rate of oxidative ATP resynthesis (i.e., improved mitochondrial efficiency).

Bailey et al. (2010) investigated the first of these possibilities using calibrated ^{31}P-magnetic resonance spectroscopy (^{31}P-MRS). This procedure permitted the in vivo assessment of absolute muscle concentration changes in phosphocreatine ([PCr]), inorganic phosphate ([Pi]), and adenosine diphosphate ([ADP]), as well as pH. The ATP supply contributed by PCr hydrolysis, anaerobic glycolysis and oxidative phosphorylation during knee-extensor exercise was also calculated. The estimated ATP turnover rates from PCr hydrolysis and oxidative phosphorylation were lower following six days of beetroot juice supplementation, with there being no change in the estimated ATP turnover rate from anaerobic glycolysis, such that there was a significant reduction in the estimated total ATP turnover rate during both low- and high-intensity exercise (Bailey et al., 2010). It is known that the ATP turnover rate in contracting muscle cells is determined principally by the activity of the actomyosin ATPases and Ca^{2+}-ATPases. NO has been shown to slow myosin cycling kinetics (Evangelista et al., 2010) and to reduce Ca^{2+}-ATPase activity

(Ishii et al., 1998). As such, elevated NO production following nitrate supplementation may have reduced skeletal muscle ATP turnover by reducing the activity of actomyosin ATPase and/or Ca^{2+}-ATPase. The intramuscular accumulation of ADP and Pi, and the extent of PCr depletion, were blunted following nitrate supplementation (Bailey et al., 2010). The smaller changes in [ADP], [Pi] and [PCr] following NO_3- supplementation would be predicted to reduce the stimuli for increasing oxidative phosphorylation (Mahler, 1985).

The accumulation of metabolites such as [ADP] and [Pi], and the rate of depletion of the finite intramuscular [PCr] reserves, are important contributors to muscle fatigue development (Allen et al., 2008). While the intramuscular [ADP], [Pi] and [PCr] were similar at exhaustion in the nitrate-supplemented and placebo conditions in the study of Bailey et al. (2010) and also Vanhatalo et al. (2011), the time taken to achieve these critical concentrations was delayed following nitrate supplementation and this, in part, may explain the improved exercise tolerance. It should be noted that while the improved muscle efficiency and reduced metabolic perturbation may be responsible for the enhanced exercise tolerance observed following nitrate supplementation, it is possible that the intervention results in a simultaneous improvement in muscle O_2 availability (Kenjale et al., 2011; Vanhatalo et al., 2011). This, too, might contribute to a blunting of muscle PCr depletion and improved exercise performance.

The second possibility, that nitrate supplementation enhances mitochondrial efficiency, has been investigated by Larsen et al. (2011). These authors isolated mitochondria from the vastus lateralis muscle of healthy humans supplemented with sodium nitrate. It was reported that nitrate supplementation reduced proton leakage and uncoupled respiration, which increased the mitochondrial P/O ratio (the amount of ATP produced/oxygen used). Importantly, the increased P/O ratio following nitrate supplementation was correlated with the reduction in whole body $\dot{V}O_2$ during exercise (Larsen et al., 2011). It appears therefore that nitrate supplementation may improve exercise efficiency by improving the efficiency of both muscle contraction (reduced ATP cost of force production) and mitochondrial oxidative phosphorylation (increased P/O ratio).

PRACTICAL APPLICATIONS

- Dietary supplementation with 5-7 mmol nitrate (~0.1 mmol/kg body mass) results in a significant increase in plasma [NO_2-] and associated physiological effects including a lower resting blood pressure, reduced pulmonary O_2 uptake during sub-maximal exercise and, perhaps, enhanced exercise tolerance or performance. This 'dose' of nitrate can readily be achieved through the consumption of 0.5 L of beetroot juice or an equivalent high-nitrate foodstuff.
- Following a 5-6 mmol 'bolus' of nitrate, plasma [NO_2-] typically peaks within 2-3 h and remains elevated for a further 6-8 h before returning to baseline after about 24 h (Webb et al., 2008). It is recommended that nitrate is consumed ~3 h prior to competition or training. A daily dose of a high-nitrate supplement is required if plasma [NO_2-] is to remain elevated.
- Most of the published studies to date have involved recreational or moderately-trained subjects and it is not known if nitrate supplementation substantially elevates plasma [NO_2-] or is ergogenic in elite athletes.
- While the ingestion of 5-6 mmol of nitrate appears to be effective, studies are ongoing to determine the 'dose-response' relationship between nitrate supplementation and changes in exercise efficiency and performance. This will provide new information on the 'optimal' loading regimen for performance enhancement.
- While nitrate supplementation appears to be ergogenic in continuous maximal activity of 5-25 min duration, possible effects on shorter-term high-intensity exercise, intermittent exercise, and longer-term endurance exercise performance have not been established.
- It is presently unclear if sustained dietary nitrate supplementation might impact upon adaptations to training: on the one hand, increased NO bioavailability might simulate mitochondrial and capillary biogenesis; on the other hand, nitrate has antioxidant properties that might potentially blunt cellular adaptations.
- Dietary or environmental exposure to nitrate has historically been considered to be harmful to human health due to a possible increased risk of gastric cancer. More recent evidence challenges this view and indicates that dietary nitrate may instead confer benefits to health (Gilchrist et al., 2010). Until more is known, it is recommended that athletes wishing to explore possible ergogenic effects of nitrate supplementation employ a natural (beetroot juice, leafy vegetables), rather than pharmacological, approach.

SUMMARY

Dietary nitrate appears to hold promise as a natural means to enhance NO bioavailability. NO production through the oxidation of L-arginine, in a reaction catalysed by the NOS enzymes, is impaired in older age and a variety of disease states and also in hypoxic tissue. The O_2-independent reduction of nitrite to NO may therefore represent an essential 'back-up' system for NO generation in situations where NOS may be dysfunctional. Dietary nitrate supplementation reduces resting blood pressure and may therefore be important in maintaining and promoting cardiovascular health. It is now well established that acute and chronic nitrate supplementation can reduce the O_2 cost of sub-maximal exercise. This improvement in muscular efficiency may be linked to a reduced energy cost of muscle contraction and/or to enhanced efficiency of mitochondrial ATP production. Since muscle efficiency is an important determinant of exercise performance, it is possible that nitrate might be classified as an ergogenic aid. Indeed, several studies indicate that, at least in recreational or moderately trained subjects, nitrate supplementation can extend exercise tolerance and improve time trial performance. However, additional work is required before the effectiveness of nitrate supplementation on performance in different types of physical activity and in different human populations is fully understood.

REFERENCES

Allen, D.G., G.D. Lamb, and H. Westerblad (2008). Skeletal muscle fatigue: cellular mechanisms. *Physiol. Rev.* 88:287-332.

Bailey, S.J., P. Winyard, A. Vanhatalo, J.R. Blackwell, F.J. DiMenna, D.P. Wilkerson, J. Tarr, N. Benjamin, and A.M. Jones (2009). Dietary nitrate supplementation reduces the O2 cost of low-intensity exercise and enhances tolerance to high-intensity exercise in humans. *J. Appl. Physiol.* 107:1144-1155.

Bailey, S.J., J. Fulford, A. Vanhatalo, P. Winyard, J.R. Blackwell, F.J. DiMenna, D.P. Wilkerson, N. Benjamin, and A.M. Jones (2010). Dietary nitrate supplementation enhances muscle contractile efficiency during knee-extensor exercise in humans. *J. Appl. Physiol.* 109:135-148.

Bescós, R., F.A. Rodríguez, X. Iglesias, M.D. Ferrer, E. Iborra, and A. Pons (2011). Acute administration of inorganic nitrate reduces VO(2peak) in endurance athletes. *Med. Sci. Sports Exerc.* 43:1979-1986.

Cermak, N.M., M.J. Gibala, and L.J. van Loon (2012). Nitrate supplementation's improvement of 10-km time-trial performance in trained cyclists. *Int. J. Sport Nutr. Exerc. Metab.* 22:64-71.

Dreissigacker, U., M. Wendt, T. Wittke, D. Tsikas, and N Maassen (2010). Positive correlation between plasma nitrite and performance during high-intensive exercise but not oxidative stress in healthy men. *Nitric Oxide* 23:128-135.

Evangelista, A.M., V.S. Rao, A.R. Filo, N.V. Marozkina, A. Doctor, D.R. Jones, B. Gaston, and W.H. Gulford (2010). Direct regulation of striated muscle myosins by nitric oxide and endogenous nitrosothiols. *PLoS One* 5:e11209.

Gilchrist, M., P.G. Winyard, and N. Benjamin (2010). Dietary nitrate-good or bad? *Nitric Oxide* 22:104-109.

Hopkins, W.G., J.A. Hawley, and L.M. Burke (1999). Design and analysis of research on sport performance enhancement. *Med. Sci. Sports Exerc.* 31:472-485.

Ishii, T., O. Sunami, N. Saitoh, H. Nishio, T. Takeuchi, and F. Hata (1998). Inhibition of skeletal muscle sarcoplasmic reticulum Ca2+-ATPase by nitric oxide. *FEBS Lett.* 440:218-222.

Jungersten, L., A. Ambring, B. Wall, and A. Wennmalm (1997). Both physical fitness and acute exercise regulate nitric oxide formation in healthy humans. *J. Appl. Physiol.* 82:760-764.

Kenjale, A.A., K.L. Ham, T. Stabler, J.L. Robbins, J.L. Johnson, M. Vanbruggen, G. Privette, E. Yim, W.E. Kraus, and J.D. Allen (2011) Dietary nitrate supplementation enhances exercise performance in peripheral arterial disease. *J. Appl. Physiol.* 110:1582-1591.

Lansley, K.E., P.G. Winyard, J. Fulford, A. Vanhatalo, S.J. Bailey, J.R. Blackwell, F.J. DiMenna, M. Gilchrist, N.Benjamin, and A.M. Jones (2011a). Dietary nitrate supplementation reduces the O2 cost of walking and running: a placebo-controlled study. *J. Appl. Physiol.* 110:591-600.

Lansley, K.E., P.G. Winyard, S.J. Bailey, A. Vanhatalo, D.P. Wilkerson, J.R. Blackwell, M. Gilchrist, N. Benjamin, and Jones, A.M. (2011b). Acute dietary nitrate supplementation improves cycling time trial performance. *Med. Sci. Sports Exerc.* 43:1125-1131.

Larsen, F.J., E. Weitzberg, J.O. Lundberg, and B. Ekblom (2007). Effects of dietary nitrate on oxygen cost during exercise. *Acta Physiol.* 191:59-66.

Larsen, F.J., E. Weitzberg, J.O. Lundberg, and B. Ekblom (2010). Dietary nitrate reduces maximal oxygen consumption while maintaining work performance in maximal exercise. *Free Radic. Biol. Med.* 48:342-347.

Larsen, F.J., T.A. Schiffer, S. Borniquel, K. Sahlin, B. Ekblom, J.O. Lundberg, and E. Weitzberg (2011). Dietary inorganic nitrate improves mitochondrial efficiency in humans. *Cell Metab.* 13:149-159.

Mahler, M. (1985). First-order kinetics of muscle oxygen consumption, and equivalent proportionality between QO2 and phosphorylcreatine level. Implications for the control of respiration. *J. Gen. Physiol.* 86:135-165.

Rassaf, T., T. Lauer, C. Heiss, J. Balzer, S. Mangold, T. Leyendecker, J. Rottler, C. Drexhage, C. Meyer, and M. Kelm (2007). Nitric oxide synthase-derived plasma nitrite predicts exercise capacity. *Brit. J. Sports Med.* 41:669-673.

Schena, F., L. Cuzzolin, L. Rossi, M. Pasetto, and G. Benoni (2002). Plasma nitrite/nitrate and erythropoietin levels in cross-country skiers during altitude training. *J. Sports Med. Phys. Fit.* 42:129-134.

Vanhatalo, A., S.J. Bailey, J. R. Blackwell, F.J. DiMenna, T.G. Pavey, D.P. Wilkerson, N.Benjamin, P.G. Winyard, and A.M. Jones (2010). Acute and chronic effects of dietary nitrate supplementation on blood pressure and the physiological responses to moderate-intensity and incremental exercise. *Am. J. Physiol.* 299:R1121-1131.

Vanhatalo, A., J. Fulford, S.J. Bailey, J.R. Blackwell, P.G. Winyard, and A.M. Jones (2011). Dietary nitrate reduces muscle metabolic perturbation and improves exercise tolerance in hypoxia. *J. Physiol.* 589:5517-5528.

Webb, A.J., N. Patel, S. Loukogeorgakis, M. Okorie, Z Aboud, S. Misra, R. Rashid, P. Miall, J. Deanfield, N. Benjamin, R. MacAllister, A.J. Hobbs, and A. Ahluwalia (2008). Acute blood pressure lowering, vasoprotective, and antiplatelet properties of dietary nitrate via bioconversion to nitrite. *Hypertension* 51:784-790.

Wilkerson, D.P., G.M. Hayward, S.J. Bailey, A. Vanhatalo, J.R. Blackwell, and A.M. Jones (2012). Influence of acute dietary nitrate supplementation on 50 mile time trial performance in well-trained cyclists. *Eur. J. Appl. Physiol.* 112:4127-4134.