

On the mechanism by which dietary nitrate improves human skeletal muscle function

Charles Affourtit^{1*}, Stephen J. Bailey², Andrew M. Jones², Miranda J. Smallwood³ and Paul G. Winyard³

¹ School of Biomedical and Healthcare Sciences, Plymouth University Peninsula Schools of Medicine and Dentistry, Plymouth University, Plymouth, UK, ² Department of Sport and Health Sciences, College of Life and Environmental Sciences, University of Exeter, Exeter, UK, ³ Institute of Biomedical and Clinical Sciences, University of Exeter Medical School, University of Exeter, Exeter, UK

OPEN ACCESS

Edited by:

Gilles Gousspillou,
Université du Québec à Montréal,
Canada

Reviewed by:

Sruti Shiva,
University of Pittsburgh, USA
Emanuele Marzetti,
Catholic University of the Sacred
Heart, Italy

*Correspondence:

Charles Affourtit,
School of Biomedical and Healthcare
Sciences, Plymouth University,
Portland Square Building, Drake
Circus, PL4 8AA, Plymouth, UK
charles.affourtit@plymouth.ac.uk

Specialty section:

This article was submitted to
Striated Muscle Physiology,
a section of the journal
Frontiers in Physiology

Received: 23 June 2015

Accepted: 14 July 2015

Published: 29 July 2015

Citation:

Affourtit C, Bailey SJ, Jones AM,
Smallwood MJ and Winyard PG
(2015) On the mechanism by which
dietary nitrate improves human
skeletal muscle function.
Front. Physiol. 6:211.
doi: 10.3389/fphys.2015.00211

Inorganic nitrate is present at high levels in beetroot and celery, and in green leafy vegetables such as spinach and lettuce. Though long believed inert, nitrate can be reduced to nitrite in the human mouth and, further, under hypoxia and/or low pH, to nitric oxide. Dietary nitrate has thus been associated favorably with nitric-oxide-regulated processes including blood flow and energy metabolism. Indeed, the therapeutic potential of dietary nitrate in cardiovascular disease and metabolic syndrome—both aging-related medical disorders—has attracted considerable recent research interest. We and others have shown that dietary nitrate supplementation lowers the oxygen cost of human exercise, as less respiratory activity appears to be required for a set rate of skeletal muscle work. This striking observation predicts that nitrate benefits the energy metabolism of human muscle, increasing the efficiency of either mitochondrial ATP synthesis and/or of cellular ATP-consuming processes. In this mini-review, we evaluate experimental support for the dietary nitrate effects on muscle bioenergetics and we critically discuss the likelihood of nitric oxide as the molecular mediator of such effects.

Keywords: dietary nitrate, nitrite, nitric oxide, oxygen cost of human exercise, cellular bioenergetics, skeletal muscle mitochondria, coupling efficiency of oxidative phosphorylation, ATP turnover

Introduction

Inorganic nitrate (NO_3^-) has long been considered an undesirable food component and pollutant of drinking water as nitrosation of secondary amines may produce carcinogenic *N*-nitrosamines (Magee and Barnes, 1956). However, the evidence that NO_3^- causes human cancers is weak and dietary NO_3^- , as e.g. found in beetroot and spinach, has instead been linked to many physiological benefits (Gilchrist et al., 2010). Humans do not only get NO_3^- from their diet as it is also generated endogenously (Tannenbaum et al., 1978) by oxidation of nitric oxide (NO) formed canonically via the L-arginine/NO synthase pathway (Moncada and Higgs, 1993). Importantly, inorganic NO_3^- can be reduced to nitrite (NO_2^-) and then NO, which offers an additional path of mammalian NO production that, unlike the canonical route, is independent of oxygen (O_2) (Lundberg et al., 2008). NO is widely believed to mediate the benefits of NO_3^- (Lundberg et al., 2009) including protection against cardiovascular

Abbreviations: AMPK, AMP-activated kinase; PCr, phosphocreatine; PKA, protein kinase A; P_{O_2} , partial oxygen tension; RNS, reactive nitrogen species; $\dot{V}\text{O}_2$, pulmonary oxygen uptake.

disease (Omar and Webb, 2014) and the metabolic syndrome (Carlström et al., 2010). It has recently been found that dietary NO_3^- lowers the O_2 cost of human exercise as less respiratory activity is required for a set rate of skeletal muscle work (Larsen et al., 2007, 2010; Bailey et al., 2009, 2010). This finding is interesting as it challenges exercise physiology dogma that the steady-state O_2 consumption for any individual is immutable at a given sub-maximal workload irrespective of age, fitness, diet, or pharmacological intervention (Poole and Richardson, 1997). This mini-review aims to evaluate the mechanistic understanding of NO_3^- effects on skeletal muscle function.

O₂ Cost of Human Exercise

In a seminal publication, Larsen and colleagues reported that a 3-day supplementation with $0.1 \text{ mmol} \cdot \text{kg}^{-1} \text{ NaNO}_3 \cdot \text{day}^{-1}$ lowered pulmonary O_2 uptake ($\dot{V}\text{O}_2$) by $\sim 3\text{--}5\%$ in humans completing sub-maximal cycling exercise (Larsen et al., 2007). Bailey and co-workers subsequently observed a 5% lower $\dot{V}\text{O}_2$ during low-intensity cycling exercise and a 16% improvement in the tolerable duration of high-intensity exercise over days 3–6 of a 6-day supplementation period with $5.6 \text{ mmol NO}_3^- \cdot \text{day}^{-1}$, administered as 500 mL NO_3^- -rich beetroot juice $\cdot \text{day}^{-1}$ (Bailey et al., 2009). Importantly, NO_3^- -depleted beetroot juice does not improve exercise economy and performance (Lansley et al., 2011) eliminating antioxidants and polyphenols (Wootton-Beard and Ryan, 2011) as exclusive “active ingredients”. NO_3^- -induced improvements have been observed in humans completing walking, running, cycling, rowing and kayaking exercise, and positive responses arise both acutely, i.e., 1–3 h after NO_3^- ingestion, and after prolonged NO_3^- supplementation over 3–15 days (Table 1). Acute (2.5 h post-ingestion) lowering of

$\dot{V}\text{O}_2$ during low-intensity exercise is progressively greater at 4.2, 8.4, and 16.8 mmol NO_3^- , whereas high-intensity exercise tolerance is unaffected by 4.2 mmol NO_3^- , but acutely improved to a similar extent by 8.4 and 16.8 mmol (Wylie et al., 2013). Therefore, short-term supplementation (≥ 3 days) with at least $5 \text{ mmol NO}_3^- \cdot \text{day}^{-1}$, or acute ingestion of at least 8.4 mmol NO_3^- , might represent an effective dietary intervention to improve the economy and performance of human locomotion, at least in healthy, moderately fit adults (Porcelli et al., 2014). Since effects on resting $\dot{V}\text{O}_2$ are equivocal (Bailey et al., 2010; Kelly et al., 2013b; Larsen et al., 2014), NO_3^- benefits may be exclusive to contracting skeletal muscles.

Physiological NO_3^- effects appear muscle-fiber-type-specific as evidenced by improved perfusion (Ferguson et al., 2014) and calcium handling (Hernández et al., 2012) of murine fast-twitch type II but not slow-twitch type I muscle. Consistent with this, NO_3^- benefit on $\dot{V}\text{O}_2$ adjustment following the onset of exercise and on tolerance to high-intensity exercise is relatively large when the contribution of type II muscle fibers to force production is increased in human skeletal muscle (Breese et al., 2013; Bailey et al., 2015). These preferential physiological effects may relate to the comparably low microvascular PO_2 in resting and contracting type II muscle (McDonough et al., 2005). Indeed, NO_3^- improves exercise economy and performance in hypoxia (Masschelein et al., 2012; Muggeridge et al., 2014) more markedly than in normoxia (Kelly et al., 2014). Importantly, NO_3^- attenuates the degree of exercise intolerance and the slowing of PCr recovery kinetics in hypoxia to the levels seen in normoxia (Vanhatalo et al., 2011). It thus appears that exercise economy and performance benefit most from NO_3^- when muscle O_2 availability is low.

Although the majority of studies in healthy adults observe NO_3^- -improved exercise economy and/or performance, the effects are attenuated in well-trained endurance athletes (Bescós et al., 2012; Peacock et al., 2012; Christensen et al., 2013; Boorsma et al., 2014; Hoon et al., 2014; Lane et al., 2014), inconsistent in diseased populations (Berry et al., 2014; Kerley et al., 2015; Leong et al., 2015; Shepherd et al., 2015; Zamani et al., 2015), and possibly different in aging humans (Kelly et al., 2013b). More generally, there is evidence of distinct NO_3^- responders and non-responders in many studies. The relative efficacy of dietary NO_3^- effects on skeletal muscle thus appears variable, which underscores the need for detailed mechanistic understanding. To aid such understanding, it is important to ascertain how the human body processes dietary NO_3^- .

Molecular Fate of Dietary NO_3^-

When humans eat NO_3^- -rich food, NO_3^- is converted to NO_2^- by nitrate reductases in commensal bacteria that reside in the posterior part of the tongue (Duncan et al., 1995). Salivary NO_2^- is rapidly protonated in the acidic environment of the stomach resulting in the formation of NO and other reactive nitrogen species (RNS) including nitrogen dioxide (NO_2), nitrous acid (HNO_2), and dinitrogen trioxide (N_2O_3) (Benjamin et al., 1994; Lundberg et al., 1994; Lundberg and Weitzberg, 2013). NO_3^-

TABLE 1 | Dietary nitrate improves the economy and/or performance of human locomotion.

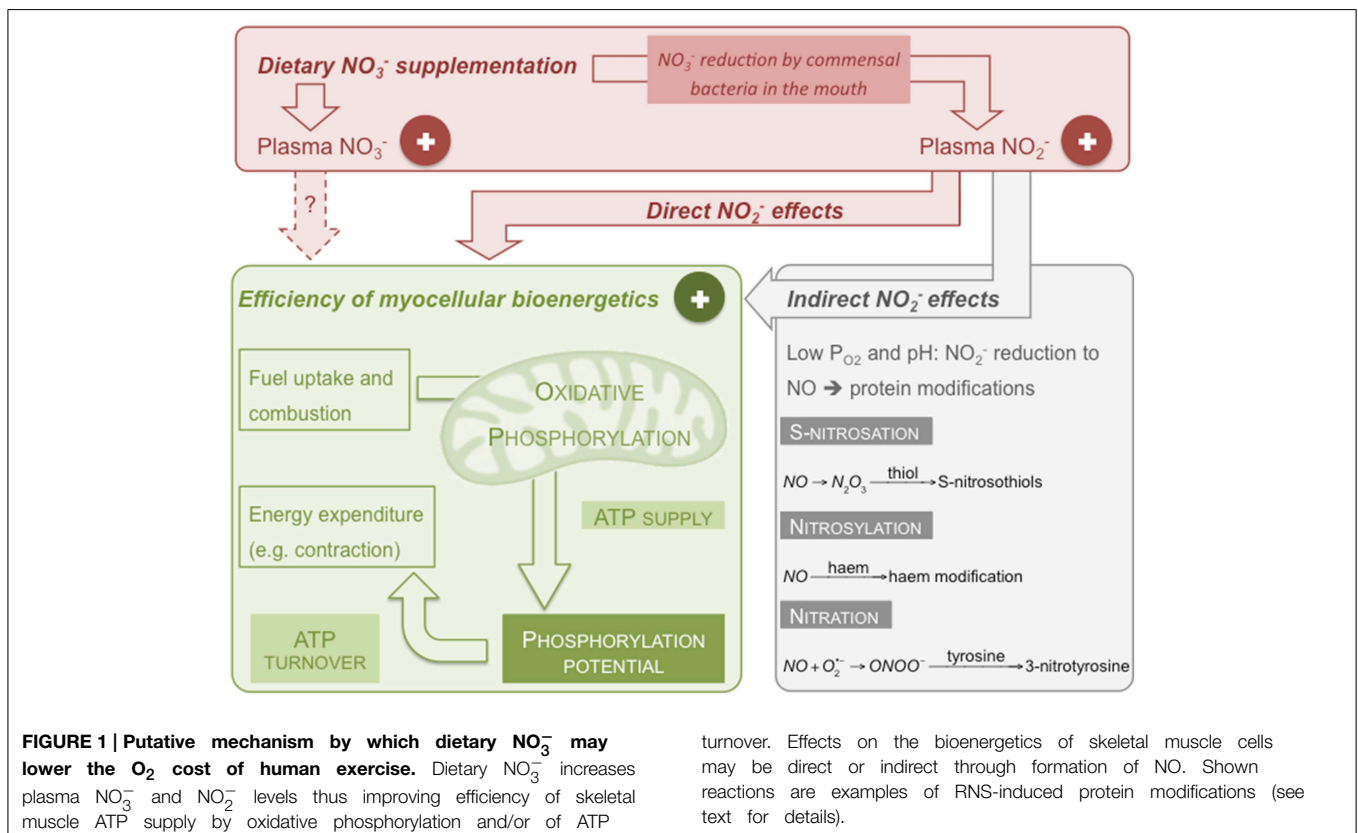
Exercise	References ^a	Exposure period	
		1–3 h	3–15 d
Cycling	Larsen et al., 2007—1st study reporting dietary NO_3^- benefit		✓
	Larsen et al., 2010	✓	
	Larsen et al., 2011		✓
	Bailey et al., 2009—1st study using beetroot juice as NO_3^- source		✓
	Vanhatalo et al., 2010	✓	✓
	Cermak et al., 2012		✓
Running	Wylie et al., 2013—Study reports dose-dependency of NO_3^-	✓	
	Lansley et al., 2011—1st study using NO_3^- -depleted beetroot juice as placebo		✓
Kayaking	Porcelli et al., 2014		✓
	Muggeridge et al., 2013	✓	
Walking	Peeling et al., 2014	✓	
	Lansley et al., 2011		✓
Rowing	Bond et al., 2012		✓

^a These studies are cited as examples—the list is not a comprehensive account of all available literature.

ingestion increases plasma NO_2^- levels in human subjects (e.g., Lundberg and Govoni, 2004; Webb et al., 2008; Bailey et al., 2009; Vanhatalo et al., 2010; Kelly et al., 2013a). Possibly catalyzed by xanthine oxidase (Zhang et al., 1998; Li et al., 2003) and/or deoxyhaemoglobin (Cosby et al., 2003; Gladwin et al., 2004; Gladwin and Kim-Shapiro, 2008), NO_2^- is reduced to NO under conditions of low oxygen tension (Figure 1). Other sites of NO_2^- reductase activity include cytochrome *c* (Basu et al., 2008) and mitochondrial respiratory complexes III (Kozlov et al., 1999) and IV (Castello et al., 2006). However, mammalian NO_2^- reductase activity has only been shown *in vitro* and in animal models (Feelisch et al., 2008; Jansson et al., 2008), and under low P_{O_2} (Li et al., 2001; Feelisch et al., 2008) and low pH (Modin et al., 2001). Generally, the low pKa of NO_2^- (3.34, Oxtoby and Nachtrieb, 1996) limits its physiological reduction, which is an inefficient process *per se* (Li et al., 2008) and thus requires high NO_2^- concentrations. Indeed, at physiological NO_2^- levels (see below), even hypoxic red blood cells do not liberate significant NO (Bryan et al., 2004). O_2 competitively inhibits NO_2^- reduction by xanthine oxidase (Li et al., 2004) and oxygenated haem effectively scavenges free NO (Feelisch et al., 2008). NO may be converted to N_2O_3 that in turn may react with free thiols to generate S-nitrosothiols (Hess et al., 2005) via an S-nitrosation reaction (Figure 1). NO is also able to modify proteins through nitrosylation, e.g., via reaction with the haem of myoglobin (Ignarro, 1991). NO furthermore binds, in a reversible and O_2 -competitive manner, to the haem of cytochrome *c*

oxidase, and in an O_2 -independent way, to the enzyme's active site copper (Giulivi et al., 2006; Brown and Borutaite, 2007; Cooper and Giulivi, 2007). Peroxynitrite (ONOO^-) arising from the reaction of NO with the superoxide anion radical may undergo a nitration reaction with tyrosine residues to form 3-nitrotyrosine (Figure 1—Radi, 2004). Importantly, tyrosine-containing proteins are also nitrated in a myeloperoxidase-catalyzed reaction using NO_2^- and hydrogen peroxide (Marquez and Dunford, 1995).

Physiological NO_2^- levels range from 50 to 500 nM in human plasma (Bryan et al., 2004; Dejam et al., 2005; Feelisch et al., 2008) and a mean concentration of 12 μM has been measured in human skin (Mowbray et al., 2009). In rodents, NO_2^- concentration varies substantially between tissues, from below quantifiable limit in the liver, heart and lung to as high as 2 μM in kidney and 3.7 μM in lymph nodes; NO_3^- varies from 1 μM in the kidney to 50 μM in the aorta (Garcia-Saura et al., 2010). In humans, the ingestion of 10 mg $\text{NaNO}_3 \cdot \text{kg}^{-1}$ has been shown to increase mean plasma NO_3^- within 90 min from 30 to 432 μM and mean plasma NO_2^- from 123 to 392 nM (Lundberg and Govoni, 2004). Similarly, 500 mL beetroot juice containing 45 mM NO_3^- on average raises mean plasma NO_3^- to 380 μM and NO_2^- to 600 nM within 30 min and 3 h of ingestion, respectively (Webb et al., 2008). Plasma NO_3^- and NO_2^- reach peak concentrations, respectively, 1–2 and 2–3 h post-ingestion, and NO_3^- gradually returns to its base level after about 24 h (Ender et al., 1964; McKnight et al., 1997; Webb et al., 2008; Wylie et al., 2013).



Mammalian tissue NO_2^- and NO_3^- both have *in vivo* half-lives of tens of minutes (Bryan et al., 2005). The half-life of NO_2^- in whole human blood is only about 110 sec (Kelm, 1999) as it is rapidly oxidized to NO_3^- ; the half-life of NO_3^- in blood is 5–8 h (Wagner et al., 1983; McKnight et al., 1997). About 60% of ingested NO_3^- is excreted by the kidneys (Green et al., 1981; Wagner et al., 1983).

The reliability of commonly reported NO_3^- and NO_2^- values very much depends on the assays used to measure these inorganic anions. The modified Griess reaction using sulfanilamide and N-1-naphthylethylenediamine dihydrochloride is a frequently used assay for measuring NO_2^- (Tsikas, 2007). Plasma NO_3^- concentrations are readily determined using this spectrophotometric assay following NO_3^- reduction to NO_2^- by cadmium (Green et al., 1982) or vanadium salts (Miranda et al., 2001). However, the Griess test lacks the sensitivity to probe the nanomolar NO_2^- levels present in human plasma. Ozone-based chemiluminescence is a preferred method of detection, which often involves deproteinisation of plasma samples by zinc sulfate precipitation before analysis (Higuchi and Motomizu, 1999). NO_2^- measurement by chemiluminescence usually involves acetic acid/sodium iodide-mediated reduction to NO, which then reacts with ozone to produce a chemiluminescence signal (Bateman et al., 2002). NO_3^- can also be measured this way by reduction to NO via reflux of the sample in vanadium chloride at 95°C. Confounding the $\text{NO}_3^-/\text{NO}_2^-$ literature, in some assays NO_3^- is reduced to NO_2^- by bacterial nitrate reductases (Sun et al., 2003) whose activity varies from batch to batch. Confusing matters further, mere “ NO_x ” values are reported to denote the sum of NO_2^- and NO_3^- levels. A last analytical note concerns the use of NO_3^- -depleted beetroot juice as placebo control in *in vivo* studies (see Section O₂ Cost of Human Exercise). It is important for experiments involving human participants to use a placebo juice that looks, tastes, and smells the same as the “real thing”. A placebo that meets these criteria can be prepared by passing beetroot juice through a Purolite a520e anion exchange column, which effectively and selectively removes NO_3^- (Gilchrist et al., 2013).

Skeletal Muscle Bioenergetics

Dietary NO_3^- benefits on the O₂ cost of exercise likely arise from increased efficiency of ATP synthesis and/or of skeletal muscle work (Figure 1). Indeed, NO_3^- increases the rate of human skeletal muscle PCr recovery after exercise in hypoxia suggesting an augmented maximum rate of oxidative ATP synthesis (Vanhatalo et al., 2011), and lowers the ATP cost of contractile force production (Bailey et al., 2010). These *in vivo* studies confirm that NO_3^- indeed affects skeletal muscle bioenergetics, but they do not disclose the underlying molecular mechanism. *In vitro* experiments with C2C12 myocytes show that beetroot juice provokes mitochondrial biogenesis and modestly increases basal cellular respiration without affecting respiratory capacity and proton leak (Vaughan et al., 2014). These observations indicate improved mitochondrial coupling efficiency as beetroot juice has increased the proportion of total O₂ consumption coupled to ATP synthesis. Although the C2C12 experiments lack an appropriate NO_3^- -depleted beetroot juice

control (see above), increased coupling efficiency of oxidative phosphorylation agrees with data reported by Larsen et al. (2011), who show that skeletal muscle mitochondria isolated from NO_3^- -supplemented subjects exhibit higher respiratory control and P/O ratios (defined in Brand and Nicholls, 2011) than mitochondria from non-supplemented controls, and that increases in P/O ratio correlate with NO_3^- -induced decreases in whole-body O₂ uptake during exercise. This increased efficiency of ATP synthesis in isolated mitochondria, however, emerges from decreased respiration linked to mitochondrial proton leak, not from stimulated O₂ uptake coupled to phosphorylation (Larsen et al., 2011). NO_3^- -lowered proton leak coincides with decreases in adenine nucleotide translocase protein and, to a lesser extent, uncoupling protein-3 (Larsen et al., 2011). It should be emphasized that these mitochondrial carriers do not necessarily contribute to proton leak (Nedergaard and Cannon, 2003; Voza et al., 2014) and that leak is expected to account for little skeletal muscle respiration at low protonmotive force (Affourtit and Brand, 2006), i.e., the bioenergetic state attained during exercise. Dietary NO_3^- also lowers the apparent affinity of mitochondrial respiration for O₂, an effect that is reproduced *in vitro*—acutely and pH-dependently—by NO_2^- (Larsen et al., 2011). Lowered affinity is attributed to an NO-induced rise in the apparent K_m of cytochrome c oxidase for O₂ (Larsen et al., 2011) but, inconsistently, NO_2^- does not affect mitochondrial respiration or efficiency (Larsen et al., 2011) like NO is expected to (Brown and Borutaite, 2007). Apparent mitochondrial respiratory affinity for O₂ depends strongly on the extent to which respiration is controlled by the enzyme reacting with O₂ (Affourtit et al., 2001)—control of cytochrome c oxidase over O₂ consumption may well have been affected by NO_2^- and pH, and also by dietary NO_3^- -induced mitochondrial changes.

It remains to be demonstrated convincingly whether or not dietary NO_3^- effects in skeletal muscle are mediated by NO. Nitrite reductase activity requires high NO_2^- levels and exceptionally low P_{O₂} and pH (see Section Molecular Fate of Dietary NO_3^-) that may indeed manifest in the ischaemic heart (Brown and Borutaite, 2007; Hendgen-Cotta et al., 2010), but are unlikely in healthy muscle. In contracting muscle, myoglobin O₂ saturation remains as high as 50% (Takakura et al., 2015) and although globins indeed exhibit nitrite reductase activity at this saturation (Huang et al., 2005), cytoplasmic NO will likely be scavenged by oxymyoglobin (Hendgen-Cotta et al., 2010). Even if O₂ were sufficiently low for NO_2^- reduction in exercising muscle, we stress that dietary NO_3^- intake remodels skeletal muscle bioenergetics in the hours to days *before* exercise (see Section O₂ Cost of Human Exercise), i.e., when the muscles are at rest. Importantly, NO_2^- also modulates cell signaling independently of NO in hypoxia and normoxia (Bryan et al., 2005). NO_2^- activates AMPK in rat aortic smooth muscle cells thus stimulating mitochondrial biogenesis, and increasing coupling efficiency and cellular respiratory control (Mo et al., 2012). NO_2^- activates PKA in cultured cardiomyocytes, stimulating mitochondrial fusion and again increasing cellular respiratory control (Pride et al., 2013). In both systems, NO_2^- improves efficiency of oxidative ATP synthesis without apparent effect on proton leak, which agrees with the beetroot juice effects on C2C12 respiration

(Vaughan et al., 2014). NO_2^- also activates PKA in cultured adipocytes, increasing mitochondrial fusion, and stimulating glucose uptake (Khoo et al., 2014). Moreover, NO_2^- increases proliferation of muscle (Totzeck et al., 2014) and epithelial cells (Wang et al., 2012).

RNS can modify proteins (see Section Molecular fate of dietary NO_3^-) and may thus improve mitochondrial coupling efficiency in various ways, e.g., by increasing proton translocation to electron transfer stoichiometries of respiratory complexes (cf. Clerc et al., 2007). By definition (Brand and Nicholls, 2011), coupling efficiency benefits from decreased proton leak and increased phosphorylation-linked respiration, as indeed reported by (Larsen et al., 2011) and (Vaughan et al., 2014), respectively. System-kinetic modeling furthermore suggests that substrate oxidation capacity, which is dependent on fuel and O_2 availability, correlates positively with coupling efficiency (Affourtit and Brand, 2009). Dietary NO_3^- may thus improve efficiency of muscle ATP synthesis, at least in part, by increasing expression of glucose transporters (Jiang et al., 2014) and/or by raising insulin availability (Nyström et al., 2012).

Dietary NO_3^- increases the contractile force of fast-twitch mouse muscle by improving calcium handling (Hernández et al., 2012) suggesting the efficiency of ATP-demanding contraction may have increased. To our knowledge, no other data are available on the mechanism by which dietary NO_3^- affects ATP turnover. However, NO_3^- supplementation may also alter efficiency of other ATP-consumers and, importantly, the relative importance of dietary NO_3^- effects on skeletal muscle ATP supply and ATP turnover remains unclear. A systems-level functional analysis of cellular energy metabolism (cf. Brand, 1998) may shed light on these issues. Using myocytes isolated from human muscle biopsies (Nisr and Affourtit, 2014) the relative effects of RNS on ATP-generating and ATP-consuming fluxes—linked through the cell's phosphorylation potential (**Figure 1**)—may be identified and quantified in an unbiased manner. A challenge of such *in vitro* analysis will be the approximation of physiologically meaningful conditions, in particular the O_2 tensions and energy

demands that prevail during the development of dietary NO_3^- benefits.

Conclusion

The striking benefit of dietary NO_3^- on the O_2 cost of exercise is of obvious interest to athletes (Jones, 2014), but may also well impact on the quality of life of aging people suffering from muscle weakness and exercise intolerance. To rationally evaluate translational potential, our mechanistic understanding of dietary NO_3^- benefits on human skeletal muscle needs to be improved. By integrating biochemistry and physiology, and studying subjects at different age, it will be important to demonstrate which reactive nitrogen species mediate dietary NO_3^- effects at the cellular level, disclose all effects of nitrogen species on myocellular bioenergetics, confirm if they are direct or indirect via action on other tissues, and quantify the relative importance of these effects.

Author Contributions

CA wrote Sections Introduction, Skeletal Muscle Bioenergetics, and Conclusion, and produced the table and figure; SB and AJ wrote Section O_2 Cost of Human Exercise; MS and PW wrote Section Molecular Fate of Dietary NO_3^- . All authors edited and approved the entire manuscript.

Acknowledgments

Research in the authors' laboratories is currently supported by the Medical Research Council (New Investigator Research Grant G1100165/1 to CA), Gatorade Sports Science Institute (Grants PEP-1330 and PEP-1420 to AJ), the Exeter Leukaemia Fund (Grant ST-06354 to AJ), the Dunhill Medical Trust (Grant R269/1112 to AJ) and the NIHR Exeter Clinical Research Facility (PW and MS). The views and opinions shown within this paper are those of the authors and do not necessarily represent those of the NIHR, NHS or the Department of Health.

References

- Affourtit, C., and Brand, M. D. (2006). Stronger control of ATP/ADP by proton leak in pancreatic beta-cells than skeletal muscle mitochondria. *Biochem. J.* 393, 151–159. doi: 10.1042/BJ20051280
- Affourtit, C., and Brand, M. D. (2009). Measuring mitochondrial bioenergetics in INS-1E insulinoma cells. *Meth. Enzymol.* 457, 405–424. doi: 10.1016/S0076-6879(09)05023-X
- Affourtit, C., Krab, K., and Moore, A. L. (2001). Control of plant mitochondrial respiration. *Biochim. Biophys. Acta* 1504, 58–69. doi: 10.1016/S0005-2728(00)00239-5
- Bailey, S. J., Fulford, J., Vanhatalo, A., Winyard, P. G., Blackwell, J. R., DiMenna, F. J., et al. (2010). Dietary nitrate supplementation enhances muscle contractile efficiency during knee-extensor exercise in humans. *J. Appl. Physiol.* 109, 135–148. doi: 10.1152/jappphysiol.00046.2010
- Bailey, S. J., Varnham, R. L., Dimenna, F. J., Breese, B. C., Wylie, L. J., and Jones, A. M. (2015). Inorganic nitrate supplementation improves muscle oxygenation, O_2 uptake kinetics, and exercise tolerance at high but not low pedal rates. *J. Appl. Physiol.* 118, 1396–1405. doi: 10.1152/jappphysiol.0114.1.2014
- Bailey, S. J., Winyard, P., Vanhatalo, A., Blackwell, J. R., Dimenna, F. J., Wilkerson, D. P., et al. (2009). Dietary nitrate supplementation reduces the O_2 cost of low-intensity exercise and enhances tolerance to high-intensity exercise in humans. *J. Appl. Physiol.* 107, 1144–1155. doi: 10.1152/jappphysiol.00722.2009
- Basu, S., Azarova, N. A., Font, M. D., King, S. B., Hogg, N., Gladwin, M. T., et al. (2008). Nitrite reductase activity of cytochrome c. *J. Biol. Chem.* 283, 32590–32597. doi: 10.1074/jbc.M806934200
- Bateman, R. M., Ellis, C. G., and Freeman, D. J. (2002). Optimization of nitric oxide chemiluminescence operating conditions for measurement of plasma nitrite and nitrate. *Clin. Chem.* 48, 570–573.
- Benjamin, N., O'Driscoll, F., Dougall, H., Duncan, C., Smith, L., Golden, M., et al. (1994). Stomach NO synthesis. *Nature* 368, 502. doi: 10.1038/368502a0
- Berry, M. J., Justus, N. W., Hauser, J. I., Case, A. H., Helms, C. C., Basu, S., et al. (2014). Dietary nitrate supplementation improves exercise performance and decreases blood pressure in COPD patients. *Nitric Oxide*. 48, 22–30. doi: 10.1016/j.niox.2014.10.007
- Bescós, R., Ferrer-Roca, V., Galilea, P. A., Roig, A., Drobic, F., Sureda, A., et al. (2012). Sodium nitrate supplementation does not enhance performance of endurance athletes. *Med. Sci. Sports Exerc.* 44, 2400–2409. doi: 10.1249/MSS.0b013e3182687e5c

- Bond, H., Morton, L., and Braakhuis, A. J. (2012). Dietary nitrate supplementation improves rowing performance in well-trained rowers. *Int. J. Sport Nutr. Exerc. Metab.* 22, 251–256.
- Boorsma, R. K., Whitfield, J., and Spriet, L. L. (2014). Beetroot juice supplementation does not improve performance of elite 1500-m runners. *Med. Sci. Sports Exerc.* 46, 2326–2334. doi: 10.1249/MSS.0000000000000364
- Brand, M. D. (1998). Top-down elasticity analysis and its application to energy metabolism in isolated mitochondria and intact cells. *Mol. Cell. Biochem.* 184, 13–20. doi: 10.1023/A:1006893619101
- Brand, M. D., and Nicholls, D. G. (2011). Assessing mitochondrial dysfunction in cells. *Biochem. J.* 435, 297–312. doi: 10.1042/BJ20110162
- Breese, B. C., McNarry, M. A., Marwood, S., Blackwell, J. R., Bailey, S. J., and Jones, A. M. (2013). Beetroot juice supplementation speeds O₂ uptake kinetics and improves exercise tolerance during severe-intensity exercise initiated from an elevated metabolic rate. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 305, R1441–R1450. doi: 10.1152/ajpregu.00295.2013
- Brown, G., and Borutaite, V. (2007). Nitric oxide and mitochondrial respiration in the heart. *Cardiovasc. Res.* 75, 283–290. doi: 10.1016/j.cardiores.2007.03.022
- Bryan, N. S., Fernandez, B. O., Bauer, S. M., Garcia-Saura, M. F., Milsom, A. B., Rassaf, T., et al. (2005). Nitrite is a signaling molecule and regulator of gene expression in mammalian tissues. *Nat. Chem. Biol.* 1, 290–297. doi: 10.1038/nchembio734
- Bryan, N. S., Rassaf, T., Maloney, R. E., Rodriguez, C. M., Saijo, F., Rodriguez, J. R., et al. (2004). Cellular targets and mechanisms of nitrosylation: an insight into their nature and kinetics *in vivo*. *Proc. Natl. Acad. Sci. U.S.A.* 101, 4308–4313. doi: 10.1073/pnas.0306706101
- Carlström, M., Larsen, F. J., Nyström, T., Hezel, M., Borniquel, S., Weitzberg, E., et al. (2010). Dietary inorganic nitrate reverses features of metabolic syndrome in endothelial nitric oxide synthase-deficient mice. *Proc. Natl. Acad. Sci. U.S.A.* 107, 17716–17720. doi: 10.1073/pnas.1008872107
- Castello, P. R., David, P. S., McClure, T., Crook, Z., and Poyton, R. O. (2006). Mitochondrial cytochrome oxidase produces nitric oxide under hypoxic conditions: implications for oxygen sensing and hypoxic signaling in eukaryotes. *Cell Metab.* 3, 277–287. doi: 10.1016/j.cmet.2006.02.011
- Cermak, N. M., Gibala, M. J., and van Loon, L. J. C. (2012). Nitrate supplementation's improvement of 10-km time-trial performance in trained cyclists. *Int. J. Sport Nutr. Exerc. Metab.* 22, 64–71.
- Christensen, P. M., Nyberg, M., and Bangsbo, J. (2013). Influence of nitrate supplementation on VO₂ kinetics and endurance of elite cyclists. *Scand. J. Med. Sci. Sports* 23, e21–e31. doi: 10.1111/sms.12005
- Clerc, P., Rigoulet, M., Leverve, X., and Fontaine, E. (2007). Nitric oxide increases oxidative phosphorylation efficiency. *J. Bioenerg. Biomembr.* 39, 158–166. doi: 10.1007/s10863-007-9074-1
- Cooper, C. E., and Giulivi, C. (2007). Nitric oxide regulation of mitochondrial oxygen consumption II: molecular mechanism and tissue physiology. *Am. J. Physiol. Cell Physiol.* 292, C1993–C2003. doi: 10.1152/ajpcell.00310.2006
- Cosby, K., Partovi, K. S., Crawford, J. H., Patel, R. P., Reiter, C. D., Martyr, S., et al. (2003). Nitrite reduction to nitric oxide by deoxyhemoglobin vasodilates the human circulation. *Nat. Med.* 9, 1498–1505. doi: 10.1038/nm954
- Dejam, A., Hunter, C. J., Pelletier, M. M., Hsu, L. L., Machado, R. F., Shiva, S., et al. (2005). Erythrocytes are the major intravascular storage sites of nitrite in human blood. *Blood* 106, 734–739. doi: 10.1182/blood-2005-02-0567
- Duncan, C., Dougall, H., Johnston, P., Green, S., Brogan, R., Leifert, C., et al. (1995). Chemical generation of nitric oxide in the mouth from the enterosalivary circulation of dietary nitrate. *Nat. Med.* 1, 546–551. doi: 10.1038/nm0695-546
- Ender, F., Havre, G., Helgebostad, A., Koppang, N., Madsen, R., and Ceh, L. (1964). Isolation and identification of a hepatotoxic factor in herring meal produced from sodium nitrite preserved herring. *Naturwissenschaften* 51, 637–638. doi: 10.1007/BF00623677
- Feelisch, M., Fernandez, B. O., Bryan, N. S., Garcia-Saura, M. F., Bauer, S., Whitlock, D. R., et al. (2008). Tissue processing of nitrite in hypoxia: an intricate interplay of nitric oxide-generating and -scavenging systems. *J. Biol. Chem.* 283, 33927–33934. doi: 10.1074/jbc.M806654200
- Ferguson, S. K., Holdsworth, C. T., Wright, J. L., Fees, A. J., Allen, J. D., Jones, A. M., et al. (2014). Microvascular oxygen pressures in muscles comprised of different fiber types: impact of dietary nitrate supplementation. *Nitric Oxide.* 48, 38–43. doi: 10.1016/j.niox.2014.09.157
- Garcia-Saura, M. F., Fernandez, B. O., McAllister, B. P., Whitlock, D. R., Cruikshank, W. W., and Feelisch, M. (2010). Dermal nitrite application enhances global nitric oxide availability: new therapeutic potential for immunomodulation? *J. Invest. Dermatol.* 130, 608–611. doi: 10.1038/jid.2009.303
- Gilchrist, M., Winyard, P. G., Aizawa, K., Anning, C., Shore, A., and Benjamin, N. (2013). Effect of dietary nitrate on blood pressure, endothelial function, and insulin sensitivity in type 2 diabetes. *Free Radic. Biol. Med.* 60, 89–97. doi: 10.1016/j.freeradbiomed.2013.01.024
- Gilchrist, M., Winyard, P. G., and Benjamin, N. (2010). Dietary nitrate – good or bad? *Nitric Oxide* 22, 104–109. doi: 10.1016/j.niox.2009.10.005
- Giulivi, C., Kato, K., and Cooper, C. E. (2006). Nitric oxide regulation of mitochondrial oxygen consumption I: cellular physiology. *Am. J. Physiol. Cell Physiol.* 291, C1225–C1231. doi: 10.1152/ajpcell.00307.2006
- Gladwin, M. T., Crawford, J. H., and Patel, R. P. (2004). The biochemistry of nitric oxide, nitrite, and hemoglobin: role in blood flow regulation. *Free Radic. Biol. Med.* 36, 707–717. doi: 10.1016/j.freeradbiomed.2003.11.032
- Gladwin, M. T., and Kim-Shapiro, D. B. (2008). The functional nitrite reductase activity of the heme-globins. *Blood* 112, 2636–2647. doi: 10.1182/blood-2008-01-115261
- Green, L. C., Ruiz de Luzuriaga, K., Wagner, D. A., Rand, W., Istfan, N., Young, V. R., et al. (1981). Nitrate biosynthesis in man. *Proc. Natl. Acad. Sci. U.S.A.* 78, 7764–7768. doi: 10.1073/pnas.78.12.7764
- Green, L. C., Wagner, D. A., Glogowski, J., Skipper, P. L., Wishnok, J. S., and Tannenbaum, S. R. (1982). Analysis of nitrate, nitrite, and [¹⁵N]nitrate in biological fluids. *Anal. Biochem.* 126, 131–138. doi: 10.1016/0003-2697(82)90118-X
- Hendgen-Cotta, U. B., Fogel, U., Kelm, M., and Rassaf, T. (2010). Unmasking the Janus face of myoglobin in health and disease. *J. Exp. Biol.* 213, 2734–2740. doi: 10.1242/jeb.041178
- Hernández, A., Schiffer, T. A., Ivarsson, N., Cheng, A. J., Bruton, J. D., Lundberg, J. O., et al. (2012). Dietary nitrate increases tetanic [Ca²⁺]_i and contractile force in mouse fast-twitch muscle. *J. Physiol.* 590, 3575–3583. doi: 10.1113/jphysiol.2012.232777
- Hess, D. T., Matsumoto, A., Kim, S.-O., Marshall, H. E., and Stamler, J. S. (2005). Protein S-nitrosylation: purview and parameters. *Nat. Rev. Mol. Cell Biol.* 6, 150–166. doi: 10.1038/nrm1569
- Higuchi, K., and Motomizu, S. (1999). Flow-injection spectrophotometric determination of nitrite and nitrate in biological samples. *Anal. Sci.* 15, 129–134. doi: 10.2116/analsci.15.129
- Hoon, M. W., Hopkins, W. G., Jones, A. M., Martin, D. T., Halson, S. L., West, N. P., et al. (2014). Nitrate supplementation and high-intensity performance in competitive cyclists. *Appl. Physiol. Nutr. Metab.* 39, 1043–1049. doi: 10.1139/apnm-2013-0574
- Huang, Z., Shiva, S., Kim-Shapiro, D. B., Patel, R. P., Ringwood, L. A., Irby, C. E., et al. (2005). Enzymatic function of hemoglobin as a nitrite reductase that produces NO under allosteric control. *J. Clin. Invest.* 115, 2099–2107. doi: 10.1172/JCI24650
- Ignarro, L. J. (1991). Signal transduction mechanisms involving nitric oxide. *Biochem. Pharmacol.* 41, 485–490. doi: 10.1016/0006-2952(91)90618-F
- Jansson, E. A., Huang, L., Malkey, R., Govoni, M., Nihlén, C., Olsson, A., et al. (2008). A mammalian functional nitrate reductase that regulates nitrite and nitric oxide homeostasis. *Nat. Chem. Biol.* 4, 411–417. doi: 10.1038/nchembio.92
- Jiang, H., Torregrossa, A. C., Potts, A., Pierini, D., Aranke, M., Garg, H. K., et al. (2014). Dietary nitrite improves insulin signaling through GLUT4 translocation. *Free Radic. Biol. Med.* 67, 51–57. doi: 10.1016/j.freeradbiomed.2013.10.809
- Jones, A. M. (2014). Dietary nitrate supplementation and exercise performance. *Sports Med.* 44, S35–S45. doi: 10.1007/s40279-014-0149-y
- Kelly, J., Fulford, J., Vanhatalo, A., Blackwell, J. R., French, O., Bailey, S. J., et al. (2013a). Effects of short-term dietary nitrate supplementation on blood pressure, O₂ uptake kinetics, and muscle and cognitive function in older adults. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 304, R73–R83. doi: 10.1152/ajpregu.00406.2012
- Kelly, J., Vanhatalo, A., Bailey, S. J., Wylie, L. J., Tucker, C., List, S., et al. (2014). Dietary nitrate supplementation: effects on plasma nitrite and pulmonary O₂

- uptake dynamics during exercise in hypoxia and normoxia. *Am. J. Physiol. Reg. Int. Comp. Physiol.* 307, R920–R930. doi: 10.1152/ajpregu.00068.2014
- Kelly, J., Vanhatalo, A., Wilkerson, D. P., Wylie, L. J., and Jones, A. M. (2013b). Effects of nitrate on the power-duration relationship for severe-intensity exercise. *Med. Sci. Sports Exerc.* 45, 1798–1806. doi: 10.1249/MSS.0b013e31828e885c
- Kelm, M. (1999). Nitric oxide metabolism and breakdown. *Biochim. Biophys. Acta* 1411, 273–289. doi: 10.1016/S0005-2728(99)00020-1
- Kerley, C. P., Cahill, K., Bolger, K., McGowan, A., Burke, C., Faul, J., et al. (2015). Dietary nitrate supplementation in COPD: an acute, double-blind, randomized, placebo-controlled, crossover trial. *Nitric Oxide* 44, 105–111. doi: 10.1016/j.niox.2014.12.010
- Khoo, N. K. H., Mo, L., Zharikov, S., Kamga-Pride, C., Quesnelle, K., Golin-Bisello, F., et al. (2014). Nitrite augments glucose uptake in adipocytes through the protein kinase A-dependent stimulation of mitochondrial fusion. *Free Radic. Biol. Med.* 70, 45–53. doi: 10.1016/j.freeradbiomed.2014.02.009
- Kozlov, A. V., Staniek, K., and Nohl, H. (1999). Nitrite reductase activity is a novel function of mammalian mitochondria. *FEBS Lett.* 454, 127–130. doi: 10.1016/S0014-5793(99)00788-7
- Lane, S. C., Hawley, J. A., Desbrow, B., Jones, A. M., Blackwell, J. R., Ross, M. L., et al. (2014). Single and combined effects of beetroot juice and caffeine supplementation on cycling time trial performance. *Appl. Physiol. Nutr. Metab.* 39, 1050–1057. doi: 10.1139/apnm-2013-0336
- Lansley, K. E., Winyard, P. G., Bailey, S. J., Vanhatalo, A., Wilkerson, D. P., Blackwell, J. R., et al. (2011). Acute dietary nitrate supplementation improves cycling time trial performance. *Med. Sci. Sports Exerc.* 43, 1125–1131. doi: 10.1249/MSS.0b013e31821597b4
- Larsen, F. J., Schiffer, T. A., Bornique, S., Sahlin, K., Ekblom, B., Lundberg, J. O., et al. (2011). Dietary inorganic nitrate improves mitochondrial efficiency in humans. *Cell Metab.* 13, 149–159. doi: 10.1016/j.cmet.2011.01.004
- Larsen, F. J., Schiffer, T. A., Ekblom, B., Mattsson, M. P., Checa, A., Wheelock, C. E., et al. (2014). Dietary nitrate reduces resting metabolic rate: a randomized, crossover study in humans. *Am. J. Clin. Nutr.* 99, 843–850. doi: 10.3945/ajcn.113.079491
- Larsen, F. J., Weitzberg, E., Lundberg, J. O., and Ekblom, B. (2007). Effects of dietary nitrate on oxygen cost during exercise. *Acta Physiol.* 191, 59–66. doi: 10.1111/j.1748-1716.2007.01713.x
- Larsen, F. J., Weitzberg, E., Lundberg, J. O., and Ekblom, B. (2010). Dietary nitrate reduces maximal oxygen consumption while maintaining work performance in maximal exercise. *Free Radic. Biol. Med.* 48, 342–347. doi: 10.1016/j.freeradbiomed.2009.11.006
- Leong, P., Basham, J. E., Yong, T., Chazan, A., Finlay, P., Barnes, S., et al. (2015). A double blind randomized placebo control crossover trial on the effect of dietary nitrate supplementation on exercise tolerance in stable moderate chronic obstructive pulmonary disease. *BMC Pulm. Med.* 15:52. doi: 10.1186/s12890-015-0057-4
- Li, H., Cui, H., Kundu, T. K., Alzawahra, W., and Zweier, J. L. (2008). Nitric oxide production from nitrite occurs primarily in tissues not in the blood: critical role of xanthine oxidase and aldehyde oxidase. *J. Biol. Chem.* 283, 17855–17863. doi: 10.1074/jbc.M801785200
- Li, H., Samouilov, A., Liu, X., and Zweier, J. L. (2001). Characterization of the magnitude and kinetics of xanthine oxidase-catalyzed nitrite reduction. Evaluation of its role in nitric oxide generation in anoxic tissues. *J. Biol. Chem.* 276, 24482–24489. doi: 10.1074/jbc.M011648200
- Li, H., Samouilov, A., Liu, X., and Zweier, J. L. (2003). Characterization of the magnitude and kinetics of xanthine oxidase-catalyzed nitrate reduction: evaluation of its role in nitrite and nitric oxide generation in anoxic tissues. *Biochemistry* 42, 1150–1159. doi: 10.1021/bi026385a
- Li, H., Samouilov, A., Liu, X., and Zweier, J. L. (2004). Characterization of the effects of oxygen on xanthine oxidase-mediated nitric oxide formation. *J. Biol. Chem.* 279, 16939–16946. doi: 10.1074/jbc.M314336200
- Lundberg, J. O., Gladwin, M. T., Ahluwalia, A., Benjamin, N., Bryan, N. S., Butler, A., et al. (2009). Nitrate and nitrite in biology, nutrition and therapeutics. *Nat. Chem. Biol.* 5, 865–869. doi: 10.1038/nchembio.260
- Lundberg, J. O., and Govoni, M. (2004). Inorganic nitrate is a possible source for systemic generation of nitric oxide. *Free Radic. Biol. Med.* 37, 395–400. doi: 10.1016/j.freeradbiomed.2004.04.027
- Lundberg, J. O., and Weitzberg, E. (2013). Biology of nitrogen oxides in the gastrointestinal tract. *Gut* 62, 616–629. doi: 10.1136/gutjnl-2011-301649
- Lundberg, J. O., Weitzberg, E., and Gladwin, M. T. (2008). The nitrate-nitrite-nitric oxide pathway in physiology and therapeutics. *Nat. Rev. Drug Disc.* 7, 156–167. doi: 10.1038/nrd2466
- Lundberg, J. O., Weitzberg, E., Lundberg, J. M., and Alving, K. (1994). Intragastric nitric oxide production in humans: measurements in expelled air. *Gut* 35, 1543–1546. doi: 10.1136/gut.35.11.1543
- Magee, P. N., and Barnes, J. M. (1956). The production of malignant primary hepatic tumours in the rat by feeding dimethylnitrosamine. *Br. J. Cancer* 10, 114–122. doi: 10.1038/bjc.1956.15
- Marquez, L. A., and Dunford, H. B. (1995). Kinetics of oxidation of tyrosine and dityrosine by myeloperoxidase compounds I and II. Implications for lipoprotein peroxidation studies. *J. Biol. Chem.* 270, 30434–30440. doi: 10.1074/jbc.270.51.30434
- Masschelein, E., Van Thienen, R., Wang, X., Van Schepdael, A., Thomis, M., and Hespel, P. (2012). Dietary nitrate improves muscle but not cerebral oxygenation status during exercise in hypoxia. *J. Appl. Physiol.* 113, 736–745. doi: 10.1152/jappphysiol.01253.2011
- McDonough, P., Behnke, B. J., Padilla, D. J., Musch, T. I., and Poole, D. C. (2005). Control of microvascular oxygen pressures in rat muscles comprised of different fibre types. *J. Physiol.* 563, 903–913. doi: 10.1113/jphysiol.2004.079533
- McKnight, G. M., Smith, L. M., Drummond, R. S., Duncan, C. W., Golden, M., and Benjamin, N. (1997). Chemical synthesis of nitric oxide in the stomach from dietary nitrate in humans. *Gut* 40, 211–214. doi: 10.1136/gut.40.2.211
- Miranda, K. M., Espey, M. G., and Wink, D. A. (2001). A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. *Nitric Oxide* 5, 62–71. doi: 10.1006/niox.2000.0319
- Mo, L., Wang, Y., Geary, L., Corey, C., Alef, M. J., Beer-Stolz, D., et al. (2012). Free Radical Biology and Medicine. *Free Radic. Biol. Med.* 53, 1440–1450. doi: 10.1016/j.freeradbiomed.2012.07.080
- Modin, A., Björne, H., Herulf, M., Alving, K., Weitzberg, E., and Lundberg, J. O. (2001). Nitrite-derived nitric oxide: a possible mediator of “acidic-metabolic” vasodilation. *Acta Physiol. Scand.* 171, 9–16. doi: 10.1046/j.1365-201x.2001.171001009.x
- Moncada, S., and Higgs, A. (1993). The L-arginine-nitric oxide pathway. *N. Engl. J. Med.* 329, 2002–2012. doi: 10.1056/NEJM199312303292706
- Mowbray, M., McLintock, S., Weerakoon, R., Lomatschinsky, N., Jones, S., Rossi, A. G., et al. (2009). Enzyme-independent NO stores in human skin: quantification and influence of UV radiation. *J. Invest. Dermatol.* 129, 834–842. doi: 10.1038/jid.2008.296
- Muggeridge, D. J., Howe, C. C. F., Spendiff, O., Pedlar, C., James, P. E., and Easton, C. (2013). The effects of a single dose of concentrated beetroot juice on performance in trained flatwater kayakers. *Int. J. Sport Nutr. Exerc. Metab.* 23, 498–506.
- Muggeridge, D. J., Howe, C. C. F., Spendiff, O., Pedlar, C., James, P. E., and Easton, C. (2014). A single dose of beetroot juice enhances cycling performance in simulated altitude. *Med. Sci. Sports Exerc.* 46, 143–150. doi: 10.1249/MSS.0b013e3182a1dc51
- Nedergaard, J., and Cannon, B. (2003). The ‘novel’ ‘uncoupling’ proteins UCP2 and UCP3: what do they really do? Pros and cons for suggested functions. *Exp. Physiol.* 88, 65–84. doi: 10.1113/eph8802502
- Nisr, R. B., and Affourtit, C. (2014). Insulin acutely improves mitochondrial function of rat and human skeletal muscle by increasing coupling efficiency of oxidative phosphorylation. *Biochim. Biophys. Acta* 1837, 270–276. doi: 10.1016/j.bbabi.2013.10.012
- Nyström, T., Ortsäter, H., Huang, Z., Zhang, F., Larsen, F. J., Weitzberg, E., et al. (2012). Inorganic nitrite stimulates pancreatic islet blood flow and insulin secretion. *Free Radic. Biol. Med.* 53, 1017–1023. doi: 10.1016/j.freeradbiomed.2012.06.031
- Omar, S. A., and Webb, A. J. (2014). Nitrite reduction and cardiovascular protection. *J. Mol. Cell. Cardiol.* 73, 57–69. doi: 10.1016/j.yjmcc.2014.01.012
- Oxtoby, D. W., and Nachtrieb, N. H., eds. (1996). *Principles of Modern Chemistry, 3rd Edn.* Fort Worth, TX: Hartcourt; Fort Worth Brace.
- Peacock, O., Tjønnå, A. E., James, P., Wisløff, U., Welde, B., Böhlke, N., et al. (2012). Dietary nitrate does not enhance running performance in elite cross-country skiers. *Med. Sci. Sports Exerc.* 44, 2213–2219. doi: 10.1249/MSS.0b013e3182640f48

- Peeling, P., Cox, G. R., Bullock, N., and Burke, L. M. (2014). Beetroot juice improves on-water 500 m time-trial performance, and laboratory-based paddling economy in national and international-level Kayak athletes. *Int. J. Sport Nutr. Exerc. Metab.* 25, 278–284. doi: 10.1123/ijnsnem.2014-0110
- Poole, D. C., and Richardson, R. S. (1997). Determinants of oxygen uptake. Implications for exercise testing. *Sports Med.* 24, 308–320. doi: 10.2165/00007256-199724050-00003
- Porcelli, S., Ramaglia, M., Bellistri, G., Pavei, G., Pugliese, L., Montorsi, M., et al. (2014). Aerobic fitness affects the exercise performance responses to nitrate supplementation. *Med. Sci. Sports Exerc.* 47, 1643–1651. doi: 10.1249/mss.0000000000000577
- Pride, C. K., Mo, L., Quesnelle, K., Dagda, R. K., Murillo, D., Geary, L., et al. (2013). Nitrite activates protein kinase A in normoxia to mediate mitochondrial fusion and tolerance to ischaemia/reperfusion. *Cardiovasc. Res.* 101, 57–68. doi: 10.1093/cvr/cvt224
- Radi, R. (2004). Nitric oxide, oxidants, and protein tyrosine nitration. *Proc. Natl. Acad. Sci. U.S.A.* 101, 4003–4008. doi: 10.1073/pnas.0307446101
- Shepherd, A. I., Gilchrist, M., Winyard, P. G., Jones, A. M., Hallmann, E., Kazimierczak, R., et al. (2015). Effects of dietary nitrate supplementation on the oxygen cost of exercise and walking performance in individuals with type 2 diabetes: a randomised, double blind, placebo-controlled cross-over trial. *Free Radic. Biol. Med.* 86, 200–208. doi: 10.1016/j.freeradbiomed.2015.05.014
- Sun, J., Zhang, X., Broderick, M., and Fein, H. (2003). Measurement of nitric oxide production in biological systems by using griess reaction assay. *Sensors* 3, 276–284. doi: 10.3390/s30800276
- Takakura, H., Furuichi, Y., Yamada, T., Jue, T., Ojino, M., Hashimoto, T., et al. (2015). Endurance training facilitates myoglobin desaturation during muscle contraction in rat skeletal muscle. *Sci. Rep.* 5:9403. doi: 10.1038/srep09403
- Tannenbaum, S. R., Fett, D., Young, V. R., Land, P. D., and Bruce, W. R. (1978). Nitrite and nitrate are formed by endogenous synthesis in the human intestine. *Science* 200, 1487–1489. doi: 10.1126/science.663630
- Totzeck, M., Schicho, A., Stock, P., Kelm, M., Rassaf, T., and Hendgen-Cotta, U. B. (2014). Nitrite circumvents canonical cGMP signaling to enhance proliferation of myocyte precursor cells. *Mol. Cell. Biochem.* 401, 175–183. doi: 10.1007/s11010-014-2305-y
- Tsikas, D. (2007). Analysis of nitrite and nitrate in biological fluids by assays based on the Griess reaction: appraisal of the Griess reaction in the L-arginine/nitric oxide area of research. *J. Chrom. B* 851, 51–70. doi: 10.1016/j.jchromb.2006.07.054
- Vanhatalo, A., Bailey, S. J., Blackwell, J. R., DiMenna, F. J., Pavey, T. G., Wilkerson, D. P., et al. (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. Reg. Int. Comp. Physiol.* 299, R1121–R1131. doi: 10.1152/ajpregu.00206.2010
- Vanhatalo, A., Fulford, J., Bailey, S. J., Blackwell, J. R., Winyard, P. G., and Jones, A. M. (2011). Dietary nitrate reduces muscle metabolic perturbation and improves exercise tolerance in hypoxia. *J. Physiol.* 589, 5517–5528. doi: 10.1113/jphysiol.2011.216341
- Vaughan, R. A., Gannon, N. P., and Carriker, C. R. (2014). Nitrate-containing beetroot enhances myocyte metabolism and mitochondrial content. *J. Tradit. Complement Med.* doi: 10.1016/j.jtcme.2014.11.033. [Epub ahead of print].
- Voza, A., Parisi, G., De Leonardi, F., Lasorsa, F. M., Castegna, A., Amorese, D., et al. (2014). UCP2 transports C4 metabolites out of mitochondria, regulating glucose and glutamine oxidation. *Proc. Natl. Acad. Sci. U.S.A.* 111, 960–965. doi: 10.1073/pnas.1317400111
- Wagner, D. A., Schultz, D. S., Deen, W. M., Young, V. R., and Tannenbaum, S. R. (1983). Metabolic fate of an oral dose of 15N-labeled nitrate in humans: effect of diet supplementation with ascorbic acid. *Cancer Res.* 43, 1921–1925.
- Wang, L., Frizzell, S. A., Zhao, X., and Gladwin, M. T. (2012). Normoxic cyclic GMP-independent oxidative signaling by nitrite enhances airway epithelial cell proliferation and wound healing. *Nitric Oxide* 26, 203–210. doi: 10.1016/j.niox.2012.03.002
- Webb, A. J., Patel, N., Loukogeorgakis, S., Okorie, M., Aboud, Z., Misra, S., et al. (2008). Acute blood pressure lowering, vasoprotective, and antiplatelet properties of dietary nitrate via bioconversion to nitrite. *Hypertension* 51, 784–790. doi: 10.1161/HYPERTENSIONAHA.107.103523
- Wootton-Beard, P. C., and Ryan, L. (2011). A beetroot juice shot is a significant and convenient source of bioaccessible antioxidants. *J. Funct. Foods* 3, 329–334. doi: 10.1016/j.jff.2011.05.007
- Wylie, L. J., Kelly, J., Bailey, S. J., Blackwell, J. R., Skiba, P. F., Winyard, P. G., et al. (2013). Beetroot juice and exercise: pharmacodynamic and dose-response relationships. *J. Appl. Physiol.* 115, 325–336. doi: 10.1152/jappphysiol.00372.2013
- Zamani, P., Rawat, D., Shiva-Kumar, P., Geraci, S., Bhuva, R., Konda, P., et al. (2015). Effect of inorganic nitrate on exercise capacity in heart failure with preserved ejection fraction. *Circulation* 131, 371–380. doi: 10.1161/CIRCULATIONAHA.114.012957
- Zhang, Z., Naughton, D., Winyard, P. G., and Benjamin, N. (1998). Generation of nitric oxide by a nitrite reductase activity of xanthine oxidase: a potential pathway for nitric oxide formation in the absence of nitric oxide synthase activity. *Biochem. Biophys. Res. Comm.* 249, 767–772. doi: 10.1006/bbrc.1998.9226

Conflict of Interest Statement: The development of a placebo beetroot juice preparation in PGW's laboratory was supported by James White Drinks Ltd. Otherwise, we confirm that this mini-review was written in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2015 Affourtit, Bailey, Jones, Smallwood and Winyard. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) or licensor are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.