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Exploring the mechanisms by which nitrate supplementation improves skeletal muscle contractile function: one fibre at a time

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Background

The beneficial role of dietary nitrate (NO_3^-) supplementation on aspects of health and exercise performance via the nitrate-nitrite-nitric oxide pathway has received a great deal of attention. A significant and growing body of literature highlights the potential beneficial effects of NO_3^- supplementation through enhanced exercise economy and tolerance, and skeletal muscle contractile function. However, the underlying mechanism(s) for these beneficial effects has yet to be fully elucidated. Furthermore, to date there has been a lack of reproducibility and consistency in the findings regarding potential mechanisms.

Recently it has been proposed that part of this mechanism of action may lie within the skeletal muscle. The skeletal muscle acts as a store of NO_3^- and nitrite (NO_2^-), with levels far exceeding those observed in the blood, which increase rapidly following the acute consumption of inorganic nitrate (Wylie *et al.* 2019). Interestingly, when skeletal muscle stores of NO_3^- and NO_2^- are elevated above a basal state they are both utilised following a bout of high intensity exercise. This suggests that following exercise these NO metabolites (NO_3^- and NO_2^-) are reduced back to nitric oxide (NO) in skeletal muscle fibres. In addition, it has been demonstrated that NO_3^- can be taken up by human primary skeletal muscles cells via the NO_3^- transporters chloride channel 1 and sialin (Srihirun *et al.* 2019), and subsequently reduced to NO_2^- and NO. Together these findings suggest that skeletal muscle is a target tissue for NO_3^- supplementation.

One of the proposed mechanisms by which dietary NO_3^- improves exercise tolerance is by influencing calcium (Ca^{2+}) handling in the skeletal muscle to delay the onset of muscle fatigue. It has now been established that NO_3^- does not appear to improve mitochondrial function in human (Whitfield *et al.* 2016) or mouse skeletal muscle (Ntessalen *et al.* 2019), making improvements in contractile function a more likely candidate as a mechanism by which exogenous NO_3^- supplementation improves aspects of exercise performance. Furthermore, increases in skeletal muscle perfusion observed following NO_3^-/NO_2^- administration could improve oxygen delivery and extraction, allowing contractile function to be maintained during fatiguing conditions. Endogenous production of NO via neuronal and endothelial nitric oxide synthases (nNOS and eNOS, respectively) has been demonstrated to influence skeletal muscle contraction in several experimental models utilising NO donors and inhibitors (Stamler & Meissner, 2001). During repeated skeletal muscle contractions, NO production is inherently upregulated, primarily through increases in nNOS activity. The subsequent effects of NO are modulated by the local tissue environment. Under low physiological P_{O_2}, NO acts to enhance contractile function and Ca^{2+} signalling whereas at ambient P_{O_2}, NO acts as an inhibitor. Similarly, it is suggested that the effects of NO_3^- supplementation are more pronounced as P_{O_2} and pH decline. It is unclear if NO_2^- derived from exogenous NO_3^- has direct physiological effects on Ca^{2+} signalling and contractile function or whether it is mediated entirely by increased NO generation. Nevertheless, exogenous supplementation with dietary nitrate appears an appropriate tool for increasing skeletal muscle NO_3^- and NO_2^- stores and enhancing NO production during exercise.

Nitrite exposure improves contractile function and delays fatigue via alterations in calcium handling

The recent study by Bailey *et al.* (2019) in *The Journal of Physiology* builds upon the work by Hernandez *et al.* (2012) who first demonstrated that 7 days of $NaNO_3^-$ supplementation resulted in increased contractile force in mouse fast-twitch fibres at low stimulation frequencies. These improvements were attributed to increases in cytosolic sarcoplasmic reticulum (SR) Ca^{2+} during tetanic contractions and an increase in the expression of calsequestrin1 and dihydropyridine receptor, two key proteins involved in Ca^{2+} uptake and release during skeletal muscle contraction. However, these findings do not account for improvements in exercise economy following acute supplementation, which would suggest the mechanism of action does not rely on structural changes due to alterations in protein expression. Bailey and colleagues proposed that acute NO_2^- administration could delay the development of fatigue in intact mouse single muscle fibres via changes in Ca^{2+} handling during repeated tetanic contractions, and suggested this would be dependent on P_{O_2} and pH. The primary finding of this study was that when skeletal muscle fibres were acutely exposed to $NaNO_2^-$ at near-physiological (2% O_2) P_{O_2} there was a delay in the development of fatigue; however, at supra-physiological P_{O_2} (20% O_2) the rate of fatigue development was increased. A clear strength of this work was the use of near-physiological P_{O_2} and co-administration of sodium lactate to mimic conditions that are likely to occur *in vivo* during skeletal muscle contraction-induced fatigue. Indeed, it was under these experimental conditions that the effect of $NaNO_2^-$ on delaying fatigue development was most prominent. As well as demonstrating that the effects of NO on skeletal muscle contractile function are both P_{O_2} and pH dependent, the authors showed that these effects on skeletal muscle function

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are underpinned by improved Ca^{2+} handling. Specifically, they demonstrate the delay in fatigue development can be explained by an NO-mediated preservation of SR Ca^{2+} pumping rate. This resulted in an attenuation in the rise of basal $[Ca^{2+}]_c$ and therefore maintenance of myofilament Ca^{2+} sensitivity.

Significance and future considerations

The findings from Bailey *et al.* (2019) build on growing body of evidence and point to a mechanism by which dietary NO_3^- may improve exercise tolerance through the modulation of Ca^{2+} handling to improve contractile function (Fig. 1). Given the biggest sources of energy consumption during exercise are actomyosin ATPases and the SR Ca^{2+} ATPases (SERCA), improvements in Ca^{2+} sensitivity and/or reductions in the ATP cost of contraction could influence fatigue development. Furthermore, this is likely to explain or contribute to the improvements in exercise tolerance and economy observed following the use of acute dietary NO_3^- supplementation. However, in contrast to previous studies (Hernandez *et al.* 2012), acute NO_2^- supplementation resulted in a reduction in isometric force production at low stimulation frequencies, as well as decreased cytosolic Ca^{2+} concentrations during both submaximal and maximal contractions. While this discrepancy may be due to the supraphysiological NO_2^- dose used in the current study, it is also possible that there are differences in the effects of chronic and acute exposure to NO donors.

In humans, a major challenge with dietary NO_3^- supplementation

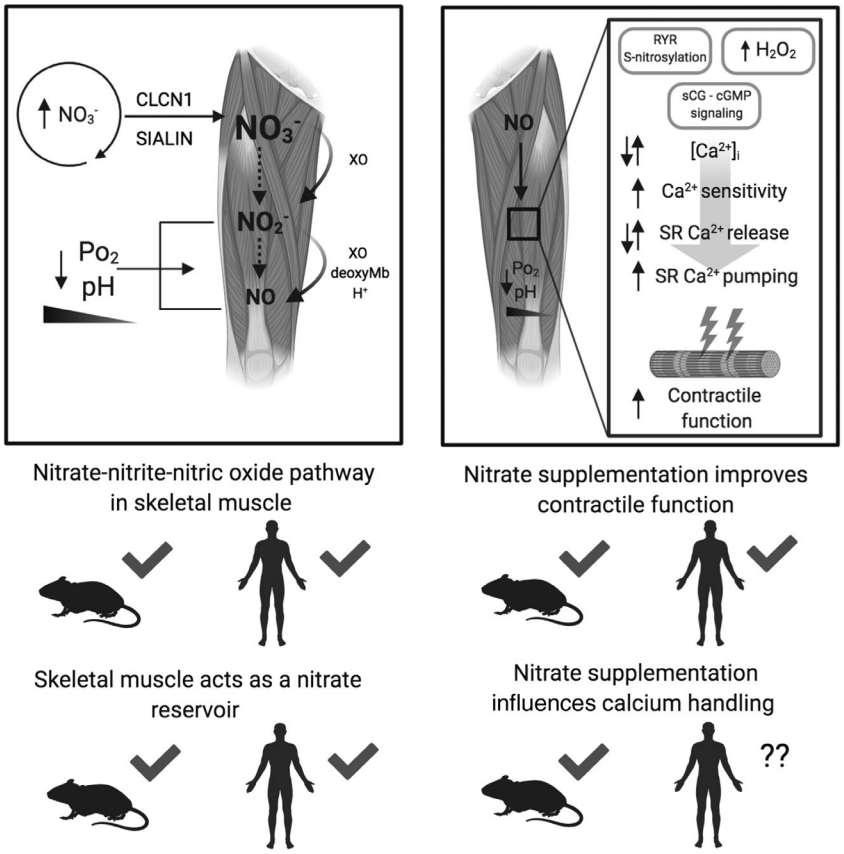

Figure 1. Current overview of nitrate supplementation and skeletal muscle calcium handling
It is now understood that nitrate supplementation is capable of increasing levels of nitrate and nitrite in the skeletal muscle, enhancing nitric oxide bioavailability during exercise. This enhanced nitric oxide production improves contractile function, with nitric oxide regulating Ca^{2+} handling. This can be achieved through several possible mechanisms; nitroslyation of the ryanodine receptor, increases in hydrogen peroxide and activation of soluble guanylate cyclase to increase cyclic guanosine monophosphate. These downstream effects lead to changes in intracellular $[Ca^{2+}]$, sarcoplasmic reticulum Ca^{2+} release and pumping, and increased Ca^{2+} sensitivity. However, there is currently no general consensus on the precise mechanism(s) of action, and further research is required. The work by Bailey *et al.* (2019) adds to a growing body of evidence that nitrate supplementation can positively influence calcium handling to improve contractile function and ultimately exercise performance. Despite significant progress in demonstrating the role of calcium handling in response to nitrate supplementation more work is required to demonstrate this mechanism *in vivo*. Ca^{2+}, calcium; $[Ca^{2+}]_i$, intracellular calcium; cGMP, cyclic guanosine monophosphate; CLCN1, chloride channel 1; deoxyMb, deoxygenated myoglobin; H^+, hydrogen ions; H_2O_2, hydrogen peroxide; NO_3^-, nitrate; NO_2^-, nitrite; NO, nitric oxide; P_{O_2}, oxygen tension; pH, potential of hydrogen; RyR, ryanodine receptor; sCG, soluble guanylate cyclase; SR, sarcoplasmic reticulum; XO, xanthine oxidase. Figure created with BioRender.com

is circumventing the rate-limiting step of the oral cavity in the NO metabolism. As a result, it is unclear whether levels of NO_2^- comparable to the present study can reach the skeletal muscle in order to elicit similar physiological effects. Indeed, the relevance of these findings to humans remains in question, given that 7 days of NO_3^- supplementation improved contractile function independently of changes in Ca^{2+} handling protein content or indices of intracellular redox status (Whitfield *et al.* 2017). Further investigation is required to determine if other aspects of Ca^{2+} homeostasis are altered in human skeletal muscle such as SERCA efficiency and Ca^{2+} sensitivity. Future studies may wish to assess the observed effects in single human skeletal muscle fibres using a similar experimental model with both slow and fast twitch muscle fibres. Overall, Bailey *et al.* (2019) have provided novel insights into the role of skeletal muscle NO_2^- in fatigue development and the mechanism responsible for the beneficial effects of using exogenous NO_3^- supplementation for exercise performance.

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Additional information

Competing interests

None declared.

Author contributions

All authors have read and approved the final version of this manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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Keywords

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