PERSPECTIVES

Dietary nitrate – a slow train coming

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Inorganic nitrate (NO_3^-) and nitrite (NO_2^-) are obligatory parts of nature's nitrogen cycle in which atmospheric dinitrogen (N_2) is fixed and made available for incorporation in plants and animals. In spite of this vital role, they are mostly known to the general public as potentially harmful constituents in our food and drinking water and among researchers as inert oxidation products of endogenous nitric oxide (NO). An increasing amount of research is now questioning this long-standing view and it is slowly becoming evident that nitrate and nitrite can be recycled back to bioactive NO, with important functions in the body (Lundberg *et al.* 2008).

Circulating nitrate and nitrite originate from two major sources: NO synthases and the diet. Leafy green vegetables, such as spinach, rocket (rucola) and beetroot, are high in nitrate and after ingestion a substantial part of this nitrate is actively taken up by the salivary glands and excreted in saliva. Oral commensal bacteria efficiently reduce nitrate to nitrite and when nitrite reaches the systemic circulation there are several pathways that further reduce nitrite to NO and other bioactive nitrogen oxides (Lundberg *et al.* 2008). This nitrate–nitrite–NO pathway can be viewed as complementary to the classical L-arginine–NO synthase pathway. One major difference, highly relevant to the article under consideration here, is that nitrite reduction is greatly enhanced during hypoxia and low pH, when NO synthases perform poorly. Therefore, it may serve as a back-up system to ensure NO bioactivity also during hypoxic/ischaemic conditions. From a therapeutic and nutritional aspect, nitrate and nitrite have been shown to reduce blood pressure (Larsen *et al.* 2006), protect against ischaemia-reperfusion (Duranski *et al.* 2005), reduce oxidative stress (Carlstrom *et al.* 2011), modulate mitochondrial function (Larsen *et al.* 2011) and reduce oxygen consumption during exercise (Larsen *et al.* 2007). The latter finding has attracted great interest from the sports community and among exercise physiologists.

Andrew Jones's group at the University of Exeter has made several important contributions to our present understanding on how inorganic nitrate improves muscular efficiency during exercise. In a series of studies they have elegantly shown that beetroot juice reduces oxygen cost during low-intensity exercise and improves high-intensity exercise tolerance (Bailey *et al.* 2009). Moreover, in a recent study this group also showed that beetroot juice improves cycling time-trial performance (Lansley *et al.* 2011). Now, in an article in this issue of *The Journal of Physiology*, they have studied the effects of beetroot juice on skeletal muscle energetics during hypoxic exercise (Vanhatalo *et al.* 2011).

They find that the nitrate-containing juice reduces muscle metabolic perturbation during hypoxic exercise and restores exercise tolerance and oxidative function. These are important findings not only relevant for exercise physiology but perhaps even more so for patients with limited oxygen delivery to the working muscle. This opens up a new avenue for dietary nitrate to improve physical capacity in diseases with limited pulmonary function or reduced circulatory capacity. Interestingly, in a recent study by Allen and coworkers, beetroot juice improved performance in patients with peripheral artery disease (Kenjale *et al.* 2011). One important and very innovative detail in Vanhatalo *et al*'s study is the use of a nitrate-depleted beetroot juice as a negative control. Since more and more research groups now use beetroot juice as a source of inorganic nitrate, this 'placebo-juice' could be of great value.

The exact mechanism(s) behind the beneficial effects of dietary nitrate on muscular efficiency are not fully understood but improved mitochondrial efficiency due to reduced uncoupling (Larsen *et al.* 2011) and better matching of tissue O_2 supply to local metabolic rate (Vanhatalo *et al.* 2011) have been suggested.

It can be anticipated that these findings will have an impact on the sports community. However, there are several unresolved questions regarding nitrate and exercise, since we do not know the exact underlying mechanism(s). Is there a dose–response relationship? Is chronic use during training periods also beneficial or even harmful? In light of recent findings showing that antioxidants (vitamins C and E) prevent health-promoting effects of physical exercise in humans (Ristow *et al.* 2009), the recently described antioxidant effects of nitrate may not necessarily be beneficial. There might be a tolerance effect although several studies point to the contrary. Future studies will hopefully clarify some of these issues.

Importantly, a word of caution is appropriate since individuals, eager to try to enhance performance, might easily confuse nitrate with nitrite or organic nitrates, with risk for fatal effects. If used by professional athletes, would intake of nitrate salts, beetroot juice or another nitrate-rich vegetable be considered as doping? If so, will there be cut-off limits in plasma levels of nitrate and nitrite in athletes?

Finally, even though the remarkable effects of nitrate on oxygen consumption during exercise are established, numerous labs are presently struggling to unravel the underlying mechanisms, which are largely unresolved. Therefore, when summarizing our current understanding, we cannot resist the temptation to paraphrase Bob Dylan: *We know something is happening, but we don't know what it is – Do we, Dr Jones?*

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