

Ergogenic effects of dietary nitrate

Citation for published version (APA):

Nyakayiru, J. D. O. A. (2019). *Ergogenic effects of dietary nitrate*. Gildeprint Drukkerijen. <https://doi.org/10.26481/dis.20190213jn>

Document status and date:

Published: 01/01/2019

DOI:

[10.26481/dis.20190213jn](https://doi.org/10.26481/dis.20190213jn)

Document Version:

Publisher's PDF, also known as Version of record

Please check the document version of this publication:

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Valorization

Scientific and societal relevance

In the sports nutrition field, meticulous research in the past decades has provided clear insight in the general areas of application for the macronutrients (i.e., carbohydrates as a main fuel, and proteins to support skeletal muscle structural adaptations) resulting in detailed recommendations for specific sports. In contrast, the efficacy of several nutritional supplements to be used as ergogenic aids or to support exercise adaptation is less well established. Dietary nitrate is an example of such a nutritional supplement that gained a lot of attention in recent years as a promising agent to enhance sports performance in athletes of both endurance and high-intensity type sports, and was even named the 'next magic bullet'. However, recommendations on the effective application of a nutritional supplement such as dietary nitrate requires extensive research that provides insight in the various factors that may modulate its efficacy. The studies described in this thesis aimed to do so by determining whether the beneficial effects of dietary nitrate can be associated with factors such as the duration of supplementation, the nitrate source, and the type of sport, while also trying to gain further insight in the *in vivo* pharmacokinetics.

The first study described in this thesis showed that even a multiday supplementation protocol with dietary nitrate does not improve performance in highly trained endurance athletes. For sport supplements in general, it is extremely relevant to not only know who might benefit, but also provide clear recommendations for those that may not benefit from it. Indeed, our findings strongly supported the ongoing paradigm shift, suggesting dietary nitrate to be less effective for endurance athletes, and perhaps more beneficial for exercise intensities that strongly recruit type II muscle fibers. We thereafter performed a study that showed that vegetable-based nitrate sources may induce greater benefits than dietary nitrate salts provided as an extracted powder. The relevance of this finding lies in the fact that although nitrate salts may be considered an easier nitrate source to process industrially into nutritional products (such as sport drinks and dietary powders), they may not be as effective as vegetable-based sources, potentially limiting their suitability as a sport supplement. The knowledge gained from these first two studies was subsequently incorporated into the third study, in which we showed that a multiday nitrate-rich beetroot juice supplementation protocol improves performance during high-intensity intermittent-type exercise in trained soccer players.

The findings from these first three studies have already been disseminated in different ways to educate and advice. For example, the knowledge has been incorporated in several sports and nutrition courses of bachelor and master students at the Maastricht University,

education of sports dietitians at the HAN University of Applied Sciences, and a course on sports nutrition for sports physicians. Furthermore, apart from the scientific publications and scientific conferences in which the observations described in the current thesis have been shared, the findings have also been spread through publications in sports and nutrition based magazines read by the general public. This approach allowed us to provide well-balanced evidence to the general public regarding the potential of dietary nitrate to promote health and exercise performance (i.e., taking our own findings as well as other recent insights into account). To specifically inform athletes and the associated (medical) staff of these athletes, we recently also started updating the dietary nitrate factsheet in collaboration with the Dutch Olympic Committee (NOC*NSF). As factsheets are considered the most comprehensive source of information for competitive athletes, providing state-of-the-art and very applicable insights regarding ergogenic aids, this was and is one of the primary aims of our pre-determined valorization plan.

Innovation

In addition to the more applied findings described in this thesis, we also provided further insight in the metabolic fate of dietary nitrate. We provide the first human evidence that nitrate is stored in skeletal muscle tissue and that this 'reservoir' can be increased with dietary nitrate supplementation. This may be an important step towards understanding how dietary nitrate can improve skeletal muscle function. In fact, we propose that the performance enhancing effect of dietary nitrate may be related to the local storage and subsequent utilization of nitrate. If this hypothesis is true, optimizing dietary nitrate supplementation for exercise performance should be aimed at establishing the dose, source and duration that most effectively increases the nitrate stored in skeletal muscle tissue. Additionally, the ability to locally utilize the nitrate stored in muscle may represent a crucial factor explaining inter-individual differences in the responsiveness to dietary nitrate. Although further work is needed to confirm these concepts, our findings clearly call for explorative research approaches with the aim to improve the local utilization of the stored nitrate, consequently increasing the effectiveness of a supplementation protocol. Furthermore, although we did not specifically focus on the clinical application of dietary nitrate, it could be speculated that the local storage of dietary nitrate may also have a functional purpose in other organs. Several organs have indeed been suggested to be capable of locally reducing nitrate and nitrite into nitric oxide, which may allow for vasodilation during ischemic and/or hypoxic events. Reducing the negative impact of such events could be crucial in maintaining the function of several organs. The fact that there is greater utilization of nitrate during low oxygen conditions further supports the potentially major role that nitrate may have in such situations, and underlines the need

to further establish the local storage and utilization of dietary nitrate beyond that in skeletal muscle tissue.

Concluding remarks

The studies described in this thesis have allowed for a further optimization of dietary nitrate supplementation strategies when aiming to improve exercise performance. Furthermore, the observed local storage of nitrate in skeletal muscle tissue provides new insights in the metabolic fate of dietary nitrate *in vivo*, and provides opportunities for future research to unravel the mechanisms behind the ergogenic effect of dietary nitrate. In addition to the relevance this may have for supplementation strategies to improve exercise performance, the observed local storage of nitrate also reveals a knowledge gap in an area with perhaps even greater implications. Extending the benefit of local storage and utilization of nitrate in skeletal muscle tissue to other organs (such as the brain and heart) provides possibilities for nitrate supplementation to attenuate tissue damage and dysfunction during an ischemic and/or hypoxic event. Confirming this hypothesis in future research will open up new avenues for the clinical application of dietary nitrate.