Dietary nitrate and sports performance

Summary

Dietary nitrate supplementation is used by many athletes to enhance sports performance. The potential ergogenic effects of nitrate are attributed to its endogenous reduction into nitrite and, subsequently, nitric oxide (NO), thereby increasing the bioavailability of this highly bioactive compound, which regulates several processes such as blood flow and muscle contraction. Nitrate supplementation seems to be more effective under conditions where oxygen is a limiting factor. Acute effects of nitrate include vasodilation and thereby increased systemic blood flow and oxygen supply, while the more 'adaptive' effects seem to concern improvements at the myocellular level, such as enhanced contractility and decreased oxygen cost of ATP production. In this thesis, we aimed to provide further insight into how nitrate supplementation can be used to enhance sports performance, under which conditions and in which athletes. One of the focus areas of this thesis was to investigate whether elite athletes can benefit from dietary nitrate supplementation.

In **chapter 2**, we present a viewpoint entitled *Can elite athletes benefit from dietary nitrate supplementation?*, in which we address the key questions concerning the proposed ergogenic effects of nitrate supplementation in elite athletes, providing a foundation for the various studies described in this thesis. As there is little known about the preferred duration of supplementation, the daily nitrate dose needed, and the nitrate source to maximize the ergogenic potential of nitrate supplementation, we next studied some of these aspects. In **chapter 3** we compared the effects of acute and 6 days of sodium nitrate supplementation on oxygen consumption during submaximal exercise and subsequent 10 km time-trial performance. However, no effects of nitrate supplementation were observed following both the acute and the 6-day intervention. We propose that this is most likely caused by the high training level of the endurance athletes included in this study, and/or by the choice for sodium nitrate as the nitrate carrier, which may be less effective than vegetable nitrate sources. Indeed in **chapter 4**, we showed that the effects of nitrate supplementation may be dependent on the NO donor chosen. Here, we provided an acute dose of nitrate from sodium nitrate, concentrated beetroot juice, spinach, and rocket salad, and investigated the increase in plasma nitrate and nitrite concentrations and the subsequent effects on resting blood pressure in a group of healthy young men and women. All four nitrate sources resulted in similar increases in plasma nitrate and nitrite concentrations, indicating an efficient absorption and endogenous bioconversion of the supplement. However, the three vegetable sources reduced resting blood pressure to a greater extent than sodium nitrate. We concluded that nitrate-rich vegetables can effectively be used as dietary nitrate supplements and may actually be more effective than so-called nitrate salts. Based on this finding we next investigated whether a high(er)

habitual vegetable and nitrate intake could explain why highly trained athletes seem to benefit less from nitrate supplementation (**chapter 5**). Based on multiple 24-h dietary recalls, we estimated the dietary nitrate intake of 553 Dutch athletes, ranging from well-trained to Olympic level. For the vast majority of athletes, nitrate intake from their habitual diet was substantially lower than any supplemental dose that would be deemed effective to induce ergogenic effects. Hence, we concluded that for most athletes the habitual dietary nitrate intake is not a sufficient substitute for nitrate supplementation and certainly does not explain why highly trained athletes seem to benefit less from nitrate supplementation.

In view of the suggestions that nitrate supplementation may be more effective for recreational athletes and during high-intensity exercise, we compared the metabolic and functional responses to beetroot juice between recreational, competitive and elite athletes, completing the exact same repeated-sprint exercise protocol (i.e., repeated Wingate tests). In **chapter 6**, we show that the plasma nitrate and nitrite concentrations at baseline and following beetroot juice supplementation did not differ between athletes with these different training levels. This is in stark contrast to previous suggestions that elite athletes might have higher baseline plasma nitrate and nitrite concentrations and/or an attenuated plasma response to nitrate supplementation. Furthermore, the lack of effect on peak power and mean power, and, perhaps more striking, the improvement in time to reach peak power following beetroot juice supplementation were not dependent on athletes' sport level. Our results indicate that beetroot juice supplementation can improve the capacity to accelerate during sprints, with no differences in the responsiveness between recreational, competitive and elite athletes performing in sports characterized by high-intensity, intermittent type exercise.

Since nitrate supplementation is more effective under conditions of low oxygen availability and low pH, we further investigated the effects of beetroot juice supplementation in elite athletes using two hypoxic settings in water polo players; the 'local hypoxia' of an intermittent performance test and the 'systemic hypoxia' of a dynamic apnea test (**chapter 7**). No effect of beetroot juice supplementation was seen for the intermittent test, that may not have created a sufficient oxygen deprivation in this group of elite athletes. For the dynamic apnea test, however, we observed a performance benefit following beetroot juice supplementation, indicating a potential benefit of nitrate supplementation for hypoxic exhaustive exercise in elite athletes.

Besides direct performance enhancements, the vasodilatory properties of nitrate may provide other beneficial effects during exercise. In the final study described in **chapter 8,** we hypothesized that nitrate ingestion could improve splanchnic blood flow and attenuate the gut injury induced by high-intensity

exercise. In contrast to what was previously shown for a different NO donor (L-citrulline), we observed no beneficial effects of nitrate supplementation, which might be related to the different NO pathway stimulated by nitrate compared to L-citrulline supplementation. We also investigated the effects of sucrose ingestion, as macronutrients are known to increase splanchnic blood flow at rest. In agreement, sucrose ingestion attenuated gut injury during and post-exercise as shown by an attenuated increase in plasma intestinal fatty acid binding protein (I-FABP) levels. We concluded that sucrose but not nitrate intake, prior to and during

high-intensity exercise can be used as a strategy to reduce gastrointestinal injury and may lower the risk of gastrointestinal complaints.

The final chapter encompasses the primary findings of this thesis and practical advises for the use of nitrate supplementation by athletes. In this relatively new field of research there are still several questions to be answered before reaching a definite consensus on how nitrate supplementation can best be used to induce its proposed ergogenic effects. However, this thesis shows that there is a potential for beneficial effects of nitrate supplementation when taking the supplementation protocol, exercise conditions, and characteristics of the athlete into account.