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The oral microbiome, nitric oxide and exercise performance

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A B S T R A C T

The human microbiome comprises ~10^{13}–10^{14} microbial cells which form a symbiotic relationship with the host and play a critical role in the regulation of human metabolism. In the oral cavity, several species of bacteria are capable of reducing nitrate to nitrite; a key precursor of the signaling molecule nitric oxide. Nitric oxide has myriad physiological functions, which include the maintenance of cardiovascular homeostasis and the regulation of acute and chronic responses to exercise. This article provides a brief narrative review of the research that has explored how diversity and plasticity of the oral microbiome influences nitric oxide bioavailability and related physiological outcomes. There is unequivocal evidence that dysbiosis (e.g. through disease) or disruption (e.g. by use of antiseptic mouthwash or antibiotics) of the oral microbiota will suppress nitric oxide production via the nitrate-nitrite-nitric oxide pathway and negatively impact blood pressure. Conversely, there is preliminary evidence to suggest that proliferation of nitrate-reducing bacteria via the diet or targeted probiotics can augment nitric oxide production and improve markers of oral health. Despite this, it is yet to be established whether purposefully altering the oral microbiome can have a meaningful impact on exercise performance. Future research should determine whether alterations to the composition and metabolic activity of bacteria in the mouth influence the acute responses to exercise and the physiological adaptations to exercise training.

1. Background

Research over the past 20 years has shifted the paradigm of our understating in how bacteria in, and on, the body contribute to human physiology. The human microbiome is recognized as a distinct and essential organ [1] and is comprised of ~10^{13}–10^{14} microbial cells. This includes the collective genomes of all microbiota that reside in tissues and biological fluids. These microbiota are predominantly bacteria but also include archaea, eukaroytes, protozoa, and viruses [2]. Microbial cells form a symbiotic relationship with the host and play a critical role in the regulation of human metabolism. Driven by recent advances in microbial metagenomics, it is becoming clearer that genetic diversity and plasticity of the human microbiome can profoundly influence the health of the human host. To date, studies that have attempted to evidence links between the microbiome and human health and disease have predominantly focused on the gastrointestinal (gut) microbiome. The gut microbiome contains an estimated 500–1000 species and 100 trillion organisms, encoding 100-fold more unique genes than the human genome [3]. On the other hand, the oral microbiome has largely been ignored until recently.

The human oral cavity is an important habitat for microbes, and a healthy mouth can harbor over 700 different species and upwards of ten billion individual bacteria that colonize the teeth and the soft tissues of the oral mucosa [4]. It has long been known that lifestyle-driven dysbiosis of the oral microbiome causes perturbations within the mouth that may result in conditions such as caries, gingivitis and periodontitis [5]. Recently, it has also been established that oral bacteria play a crucial role in the human nitrogen cycle by reducing salivary nitrate to nitrite. When swallowed, the nitrite can be converted to nitrogen oxides via several enzymatic and non-enzymatic processes. One of these molecules, nitric oxide (NO), is a key signaling molecule that has antimicrobial effects in organs such as the stomach and is a potent vasodilator and a cytoprotective molecule across all tissues. Given that human cells have a limited capacity to reduce nitrate, there is intriguing preliminary evidence to suggest that the composition and activity of bacteria inside the mouth may intrinsically contribute to the regulation of cardiovascular and metabolic health. It is also well-established that NO mediated signaling in skeletal muscle can modulate contractile function, hormone regulation, and local microcirculation [6]. As a consequence, therefore, the activity of the oral microbiome may also influence acute exercise performance and potentiate the adaptive responses to chronic exercise training.

Below, we discuss the role of nitrate-reducing bacteria in maintaining NO bioavailability and regulating physiological homeostasis. Further, we review recent research which has explored the impact of interventions that are designed to either disrupt or augment the oral microbiome. Where previous review articles have concentrated solely on the role of the oral microbiota in the context of cardiovascular health

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[7], our focus is on the novel possibility that alterations to microbial activity may be either ergogenic or ergolytic. Finally, we discuss future directions and the key research studies that need to be undertaken in order to better understand the importance of the symbiotic relationship between the microbiome and human host during exercise.

2. Nitric oxide production pathways

The discovery of NO originated from the observation that L-arginine was required for nitrite production involving a group of enzymes called NO synthase (NOS) [8]. NOS enzymes are homodimers that are responsible for catalyzing the five electron, multi-step oxidation of the guanidino nitrogen of L-arginine into NO with L-citrulline as a bi-product. These enzymes require multiple bound cofactors/prosthetic groups including flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN), heme, glutathione, nicotinamide adenine dinucleotide phosphate (NADPH), tetrahydrobiopterin (BH_4) and Ca^{2+}-calmodulin (Fig. 1).

Several agonists exist that signal the endothelial cells to produce NO including sheer stress, acetylcholine, histamine and others. However, this pathway becomes dysfunctional with age due to NOS uncoupling and in most cases, loss of NO production in the endothelial cells occurs due to oxidative stress and oxidation of tetrahydrobiopterin (BH4) [9]. This is important because endothelial dysfunction is the earliest event in the onset and progression of cardiovascular disease [10,11]. Therefore, understanding how the body makes NO and recognizing how these pathways become dysfunctional and unproductive will allow us to define the context for safe and effective interventions. For example, population-based studies show that sedentary behavior and physical inactivity are associated with biomarkers of endothelial dysfunction [12] whereas exercise is widely accepted to have far reaching and myriad health benefits [13]. Some of the cardioprotective benefits of exercise may be underpinned by modulation of NO pathways as there is sound evidence that NO levels are increased via exercise-induced sheer stress [14]. Alternatively, NO production can be increased through the diet, provided effective nitrate-reducing bacterial communities are present [15,16].

3. Oral microbiome and nitric oxide generation

In the human body, nitrate is generated from the oxidation of NO and from the absorption of nitrate contained in food and beverages [17]. The vast majority of dietary nitrate is derived from green leafy and root vegetables, although there are other sources, including drinking water and preservatives [18]. The salivary glands extract nitrate from the plasma and concentrate this inside the oral cavity via active transport by sialin [19]. In basal conditions, nitrate concentration is ~10 times higher in the saliva (100–500 μM) than in the plasma, and can rise to 5–8 mM following a nitrate-rich meal [20]. The oral microbiome contains several species of bacteria that selectively express a nitrate reductase enzyme to perform a 2-electron reduction of nitrate to nitrite [15]. There is disparity in the reporting of nitrate-reducing species, perhaps due to methodological differences in oral sampling area, DNA extraction method, primer design, and gene database selection. However, they are generally considered to include representatives of *Actinomyces, Coynebacterium, Haemophilus, Kingella, Neisseria, Rothia, and Veillonella* [21–23]. This produces a nitrite-enriched saliva that, when swallowed, becomes protonated (pKa nitrite is 3.4) in the acid environment of the stomach to generate NO gas [24]. NO can be absorbed across gastric mucosal and/or is oxidized to nitrite or binds to glutathione to generate S-nitrosoglutathione (GSNO) [10]. Both nitrite and GSNO prolong the bioactive half-life of NO from 2 milliseconds out to tens of minutes and hours [25]. Numerous studies have shown that nitrite produced from bacterial nitrate reduction is an important storage pool for NO in blood and tissues when NOS-mediated NO production is insufficient [26–30].

However, even if an individual has nitrate reducing bacteria in their oral microbiome, this does not always result in a direct benefit from dietary nitrate because there are additional factors which can affect the absorption of nitrite. For example, stomach acid is required for the optimal effects of salivary nitrite that results from consuming dietary nitrate. Nitrite concentration in the saliva from reduction of dietary nitrate when swallowed becomes protonated (nitrite pKa ~3.4) to form nitrous acid which can spontaneously release NO [31]. However, proton pump inhibitors (PPIs) and other antacids, by inhibiting stomach acid production and increasing gastric pH, prevent the formation of nitrous acid from salivary nitrite, and, thus, NO release. Furthermore, administration of PPIs has been shown to block the blood pressure lowering effects of orally administered sodium nitrite [32], and blunt the reduction of nitrite to NO by antioxidants in the stomach [33].

While it is unequivocal that the oral microbiome is critical for nitrate reduction, it is less clear how diversity in its compositional structure might influence inter-individual and within-individual variability in NO metabolism. Given that the oral microbiota are continuously exposed to fluctuating environment, it is no surprise that they display profound phenotypic plasticity [34]. The abundance of bacterial species can alter rapidly in response to alterations in environmental pH, carbohydrate availability, oxygen tension and the redox environment, and exposure to antimicrobials such as fluoride and mouthwashes [35]. We have previously demonstrated that the coefficient of variation for the relative

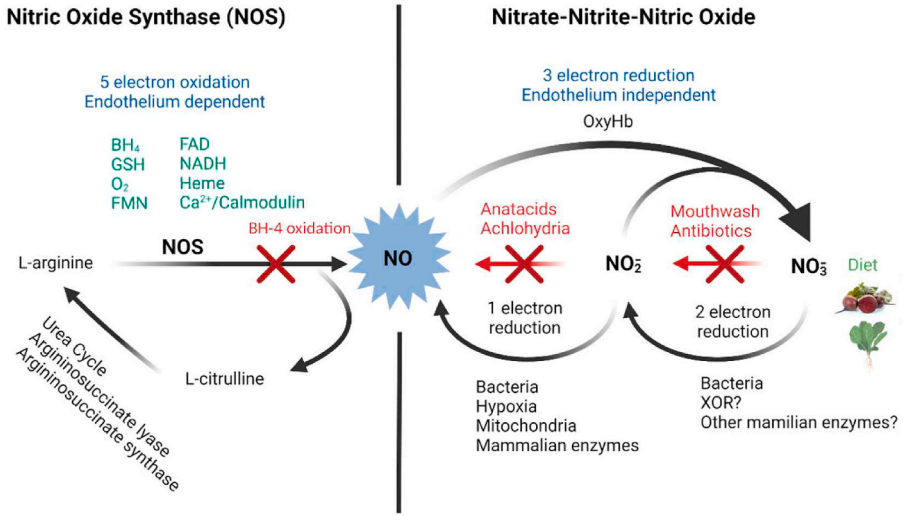

Fig. 1. There are two production pathways the human body generates NO. Pathway 1 involves oxidation of L-arginine to NO through a complex series of reactions involving nitric oxide synthase (NOS) enzymes. This pathway becomes dysfunctional with age. Pathway 2 involves the provision of nitrate and nitrite found in our food supply and generated from oral nitrate reducing bacteria. This pathway becomes disrupted with insufficient nitrate ingestion, antiseptic mouthwash/antibiotic use as well as antacid therapy. One can compensate for the other but when both systems fail, NO based signaling is completely inhibited and disease ensues. Created with BioRender.com.

abundance of nitrate-reducing species of bacteria was between 35 and 132% when sampled over three consecutive weeks [36]. This is despite controlling for diet and exercise in the week prior to measurement and repeating the same oral health care regimen on the morning of each trial. This profound variability of the microbiome is important as we have also shown that the rate and magnitude of salivary nitrite production following a nitrate dose is associated with the cumulative relative abundance of nitrate-reducing species on the dorsal surface of the tongue [16]. Interventions which cause significant modifications to the oral microbiome may, therefore, be reasonably expected to alter the generation of NO production via this pathway.

4. NO and exercise performance

Cardiovascular research has identified a potential role for the oral microbiome in human health via the conversion of dietary nitrate into nitrite, providing bioavailability for subsequent conversion to NO [37]. Continuous generation of NO is essential for the integrity of the cardiovascular system, and decreased production or bioavailability of NO is central to the development of many disorders [38]. NO is also an important ergogenic molecule that controls oxygen delivery and oxygen utilization for ATP production as well as mitochondrial biogenesis [39].

It is well-established that supplementing the diet with exogenous nitrate will, in most cases, markedly increase the bioavailability of NO. In the last 15 years, a plethora of studies have been undertaken to establish whether this can translate to a meaningful positive impact on exercise performance. A recent systematic review and meta-analysis suggests that nitrate supplementation exerts clear ergogenic effects (~3%) in healthy men across a range of exercise modalities and environmental conditions [40]. The myriad physiological mechanisms that underpin these effects are discussed at length elsewhere in this special issue. Of note, however, is that the ergogenic effects of nitrate supplementation are not consistently observed in highly trained endurance athletes. Some have speculated that this may be a consequence of the physiological adaptations to endurance training which better preserve muscle oxygenation status during exercise [41]. It is also reasonable to assume that the aforementioned intra- and inter-individual differences in the oral microbiome may contribute to variability in the response to nitrate supplementation between individuals.

An extensive body of evidence shows that a unique combination of exercise, diet, and body composition promote a more "health associated" gut microbiome in athletes that can modulate mucosal immunity and improve gastrointestinal barrier function [42]. It remains to be established whether elite athletes develop similar adaptations to their oral microbiome although there is emerging evidence that supports this hypothesis. Thomas and colleagues [43] reported a positive association between oral nitrate reduction capacity (the magnitude by which a nitrate solution is reduced to nitrite inside the oral cavity) and parameters of aerobic exercise capacity. These preliminary data do suggest that exercise training (and associated changes in diet and lifestyle) can alter the oral microbiome such that it augments NO production from endogenous nitrate stores and nitrate obtained through the diet. Given the crucial role of NO in physiological function, further research is clearly warranted to better understand how exercise training influences the diversity and activity of bacterial species within the oral cavity.

5. Shifting the oral microbiome

Of all body sites the oral microbiome is the second most complex ecosystem in humans [44]. Its complexity is likely due to continuous exposure to the range of internal and external pressures it is subject to. Whilst a full review of all factors which contribute to fluctuations in the oral microbiome is beyond the scope of this review, we have summarized some of the main contributors in Fig. 2. The following sections detail the effect of diet, mouthwash, probiotics, and the oral health status of athletes on the composition of the oral microbiome and nitrate reduction capacity. This discussion is highly relevant because understanding how to harness the oral microbiome to enhance NO production could have profound effects on human health and human performance.

5.1. Diet

Diet is a potent modulator of the oral microbiome with differences in microbial composition observed in cohorts who have distinct dietary patterns, such as hunter gatherers, traditional farmers, western populations, vegetarians and vegans [45–47]. Supplementing the diet with nitrate-rich beetroot juice has also been shown to significantly alter the oral microbiome with fairly consistent patterns of change reported across studies [48–50]. The majority of studies show that the relative abundance of both *Neisseria* and *Rothia* increase in the saliva following

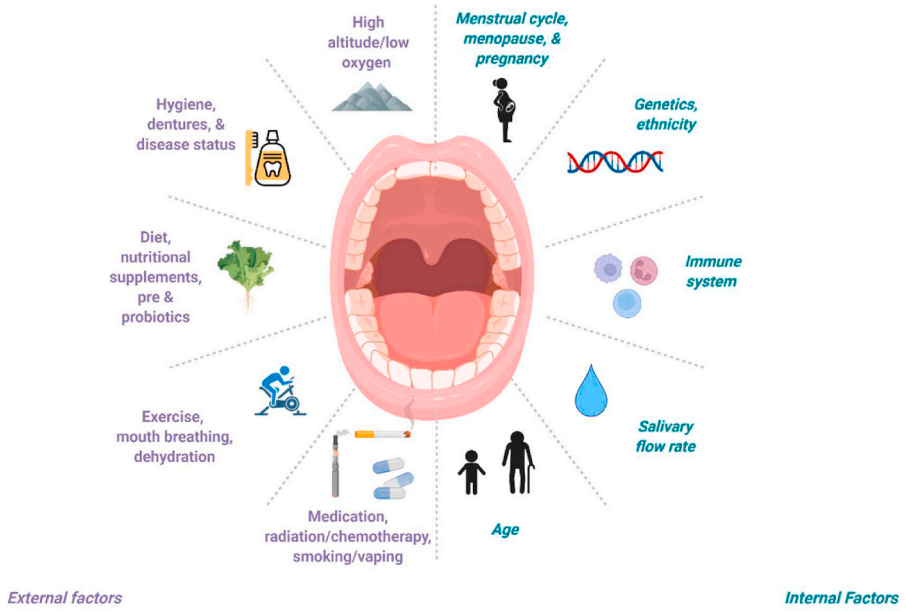

Fig. 2. Several external and internal factors are thought to influence the structure and function of the oral microbiome. External factors (originating outside the oral cavity) are on the left of the schematic (purple text) and internal factors are on the right side (green italics). Created with BioRender.com.

nitrate supplementation [49,50]. These data have also been replicated *in vitro* where 6.5 mM of nitrate solution was shown to promote the proliferation of these species in a salivary biofilm [51]. These alterations to microbial composition are significant given that *Niesseria* and *Rothia* are key nitrate-reducing species, and they are associated with good oral health. It is plausible, therefore, that dietary-induced alterations to the oral microbiome may positively influence exercise performance by augmenting NO production. While this would support previous suggestions that chronic supplementation of dietary nitrate may be more likely to improve exercise performance than a single acute dose [52], a recent meta-analysis reported that the number of days of supplementation did not influence the ergogenic effect [40]. Indeed, there is emerging evidence to suggest that the abundance of *Rothia* responds rapidly (within 4 h) to acute nitrate supplementation [53].

It should be acknowledged that a high nitrate diet has previously been raised as a safety concern due to reports that it may have adverse effects on human health. From the mid-twentieth century, a high level of nitrate in drinking water was linked to cases of methemoglobinemia, a potentially fatal blood disorder in infants [54]. While more recent evidence is less conclusive that nitrate in drinking water is a significant risk factor in the development of methemoglobinemia, it does continue to be recognized as a possible of co-factor [55]. Diets high in potassium nitrate or sodium nitrite have been suggested to increase the risk of carcinogenesis [56], possibly to due to the formation of nitrosamines following ingestion. However, when nitrate is consumed from vegetable sources, nitrosation is inhibited by the high antioxidant content [57]. Indeed, while high nitrate diets, such as, Dietary Approaches to Stop Hypertension (DASH) diet well exceed the recommended acceptable daily intake for nitrate, the evidence overwhelmingly suggests they are cardioprotective and associated with lower cancer risk [58,59]. Furthermore, there does not appear to be any epidemiological evidence linking a high nitrate vegetable intake to cancer risk [60].

While there is a paucity of well-controlled experimental trials which report the effects of other specific dietary interventions on the oral microbiome, some findings are now beginning to emerge. Murtaza and colleagues [61] observed that a low carbohydrate, high fat (LCHF) diet decreased the relative abundance of *Haemophilus, Neisseria and Prevotella* spp. and increased the relative abundance of *Streptococcus* spp. in a cohort of elite endurance race walkers. This three-week dietary intervention also increased the rate of whole body oxygen utilization ($\dot{V}O_2$) and negated the performance benefits of an intensified exercise training programme [62]. While these effects are predominantly driven by a shift in substrate oxidation (from carbohydrate to fat), it remains a possibility that impaired NO production could be a contributing factor. There are some preliminary data to support this notion with one report (as yet unpublished) suggesting that a high fat diet impairs nitrate to nitrite reduction and high-intensity exercise performance [63]. Collectively, these data provide clear evidence that the oral microbiome is malleable to change via specific dietary interventions, but further research is required to decipher the implications for NO production and exercise performance.

5.2. Probiotics

Our increased understanding of the importance of the microbiome to health has resulted in an upsurge in research investigating ways to adapt the microbiome to harness its potential therapeutic benefits. Probiotics are defined as "*live microorganisms that, when administered in adequate amounts, confer a health benefit on the host*" [64] and are one method that has been widely investigated in this context [65]. To date, the vast majority of research has examined the clinical effects of probiotic strains which are known to colonize the gut such as *Lactobacillus* and *Bifidobacterium* species. Aside from altering the composition of the gut microbiome, myriad effects of probiotic supplementation have been reported which include an improved immune function [65], prevention of antibiotic-associated diarrhoea [66], reduced blood pressure, blood glucose and insulin resistance [67], and an improvement in total cholesterol and low-density lipoprotein levels [68]. A smaller number of studies have also explored the effects of probiotics on parameters of exercise performance. For example, Pugh and colleagues [69] reported that a multi-strain probiotic appears to reduce metabolic perturbations in skeletal muscle during marathon running, potentially due to better maintenance of gut integrity. Others have shown that probiotics can reduce the incidences of gastrointestinal issues during endurance exercise [70]. Nevertheless, it is not currently possible to unravel the precise mechanisms of action from this small number of studies.

Most liquid-based oral probiotics are unlikely to elicit measurable effects in the oral cavity as the microbiota do not survive gastric passage [71]. However, specific oral probiotics do exist in the form of lozenges and the effects on various oral and systemic conditions have been explored. The collective evidence demonstrates that probiotics may decrease oral pathogens, protect against tooth decay and improve oral health [72] although data from long term randomized clinical trials are lacking. To our knowledge, no previous study has reported the effects of oral probiotics on the nitrate-nitrite-NO pathway or on parameters of exercise performance. However, some patents have been filed which detail the creation of new probiotics to enhance this pathway, suggesting this is an emerging area of research development and commercial interest [73,74]. It is certainly conceivable that the introduction of a probiotic to the oral cavity could promote the growth of nitrate-reducing species and augment NO production via the nitrate-nitrite-NO pathway. Alternatively, probiotics based on other species may alter the metabolic activity of resident flora by producing bacteriocin like inhibitory substances such as dextranase and urease which will increase salivary pH [71]. An increase in salivary pH has also been reported following dietary nitrate supplementation and is thought to increase the proliferation of some species of nitrate-reducing bacteria [48]. Whilst promising evidence exists to support the potential benefit of altering the oral microbiome to improve performance and health it should be recognized that manipulation of an established microbial ecosystem via probiotics, diet or other interventions is complex and may have unintended consequences [75]. Robust clinical studies are clearly required to determine the effect of specific interventions.

5.3. Antiseptic mouthwash

Anything that disrupts the oral microbiome and its bacterial communities will disrupt NO production with potential negative impacts on cardiovascular health and exercise performance. For the past 15 years, strong evidence has accumulated revealing that using antiseptic mouthwash disrupts the oral microbiome and as a result, disrupts NO production. Kapil and colleagues demonstrated that use of antiseptic mouthwash for 7 days caused a decrease in salivary and plasma nitrite with a concomitant increase in systolic and diastolic blood pressure [76]. Others have shown that mouthwash inhibits an increase in salivary and plasma nitrite from nitrate ingestion [77] demonstrating that killing oral bacteria attenuates the NO-dependent biological effects of dietary nitrate. Bescos and colleagues [78] recently showed that 7-days of Chlorhexidine altered the oral flora resulting in a more acidic oral environment and lower nitrite availability in healthy individuals. Furthermore, studies have demonstrated that even weaker strength mouthwashes lead to lower saliva [79–81] and plasma nitrite [79,80] levels in healthy volunteers. While these authors did not perform metagenomic sequencing, it is reasonable to assume this disruption to the oral microbiome suppressed nitrate reduction.

Over 180 million Americans use mouthwash on a daily basis and in 2015 alone, approximately 269 million antibiotic prescriptions were dispensed from outpatient pharmacies in the United States, enough for five out of every six people to receive one antibiotic prescription each year [82]. With this high prevalence of antibiotic and antiseptic mouthwash use in the US it is possible that over half of the population

has a limited capacity to reduce dietary nitrate. This means that although they may be consuming what is considered a healthy diet with sufficient nitrate, they are unable to benefit from increased NO production due to lack of nitrate reduction by bacteria. In addition, there is emerging evidence that mouthwash use may be related to increased antimicrobial resistance and cross resistance to antibiotics [83], however, a full discussion of this issue is beyond the scope of this review.

Rinsing with antiseptic mouthwash prior to a treadmill run has also been shown to impair post-exercise muscle oxygenation, likely due to a reduced NO production and consequent effects on vasodilation [84]. This presents the intriguing possibility that disruption of the oral microbiome with antiseptic mouthwash may have deleterious effects on exercise performance in addition to negatively impacting population health via increased incidence of hypertension [85]. What is not clear at present is exactly how long the microbiome takes to restore to its baseline structure and function following mouthwash use. There is some limited evidence that nitrate reducing function is restored 3-days after mouthwash use [86], however, the exact timescale has not been confirmed and metagenomic analysis has not been performed to examine community dynamics in the recovery phase. While these data could help to inform strategic timing of mouthwash use around competition schedules, it is important to note that most mouthwashes are recommended for daily use and, at this dosing frequency, incidence of hypertension, increased mortality in hospital settings and impaired nitrate reduction are observed [77,85,87–89].

6. Athlete oral health

Although the effects of exercise are overwhelmingly positive for both physical and mental health, it is now apparent that elite athletes often have poor oral health and are at higher risk of common oral diseases such as caries and periodontitis [90]. This surprising phenomenon cannot be attributed to compromised oral hygiene as athletes have been shown to partake in normal oral health-related behaviors [91]. Whilst the precise pathogenesis has yet to be established, suggested underlying causes include a high habitual carbohydrate intake, a compromised immune-inflammatory response from exercise-induced immune suppression, frequent dehydration and reduced salivary flow rate resulting from demanding training regimes [92]. It is certainly well-established that sugar fermentation carried out by acidogenic bacteria is the causative factor in development of dental caries. For the athlete, suboptimal oral health may have a devastating effect on training and competitive performances, either due to direct pain or indirect effects such as increased systemic inflammation or psychosocial impacts [92]. Nevertheless, these consequences are difficult to prevent in the competitive athlete due to ergogenic effects of carbohydrate rich diets and intense training schedules. There is, therefore, a clear unmet need for preventative strategies to address this issue.

Emerging evidence suggests that dietary nitrate supplementation may combat poor oral health via prebiotic actions. Given the reported ergogenic effects of nitrate, this dietary intervention may be doubly appealing to athletes. As previously described, dietary nitrate supplementation consistently results in modifications to the composition of the oral microbiome [48–50]. Importantly, these adaptations include a proliferation of bacterial species that are associated with good oral health and a reduced abundance of species that are commonly associated with oral diseases such as periodontitis and caries [93–98]. Nitrate supplementation also increases the pH of saliva [48], likely because saccharolytic caries associated species degrade carbohydrates from the diet with strong acids produced as bi-products [99]. These findings are highly relevant given that a salivary pH sustained below 5.5 will result in de-mineralisation of the teeth [100]. Recent work in our lab has also demonstrated that a single dose of nitrate-rich beetroot juice attenuated the decline in salivary pH that followed ingestion of a carbohydrate-rich sport drink [101]. These effects were observed both before and after an extended bout of exercise. Thereafter, Rosier and colleagues published data which sheds light on the mechanisms that underpin these effects. In this eloquent study, the authors demonstrated that nitrate can acutely limit acidification when sugars are fermented, which appears to result from lactate usage by nitrate-reducing bacteria [53].

There is extensive evidence to support the notion that a nitrate-rich diet can help prevent oral disease. Doel and colleagues [22] reported that children with a higher abundance of nitrate-reducing bacteria such as *Veillonella atypica* in the oral cavity were better able to reduce nitrate and were significantly less likely to experience caries. In agreement, it has also been shown that adults and children with higher salivary nitrite have a lower incidence of caries [102,103]. Although nitrate has been associated with positive effects on markers of oral health, very few studies have explored whether this is an effective intervention to treat oral disease. Nevertheless, the preliminary evidence is encouraging. Jockel-Schneider and colleagues showed that two-weeks of nitrate-rich lettuce juice increased the abundance of *Rothia* and *Neisseria* [93] and decreased gingival inflammation in periodontal patients [104]. Unlike treatment with antibiotics or mouthwash, this intervention appears to treat the root cause of the disease but also preserve the entero-salivary nitrate-nitrite-NO pathway. It must be acknowledged, however, that while nitrate shows some early promise as a prebiotic to support the oral health of athletes, these effects (and the consequent effects on training and performance) require exploration in prospective clinical trials.

7. Perspectives and future direction

Despite the exponential growth in the study of the microbiome, our understanding of the symbiotic relationship between bacteria and the human host is embryonic. What is well understood is that the actions of nitrate-reducing bacteria in the oral cavity are essential for NO production and, as a consequence, these microbiota play a defining role in maintaining cardiovascular health. As we begin to understand more about the important role of NO in the regulation of muscle blood flow, muscle contraction, and metabolic function it also seems likely that there is an inextricable link between the oral microbiome and exercise capacity. There is, therefore, much value in further exploration of how the microbiome influences acute exercise performance and the response to training.

It is noteworthy that much of the research on the oral microbiome has been undertaken using metagenomics techniques that enable the abundance of bacterial species to be calculated in any given environment. While these data reveal how the relative composition of the microbiota shift in response to various stimuli, less is known about how the *activity* of the bacteria respond. This is crucial to advance our understanding of how diet, probiotics, exercise and other interventions influence the oral microbiome and to establish the mechanisms by which downstream effects on health and exercise performance are attained. Research using multi-omics techniques coupled with *in vivo* assessments of NO bioavailability and muscle function would certainly provide more extensive insight in this field.

It also remains to be established whether some interventions that induce changes to the oral microbiome could elicit either ergolytic (e.g. antiseptic mouthwash) or ergogenic (e.g. probiotics) effects and influence the adaptations to exercise training. In the case of mouthwash, these data would be particularly informative to athletes who may use this treatment in a misguided notion that is beneficial to their health. Additional data on how probiotics affect the acute and chronic responses to exercise would also stimulate further interest in this area and potentially advance the development of commercially-available products. Lastly, there is an urgent need to find low cost, safe, effective treatments for oral diseases, particularly in elite athletes. Given the promising findings in clinical groups, a randomized controlled trial to determine the effects of nitrate supplementation on the oral health status of athletes is clearly warranted.

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