Modulation of oral microbiota: a new frontier in exercise supplementation

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Abstract

Research on the human microbiome has flourished over the last decade due to the essential role that bacterial communities have in host physiology and health. Recent studies have indicated that exercise is an important modulator of the composition and activity of the gut microbiome. However, microbiota is present throughout the whole gastrointestinal tract, the oral cavity being the first place where a large community of microorganisms is found. Recent evidence has established the first link between the oral microbiome and the physiological response to exercise, which opens a new way to explore host-microbiota interactions. The oral cavity is essential for many other physiological processes such as food digestion, the sensory experience of eating and palatability and immunity. Within the oral cavity, saliva is a key element for regulating all these functions as well as to protect the oral environment providing a lubricating mucoid secretion with organic and inorganic constituents. Here, we review what is known about the potential link between exercise and the oral microbiome including its role in oral health. We also discuss the effect of diet and the impact of pre and probiotics on the oral microbiome and their potential ergogenic effect.

Key words

Exercise; saliva; oral microbiome; oral health; diet

Abbreviations

ADS: arginine deiminase pathway

ATP: adenosine triphosphate

Ig A: immunoglobulin A

IL-6: interleukin-6

1. Introduction

Research over the last two decades has elucidated the key role of exercise for the prevention and management of the majority of the pathophysiological states including metabolic, immune and mental diseases, and cancer (1). The health benefits of exercise are mediated by multiple mechanisms, but, there is growing evidence suggesting that, at least part of these benefits are associated with changes on the composition and activity of bacteria colonizing the gut in response to physical stress (2). These changes on the gut microbiome seem to be related to positive changes on the metabolic and immune response of the human host (3-5). However, although the majority of research on exercise has been focussed on the gut microbiome, it is now evident that large communities of bacteria are present throughout the gastrointestinal tract (6). The oral cavity is the first place where large communities of microorganisms are found within the gastrointestinal tract, harbouring the second most complex microbiome in the body after the gut (7). The oral cavity is not a homogenous environment for the resident microbiota as offers distinct habitats (sub-gingival and supra-gingival plaque, keratinised gingiva, palate, buccal mucosa, throat, tonsils, tongue, saliva), and each habitat provide a specific set of environmental conditions and nutrients, resulting in a highly distinct set of species within each tissue site (8). Consequently, research investigating the oral microbiome is growing rapidly to gain more understanding regarding the role of oral bacteria in oral and general health (8, 9).

In addition to the microbial community, the oral cavity is essential for many other physiological processes such as physical and chemical digestion of food, the sensory experience of eating and palatability and the body's defence against infectious agents (10). Saliva is a key element for regulating all these functions, as well as protecting the oral environment to provide a lubricating mucoid secretion with organic and inorganic constituents (11). Furthermore, saliva contributes to the colonisation of microorganisms in the oral cavity and shapes the composition of the resident microbiota (12). Thus, the main goal of this review article is to evaluate the available evidence on the impact of exercise on the oral cavity, with especial emphasis on

saliva and the oral microbiome. We also discuss the effect of diet and the impact of pre and probiotics on the oral microbiome and their potential ergogenic effect.

2. Saliva production and composition

About 90% of saliva is produced by the three paired major salivary glands (parotid, submandibular and sublingual), and the remaining 10% by several minor salivary glands located in the oral mucosa (labial, palatine, buccal and lingual glands) (12). A normal flow of saliva is important to ensure lubrication of the teeth and oral mucous membranes however, the flow rate of saliva is dependent on multiple factors, such as the type and size of gland which is activated, hydration and nutritional status and gender as well as the time of the day (11). Under healthy and resting physiological conditions, the mean unstimulated whole salivary flow rate is between 0.3–0.4 ml/min (13). An unstimulated whole salivary flow rate of < 0.1 ml/min is considered pathologically low and designated hyposalivation (14). Overall, in healthy people the total volume of saliva secreted per day is between 0.5–1.0 litres, including saliva stimulated by food and drink consumption (12, 15).

The composition of saliva is also dependent on the flow rate, but under normal physiological conditions, water (99%) is its main constituent (16). The fluid characteristics of saliva are essential for the mechanical rinsing of the oral cavity, for dissolving taste substances and transporting them to taste receptor sites, protection of the taste buds, food bolus formation, clearance of food debris and microorganisms, and facilitation of mastication and swallowing as well as speech (11, 15, 17). Saliva contains an small fraction (1%) of solid constituents including electrolytes such as sodium, chloride, potassium, calcium, magnesium, phosphate and bicarbonate, and proteins such as proline-rich proteins, α-amylases, mucins, cystatins, histatins, statherin and host defence peptides (18, 19) . Importantly, the organic and inorganic fractions of saliva can supply information about alterations in enzymatic activity in response to oral disease (20). Saliva also contains a large amount of bacteria (100 million bacterial

cells/millilitre), forming complex communities of microorganisms with different properties (12). Salivary components are the primary nutritional source for these microorganisms and are required for the development of a balanced microbiome (19). A large number of salivary components, including secretory immunoglobulin A (Ig A), lactoferrin, lactoperoxidase, lysozyme, statherin and histatins, directly and indirectly regulate the microbiome, keeping it in balance (8). Traditionally, oral bacteria have been associated with the risk of oral disease, but this view has substantially changed over the last few years (9). Current approaches, using genomic, proteomic and metabolomics techniques to identify and quantify the microorganisms in saliva, have revealed a much more complex ecosystem than previously appreciated, with some of these bacteria playing a key role on the health of the human host. These two recent reviews provide more detail about the basic composition and function of human saliva (12, 19).

3. Saliva composition and exercise

Saliva production and composition may vary substantially according to different stimulus, exercise being a key modulator of both factors. Part of the effects of exercise on saliva are mediated through the autonomic nervous system but this may also depend on the type, intensity and duration of exercise (21, 22). The major salivary glands are innervated by sympathetic as well as parasympathetic nerves, with sympathetic signalling being the most predominant during exercise (23). Increased sympathetic activity can lead to an increase in salivary protein expression and more mucoid saliva (23), with transient changes in other compounds such as cortisol, lactate and electrolytes (23).

Regarding salivary proteins, a wide consensus exists indicating that exercise, especially at high intensities (> 70% VO_{2max}), raises the concentration of α-amylase, which is considered a marker of evoked sympathetic stimulation (21, 24). Recent research has also shown that exercise increases the concentration of other salivary proteins from the cystatin and mucin

families (25, 26). On the other hand, acute exercise can reduce the concentration of some salivary proteins such as Ig A (23, 27). This response has been commonly associated with an 'open window' theory, suggesting that the host protection is compromised over the first period of exercise recovery due to lower count of immune proteins (e.g. Ig A) (28). This may provide an opportunity for viruses and bacteria to trigger opportunistic infections (28). However, while this refers to an acute response to exercise, the chronic response with regards to salivary Ig A levels and immune function, may differ. A study by Akimoto et al (29) indicated that an exercise intervention at moderate intensity for a year was effective to increase salivary Ig A levels in older people. Additionally, exercise can reduce the risk of infections by increasing salivary cystatins levels since they inhibit the binding of bacteria to buccal epithelial cells and bacterial growth (30, 31).

4. The saliva microbiome

The oral cavity harbours the second most complex microbiome in the body, after the gut (7), which has an active role in physiological, nutritional and defensive development of the human host (8). The oral microbiome also faces challenges that are not experienced by the gut microbiome, since the host has the option to maintain good oral hygiene. Oral care procedures such as tooth brushing, tongue scrapping, flossing and oral rinses may have a large impact on colonising oral bacteria since they can reduce the formation of biofilms (32), which are aggregates of mixed species that can growth on different surfaces of the oral cavity. The oral cavity is not a homogenous environment for the resident microbiota as offers distinct habitats (sub-gingival and supra-gingival plaque, keratinised gingiva, palate, buccal mucosa, throat, tonsils, tongue, saliva) that do not suit all the microorganisms (33). For example, the microbial composition in saliva seems to be similar to the microbial profile in the tongue and buccal mucosa, but, it does not correlate with the dental plaque (supra and sub-gingival microbiome) (34). From a technical view, the collection of dental plaque samples requires good sampling skills and adequate equipment compared to saliva sampling (35). Consequently, saliva

sampling is more common for studying the oral microbiome because it is easier, non-invasive and cheaper than dental plaque, and it provides a more general view of the microbiome. Additionally, it allows the measurement of the salivary flow rate, which is an important factor to consider in regards to oral health (17). However, we must keep in mind that the best approach to have a general view of the oral microbiome is to collect and analyse samples from different habitats including dental plaque. Furthermore, it is important to pay attention to the method of sampling. For example, there are different methods for the collection of saliva such as mouth rinses, oral swaps and spitting methods (stimulated and non-stimulated) that can lead to variations in the final results as indicated in a study comparing four different types of mouth rinses (36). Spitting methods (stimulated and non-stimulated saliva collection) seem more reliable since they show similar bacterial profiles (35, 37), however, we recommend that one consistent oral collection method should be used for all oral microbiome comparisons to make studies more reliable. This review will focus on the saliva microbiome given our experience on this, but, as indicated above, further research is needed looking at different oral habitats and other microorganisms colonising the mouth such as fungi and viruses.

Firmicutes are the most abundant phyla and *Streptococci* the main genus from this family in saliva (33). At the species level *S. mitis*, *S. oralis*, *S. salivarius* and *S. sanguinis* are the most abundant from this genus (Figure 1) (33). Bacteroidetes are the second most abundant phyla being *Prevotella* the main genus of this family of bacteria found in healthy saliva, with *P. melaninogenica* as the main species (Figure 1). Firmicutes and Bacteroidetes comprise over 70% of the total amount of bacteria in saliva from healthy humans, with Firmicutes such as the species *Streptococcus mutans* (tooth decay) and Bacteroides, such as the species *Porphymonas gingivalis* (gum disease) associated with some oral diseases. Then, Actinobacteria, Proteobacteria and Fusobacteria are the next most abundant phyla. Figure 1 shows the main genus and species of these phyla that are presented in human saliva.

The composition and activity of oral bacteria is particularly important because it can instigate both oral and systemic diseases. Within the oral cavity, commensal bacteria turned

opportunistic bacteria are known to cause many diseases or conditions, such as dental caries (tooth decay), gingivitis, periodontitis (gum disease), peri-implantitis, endodontic infections, and tonsillitis (38). Regarding the link between oral and systemic disease, teeth damage and epithelial damage in the mouth may facilitate the entry of oral bacteria into the bloodstream. Then, bacteria may harm blood vessels or cause clots by releasing toxins that resemble proteins found in artery walls or bloodstream (39). Importantly, several species of oral bacteria such as *Porphyromonas gingivalis* (40, 41) have been found in atheroma plaques (40, 41), and have also been linked to the synthesis of amyloid-beta protein in the brain, which is the component of amyloid plaques in the brain responsible for Alzheimer's disease (42). Other species of *Streptococcus such as S. mutans*, which is associated with caries, has also been found in the brain of people who had a stroke (43). *Streptococcus gordonii* have also been linked to infective endocarditis, which is a rare but life-threatening disease (44). *Fusobacterium nucleatum* species from the oral cavity are also considered an important promoter factor on colorectal cancer (45). Thus, a healthy oral ecosystem is essential to avoid the leak of oral bacteria into the bloodstream that can seriously harm systemic health due to excessive inflammatory reaction, immunosuppression of host, anti-apoptotic activity or secretion of carcinogens (46).

Research performed over the last decade is providing unprecedented knowledge to understand the role oral bacteria in human health (8, 47). For instance, it is now recognised that, oral bacteria are a key driver of the nitrate/nitrite/nitric oxide pathway. This is an alternative pathway of nitric oxide synthesis in the body and it is dependent on the presence of nitrate-reducing oral bacteria in the mouth (48). Nitric oxide is a gaseous molecule involved in a vast array of physiological functions such as vasodilation, neurotransmission and immunity (49, 50). Oral bacteria can reduce inorganic nitrate that is ingested in food or endogenously produced in the body into nitrite (51). Importantly, the human body lacks specific and effective nitrate reductase enzymes so bio-activation of nitrate to nitrite relies on oral bacteria (52-55), and some species in the gut such as *Escherichia coli*, within the

Proteobacteria phylum, that have been also reported to have the ability to reduce nitrate into nitrite (56). Once nitrite is swallowed, it is rapidly absorbed across the upper gastrointestinal tract (51) to the bloodstream where it can be reduced back to nitric oxide by several enzymes and proteins (48). This oral nitrate/nitrite/nitric oxide pathway seems to be essential to enhance vasodilation and to keep blood pressure within normal physiological ranges (57). The vasodilator effects of inorganic nitrite have been shown in studies infusing nitrite intravenously and reporting a rapid blood-pressure lowering response (58). Other studies (53, 59, 60), but not all (54, 61), inhibiting oral nitrite synthesis with antibacterial mouthwash, reported an increase in blood pressure under resting conditions. We have also shown that the nitrate-reducing activity of oral bacteria is essential to reduce the blood pressure after a single bout of exercise in healthy people, which is a common physiological response known as post-exercise hypotension (55). Furthermore, the capacity of oral bacteria to convert nitrate into nitrite may be essential to sustain oral health by reducing oral acidity (62). In regards to the potential link between dietary nitrate consumption, particularly in water, and the risk of cancer, although this has been described in literature for over 40 years, it is still an open debate (63). Interestingly, the main dietary source of inorganic nitrate are vegetables (64), yet there is a lack of evidence indicating a link between the consumption of high-nitrate vegetables and cancer (65). According to this, other dietary factors such as lower vitamin C intake (and probably a lack of other antioxidants), in combination with higher nitrate/nitrite intake from non-vegetable sources, could be the main risk factors of cancer (66).

5. Exercise and the oral microbiome

Research on exercise and the human microbiome has mainly focussed on gut bacteria, with several studies reporting different compositions and activities of the gut microbiome in trained individuals compared to sedentary ones (4, 5, 67-73). These changes have been related to greater production of short-chain fatty acids (butyrate) in the gut and better cardiovascular fitness (69, 73, 74). More recently, research has revealed that endurance exercise stimulates

the gut bacteria to metabolize exercise-induced lactate to propionate, and this seems to be associated with better exercise capacity (75, 76).

In contrast to the gut microbiome, research on exercise and the oral microbiome until recently has been largely unexplored. In attempt to address this gap, we have recently performed two studies investigating the effects of exercise on the nitrate-reducing activity of oral bacteria, with regards exercise performance and cardiovascular health (55, 77). In the first study, we determined that the nitrate-reducing activity of oral bacteria was positively correlated with cardiovascular fitness of healthy people (77). This finding may suggest a modulatory effect of exercise on the composition and/or activity of the oral microbiome, in order to enhance nitric oxide availability under different physiological conditions. However, a key limitation of our study was the lack of genomic analysis that did not allow us to determine whether higher oral nitrite synthesis related to higher abundance and/or activity of nitrate-reducing species of oral bacteria. Although a recent study by Kapil et al. (78) found that higher production of oral nitrite was more likely to be related to higher activity of nitrate-reducing oral bacteria, than abundance of them, in healthy women.

In the second study, we found that the nitrate-reducing activity was essential to reduce the blood pressure after a single bout of exercise in healthy people, but this was not associated with changes in the abundance and composition of the oral microbiome (55). However, we could not exclude the possibility of changes in the oral microbiome occurred because we used antibacterial mouthwash with chlorhexidine to inhibit the nitrate-reducing activity of oral bacteria. Importantly, a key limitation of the 16S rRNA gene sequencing is that it is not able to differentiate between live and dead bacteria, and there was a short time frame between the sampling (about 3 hours). We have observed significant changes in the abundance and nitrate-reducing activity when the same mouthwash was used for a week (24). Future studies combining meta-omics approaches, including 16S rRNA gene sequencing, metagenomics, metatranscriptomics and metaproteomics are thus now needed, not only to better understand

the impact of exercise on the oral microbiome, but to determine the composition and activity of oral bacteria under different physiological situations.

We believe that chronic exercise may promote positive changes in the composition and activity of the oral microbiome, much like studies on the gut microbiome have reported (4, 5, 67-73). Both microbiomes seem to be very well linked and it has been suggested that about 45% of the bacteria of the large intestine and the oral cavity overlap (79). This may be related to saliva and food ingestion. About 0.75 to 1.0 litres of saliva are swallowed daily in healthy people (19). The ingested saliva contains a large amount of oral bacteria that are destroyed in the acidic environment of the stomach. However, those bacteria tolerating the harsh pH of the stomach can reach and proliferate within the gastrointestinal tract (80).

There are several exercise-induced mechanisms that can potentially re-shape the oral microbiome, for instance, alterations of the acid/base balance (4). Exercise at moderate-high intensities also elicits changes on salivary ions such as sodium (Na^+), potassium (K^+) and hydrogen (H^+) in response to sympathetic stimulation, which leads to transient changes on salivary pH (26, 81). Furthermore, exercise also increases lactate concentration in saliva, as we and others have found, after a single bout of exercise at high intensity (23, 77). The traditional view of lactate as a metabolic residue has substantially changed over the last few years, and it is now evident that lactate plays a key role in delivery of oxidative and gluconeogenic substrates, as well as cell signalling (82). Furthermore, lactate is a major source of carbon and energy source for some bacterial species (83). A recent study by Scheiman et al (75) found that serum lactate produced during exercise was metabolized by gut bacteria producing propionate, a short chain fatty acid that can be used to provide energy to muscle cells (75).

Other exercise-induced mechanisms suggested as modulating the gut microbiome, and probably exercise performance, with potentially similar effects on the oral microbiome, are synthesis of anti-inflammatory myokines such as interleukin-6 (IL-6), increased body temperature and epithelial permeability and changes in blood flow distribution during exercise

(2). Last but not least, despite a large body of evidence, research tends to be focussed on the modulation of the human microbiome through lifestyle factors such as exercise. The relative contribution of the host genetics may be also important in shaping the body's microbiome, particularly in heterogeneous populations such as elite athletes (84). However, further research is needed to elucidate the contribution of environmental and genetic factors on the human microbiome with regards exercise performance as well as human health.

6. Exercise and oral health

Current knowledge about the effect of exercise on oral health is controversial. Data from observational studies suggests that elite athletes have similar or even greater prevalence of oral disease, including dental erosion, dental caries and periodontitis compared to the general population (85). However, the methodology of previous studies (cross-sectional) have considerable limitations for deriving the causal relationships between exercise and oral disease. It is likely that a greater prevalence of oral disease in elite athletes may be related to secondary factors such as diet and hydration (85, 86). For instance, current dietary sport guidelines recommend the consumption of a diet rich in carbohydrates including products with high content of sugars such as energy bars and sport gels, because glucose is the main energy fuel used by muscles during high intensity exercise (87).

With respect to dental caries, carbohydrates are fermentable and provide a substrate for bacterial growth of *Streptococcus mutans*, which in turn produce acid, leading to tooth decay if athletes do not have good oral hygiene habits (88-90). Sports drinks may also further increase the risk of dental caries and erosion due to their low pH (< 5.5) and sugar content, which may promotes demineralization of the enamel, although current literature on this is conflictive (91). Exercise-induced dehydration is another factor that may increase the impact of carbohydrates on caries and acidic drinks on erosion by reducing salivary flow, and therefore, impairing the protective properties of saliva (89).

Periodontitis is a chronic multifactorial inflammatory disease causing progressive destruction of the soft tissues and bone surrounding teeth, ultimately leading to tooth loss (92). High prevalence of moderate-severe periodontitis (> 40%) has been reported in a recent study in British elite athletes (n = 352) from different sports despite the majority of them reported positive oral health-related behaviours such as brushing their teeth at least twice daily and using additional methods for oral hygiene such as using dental floss and interdental brushes and fluoride mouthwash (93). Although this study linked these results to some detrimental dietary habit such as high sugar and sport drink consumption, it did not take into account other important factors such as the use of oral care products containing antimicrobial compounds. From this viewpoint, we have recently found that the use chlorhexidine mouthwash for a week promotes the abundance of acidogenic oral species causing a reduction of salivary pH (24), which is a risk factor of periodontitis. Over-the-counter use of different type of mouthwashes is common in the general population (e.g. to reduce halitosis), but there is a lack of research investigating the potential adverse effects of the long-term daily use of these products and especially those containing antibacterial ingredients. Importantly, recent studies have linked the use of oral mouthwash with increased risk of diabetes, hypertension and greater mortality rates in hospitalized patients (59, 94, 95). Thus, further attention is needed in regards to the use of antibacterial products by athletes as they may have detrimental effects on their oral health at long term.

Other exercise-induced factors that may have a detrimental effect on oral health are drying of the mouth due to high airflow during activities such as cycling and running that may reduce the protection of the teeth (85, 86). Some athletes also report frequent vomiting as a result of pre-competition anxiety, again creating decreased oral pH and potentiating acid erosion of enamel (85, 86).

On the other hand, and in contrast to the view linking exercise to oral disease, there is also evidence suggesting that exercise is protective against oral problems (96-102). This may be mediated through different mechanisms. For instance, by enhancing the production of anti-

inflammatory peptides (myokines) in the muscle (103). Myokines seem to play a key role to strengthen the immune-metabolic response to exercise through the release of humoral factors capable of interacting with other distal tissues (104). Exercise also stimulates the secretion of adrenal hormones cortisol and adrenaline owing to the activation of the sympathetic nervous system (103). Cortisol is known to have potent anti-inflammatory effects (105). Furthermore, exercise may also modulate the composition and activity of oral bacteria by promoting changes in the acid/base conditions of the oral environment as indicated previously (23, 92). Exercise-induced lactate may be another factor that can stimulate changes in the composition and activity of the oral microbiome, as has been shown in the gut (75). Increased nitrite availability has been also suggested as another protective factor of the mouth because it may help to decrease biofilm formation and plaque accumulation by susceptible species (106).

The role of the oral cavity on exercise performance has also been reported in studies using carbohydrate rinses. Several of these studies (107-111), but not all (112-114), found that the presence of carbohydrates in the mouth, without swallowing, was effective at enhancing cardiovascular performance in healthy people. This interesting effect has been related to the activation of different brain regions through taste transduction pathways, linked to a central mechanism that controls the recruitment of motor units during exercise (108). However, the role of oral bacteria on these taste receptors and central mechanism is not understood. This is interesting since a potential interaction between gut bacteria and brain has been described by means of neural, endocrine, immune and humoral links (115), but, this has not been explored with regard to the oral microbiome. From this viewpoint, recent studies have shown that the oral microbiome of patients with neurodegenerative diseases such as Parkinson's and Alzheimer's diseases differ to the microbiome of healthy individuals (116, 117). However, whether this a cause or consequence of these conditions it remains to be determined. Importantly, exercise can reduce the risk of developing neurodegenerative disease by almost 50%, as well as, slow down its progression in patients that already have it (118, 119). New studies are required to clarify whether these benefits are mediated, at least partially, by

changes in the composition and activity of oral bacteria. A better understanding about the potential axis between dynamic activity of oral bacteria and brain function may provide fundamental knowledge for treating neurological diseases such as Alzheimer's and Parkinson's disease more efficiently in the future.

7. The impact of dietary compounds and pre/probiotics on the oral microbiome

Diet is suggested to play a fundamental role in shaping the human microbiome, but research has mainly focussed in the effect of diet on the gut microbiome (120). In attempt to address this gap, we have investigated whether two different dietary patterns, including a plant-based diet and an omnivore diet, were related to a different composition and nitrate-reducing activity of oral bacteria (54). We did not find differences between people following these dietary patterns for at least a year. In agreement with this, other studies have reported no differences in the composition of oral bacteria between vegetarians and omnivores (121). Whilst these findings suggest a limiting effect of diet in changing the composition of the oral microbiome, some caution is needed at present, as other studies report changes in the composition of oral bacteria associated with some dietary compounds (122). It is also important to note that studies comparing vegetarian and omnivore diets may not be a good representation of the effect of diet on the microbiome if they matched the participants by physiological and metabolic factors because the inclusion of animal protein should not be necessarily associated with a less healthy dietary pattern and physiological status as we have recently shown (54). On the other hand, recent studies have indicated different compositions and abundance of oral bacteria, as well as higher prevalence of periodontitis, in subjects with obesity and type 2 diabetes, but they could not establish whether this was related to diet (123, 124). Thus, new studies are needed investigating more in depth the role of diet on the oral microbiome.

Recent studies have also shown that some dietary compounds are able to induce changes on the oral microbiome. For instance, the ingestion of concentrated beetroot juice, which is an

important source of polyphenols and inorganic nitrate, has been related to changes in the diversity and abundance of oral bacteria (125, 126). The composition of tap water has also been related to different oral microbial profiles (55). Regarding exercise and diet, a recent study reported that a diet rich in fat (ketogenic diet) was associated with reductions in the relative abundance of bacteria within phyla Bacteroidetes and Proteobacteria and increased levels of Firmicutes in elite walker athletes, compared to a diet rich in carbohydrates (127). Interestingly, these oral microbial changes were accompanied by a reduction in exercise efficiency, which is a key factor in endurance performance (128). Perhaps, this detrimental physiological response may be explained by the lower formation of nitrite in the oral cavity induced by the reduction of Bacteroidetes and Proteobacteria. These phyla contain some of the main nitrate-reducing species as we and others have demonstrated (54, 129, 130). In agreement with this view, some studies have reported an improvement in exercise efficiency (low oxygen demands) after increasing circulatory levels of nitrite using dietary supplements of inorganic nitrate, but this has not been related to changes on the oral microbiome (131-133). Further research is needed to confirm the impact of diet on the composition and activity of oral bacteria with regard exercise performance and also cardiovascular health.

The use of oral products containing pre or probiotics has been suggested as a promising approach to modulate the oral microbiome in a way that can help to protect host health (134). Following this, a recent study using a probiotic containing Streptococcus (*S. thermophiles*) and *Lactobacillus* (*L. delbrueckii*, *L. bulgaricus*, *L. paracasei*) strains acutely found an increase in the overall diversity of the oral microbiome, but the structure of the microbiome did not change within 24 hours after the ingestion (135). Another study found that the use of a probiotic containing *Bifidobacterium* (*B. animalis* and *B. lactis*) strains for 30 days induced positive clinical outcomes that were related to changes in the oral microbiome and immunological markers in patients with periodontitis (136). Interesting findings were also reported by a study showing that a probiotic containing *Lactobacillus salivarius* decreased the expression of cancer cells and induced apoptosis in a dose-dependent manner in rats (137). Regarding

prebiotics, the amino acid Arginine, which is one of the main salivary components responsible for the control of pH, has shown promising effects to reduce the risk of caries in *in vitro* and clinical studies. In the oral cavity, Arginine is metabolized mainly by the arginine deiminase pathway (ADS) of certain oral bacteria to produce citrulline, ornithine, carbon dioxide, adenosine triphosphate (ATP), and ammonia (138). Ammonia production from arginine metabolism serves as a mechanism used by oral bacteria for maintaining a relatively neutral environmental pH that favours the persistence of ADS-positive (ADS^+) bacteria while being competitive against caries pathogens (139).

As indicated previously, a study by Scheiman et al (75) described a potential mechanism that can enhance exercise capacity through a pathway, where lactate produced during exercise in muscle cells is metabolized by *Veillonella atypica* in the gut, in turn producing propionate, a short chain fatty acid that can be used to provide energy to muscle cells. The specific identification of *Veillonella* as an exercise-enhancing microbe caught the attention of other scientists, as this genus is also essential in the oral nitrate/nitrite/nitric oxide pathway. This pathway is also involved in improving human exercise performance so it has been suggested that the modulation of this genus in the oral cavity through pre or probiotics may enhance exercise performance in humans (140). However this should be investigated more in depth by new studies investigation the role of *Veillonella* supplements in exercise performance.

8. Conclusion

The traditional view of oral bacteria contributing to oral disease has substantially changed over the last few years, with it now being accepted that the majority of bacteria colonizing the mouth are not only essential to sustain oral, but also general health. Exercise-induced physical stress may be promoting a ‘healthy’ and more diverse oral microbiome through different mechanisms. However, we are still in the infancy of this research, and further studies combining different laboratory approaches such as microbial gene sequencing,

metagenomics, metatranscriptomics and metaproteomics, are needed to decipher whether exercise protects against oral disease, and whether exercise helps to lower the risk of other systemic conditions, such as cardiovascular and neurodegenerative diseases, via shifts in the oral microbiome. Furthermore, new studies have to decipher whether the modulation of the oral microbiome using pre or probiotics may enhance exercise capacity in humans and those mechanisms involved.

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Figure Legends

Figure 1: Relative abundance of main phyla, genus and species in saliva of healthy people (original, non-published data from the authors).