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Effects of dietary nitrate supplementation on oral health and associated markers of systemic health: a systematic review

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ABSTRACT

Poor oral health can impact an individual's ability to eat and has been associated with an increased risk of non-communicable diseases. While the benefits of nitrate consumption on oral health were first proposed more than 20 years ago, no systematic review has been published examining effects of dietary nitrate on oral health. This systematic review investigated the effects of dietary nitrate on markers of oral health *in vivo* in randomized controlled trials (RCTs). Five databases (PubMed, The Cochrane Library, CINAHL, MEDLINE, and SPORTDiscus) were searched from inception until March 2023. Nine articles reporting data on 284 participants were included. Dietary nitrate was provided *via* beetroot juice in most studies. The duration of the interventions ranged from one day to six weeks. Dietary nitrate supplementation increased the relative abundance of several individual bacterial genera including *Neisseria* and *Rothia*. Dietary nitrate supplementation increased salivary pH and decreased salivary acidification following consumption of a sugar-sweetened beverage. Furthermore, dietary nitrate supplementation resulted in a decrease in the gingival inflammation index. The results of this systematic review suggest that dietary nitrate could represent a potential nutritional strategy to positively modify oral health by impacting the oral microbiome, altering salivary pH, and minimizing gingival inflammation.

Introduction

Dietary inorganic nitrate is a polyatomic ion abundant in green leafy vegetables (e.g., spinach, lettuce and rocket) and several root vegetables, including beetroot (Santamaria 2006). This compound is also found in herbs, certain fruits, legumes, grains, processed meat products, and in tap water. When dietary nitrate is ingested (e.g., in the form of a nitrate-rich food or supplement), it is absorbed in the intestines, enters the bloodstream, and then takes several pathways throughout the body (Blekkenhorst et al. 2018). While the kidneys excrete ~75% of nitrate, approximately 25% returns to the oral cavity *via* the salivary glands, where it is converted from nitrate into nitrite by nitrate-reducing anaerobic bacteria that principally exist on the dorsal surface of the tongue (Blekkenhorst et al. 2018; Milton-Laskibar, Alfredo Martínez, and Portillo 2021). Once swallowed, nitrous acid is formed in the stomach's acidic environment, which further decomposes to form nitric oxide (NO) and other nitrogen oxides (Blekkenhorst et al. 2018). A portion of the nitrite passes into systemic circulation, where it

can be further reduced to NO *via* various enzymatic and nonenzymatic pathways.

NO is involved in a range of processes in the body and consumption of dietary nitrate, by increasing NO bioavailability, can lead to a range of physiological changes which can positively impact health. For example, several randomized controlled trials have shown that dietary nitrate reduces blood pressure (Asgary et al. 2016; Bailey et al. 2009; Jackson et al. 2018; Jonvik et al. 2016; Webb et al. 2008) and, consequently, might be applied as a potential adjunct therapy alongside traditional antihypertensive medications (Griffiths et al. 2023). Furthermore, dietary nitrate supplementation has been found to decrease platelet aggregation (Siervo et al. 2018; Velmurugan et al. 2016), enhance endothelial function (Babateen et al. 2023; Velmurugan et al. 2016), and reduce arterial stiffness (Kim et al. 2014; Velmurugan et al. 2016).

It has been known for some time that the oral microbiome, which is the second most diverse microbial community in the human body and includes more than 700 known species, is crucial for the reduction of nitrate to nitrite in the body (Kilian

et al. 2016; Shapiro, Hotchkiss, and Roe 1991). Indeed, destroying the oral microbiome *via* rinsing with chlorohexidine mouthwash can block the conversion of nitrate into nitrite and, subsequently, NO, such that nitrate consumption does not impact downstream physiological processes (Babateen et al. 2019). Likewise, it has been known for some time that disturbances in the composition of the oral microbiome may lead to many diseases, including cardiovascular disease (Blekkenhorst et al. 2018). However, more recently, it has become apparent that the consumption of nitrate may also impact the oral microbiome and, potentially, other processes in the mouth. Early studies showed that salivary nitrate and nitrite levels were inversely associated with caries levels and the etiology of gingivitis (Allaker et al. 2001; Doel et al. 2004). It was suggested that increasing the consumption of vegetables rich in nitrate has an important role in promoting nitrate-reducing bacteria, which contributes to the beneficial effects on oral health (Doel et al. 2004). As such, in recent years, researchers have shifted their focus to examining the influence of nitrate on oral health markers, moving beyond the mouth's role as a nitrate processing site. This research has demonstrated the ability of dietary nitrate to affect the oral microbiota (Vanhatalo et al. 2018, 2021; Velmurugan et al. 2016), influence salivary pH (Hohensinn et al. 2016; Rosier et al. 2021), and potentially impact conditions such as halitosis (Rosier et al. 2022).

Although the impact of nitrate on oral health is attracting increasing attention, to our knowledge, no systematic review has been published examining controlled trials of the effect of dietary inorganic nitrate on oral health. However, this could be valuable to summarize current state of the research, identifying potential effect modifiers or responsive groups to nitrate supplementation (e.g., athletes or individuals who have oral health problems), as well as gaps for future research. Therefore, this study aims to examine the effects of dietary nitrate on *in vivo* markers of oral health in randomized controlled trials. As a secondary outcome, we will also explore how nitrate-related modifications in oral health impact systemic health parameters (e.g., blood pressure, arterial stiffness, and cognitive function), given the previously reported links between oral and systemic health in the wider literature.

Methods

Register and protocol

The current systematic review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines, and a study protocol was prepared before the initiation of the systematic research according to the PRISMA Protocol (PRISMA-P) guidelines (Page et al. 2021). The systematic review was registered prior to database searches with the International Prospective Register of Systematic Reviews (PROSPERO) on March 29, 2023 (CRD42023411159). This systematic review followed the pre-registration.

Search strategy

The selected studies were identified through a comprehensive systematic search without language restrictions from inception through to March 2023 in five databases: PubMed, The Cochrane Library, as well as CINAHL, MEDLINE, and SPORTDiscus *via* EBSCOhost. Search terms related to dietary nitrate, key nitrate-rich foods (e.g., beetroot, spinach and lettuce) and oral health markers were used, with MeSH terms utilized wherever appropriate (details of the search strategy for each database can be found in Supplementary Table S1). The search results were exported to Endnote and then to Covidence, an online systematic reviewing platform. Duplicate search results were excluded prior to screening retrieved articles.

Study selection

Three investigators (S.A., O.M.S. and A.W.) examined the titles and abstracts of the identified studies to evaluate eligibility for inclusion in the review, with each article independently screened by two reviewers. Potential studies that could not be excluded from the review based on an appraisal of title and abstract were carried over to the full-text stage of the review for evaluation. Eligible studies were subject to full-text evaluation by two investigators (S.A. and O.M.S.) to determine final eligibility for inclusion in the systematic review. Once qualified studies were identified, a manual review of the references in those studies was performed to identify other potentially relevant articles (S.A). An additional reviewer (K.B.) was consulted if a unanimous opinion could not be reached between two reviewers at any stage in the review.

Study inclusion/exclusion criteria were based on the use of participant, intervention, comparator, outcome and study design (PICOS) criteria, as follows:

Participants: Adult participants (≥ 18 years), irrespective of health status.

Intervention: Oral dietary nitrate in humans as a supplement, juice, vegetable, or other forms, e.g., nitrate salts.

Comparator: A low nitrate or nitrate-free intervention, as defined by the relevant study authors, as a comparison. When nitrate was provided in combination with another lifestyle, clinical or pharmacological intervention, a study was only eligible for inclusion provided that the study included a comparable, valid control group. For example, if nitrate was combined with exercise, the control group must undergo an identical exercise intervention to allow isolation of the effects of nitrate alone.

Outcome: Studies reporting findings regarding *in vivo* markers of oral health (e.g., oral microbiome, dry mouth, saliva pH, saliva flow, dental caries, periodontal disease and halitosis).

Study design: RCTs involving two or more arms (e.g., a comparison against a placebo condition with low nitrate or no nitrate) were included. Both cross-over and parallel group designs were eligible for inclusion.

Data extraction

Data extractions were performed by the first author (S.A.) using a pre-piloted data extraction sheet. The following data were extracted from studies that met the specified inclusion criteria: name of the author(s), year of publication, the country in which analysis was conducted, study design and

blinding, age, gender, number of participants, number of arms, type of dietary nitrate and placebo, duration of intervention, and dose. In addition, oral health markers (e.g., microbiome composition, salivary pH, gingivitis, saliva flow rate and lactate) and related systemic health markers (such as diastolic and systolic blood pressure, heart rate, arterial stiffness and cognitive health) were also extracted to explore whether changes in oral health with nitrate contribute to changes in systemic health. Where numerical data were missing (e.g., mean and standard deviation values), they were extracted from figures using digitization software (WebPlotDigitizer, Version 4.3). In addition, where required, the authors of the included papers were contacted for further information. Two investigators (O.M.S and A.W.) checked the data extraction for accuracy and made edits where required.

Risk of bias assessment

The risk of bias was assessed using the Revised Cochrane risk-of-bias tool for randomized trials (RoB 2) (Sterne et al. 2019). This tool focuses on five areas related to: (1) bias arising from the randomization process, (2) bias due to deviations from intended interventions, (3) bias due to missing outcome data, (4) bias in outcome measurement and (5) bias in the selection of the reported outcome. An overall bias score for each study was provided. The risk of bias was independently assessed by one researcher (S.A) and checked for accuracy by another (A.G).

Quality of evidence

The quality of evidence for the effects of dietary nitrate on oral health was appraised by two independent researchers (SA and OMS) using the Grades of Recommendation, Assessment, Development, and Evaluation (GRADE) framework (Guyatt et al. 2008). Conflicts were resolved by discussion between the two researchers. Domains considered in the GRADE appraisal include risk of bias, inconsistency of results, indirectness of evidence, imprecision of results, and publication bias.

Results

Search results

The initial literature search yielded 5,525 studies. Following the removal of 2,437 duplicates, 3,088 titles and abstracts were screened. 17 studies were identified as potentially suitable for inclusion, and full texts were retrieved for further evaluation. Finally, following a full text review, nine studies were considered eligible for inclusion. Figure 1 depicts an overview of the
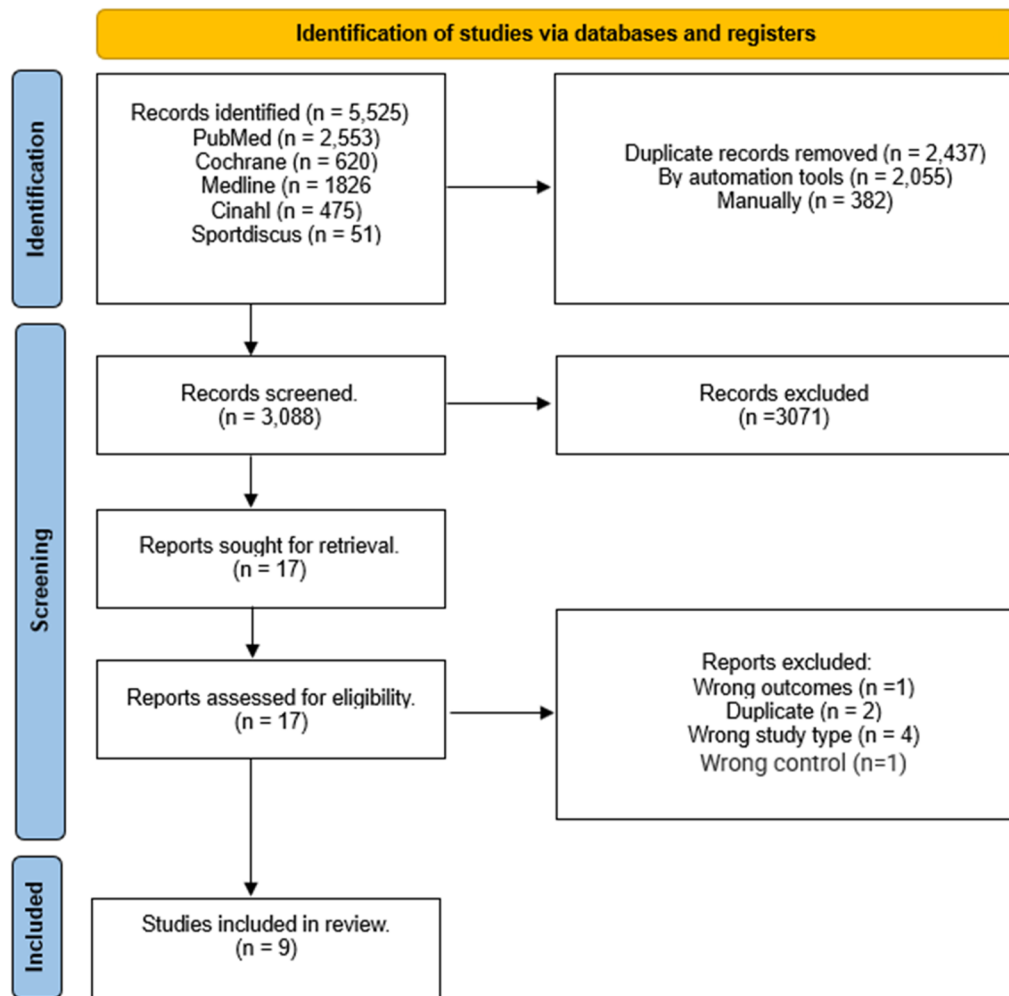

Figure 1. PRISMA Flow diagram of the studies included in this systematic review.

screening procedure, the study selection, and the main reasons for study exclusion at the full text appraisal stage.

Study characteristics

The systematic review included nine randomized controlled trials including a total of 284 participants (nitrate $n=185$, placebo $n=183$), aged 18–80 years, reporting the effects of dietary nitrate supplementation on markers of oral health (M. Burleigh et al. 2019; M. C. Burleigh et al. 2020; Hohensinn et al. 2016; Jockel-Schneider et al. 2016, 2021; Rosier et al. 2021; Vanhatalo et al. 2018, 2021; Velmurugan et al. 2016). The characteristics of all nine studies are presented in Table 1.

The majority of the studies focused on the effect of dietary nitrate on healthy participants with no oral health complaints ($n=6$) (M. Burleigh et al. 2019; M. C. Burleigh et al. 2020; Hohensinn et al. 2016; Rosier et al. 2021; Vanhatalo et al. 2018, 2021), while two studies were conducted on individuals with gingival inflammation (Jockel-Schneider et al. 2016, 2021) and another study on individuals with hypercholesterolemia (Velmurugan et al. 2016). Six studies used nitrate-rich beetroot juice as a nitrate source (M. Burleigh et al. 2019; M. C. Burleigh et al. 2020; Hohensinn et al. 2016; Vanhatalo et al. 2018, 2021; Velmurugan et al. 2016), two studies used nitrate-rich lettuce juice (Jockel-Schneider et al. 2016, 2021), and a nitrate-rich beetroot supplement dissolved in mineral water was used in one study (Rosier et al. 2021). Five studies were conducted in the United Kingdom (M. Burleigh et al. 2019; M. C. Burleigh et al. 2020; Vanhatalo et al. 2018, 2021; Velmurugan et al. 2016), two in Germany (Jockel-Schneider et al. 2016, 2021), one in Spain (Rosier et al. 2021), and one in Austria (Hohensinn et al. 2016).

The paper by Rosier et al. (Rosier et al. 2021) involved three independent studies. The first study was carried out using a nitrate-rich supplement containing dry beetroot extract, molybdenum, and vitamin C, and the second and third studies used beetroot extract containing nitrate without any other ingredients. However, only two studies were reported in this review (the first and third). The second study lacked a control group and, therefore, was not eligible for inclusion.

The duration of the interventions ranged from one day to a maximum of six weeks. Doses of dietary nitrate ranged from ~3.22 mmol/d to ~19.2 mmol/d. In most investigations, participants were instructed to avoid mouthwash use during the study (M. Burleigh et al. 2019; M. C. Burleigh et al. 2020; Hohensinn et al. 2016; Jockel-Schneider et al. 2016, 2021; Rosier et al. 2021; Vanhatalo et al. 2018, 2021). Three studies included smoking participants (Jockel-Schneider et al. 2016, 2021; Rosier et al. 2021), while smoking was an exclusion criterion in three other studies (Hohensinn et al. 2016; Vanhatalo et al. 2018, 2021), and the remaining three studies did not report any relevant information in this regard (M. Burleigh et al. 2019; M. C. Burleigh et al. 2020; Velmurugan et al. 2016).

The effects of dietary nitrate on the oral microbiome were investigated in six studies (M. Burleigh et al. 2019; Jockel-Schneider et al. 2021; Rosier et al. 2021; Vanhatalo et al. 2018, 2021; Velmurugan et al. 2016), while one study examined its impact on gingivitis (Jockel-Schneider et al. 2016), four studies on salivary pH (M. Burleigh et al. 2019; M. C. Burleigh et al. 2020; Hohensinn et al. 2016; Rosier et al. 2021), and one study on ammonium and lactate formation (Rosier et al. 2021). Five studies used unstimulated saliva (M. Burleigh et al. 2019; Rosier et al. 2021; Vanhatalo et al. 2018, 2021; Velmurugan et al. 2016), two studies used stimulated saliva (Jockel-Schneider et al. 2016, 2021) and one reported using both stimulated and unstimulated saliva samples (M. C. Burleigh et al. 2020). One of the included studies did not report whether the saliva samples were stimulated or unstimulated (Hohensinn et al. 2016). In addition, the links between oral and systemic health changes (e.g., blood pressure and cognitive function) were reported in two studies.

Principal findings

Oral health markers
Oral microbiome. Six studies investigated the effects of dietary nitrate supplementation on the diversity and/or relative abundance of the oral microbiome (Table 2). Various methods were used to collect oral microbiome samples. One study collected microbiome samples *via* a tongue dorsum swab (M. Burleigh et al. 2019); three collected them *via* saliva (Rosier et al. 2021; Vanhatalo et al. 2021; Velmurugan et al. 2016), and one collected them from periodontal pockets (Jockel-Schneider et al. 2021). One study used two different methods to measure the oral microbiome - *via* saliva at baseline and *via* a tongue swab at the endpoint. The intervention arm and placebo were then compared at the endpoint (Vanhatalo et al. 2018). Five studies assessed the oral microbiome using Illumina sequencing (M. Burleigh et al. 2019; Jockel-Schneider et al. 2021; Rosier et al. 2021; Vanhatalo et al. 2018, 2021). In contrast, one study used 454 pyrosequencing (Velmurugan et al. 2016). In one study, the Illumina sequencing method was used to evaluate relative abundance of bacteria, and the qPCR method was used to detect absolute abundance of *Rothia* bacteria (Rosier et al. 2021).

Diversity of oral microbiome. Of the six studies reporting on oral microbiome, four reported the effects of nitrate supplementation on the diversity of the oral microbial community. In contrast, two studies did not report the impact of nitrate on the diversity of the oral microbiome (Rosier et al. 2021; Velmurugan et al. 2016). The Shannon index was used as a measure of alpha diversity in three studies (M. Burleigh et al. 2019; Vanhatalo et al. 2018, 2021), and did not show a significant difference between the nitrate and placebo groups following supplementation. In a study by Jockel-Schneider et al. (Jockel-Schneider et al. 2021), species richness showed a significant decrease in the nitrate condition but no significant change in the

Table 1. Characteristics of the studies included in the systematic review of the effects of dietary nitrate on oral health.

Authors	Study Design	Country	Health Status	Sample Size	Age (Years)	Gender (M/F)	Duration	Type of dietary nitrate/placebo	Nitrate dose (mmol/day)	Saliva sample	Key Outcomes
(Velmurugan et al. 2016)	RCT, parallel, double blind	UK	Hypercholesterolemic patients	67	18-80	24/43	6 weeks	Beetroot juice / Depleted beetroot juice	~6.0	Unstimulated saliva	Oral microbiome, BP, FMD, HR, PWV, BAD, PMA level, P-selectin concentrations
(Vanhatalo et al. 2018)	RCT, crossover, double-blind	UK	Healthy	18	18-79	7/11	10 days	Beetroot juice / Depleted beetroot juice	~ 12.4	Unstimulated saliva	Oral microbiome, SFR-Q, BP, PWV
(Vanhatalo et al. 2021)	RCT, crossover, double-blind	UK	Healthy	30	70-80	13/17	10 days	Beetroot juice / Depleted beetroot juice	~ 19,2	Unstimulated saliva	Oral microbiome, SFR-Q, BP, CF,
(Hohensinn et al. 2016)	RCT, parallel, single blind	Austria	Healthy	46	18-35	NR	14 days	Beetroot juice / Depleted beetroot juice	~6.45	NR	Salivary pH
(Rosier et al. 2021) (study1)	RCT, crossover, blind	Spain	Healthy	12	25-60	5/7	one day	Nitrate rich beetroot-supplement dissolved in mineral water / Nitrate-poor placebo dissolved in mineral water	~4.03	Unstimulated saliva	Salivary pH, Lactate levels, Ammonium levels.
(Rosier et al. 2021) (study3)	RCT, crossover, blind	Spain	Healthy	6	25-33	3/3	one day	Nitrate rich beetroot-supplement dissolved in mineral water /Water	~3.54	Unstimulated saliva	Oral microbiome, Salivary pH, Lactate levels, Ammonium levels.
(Jockel-Schneider et al. 2016)	RCT, parallel, double blind	Germany	Gingival inflammation patients	44	46-77	16/28	14 days	Lettuce juice / Depleted lettuce juice	~3.22	Stimulating saliva	Gingival index, Plaque control record
(M. Burleigh et al. 2019)	RCT, crossover, single-blind	UK	Healthy	11	30±7 (mean)	All M	7 days	Beetroot juice / Depleted beetroot juice	~12.4	Unstimulated saliva	Oral microbiome, Salivary pH, BP, FMD, RHR
(M. C. Burleigh et al. 2020)	RCT, crossover, double -blind	UK	Healthy	11	30±7 (mean)	All M	one day	Beetroot juice / Depleted beetroot juice or Water	~12.4	Stimulating saliva/ Unstimulated saliva	Salivary pH, Saliva flow rate, HR
(Jockel-Schneider et al. 2021)	RCT, parallel, double blind	Germany	Gingival inflammation patients	37	46-77	16/28	14 days	Lettuce juice / Depleted lettuce juice	~3.22	Stimulating saliva	Oral microbiome

RCT; Randomized controlled trial, NR; Not reported, SFR-Q; Salivary flow rate questionnaire, BP; Blood pressure, FMD; Flow mediated dilation, RHR; Resting heart rate, HR; Heart rate, PWV; Pulse Wave Velocity, BAD; Brachial artery diameter, PMA level; platelet-monocyte aggregate, CF; Cognitive function.

Table 2. Results summary of randomized controlled trials investigating effects of the dietary nitrate supplementation versus placebo on diversity and relative abundance of the oral microbiome. An upwards arrow indicates a significant increase in the nitrate treatment versus placebo, a downwards arrow represents a significant decrease in the nitrate treatment versus placebo, and a horizontal arrow indicates no significant difference between nitrate and placebo.

Authors	Diversity	Phylum	Direction of change (nitrate vs. placebo)	Genus	Direction of change	Species	Direction of change	The type of abundance	Sample collection method
Burleigh et al. 2019	**Shannon index:** There was no significant difference between the groups before or after the intervention.	Bacteroidota	↔	Prevotella	↓	Prevotella melaninogenica	→	Relative	Via Tongue swab
		Bacillota	↔	Neisseria	↑	Actinomyces hyovaginalis	→		
		Fusobacteria	↔	Streptococcus	↓	Neisseria subflava	↑		
		Actinomycetota	↔	Actinomyces	→				
		Pseudomonadota	↑						
Jockel-Schneider et al. 2021[a]	**Alpha diversity:** A significant decrease was observed after the intervention in the intervention group in species richness compared to the baseline, while there was a non-significant increase in the placebo group. **Beta diversity:** The microbial makeup significantly changed in the nitrate condition.	NR	NR	Prevotella_7	→	NR	NR	Relative	Via Periodontal pockets
				Fusobacterium	→				
				Neisseria	←				
				Butyrivibrio_2	←				
				Rothia	↑				
				Treponema_2	→				
				Other	→				
Rosier et al. 2021 (study3)	NR	NR	NR	Capnocytophaga	↔	Prevotella salivae	↔	Relative/Absolute	Via Saliva
				Prevotella	↔	Rothia mucilagniosa	←		
				Neisseria	↔	Rothia dentocariosa	←		
				Haemophilus	↔	Corynebacterium durum	→		
				Veillonella	↔				
				Streptococcus	↔				
				Atopobium	↔				
				Rothia	←				
				Campylobacter	↔				
Vanhatalo et al. 2018	**Shannon index:** No significant difference in diversity were observed between Beetroot Juice and Placebo conditions.	Bacteroidota	→	Blattabacterium	←	Capnocytophaga ochracea	→	Relative	Via Tongue swab
		Bacillota	↔	Capnocytophaga	←	Prevotella melaninogenica	→		
		Fusobacteria	↔	Prevotella	→	Neisseria lactamica	←		
		Actinomycetota	↔	Neisseria	←	Neisseria meningitidis	←		
		Pseudomonadota	↑	Haemophilus	↔	Nitrosococcus halophilus	←		
				Blautia	←	Enterococcus faecalis	←		
				Enterococcus	←	Megaspaera elsdenii	→		
				Lactobacillus	←	Veillonella parvula	→		
				Megasphaera	→	Carnobacterium maltaromaticum	←		
				Veillonella	→	Clostridium difficile	→		
				Streptococcus	↔	Ruminococcus torques	→		
				Atopobium	←	Atopobium parvulum	←		
				Rothia	→	Rothia mucilagniosa	→		
				Spirochaeta	↔	Sanguibacter keddiei	→		
				Campylobacter	↔	Spirochaeta Africana	↔		
				Other	→	Other	→		

(Continued)

Table 2. Continued.

Authors	Diversity	Taxonomic composition						The type of abundance	Sample collection method
		Phylum	Direction of change (nitrate vs. placebo)	Genus	Direction of change	Species	Direction of change		
Vanhatalo et al. 2021	**Shannon and the Chao-1 index:** No significant difference in diversity was observed between Beetroot Juice and Placebo conditions.	Bacteroidota	→	NR	NR	Prevotella intermedia	→	Relative	Via Saliva
		Bacillota	→			Prevotella melaninogenica	→		
		Fusobacteria	→			Capnocytophaga orchracea	←		
		Actinomycetota	↔			Flavobacterium indicum	←		
		Pseudomonadota	←			Neisseria lactamica	←		
						Neisseria meningitidis	←		
						Nitrosococcus halophilus	←		
						Ornithobacterium rhinotracheale	←		
						Leptothrix cholodnii	←		
						Bacillus amyloliquefaciens	→		
						Ruminococcus bromii	→		
						Clostridium difficile	→		
						Megasphaera elsdenii	→		
						Ruminococcus torques	→		
						Veillonella parvula	→		
						Tetragenococcus halophilus	↔		
						Butyrivibrio proteoclasticus	→		
						Clostridium phytofermentans	→		
						Roseburia hominis	←		
						Variovax paradoxus	→		
						Atopobium parvulum	←		
						Rothia mucilaginosa	←		
						Phycisphaera mikurensis	←		
						Spirochaeta Africana	→		
Velmurugan et al. 2016	NR	NR	NR	NR	NR	Rothia mucilaginosa	↔	Relative	Via Saliva
						Neisseria flavescens	←		

[a]Denotes a significant difference from the baseline, NR; Not reported.

placebo condition ($p > 0.05$). Meanwhile, beta diversity (weighted UniFrac distances) was significantly different when compared before and after the nitrate, but not placebo, supplementation, reflecting a significant shift of the composition of the microbial communities within the nitrate group (Jockel-Schneider et al. 2021). It should be noted that Jockel-Schneider et al. (Jockel-Schneider et al. 2021) did not compare alpha or beta diversity between nitrate and placebo conditions, and just conducted within group (i.e., pre-to-post test) comparisons. In both investigations by Vanhatalo et al. (Vanhatalo et al. 2018, 2021), the use of non-metric multidimensional scaling (NMDS), an analytic method to evaluate microbiome similarity between conditions, revealed a significant difference in the oral microbial community post-supplementation between the nitrate and placebo conditions (Vanhatalo et al. 2018, 2021)

Phylum. Three studies explore the effects of dietary nitrate supplementation on the relative abundance of bacteria at the phyla level: all three studies reported a significant increase in *Pseudomonadota* (formerly *Proteobacteria*) (M. Burleigh et al. 2019; Vanhatalo et al. 2018, 2021). A decrease in *Bacteroidota* (formerly *Bacteroidetes*) was seen in two studies (Vanhatalo et al. 2018, 2021) with nitrate supplementation. One study reported a decrease in *Fusobacteria* and *Bacillota* (formerly *Firmicutes*) with nitrate supplementation (Vanhatalo et al. 2021), while *Actinomycetota* (formerly *Actinobacteria*) was unchanged in three studies (M. Burleigh et al. 2019; Vanhatalo et al. 2018, 2021) (Figure 2).

Genus. Four studies examined the effects of dietary nitrate supplementation on the relative abundance of bacteria at the genus level. Nitrate supplementation significantly affected the abundance of 19 genera of bacteria (Figure 3), particularly *Neisseria, Rothia, Prevotella* and *Streptococcus. Neisseria* was one of the genera most commonly impacted *via* nitrate interventions, with its relative abundance increasing significantly in three studies. Vanhatalo et al. (Vanhatalo et al. 2018) observed a significant increase compared to the control condition, and Jockel-Schneider et al. (Jockel-Schneider et al. 2021) reported an increase compared to the baseline. A crossover study by Burleigh et al. (M. Burleigh et al. 2019) documented an increase in the relative abundance of *Neisseria* in both arms of the trial; nevertheless, the increase was significantly higher in the nitrate supplementation arm compared with the placebo arm. In contrast, no significant change in relative abundance of *Neisseria* was observed in one study by Rosier et al. (Rosier et al. 2021).

Two independent investigations (Jockel-Schneider et al. 2021; Vanhatalo et al. 2018) demonstrated a significant increase in the relative abundance of *Rothia*. Rosier et al. (Rosier et al. 2021)also reported an increase in absolute abundance of *Rothia* by qPCR four hours after nitrate supplementation compared with water.

Prevotella abundance decreased after dietary nitrate interventions in two of the three independent studies which measured this genera. Vanhatalo et al. (Vanhatalo et al. 2018) reported a statistically significant decrease of 60%. In similar results, Burleigh et al. (M. Burleigh et al. 2019) found a decrease in the intervention group (from 34% ± 17% to

Phylum

Figure 2. Summary of findings from randomized controlled trials exploring the effect of dietary nitrate on the relative abundance of different bacterial phyla. The vertical axis depicts the measured phyla from the included studies, while the horizontal axis illustrates the total number of studies that assessed each phylum separately. Green bars represent a significant increase, red bars represent a significant decrease, and grey bars represent no significant change.
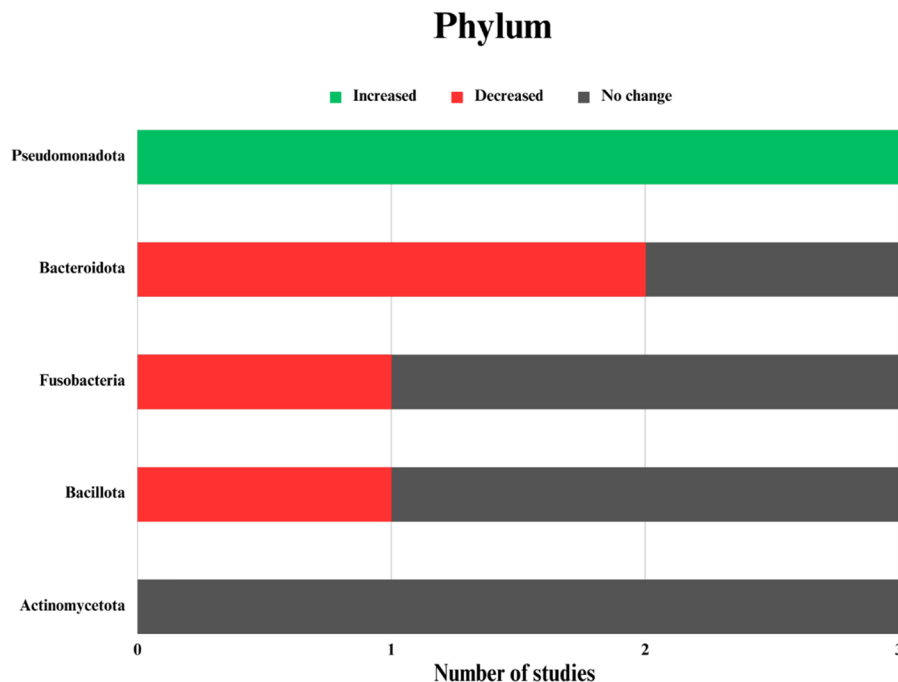
Genus

Figure 3. Summary of findings from randomized controlled trials exploring the effect of dietary nitrate on the relative abundance of different bacterial genera. The vertical axis depicts the measured genera from the included studies, while the horizontal axis illustrates the total number of studies that assessed each genus separately. Green bars represent a significant increase, red bars represent a significant decrease, and grey bars represent no significant change.
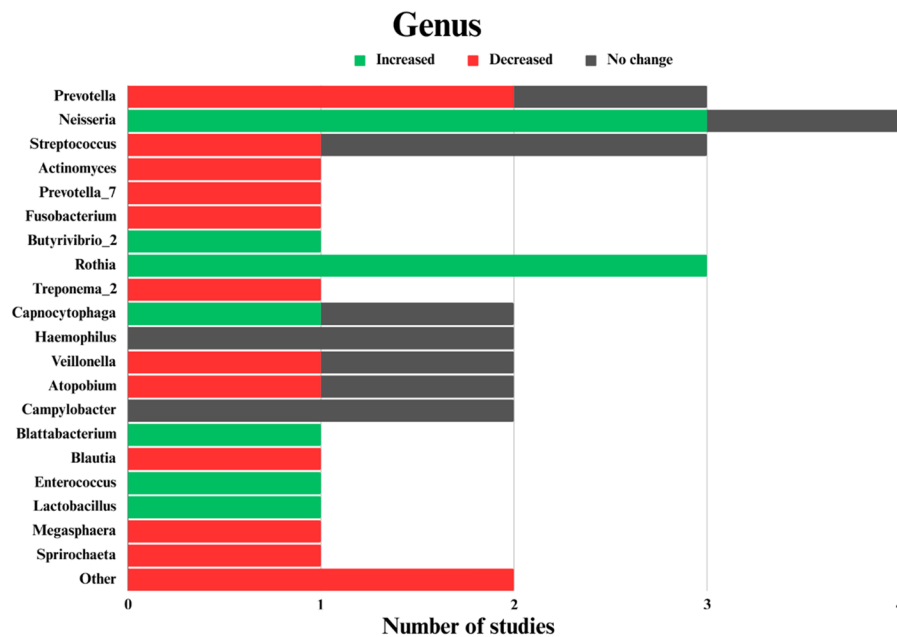
23% ± 11%), while there was an increase in the control group (from 26% ± 16% to 31% ± 14%). In contrast, Rosier et al. (Rosier et al. 2021) recorded no changes in *Prevotella*.

Regarding *Streptococcus,* one of the trials demonstrated a significant decrease in the relative abundance of this bacterial genus in the nitrate group (from 9 ± 6% to 6 ± 4%), while showing an increase in the control group (6 ± 4% to 8 ± 3%), which consumed nitrate-depleted beetroot juice (M. Burleigh et al. 2019). However, there was no significant change in relative abundance of *Streptococcus* in the two other studies (Rosier et al. 2021; Vanhatalo et al. 2018).

Vanhatalo et al. (Vanhatalo et al. 2018)observed a significant decrease in the relative abundance of *Veillonella* by 65%; however, its abundance did not change in another study (Rosier et al. 2021). The impact of nitrate supplementation on the relative abundance of other bacteria is presented in Table 2. Typically, the extent (%) of the change was not reported by authors, and therefore only direction of change is reported.

Species

At the species level, five studies provided data on the abundance of 36 bacterial species which were affected by nitrate supplementation, with the most commonly reported species being *Prevotella melaninogenica, Rothia mucilagniosa, Neisseria lactamica, Neisseria meningitidis*, and *Nitrosococcus halophilus* (Figure 4). An analysis of the 16S rRNA gene has been used to classify the bacteria in all studies; this is considered an exploratory analysis for the purpose of this review, given potential risk of misclassification (Hiergeist et al. 2023). Three independent randomized studies (M. Burleigh et al. 2019; Vanhatalo et al. 2018, 2021) demonstrated a significant decrease in the relative abundance of *P. melaninogenica*

12%, 67%, and 56%, respectively, after nitrate supplementation. In one of the studies, although the abundance of *R mucilagniosa* increased in the nitrate group versus placebo, it was not significant (Velmurugan et al. 2016). Vanhatalo et al. (Vanhatalo et al. 2018) reported a 234% increase in the relative abundance of *R mucilagniosa* with nitrate supplementation. Another study recorded an increase of 259% (Vanhatalo et al. 2021). This was also supported by Rosier et al.(Rosier et al. 2021), who found a significant increase in the relative abundance of *R.mucilagniosa* after nitrate ingestion. Vanhatalo et al.(Vanhatalo et al. 2018) documented an increase in the relative abundances of *N. lactamica, N. meningitidis,* and *N. halophilus* after nitrate ingestion. Consistent with this finding, another study of older adults by the same authors reported a statistically significant increase in the relative abundance of these species by 175%, 305%, and 115%, respectively, after nitrate ingestion (Vanhatalo et al. 2021). In contrast, the relative abundance of *Veillonella parvula* decreased in two studies by 65% and 63%, respectively, along with other species, including *Ruminococcus torques, Atopobium parvulum,* and *Clostridiodes difficile* (formerly *Clostridium difficile*) (Vanhatalo et al. 2018, 2021).

Salivary pH, metabolites and flow rate

Four studies investigated the effects of dietary nitrate supplementation on salivary pH (M. Burleigh et al. 2019; M. C. Burleigh et al. 2020; Hohensinn et al. 2016; Rosier et al. 2021). One of these studies (Rosier et al. 2021) also evaluated the effects of dietary nitrate on salivary metabolites (lactate and ammonium) in two independent trials, and another study examined the effects of dietary nitrate on stimulated and unstimulated salivary flow rates (M. C. Burleigh et al. 2020). Table S2 in the supplemental materials summarizes the impact
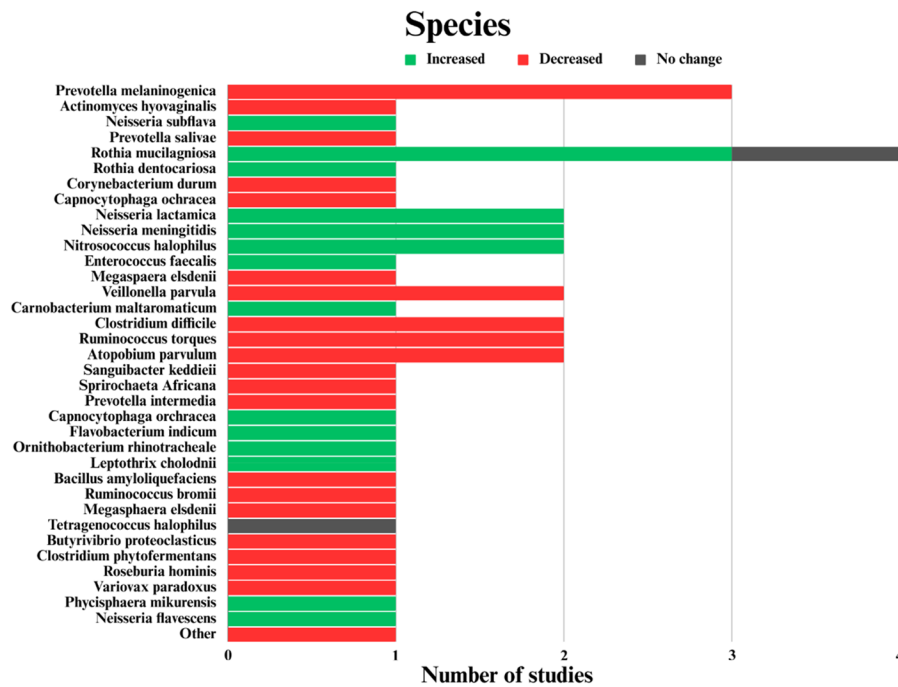
Figure 4. Summary of findings from randomized controlled trials exploring the effect of dietary nitrate on the relative abundance of different bacterial species. The vertical axis depicts the measured species from the included studies, while the horizontal axis illustrates the total number of studies that assessed each genus separately. Green bars represent a significant increase, red bars represent a significant decrease, and grey bars represent no significant change.

of nitrate supplementation on salivary pH, metabolites and salivary flow rate from these studies.

In the crossover study conducted by Burleigh et al. (2019), there was a significant increase in salivary pH following 7-days nitrate supplementation (baseline: 7.13 ± 0.54, post-nitrate: 7.39 ± 0.68) whereas salivary pH was unchanged in the placebo condition (baseline: 7.23 ± 0.17, post-placebo: 7.00 ± 0.28). Likewise, another study showed a significant increase in salivary pH levels with nitrate supplementation, with salivary pH reaching ~7.5 on the 7th and the 15th days of supplementation (Hohensinn et al. 2016). However, during a 4-week observation phase, which was conducted after the initial 2-week period of supplementation and during which nitrate supplementation was withdrawn, salivary pH returned to baseline levels (~7.0).

In an acute supplementation study by Burleigh et al. (2020) in which nitrate was provided 1 h prior to each experimental trial, there was no change in salivary pH in a group of athletes ($n = 11$) after consumption of nitrate-rich beetroot juice (M. C. Burleigh et al. 2020). However, when participants subsequently ingested a carbohydrate beverage, nitrate supplementation prevented the decline in salivary pH which was otherwise apparent in the placebo condition (i.e., it mitigated the carbohydrate-dependent salivary acidification).

These findings were similar to those reported by Rosier and colleagues in their study, which included two independent experiments. In the first experiment, despite the significant decrease in salivary pH in both arms following sucrose rinse, pH dropped 0.23 points less when using the supplement rich in nitrate compared with the placebo (Supplementary Table S2) (Rosier et al. 2021). Salivary pH levels were positively correlated with salivary nitrate

concentrations ($r = 0.436$, $p < 0.05$). Lactate production was also negatively correlated with salivary nitrate concentrations ($r = -0.508$, $p < 0.05$). In the second experiment, despite the trend toward a smaller post-sucrose decrease in salivary pH in the nitrate supplement arm, it was not statistically significant compared to the control group that consumed water (Rosier et al. 2021). Less lactate was formed after 4 h of nitrate ingestion compared to the control group. The researchers in this study did not find a significant increase in salivary ammonia in any of their clinical studies.

One study investigated the effects of nitrate supplementation on stimulated and unstimulated salivary flow rates (M. C. Burleigh et al. 2020). This study included four experiments. Participants consumed different liquids an hour before the experiment, either water (positive and negative control), beetroot juice, or placebo beetroot juice. Then, the participants consumed a carbohydrate supplement before, during, and after exercise, except for the negative control, who consumed water instead of the carbohydrate supplement. The stimulated salivary flow rate decreased significantly after exercise in positive control and placebo trials. In contrast, there was no significant decrease in the stimulated salivary flow rate in the nitrate trial, indicating that nitrate-rich beetroot juice might play a role in maintaining saliva production during exercise (Supplementary Table S2). In comparison, there were no significant variations in unstimulated salivary flow rates before and after exercise in any trial.

Impact on gingival inflammation

One study that involved periodontal recall patients with mild-to-moderate chronic gingivitis examined the effect of 14-days nitrate supplementation on gingival inflammation, as
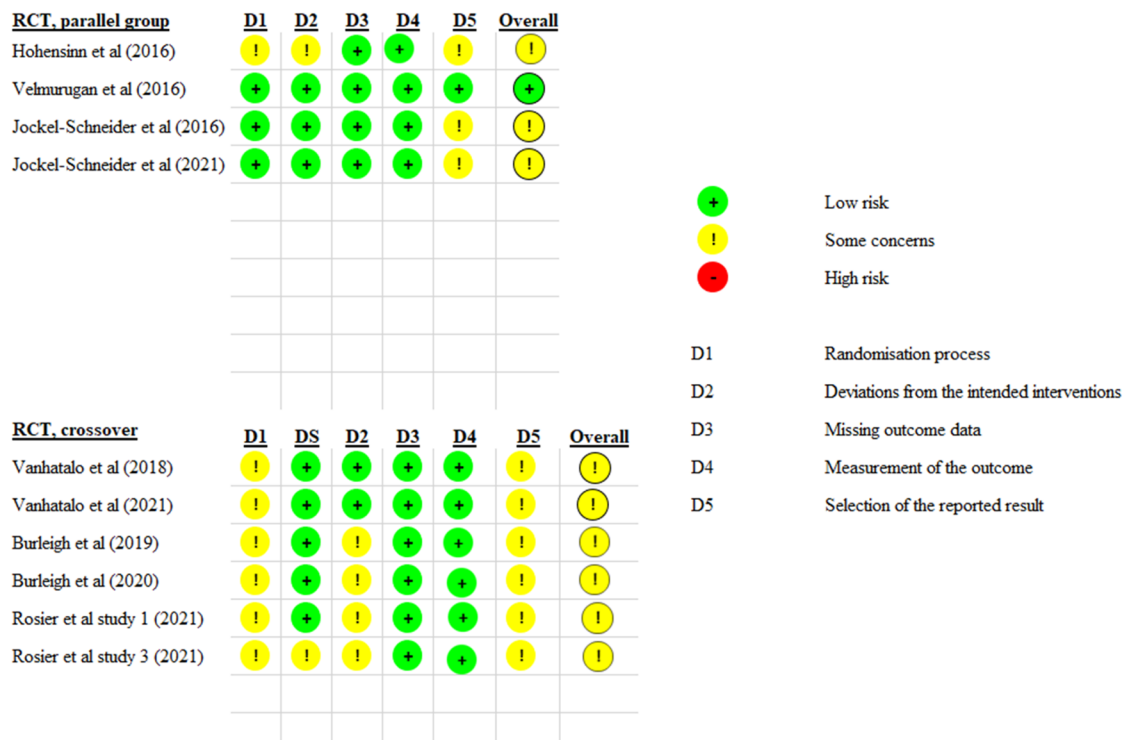
Figure 5. Findings of the bias risk assessment for the randomized controlled trials included in this systematic review.

determined by a gingival inflammation index which involves a visual assessment on the buccal aspect of all teeth (Jockel-Schneider et al. 2016). All patients received periodontal supportive treatment before being assigned to either the intervention or the placebo arm. Dietary nitrate supplementation resulted in a significantly greater decrease in the gingival inflammation index compared with placebo. However, plaque coverage on the teeth – evaluated using a plaque control record – did not differ significantly between conditions.

Impact on associated health outcomes

Several studies examined how nitrate-related modifications in oral health impacted markers of systemic health (Supplementary Table S3), including two studies which reported associations between the changes in the oral microbiome and health markers (Vanhatalo et al. 2018, 2021).

Blood pressure

In the study by Vanhatalo et al. (2021), nitrate supplementation significantly decreased systolic blood pressure compared with placebo (124 mmHg ± 14 vs. 129 mmHg ± 14), with no significant change in diastolic blood pressure or mean arterial pressure. Interestingly, there was a significant inverse correlation between microbiome module MM6 (*Rothia mucilaginosa* and *Streptococcus*), which was sensitive to nitrate supplementation, and mean arterial blood pressure (r=−0.44).

Pulse wave velocity

In the study conducted by Vanhatalo et al. (2018), there was no significant difference in pulse wave velocity (PWV)

(9.52 m/s ± 8.32 vs. 8.09 m/s ± 5.79) between the trial conditions overall. When analyses were divided by age, it was shown that pulse wave velocity increased in older participants (4.69 m/s) and decreased in younger participants (-6.86 m/s) following nitrate supplementation. In this study, pulse wave velocity was positively correlated with the relative abundance of *Micrococcales* (r=0.48), *Rothia* (r=0.45), *R. mucilaginosa* (r=0.41) and *campylobacter concisus* (r=0.42).

Cognitive function

Vanhatalo et al. (2021)reported a significant decrease in in the number of errors in the Rapid Visual Information Processing test (RVP) test for sustained attention (124 ± 14 vs. 129 ± 14) after 10 days of nitrate supplementation compared with placebo. However, performance in other cognitive tests (Stroop test, number recall and serial subtractions, choice-reaction time) was no different between nitrate and placebo conditions. Moreover, there was a significant correlation between the MM5 microbiome module (*Neisseria* and *Haemophilus*) and reaction time in the information processing cognitive function test (r=−0.39).

Risk of bias assessment

Overall, the risk of bias in studies was mixed (Figure 5). One study had a low risk of bias overall (Velmurugan et al. 2016), while the rest were rated as having some bias concerns. The main reasons for this evaluation were insufficient information about the randomization procedure (70% of the studies), the effectiveness of the blinding process (40% of

the studies), and the absence of a predetermined and published protocol (90% of the studies). No study was considered to have a high risk of bias.

Quality of evidence

GRADE assessments suggested that the quality of evidence for all oral health outcomes was low (Supplementary Table S4). There were some concerns over the risk of bias for all outcome measures. We judged there to be serious indirectness for all outcomes except for gingival inflammation given most studies included healthy participants (rather than those with oral health complaints, which may be a more relevant population) and outcomes may reflect indirect markers of oral health which could be of less relevant in practice to dentists/clinicians. We judged there to be serious imprecision for all outcomes, due to the small number of studies exploring each outcome and the typically low sample size of these investigations ($n = 6$ to 67). Although there were more studies ($n = 6$) focusing on the oral microbiome, not all investigations looked at the same bacteria, which increased the risk of imprecision.

Discussion

This study is the first review to systematically evaluate the effects of dietary nitrate supplementation on oral health in adults. The results of the studies in this review showed that dietary nitrate supplementation affected the abundance of individual genera and species of bacteria in the oral cavity. Moreover, dietary nitrate appeared to reduce the acidification of saliva and decreased gingival inflammation.

The current review found that dietary nitrate interventions had consistent effects on the abundance of some individual genera and species. At the genera level, three trials revealed an increase in the relative abundance of *Neisseria* (M. Burleigh et al. 2019; Jockel-Schneider et al. 2021; Vanhatalo et al. 2018). Similarly, *Rothia* abundance increased in three trials (Jockel-Schneider et al. 2021; Rosier et al. 2021; Vanhatalo et al. 2018). Although species have slightly different sequences and multiple copies of the 16S rRNA gene, which may make their classification difficult (Hiergeist et al. 2023), this may be driven by an increase in the relative abundance of several different species within these genera, such as *Neisseria lactamica* and *Neisseria meningitidis*, which each increased in two studies after nitrate supplementation, and *Neisseria flavescens* and *Neisseria subflava*, which each increased in one study. Furthermore, *Rothia mucilaginosa* increased in three studies and *Rothia dentocariosa* in one study. *Prevotella* was the most prominent genus that decreased following nitrate supplementation (M. Burleigh et al. 2019; Vanhatalo et al. 2018), which appeared to be driven by a decrease in the relative abundance of two species, namely *Prevotella melaninogenica* in three studies and *Prevotella subflava* in one study.

Rothia, *Neisseria*, *Prevotella* and *Veillonella* have been demonstrated to contribute toward nitrate reduction in the oral cavity (Doel et al. 2005; Hyde et al. 2014; Sato-Suzuki et al. 2020). Therefore, dietary nitrate may play an important role in affecting oral health through these bacteria by increased abundance of some nitrate reducers (e.g., *Rothia* and *Neisseria*) and decreased abundance of others (e.g., *Prevotella*). It is possible that these bacteria respond differently to nitrate supplementation because of the impacts of nitrate on salivary pH (nitrate supplementation typically raised salivary pH) (Hohensinn et al. 2016). For example, *Prevotella* thrives in an acidic environment in the oral cavity (pH ranges 5.5–6) (Kianoush et al. 2014). According to several investigations, a higher abundance of *Rothia* is associated with better oral health, being free of dental caries (Agnello et al. 2017; Baker et al. 2021), and possessing healthy gums (Feres et al. 2021). In addition, a higher abundance of certain *Rothia* species, specifically *R. mucilaginosa*, has been observed in halitosis-free individuals (Carda-Diéguez et al. 2021a).

Several studies have presented contradictory findings concerning the relationship between *Neisseria* species and dental health. Certain studies indicate that a higher abundance of *Neisseria* species' is associated with dental caries (Agnello et al. 2017; Zheng et al. 2018). However, other studies suggest its association with caries-free individuals (Asakawa et al. 2018; Qudeimat et al. 2021; Zhang et al. 2021). It is important to highlight that *Neisseria* appears in early dental plaque and then disappears as the plaque matures (Takeshita et al. 2015). Therefore, these contradictory results may be due to different sampling time points, techniques, or definitions of caries across studies.

In contrast, the abundance of *Prevotella* (which typically decreased across studies consequent to nitrate ingestion), especially *P. melaninogenica*, has been positively associated with dental caries (Agnello et al. 2017; Baker et al. 2021; Qudeimat et al. 2021) and halitosis (Carda-Diéguez et al. 2021b). However, while the increased abundance of some *Prevotella* species is not associated with dental caries (e.g., *Prevotella melaninogenica*), other *Prevotella* species are indeed more abundant in the presence of caries (e.g., *Prevotella amnii*) (Wang et al. 2019). Further studies using more advanced sequencing approaches (e.g., metagenomics) would be valuable to provide more granular insight into the impact of nitrate at the species level.

Interestingly, nitrate interventions did not increase the relative abundance of *Veillonella* or *Streptococcus* in studies included in this review. Several investigations have reported an increased relative abundance of these genera and their species, especially *Streptococcus mutans*, among individuals with dental caries compared with healthy individuals (Baker et al. 2021; Qudeimat et al. 2021; Xu et al. 2014).

Despite reasonably consistent effects of nitrate supplementation on the relative abundance of specific bacterial genera/species, which exemplifies the ability of nitrate to help 'shape' the community of bacteria in the oral cavity, there was no consistent influence on the oral microbial community diversity compared with placebo. Differences in findings between studies could relate to between-study differences in methodology (e.g., sampling site, analytic approach, supplement provided, participant cohort).

One of the factors affecting oral health is the pH of saliva (Widmer 2010). In this review, nitrate prevented a decrease in salivary pH after carbohydrate consumption or sucrose rinses (M. C. Burleigh et al. 2020; Rosier et al. 2021), while increasing resting/basal salivary pH in two other studies following nitrate supplementation (M. Burleigh et al. 2019; Hohensinn et al. 2016). Among the studies that investigated nitrate's effect on the acidity of saliva, two studies reported an increase in the relative abundance of *Neisseria* and *Rothia*, respectively (M. Burleigh et al. 2019; Rosier et al. 2021). Low salivary pH is associated with dental caries, as tooth enamel is demineralized at a pH of 5.5 or less (Widmer 2010). Moreover, lower salivary pH values have been reported with periodontitis, and it increases after treatment (Lăzureanu et al. 2021). Another one of the studies in this review demonstrated a direct effect of dietary nitrate intake on improving gum health in gingivitis patients after two weeks compared to baseline and it did not record any improvement in the placebo group (Jockel-Schneider et al. 2016). This improvement could be related to adjusting the salivary pH; however, this was not measured in the study. Dietary nitrate seems to inhibit the acidification of saliva, along with changes in the relative abundance of nitrate-reducing bacteria, such as *Neisseria* and *Rothia*.

The possible mechanism is that nitrate-reducing bacteria, such as *Rothia* and *Neisseria*, during nitrate reduction, use lactate as an electron donor and carbon source (Rosier et al. 2021), thus reducing the acidic environment in the oral cavity. This was demonstrated in the results of *in vitro* and clinical studies conducted by Rosier et al. in which lactate production decreased with nitrate supplementation while preventing a decrease in salivary pH and increased *Rothia* abundance (Rosier et al. 2021). Similar findings were also reported in another *in vitro* study by these authors, which showed the same results in addition to an increase in the abundance of *Neisseria* (Rosier et al. 2020). Increased nitrate intake was negatively associated with lactate production and positively associated with salivary pH and the relative abundance of *Neisseria* and *Rothia* (Rosier et al. 2021). Dental caries among individuals is associated with increased lactate production (Shimizu, Igarashi, and Takahashi 2008). This acid is the end product of sugar metabolism and is involved in developing dental caries (Poza-Pascual et al. 2021). These results may be particularly important for those who use sports drinks or follow a Western diet (Griffiths et al. 2022), which results in increased lactate production due to sugar fermentation and lower saliva pH, predisposing the mouth to diseases of the teeth and gums. Another mechanism is that nitrate consumption reduces the abundance of bacteria that often cause caries, such as *Streptococcus*, which may contribute to suppressing the decrease in salivary pH. For example, one study observed an increase in saliva pH, simultaneously with a reduction in the relative abundance of *Streptococcus* following nitrate (M. Burleigh et al. 2019).

Dietary nitrate has been shown to be important in improving many health outcomes. For instance, it reduces blood pressure (Kapil et al. 2015), atherosclerosis, and platelet aggregation, and enhances endothelial function (Velmurugan et al. 2016). Additionally, in a recent population-based cohort study, dietary nitrate intake from vegetable sources was associated with a reduced risk of dementia (De Crom et al. 2023). Changes in the oral microbiota may mediate some of these effects. A study by Vanhatalo et al. (2021) showed stable associations between oral microbiome units, cognitive function, and cardiovascular health. Dietary nitrate intervention improved sustained attention, and information processing speed was also associated with the microbiome module (MM5) comprising *Neisseria-Haemophilus* (Vanhatalo et al. 2021).

The oral cavity is a complex environment that is affected by a number of factors including diet, salivary pH, saliva flow rate, smoking, medication intake, and others. However, long-term oral health maintenance may contribute to a reduced risk of cardiovascular disease or dementia by increasing the bioavailability of nitric oxide (Stephan et al. 2017). Likewise, poor oral health can lead to poor nitrate metabolism and, thus, decreased bioavailability.

Strengths and limitations

This review has several strengths, including pre-registration of the protocol on PROSPERO to increase transparency, and adherence to the PRISMA guidelines. In addition, this review focused on RCTs, which provide causal evidence for an impact of nitrate supplementation on oral health. This review also employed a comprehensive search strategy, which was devised by an information specialist. Nevertheless, it is possible that might not have captured all relevant literature in our searches. A limitation of our review is that we did not quantitatively synthesize the findings from studies *via* meta-analysis. However, we felt this was not appropriate here due to considerable between-study variability in the experimental design and the limited number of studies exploring consistent outcome measures. The studies reviewed also had several limitations, the most important of which is that all studies included in the review were of relatively short duration, not exceeding six weeks. Future studies are warranted to improve our understanding of the long-term effects of these supplements. Moreover, there was considerable variability in the methods used to evaluate oral microbiome composition and saliva sample collection methods across the studies. There were also difficulties related to using short-read 16S rRNA gene sequence analysis, which may have led to the difficulty of classifying some genera. Most species contain multiple copies of the 16S rRNA gene, with slightly different sequences, which may cause bias (Hiergeist et al. 2023). At the same time, some species observed across studies were unlikely to be permanent colonizers of the oral cavity (e.g., *Clostridiodes difficile* and *Ruminococcus torques*), therefore they may have been misclassified (Sánchez-Pellicer et al. 2021). Moreover, it is noted that most studies used beetroot juice as a supplement, which is consistent with the wider literature exploring the health effects of nitrate. One advantage of this approach is that a nitrate-depleted beetroot juice is also available, which allows double-blind investigations to be conducted with the provision of a supplement which is otherwise identical to the nitrate-rich beetroot juice. However, whole foods (whole

vegetables) are the source of most dietary nitrate, which is cheaper and more widely available than beetroot juice (Griffiths et al. 2023). Not using whole foods as a source means that some important mechanisms, such as chewing food in the mouth, may not be present in contrast to the use of juices. This process may have an effect on oral health through chewing and increased saliva secretion (Inui 2014). In addition, some studies reported changes in different bacteria (e.g., noting the number of bacteria affected by nitrate supplementation) but did not provide information on the specific bacteria that were altered after supplementation (Velmurugan et al. 2016). Two studies used a within-group comparison analysis rather than a comparison between the two study groups (Jockel-Schneider et al. 2016, 2021). In one study, it is worth noting that the placebo group ($n = 19$) included three smokers, while all participants recruited in the intervention group ($n = 20$) were nonsmokers, which may have affected the results (Jockel-Schneider et al. 2016, 2021). Several investigations have shown the effect of smoking on the oral microbiome. For instance, the abundance of *Neisseria* is reduced, and the abundance of *Prevotella* and *Veillonella* is increased among smokers compared to nonsmokers (Jia et al. 2021; Thomas et al. 2014). Finally, three studies limited the consumption of foods containing nitrate (Jockel-Schneider et al. 2016, 2021; Rosier et al. 2021). This might lead to an overestimation of the effects of nitrate on health parameters compared with a 'real world' setting in which participants may be consuming higher background levels of this compound.

Conclusion

This review investigated the effects of dietary nitrate on markers of oral health in adults. Despite the limited number of studies included in this review nitrate appears to have reasonably consistent effects on the oral microbial ecology, such as increasing the relative abundance of *Neisseria* and *Rothia*, decreasing the abundance of *Prevotella*, which implies potential beneficial changes to oral health. Dietary nitrate interventions were also demonstrated to raise salivary pH and prevent salivary acidification. However, the quality of evidence overall was low (according to GRADE methodology) and, moving forward, additional longer-term studies are warranted, including in groups who already have poor oral health (e.g., caries and gingivitis). It is also necessary to identify whether whole food sources of nitrate elicit similar effects to those reported with beetroot/lettuce juice. Nevertheless, the current evidence is promising and provisionally suggests that dietary nitrate may represent a simple, yet effective nutritional strategy to positively impact a range of oral health parameters.

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