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Citation for published version:

Shannon, OM, Easton, C, Shepherd, AI, Siervo, M, Bailey, SJ & Clifford, T 2021, 'Dietary nitrate and population health: A narrative review of the translational potential of existing laboratory studies', *BMC Sports Science, Medicine and Rehabilitation*, vol. 13, 65. <https://doi.org/10.1186/s13102-021-00292-2>

Digital Object Identifier (DOI):

[10.1186/s13102-021-00292-2](https://doi.org/10.1186/s13102-021-00292-2)

Link:

[Link to publication record in Heriot-Watt Research Portal](#)

Document Version:

Publisher's PDF, also known as Version of record

Published In:

BMC Sports Science, Medicine and Rehabilitation

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REVIEW

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Dietary nitrate and population health: a narrative review of the translational potential of existing laboratory studies

Oliver M. Shannon¹, Chris Easton², Anthony I. Shepherd³, Mario Siervo⁴, Stephen J. Bailey⁵ and Tom Clifford^{5*} 

Abstract

Background: Dietary inorganic nitrate (NO_3^-) is a polyatomic ion, which is present in large quantities in green leafy vegetables and beetroot, and has attracted considerable attention in recent years as a potential health-promoting dietary compound. Numerous small, well-controlled laboratory studies have reported beneficial health effects of inorganic NO_3^- consumption on blood pressure, endothelial function, cerebrovascular blood flow, cognitive function, and exercise performance. Translating the findings from small laboratory studies into 'real-world' applications requires careful consideration.

Main body: This article provides a brief overview of the existing empirical evidence basis for the purported health-promoting effects of dietary NO_3^- consumption. Key areas for future research are then proposed to evaluate whether promising findings observed in small animal and human laboratory studies can effectively translate into clinically relevant improvements in population health. These proposals include: 1) conducting large-scale, longer duration trials with hard clinical endpoints (e.g. cardiovascular disease incidence); 2) exploring the feasibility and acceptability of different strategies to facilitate a prolonged increase in dietary NO_3^- intake; 3) exploitation of existing cohort studies to explore associations between NO_3^- intake and health outcomes, a research approach allowing larger samples sizes and longer duration follow up than is feasible in randomised controlled trials; 4) identifying factors which might account for individual differences in the response to inorganic NO_3^- (e.g. sex, genetics, habitual diet) and could assist with targeted/personalised nutritional interventions; 5) exploring the influence of oral health and medication on the therapeutic potential of NO_3^- supplementation; and 6) examining potential risk of adverse events with long term high- NO_3^- diets.

Conclusion: The salutary effects of dietary NO_3^- are well established in small, well-controlled laboratory studies. Much less is known about the feasibility and efficacy of long-term dietary NO_3^- enrichment for promoting health, and the factors which might explain the variable responsiveness to dietary NO_3^- supplementation between individuals. Future research focussing on the translation of laboratory data will provide valuable insight into the potential applications of dietary NO_3^- supplementation to improve population health.

Keywords: Nitrate, Beetroot juice, Population health, Epidemiology, Randomised controlled trials, Blood pressure, Exercise performance, Translation

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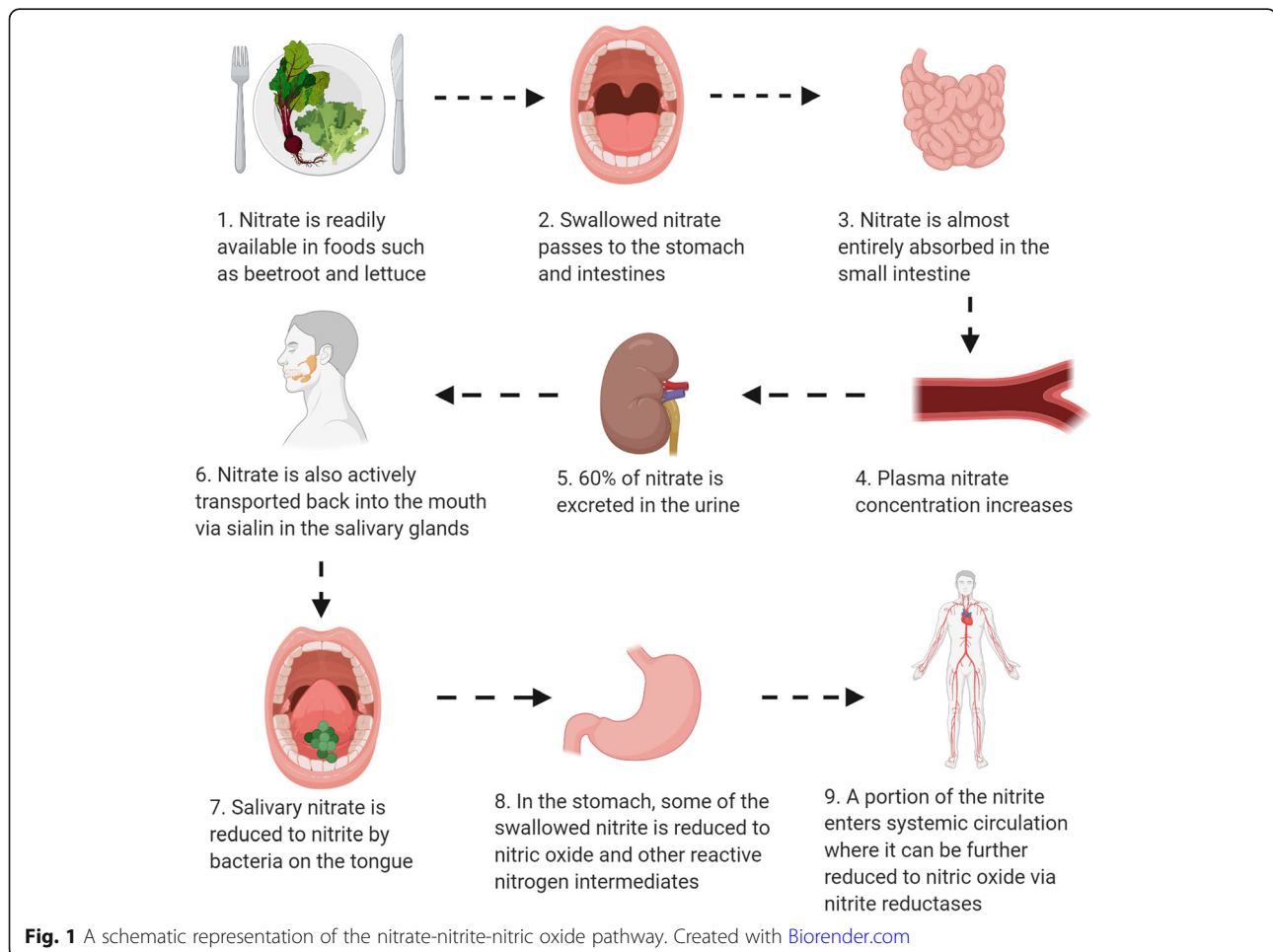
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Background

Dietary inorganic nitrate (NO_3^-) is a polyatomic ion present in large quantities in green leafy vegetables and certain root vegetables such as beetroot [1]. In recent years, inorganic NO_3^- has attracted substantial attention as a potential health promoting and exercise performance-enhancing dietary compound. These effects have largely been attributed to its ability to serve as a substrate for the ubiquitous gasotransmitter, nitric oxide (NO; Fig. 1) [2]. Following consumption, inorganic NO_3^- is absorbed in the upper gastrointestinal tract, increasing plasma NO_3^- concentration [3]. In the blood, exogenous NO_3^- mixes with endogenous NO_3^- produced via oxidation of NO. Most (~60%) of the ingested NO_3^- is excreted in the urine [4]. However, ~25% is actively taken up by the salivary glands via the transporter protein sialin [5], and secreted into the oral cavity, where it is reduced to nitrite (NO_2^-) by facultative anaerobic bacteria residing primarily on the dorsal surface of the tongue [6, 7]. Salivary (in the saliva) NO_2^- is then swallowed and a portion is converted into NO and other nitrogen oxides in the acidic environment of the stomach

[2, 8, 9]. A further portion of the swallowed NO_2^- reaches the systemic circulation, where it can be transported to various tissues and reduced to NO by a range of enzymatic and non-enzymatic catalysis [2, 3]. By increasing the bioavailability of NO and other nitrogen oxides, which play a role in the regulation of multifarious physiological processes, inorganic NO_3^- has the capacity to elicit far-reaching effects in the human body.

One of the most well-documented effects following inorganic NO_3^- consumption is a decrease in blood pressure (BP), an effect which was first demonstrated by Larsen and colleagues from the Karolinska Institute in 2006 [10]. This group reported that 3 days of supplementation with NO_3^- salts (0.1 mmol/kg/d sodium NO_3^-) reduced diastolic and mean arterial BP by -3.7 and -3.2 mmHg, respectively, in young healthy adults. A number of independent research groups [11–16] has substantiated these promising findings across a range of participant cohorts and using various supplementation strategies, including the provision of whole and juiced vegetables, especially beetroot juice [17]. Over the past 10 years, as this burgeoning research area has expanded,



various other potentially beneficial effects of inorganic NO_3^- consumption have also emerged. Notably, NO_3^- has been shown to improve a range of cardiovascular risk factors [17], increasing endothelial function [14, 18–21], decreasing arterial stiffness [15, 20, 22, 23], and reducing platelet aggregation [20, 24, 25]. Some [26–28], but not all [18, 29–31] studies have also shown beneficial effects of inorganic NO_3^- on cognitive function – effects which may be underpinned by alterations in cerebrovascular blood flow [31–33] and could be of value to a range of clinical and healthy populations [34]. Likewise, NO_3^- has been identified as a potential prebiotic for the oral microbiome [35], with the potential to positively impact oral health [36]. Moreover, NO_3^- consumption has been demonstrated to improve performance during continuous [12, 13, 29, 37–42], intermittent [43–45] and strength-based [46, 47] exercise, especially in untrained and recreationally active individuals [40, 48–50]. The mechanisms for the ergogenic effects of NO_3^- have not been fully resolved, but may include: 1) improvements in mitochondrial efficiency (reported by some [51], but not others [52]); 2) enhanced muscle contractile efficiency/ function [53–56]; and 3) augmented tissue blood flow, particularly to areas of low oxygen tensions such as type II muscle fibres (demonstrated in animal models [57, 58], but with less convincing data in humans [59–63]).

Current research has provided valuable insight into optimisation of NO_3^- supplementation strategies (e.g. pharmacokinetics, dose-response and supplementation duration) [13, 64, 65] and mechanisms of action [51, 53, 57, 66]. Nevertheless, more research is needed to understand whether findings from typically small, well-controlled laboratory studies are likely to translate into clinically relevant improvements in population health. This article highlights key areas for further research that could help in this regard. Such research is warranted to help guide practitioners, influence policy, and form guidelines for the effective and safe consumption of inorganic NO_3^- .

Main text

Research focus 1: large-scale, longer duration trials

Although NO_3^- consumption has been linked with a range of positive health outcomes, the majority of trials exploring the salutary effects of inorganic NO_3^- have involved short-term supplementation regimens, typically a few days in duration. Only a handful of trials have explored the medium- to longer-term effects of NO_3^- consumption (4 weeks to 6 months), usually focusing on BP or endothelial function as an outcome. Whilst not a universal finding [67, 68], beneficial effects of medium- to longer-term NO_3^- supplementation protocols have been reported in some trials [69–71]. For example, Siervo

et al. [69] found that 2 months supplementation with NO_3^- -rich beetroot juice (~ 6.5 mmol/d NO_3^-) decreased 24-h systolic and diastolic BP by -10.8 and -5.4 mmHg, respectively, in a Sub-Saharan African setting. Similar effects were also observed when NO_3^- rich beetroot juice was co-ingested alongside folate. In another study, Mills and colleagues [70] showed that 6 months consumption of NO_3^- -rich beetroot juice (~ 11 mmol/d NO_3^-) decreased central systolic pressure by -2.6 mmHg. Likewise, Kapil et al. [71] reported reductions in 24-h systolic and diastolic BP (7.7 and 5.2 mmHg, respectively) and improved endothelial function and arterial stiffness with 4 weeks NO_3^- -rich beetroot juice supplementation (6.4 mmol/d NO_3^-) with no change after placebo. Although focusing on different outcomes to the above trials, a study by Thompson et al. [72] also showed greater adaptations to sprint interval training in individuals consuming NO_3^- rich beetroot juice (13 mmol/d) over a 4-week period, providing further evidence of a benefit of this supplement when given over prolonged periods.

By contrast, studies by Blekkenhorst [67] and Sundqvist [68] observed no effects of 4- and 5-week NO_3^- interventions on BP. The lack of effect in these studies could be related to the relatively low NO_3^- doses administered (2.4 and 4.8 mmol/d NO_3^- , respectively). Conversely, the source of NO_3^- (vegetables or NO_3^- pills rather than NO_3^- -rich beetroot juice) could be relevant in explaining the lack of effect in these studies, given different foods providing equivalent doses of NO_3^- appear to have divergent effects on plasma NO_2^- concentration and BP [16], which could be linked to the (poly) phenol and ascorbate content of these foods [73]. Indeed, in most studies to date NO_3^- has been administered as beetroot juice, which is also rich in a constellation of different bioactive compounds, particularly (poly) phenols and the betalains [74]. Independent of NO_3^- , betalains have been shown to possess antioxidant [75], anti-inflammatory [76], and vasodilatory [77] properties, although studies in humans are still scarce. To isolate the effects NO_3^- from other compounds in beetroot juice, researchers often compare the effects of a NO_3^- rich beetroot juice to a taste-, smell- and appearance-matched NO_3^- depleted juice. One limitation of this strategy is that it cannot account for any synergistic interactions between NO_3^- and the other bioactive compounds that may augment the physiological effects of beetroot juice; in other words, we cannot be certain if the positive effects in these studies are simply due to NO_3^- or its interactions with the other bioactive compounds present. Thus, studies chiefly aimed at untangling the mechanistic effects of NO_3^- may prefer to administer NO_3^- in the form of NO_3^- salts instead of food-based supplements that contain other compounds.

Overall, additional comparisons of NO_3^- rich beetroot juice and sodium NO_2^- or NO_3^- supplements are required. When interpreting the findings of the studies discussed in this review, it is important the reader is aware that studies with NO_3^- salts and NO_3^- rich beetroot juice do not contain the same compounds and therefore different effects are possible. Notwithstanding, as discussed in *Section 4* of this review, cross-talk between NO_3^- and other dietary components or participant-level differences in the response to NO_3^- could also account for the lack of effect of NO_3^- in the studies of Blekkenhorst [67] and Sundqvist [68].

Based around the current evidence it is likely that, under the right circumstances (which remain to be fully elucidated), consumption of inorganic NO_3^- could elicit longer-term health benefits. In order to fully appreciate the potential applications of NO_3^- on population health, large-scale (e.g. $n > 1000$), longer duration (e.g. 2–5 years) trials which focus not only on risk factors (e.g. BP, endothelial function, cognitive function), but also incidence of key non-communicable diseases (e.g. CVD, dementia) are warranted. Specific considerations for the design of such studies are provided in Table 1. Whilst likely to be logistically complex and require substantial financial backing from funders, this research could be justified by the promising evidence from short-term trials and the potential application of findings to ease the unsustainable societal and financial burden of conditions such as CVD (annual global costs ~\$863 billion [78]) and dementia (annual global cost ~\$1 trillion [79]). Prior to undertaking this research, it is essential to obtain more data on the feasibility and acceptability of different strategies to increase habitual NO_3^- intake by a sufficient quantity and for a sufficient period to obtain long lasting health benefits. This information is critical for the design of feasible longer-term trials and translation to the general population, and will be explored in more detail in the next section.

Research focus 2: feasibility and acceptability of different strategies to facilitate prolonged, increased consumption of nitrate

To date, a limited number of studies have reported data on the feasibility and acceptability of beetroot juice as a vehicle for increasing habitual NO_3^- intake. Mixed findings have been reported. For example, Ormesher and colleagues [80] gave 40 pregnant women 70 mL/d concentrated beetroot juice (~400 mg of NO_3^-) and, after 8 days of ingestion, 97% of participants indicated they would consume the supplement again, if they were experiencing benefits. However, only 62% of participants reported finding it easy to consume the beetroot juice and just over half of the participants rated the drink as palatable (54%). These findings suggest that longer-term consumption of beetroot juice may be difficult in this cohort, which could impede longer-term adherence. More recently, Kandhari et al. [81] evaluated the feasibility of a 60-day concentrated beetroot juice and folate intervention to treat hypertension in Sub-Saharan Africa. No serious adverse events were reported, and compliance was >90%, suggesting beetroot juice was well accepted in this population. In addition, all participants rated the taste as “good” or “very good” and most participants (~87%) indicated a preference for beetroot juice over BP medication. The studies by Ormesher et al. [80] and Kandhari et al. [81] both administered the same brand of concentrated beetroot juice, such that the different findings cannot be attributed to a different type of supplement administered. Alternatively, it is possible that the different findings of Ormesher et al. [80], which was conducted in the UK, and Kandhari et al. [81], which was conducted in Tanzania, reflect cultural/ regional differences in food preference. However, it is noteworthy that participants in the Ormesher et al. [80] study were also pregnant, which may have further

Table 1 Key considerations for future randomised controlled trials exploring the health effects of NO_3^- ingestion

Consideration	Recommendation
Dose	Consumption of a NO_3^- dose ≥ 8 mmol
NO_3^- form	Provision of NO_3^- salts or vegetables, with NO_3^- content independently verified
Study duration	Longer duration (e.g., months-to-years) warranted
Participant cohort	‘At risk’ cohort studied (e.g., individuals with hypertension for studies exploring effects of NO_3^- on cardiovascular disease risk)
Genetics/ microbiome	Consider recruitment of T allele carriers with G894T polymorphism in the eNOS gene
Microbiome	Consider recruitment of individuals with greater abundance of NO_3^- reducing oral bacteria
Mouthwash	Avoidance of mouthwash prior to and during the study
Dietary controls	Avoidance of thiocyanate and sulphate rich foods in conjunction with NO_3^-
Other lifestyle factors	Avoidance of smoking
Outcomes	Inclusion of hard clinical endpoints (e.g., CVD or dementia incidence) to build upon promising findings on risk factors for these conditions

contributed towards the difference in palatability given pregnancy is known to influence taste [82].

In another study, Babateen et al. [83] examined the feasibility of different doses of concentrated beetroot juice in overweight and obese older adults over a 13-week period. Compliance was high, no adverse events were reported, and the attrition rate was 19%, which is similar or lower than the dropout rates reported in other human intervention trials [84, 85]. Collectively, these studies suggest that beetroot juice may represent an acceptable strategy to facilitate increased consumption of NO_3^- , at least in certain cohorts. However, future studies need to evaluate the feasibility and acceptability of beetroot juice consumption over longer periods (e.g., > 6 months) and in other populations.

Concentrated, commercially available beetroot juice shots have the advantage of being readily available (they are now sold in many major supermarket chains) and contain a standardised dose of NO_3^- sufficient to influence myriad health outcomes. This form of beetroot juice has also been shown to be more effective at reducing BP (and presumably eliciting other physiological changes) than non-concentrated beetroot juice when the same dose is administered [86]. In addition, as mentioned in the previous section, beetroot juice also contains other bioactive compounds that may contribute to overall health. Nevertheless, as participants do not always enjoy the taste of beetroot juice and the relatively high cost of commercially available beetroot 'shots' (~£1–2 or \$2–3 each) may be prohibitive to some users, it is essential for researchers to explore the feasibility and acceptability of other strategies to increase NO_3^- consumption. This could include other NO_3^- -rich foods (e.g. lettuce, rhubarb, spinach, radish), gels, powders, crystals, capsules and non-beetroot drinks. To this end, both Blekkenhorst et al. [67] (> 98% compliance) and Sundqvist et al. [68] (> 97% compliance) demonstrated excellent compliance to 4 and 5 week interventions, respectively, with NO_3^- -rich vegetables, which were well tolerated with minimal side effects. Importantly, Sundqvist et al. [68] reported similar compliance between NO_3^- -rich vegetables and NO_3^- -containing pills (> 97% vs. > 98%). Nevertheless, neither Blekkenhorst et al. [67] nor Sundqvist et al. [68] reported beneficial physiological effects of their interventions, which could be related to the relatively modest NO_3^- doses provided (~ 2.4 and 4.8 mmol/d respectively) or other methodological factors which were discussed in *Section 1* of this review. A comprehensive investigation of patient preferences and the real and perceived barriers of adopting a high- NO_3^- diet or consuming NO_3^- -rich supplements warrants further investigation. In addition, studies need to determine the amount of NO_3^- -rich vegetables required to elicit beneficial physiological effects, whether this is achievable for

different populations, and whether effects are superior to non-vegetable NO_3^- sources. Finally, it is worth exploring whether there are regional and population preferences, as this knowledge could be used to develop more targeted NO_3^- products.

Research focus 3: nitrate intake and health outcomes in epidemiological studies

The role of dietary NO_3^- for human health has gradually shifted over the last five decades. Indeed, this compound was initially considered as a risk factor for cancer, endocrine disorders and infant methaemoglobinaemia. However, the stigma attached to dietary NO_3^- has gradually dwindled, and NO_3^- is now viewed by many a potential health-promoting compound (see *Section 6* for further details). The initial results suggesting a harmful role of dietary NO_3^- intake (from food) were mostly derived from animal models and weakly designed epidemiological studies which have had a prominent, almost demonizing, influence on defining the role of dietary NO_3^- for human health [87]. These initial studies informed the still contentious WHO nutritional recommendations for dietary NO_3^- intake in humans which was set at 3.7 mg/kg body weight [88]. The perception of dietary NO_3^- as a risk factor started to change with the discovery of the role of NO_3^- as key substrate for the NO_3^- - NO_2^- - NO pathway and the evidence of a beneficial effect of NO_3^- on health parameters such as BP.

After the study by Larsen et al. [10] in 2006, which first demonstrated a BP lowering effect of sodium NO_3^- , there was a rapid surge in research testing the effects of dietary NO_3^- on health outcomes [89]. However, the research strategy in the last decade has almost taken an inverse approach to that typically adopted in nutritional science as the conduction of clinical trials have surpassed epidemiological investigations, which are generally considered as a first step to validate research hypotheses [90–92]. One of the primary reasons for the inverse trend is the lack of reliable and representative food databases of NO_3^- content to support an accurate dietary assessment [93]. An additional limitation is the severe lack of validation studies testing the accuracy of dietary assessment methods against valid biomarkers of NO_3^- intake (e.g. 24-h urinary NO_3^- concentrations) [94]. This is compounded by the fact that the NO_3^- content of vegetables will vary by farming method (whether NO_3^- fertiliser is used or not), growing conditions, time of year the crop is harvested, and storage conditions [1], such that there is likely to be a degree of error in estimated NO_3^- intake values [95]. Several research groups have developed independent databases by collecting data on NO_3^- food content from published sources in an attempt to obtain valid estimates of NO_3^- intake and evaluate associations with health outcomes [96, 97].

Although this is a step in the right direction, it remains difficult to accurately estimate long-term habitual dietary NO_3^- intake for the reasons mentioned above. In addition, NO_3^- concentrations measured in biological fluids have been used in some analysis as indirect markers of NO_3^- intake [98]. Whether these objective markers of NO_3^- intake show stronger links with health outcomes compared with subjective, self-reported NO_3^- intake values, is the subject of ongoing research. A summary of the key non-cancer related epidemiological studies testing the association of inorganic NO_3^- with health outcomes is provided in Table 2.

The first studies to evaluate the association between dietary NO_3^- intake and health outcomes were conducted in 2016 in Iran (two studies) [99, 101] and in the United States (one study) [100]. The former evaluated the association of vegetable NO_3^- intake with risk of chronic kidney disease in the Tehran Lipid and Glucose Study and found a higher prevalence of chronic kidney disease (CKD) at baseline (cross-sectional analysis) in the high- NO_3^- intake group whereas no significant association with CKD risk was observed after a 3-year follow up [101]. Using the same dataset, Bahadoran et al. [99] found that dietary NO_3^- intake, overall and from animal sources, was not associated with prospective risk of diabetes. The US study was conducted in a very large sample (> 100,000 participants) and assessed dietary NO_3^- intake in the Nurses' Health Study and the Health Professionals Follow-up Study [100]. The results showed a significantly lower risk of primary open-angle glaucoma in participants with higher NO_3^- intake [100]. However, a subsequent analysis conducted in the Nurses' Health Study found a non-significant association between dietary NO_3^- and prospective risk of coronary heart disease [111]. More recently, several cross-sectional and longitudinal studies have observed significant associations between high NO_3^- intake or urinary NO_3^- concentrations (as a proxy for NO_3^- intake) with cardiovascular outcomes including lower BP [107], risk of hypertension [109], common carotid intimal medial thickness [103], congestive heart failure [114] and CVD mortality [109]. Conversely, higher plasma NO_3^- concentrations in the Framingham Offspring Study [106] were associated with an increased risk of all-cause mortality, which may be explained by the rise in plasma NO_3^- concentrations in participants with impaired kidney function included in the analysis and highlights the potential risk of reverse causality in these investigations. The improvements in physical performance and cognition observed in some of the NO_3^- supplementation trials were also explored in two cross sectional studies [98, 112]. Improved hand-grip strength and timed up and go tests (a test of functional mobility) were observed in middle-aged and older Australian participants with a

higher NO_3^- intake [112] whereas NO_3^- concentrations measured in spot urine samples were not associated with improved cognition in 1015 older Americans participants enrolled in the National Health and Nutrition Examination Survey [98]. The NIH workshop on dietary NO_3^- held in 2016 [115] advocated for more epidemiological research to be conducted to better define the predictive role of dietary NO_3^- consumption for the prevention as well as treatment of chronic diseases. The consensus statement also encouraged the development of detailed and country-specific NO_3^- food composition tables for a more accurate assessment of the exposure to dietary NO_3^- [115]. The current epidemiological evidence points towards a protective role of dietary NO_3^- intake for cardiovascular events and mortality whereas the predictive role for cancer risk is still undefined as latest meta-analyses on the topic indicate a lack of association between dietary NO_3^- consumption and cancer risk [116, 117]. There is still scarce or no data from prospective studies on the association of dietary NO_3^- intake with other chronic conditions with established links with $\text{NO}_3^-/\text{NO}_2^-$ and NO pathways such as diabetes, hypertension, physical disability or dementia. Further epidemiological studies in this area are therefore warranted. Such research will complement the findings from RCTs, by providing information on the effectiveness of a NO_3^- for disease reduction in real-world circumstances with greater sample sizes and longer follow up than is logistically feasible in most RCTs [90, 91].

Research focus 4: inter-individual differences in the response to nitrate

At the individual participant level, several groups have suggested the existence of possible 'responders' and 'non-responders' to NO_3^- , irrespective of the vehicle used to provide this inorganic anion [64, 118, 119]. It is important to note that random within-subject variation could explain much of the variability in response to NO_3^- supplementation between individuals [120, 121]. Similarly, issues may also arise when attempting to establish whether an individual is a dependable 'responder' or 'non-responder' on different occasions [122, 123]. Nevertheless, several factors have been identified which could explain genuine differences in the response to NO_3^- between individuals. These include individual characteristics such as age [124, 125], health [126] and exercise training status [40, 49], sex [14], genetic factors [127], and differences in the oral microbiome (explored further in Section 5 of this review). In addition, between-participant differences in potentially plastic lifestyle factors such as smoking status [128], use of mouthwash [129], and habitual diet [49, 130] might also impact an individual's response to NO_3^- . We briefly review the impact of these variables on the effects of NO_3^- below.

Table 2 Key epidemiological studies exploring associations between inorganic nitrate consumption and non-cancer related health outcomes

Author, year	Population Size	Study Design	Duration of Follow up (y)	Nitrate Assessment	Health Outcome	Key Findings
Bahadoran et al. [99]	4920	Prospective (Tehran Lipid and Glucose Study)	5.8	FFQ	Type 2 Diabetes (T2D)	No significant association between NO_3^- intake and the risk of T2D in fully adjusted model
Kang et al. [100]	Nurses' Health Study (63,893 women) Health Professionals Follow-up Study (41,094 men)	Prospective	~ 30 years for both	FFQ	Primary open-angle glaucoma (POAG)	Higher dietary NO_3^- and green leafy vegetable intake was associated with a lower POAG risk, particularly POAG with early paracentral VF loss at diagnosis.
Mirmiran et al. [101]	1546	Prospective (Tehran Lipid and Glucose Study)	3	FFQ	Chronic Kidney Disease (CKD)	At baseline, higher intake of high-vegetable NO_3^- intake was associated with a 48% higher chance of having CKD (OR 1.48, 95% CI 1.05–2.13). After 3 years of follow-up, there was no significant association with the occurrence of CKD
Bleckenhorst et al. [102]	1227	Prospective (Perth Longitudinal Study of Aging in Women)	15	FFQ	Atherosclerotic vascular disease (ASVD) mortality	A high vegetable NO_3^- intake was associated with a lower risk of ASVD (HR: 0.79 95% CI: 0.68, 0.93, $P = 0.004$) and all-cause mortality (HR: 0.87 95% CI: 0.78, 0.97, $P = 0.011$)
Bondonno et al. [103]	1226	Prospective (Perth Longitudinal Study of Aging in Women)	14.5	FFQ	CCA-IMT, plaque severity and risk of an ischemic cerebrovascular disease event	Higher intake of vegetable NO_3^- was associated with 17% lower risk of cerebrovascular disease events ($P = 0.02$) and lower CCA-IMT ($P = 0.002$).
Gumanova et al. [104]	1087	Cross-sectional (Stress Aging and Health Study)	–	Plasma NOx	Diabetes type II, hyperthyroidism, coronary heart disease, gout and thrombosis/stroke, osteoporosis, cancer	NOx over 44.7 μM were associated with increased prevalence of diabetes type II, hyperthyroidism, coronary heart disease, gout and thrombosis/stroke
Kuhnle et al. [105]	7598	Cross-sectional (EPIC Norfolk)	–	Drinking water NO_3^- concentrations	Blood pressure (BP)	At low sulfate concentrations, NO_3^- was inversely associated with BP (– 4 mmHg in top quintile) whereas this was reversed at higher concentrations (+ 3 mmHg in top quintile)
Maas et al. [106]	2855	Prospective (Framingham Offspring Study)	17.3	Plasma NO_3^-	All-cause mortality and incident CVD	Plasma NO_3^- was weakly associated with an increased risk of death (HR, 1.16; 95%CI, 1.00–1.35 $P = 0.057$) but not with incident CVD
Smallwood et al. [107]	919	Cross-Sectional (InChianti)	–	24-h urinary NO_3^-	Blood pressure	Systolic blood pressure in the ≥ 2 mmol urinary NO_3^- excretion group was 3.9 (CI: – 7.1 to – 0.7) mm Hg lower than in the comparison < 1 mmol excretion group.
Liu et al. [108]	2900	Prospective (Blue Mountains Eye Study)	15	FFQ	CVD mortality	In multivariable-adjusted analysis, participants in quartile 4 [> 137.8 mg/d; HR 0.63 (95% CI 0.41, 0.95)] of vegetable NO_3^- intake had lower hazards for CVD mortality compared to participants in quartile 1 (< 69.5 mg/d)

Table 2 Key epidemiological studies exploring associations between inorganic nitrate consumption and non-cancer related health outcomes (Continued)

Author, year	Population Size	Study Design	Duration of Follow up (y)	Nitrate Assessment	Health Outcome	Key Findings
Mendy et al. [109]	17,618	Prospective (NHANES)	4.3	Urinary NO ₃ ⁻ in spot urine samples	Hypertension and CVD prevalence and all-cause mortality	1-unit increase in log-transformed urinary NO ₃ ⁻ was associated with a > 30% decrease in the odds of hypertension (odds ratio, 0.67; 95% confidence interval [CI], 0.55–0.81), stroke (OR, 0.61, 95% CI, 0.43–0.87) and cardiovascular mortality (HR, 0.44; 95% CI, 0.26–0.73)
Jackson et al. [110]	5324	Prospective (Australian Longitudinal Study on Women's Health)	15	FFQ	Incidence of self-reported CVD-related complications	Women reporting higher total dietary NO ₃ ⁻ intakes (Q4 > 78.2 mg/d) and vegetable NO ₃ ⁻ intakes (Q4 > 64.4 mg/d) were 25 and 27% reduced risk of developing CVD-related complications, respectively.
Jackson et al. [111]	Nurses' Health Study and Health (62,535 women)	Prospective	26	FFQ	Coronary heart disease	Dietary NO ₃ ⁻ intake was not related to risk of CHD after adjustment for other lifestyle and non-vegetable dietary factors
Sim et al. [112]	1420	Cross-sectional (Perth Longitudinal Study of Aging in Women)	–	FFQ	Hand-grip strength and time up and go (TUG)	Higher NO ₃ ⁻ intake (31.2 mg/d) was associated with lower odds for weak grip strength (OR 0.84, 95% CI 0.74–0.95, <i>P</i> = 0.005) and slow TUG (OR 0.86, 95% CI 0.76–0.98, <i>P</i> = 0.021)
Riddell et al. [113]	2656	Prospective	1.5	Urinary NO ₃ ⁻ to creatinine ratio (uNCR)	Prediction of renal transplant rejection	Overall uNCR was highly variable with no diagnostic threshold for kidney transplant rejection
Wu et al. [114] 2020	14,894	Cross-sectional (NHANES)	–	Urinary NO ₃ ⁻ in spot urine samples	Congestive heart failure, coronary heart disease, angina pectoris, myocardial infarction	Significant association between urinary NO ₃ ⁻ and congestive heart failure (OR = 0.651, 95% CI 0.507–0.838, <i>P</i> < 0.001)
Pereira et al. [98]	1015	Cross-sectional (NHANES)	–	Urinary NO ₃ ⁻ in spot urine samples	Cognitive function	Urinary NO ₃ ⁻ concentrations were not associated with cognitive performance on any of the cognitive tests.

EPIC European Prospective Investigation of Cancer, FFQ Food Frequency Questionnaire, CCA-IMT Common Carotid Intimal Medial Thickness, NO₃⁻ Nitrate, NO₂⁻ nitrite, NO_x Nitrate + Nitrite Concentration, CVD Cardiovascular Disease, OR Odds Ratio, HR Hazard Ratio, NHANES National Health and Nutrition Examination Survey, uNCR Urinary nitrate to creatinine ratio

Individuals with lower aerobic fitness levels may respond more favourably to NO₃⁻ supplementation [40, 131]. This theory stemmed from several studies reporting that while NO₃⁻ supplementation from any source enhanced exercise performance in recreational level athletes ($\dot{V}O_{2peak}$ 40–60 ml/kg/min), such effects were less pronounced or non-existent in well-trained and elite endurance athletes (typically manifesting a $\dot{V}O_{2max}$ > 60 ml/kg/min) [132–135]. Porcelli et al. [40] provide the most convincing evidence to support this notion and demonstrated that, when all other methodological factors such as the exercise test and NO₃⁻ dose are held content, individuals with a higher aerobic fitness status are less responsive to the ergogenic effects of NO₃⁻. Indeed, those authors reported beneficial effects of sodium NO₃⁻ on 3 km running performance in individuals with low ($\dot{V}O_{2peak}$: 28.2–44.1 ml/kg/min), and moderate ($\dot{V}O_{2peak}$: 45.5–57.1 ml/kg/min), but not high ($\dot{V}O_{2peak}$: 63.9–81.1 ml/kg/min) aerobic fitness levels. Several possible explanations have been

put forth to try and explain why high fitness levels might render NO₃⁻ supplementation less effective, and these are discussed in detail elsewhere [40, 131, 136]. One prominent explanation is that elite endurance athletes might produce more NO via the canonical NOS pathways and are therefore less reliant on NO₃⁻ as a substrate for NO generation [132]. Furthermore, recent evidence indicates that NO₃⁻ might elicit preferential effects on type II compared with type I muscle fibres [54, 57, 58]. Well-trained endurance athletes might therefore benefit less from NO₃⁻ supplementation given a lower proportion of type II, and a higher proportion of type I, muscle fibres compared with recreationally active individuals [137, 138]. In contrast, some studies have shown a beneficial effect of NO₃⁻ in well-trained athletes [42, 139–141]. Jonvik et al., (2015) suggested that methodological limitations of some studies could at least partly explain the null findings in some studies with elite athletes. Notably, there are far less studies assessing the effects of NO₃⁻

supplementation, irrespective of vehicle, in well-trained athletes in comparison to healthy, physically active, individuals. This is likely because well-trained athletes are only a small fraction of the population, and are logistically harder to test and recruit to studies due to their desire to avoid potential training interruptions. Thus, more research is still required to ascertain the influence of aerobic fitness levels on the responsiveness to NO_3^- supplementation.

Women are underrepresented in research into the health effects of dietary NO_3^- [142]. Nevertheless, preliminary evidence suggests potentially differential effects of NO_3^- (at least in regard to the effects of NO_3^- on BP) between the sexes, which warrants further investigation. Women have been demonstrated to have greater oral NO_3^- reducing capacity than men due to an oral microflora composition that is more conducive for NO_3^- reduction to NO_2^- [143]. Nevertheless, Kapil et al. [14] and Coles and Clifton [144] both demonstrated BP-lowering effects of NO_3^- (potassium NO_3^- and beetroot juice, respectively) in men with higher baseline BP and lower plasma NO_2^- concentrations but not in women. Likewise, in a meta-analysis by Jackson et al. [17], BP reductions with NO_3^- were greater in studies with more male participants. Those authors speculated that this could be related to a greater vascular production of NO in pre-menopausal women due to oestrogen-related release and activity of NO [145], diminishing the response to supplemental NO_3^- in women compared with men.

Although studies remain scarce, there is some evidence that the heterogeneous responses to NO_3^- supplementation are partly explained by polymorphisms in the eNOS gene. This was first explored by Hobbs et al., [127], who examined the effects of NO_3^- supplementation on BP in patients with and without a specific polymorphism in the eNOS gene (G894T), which has been suggested to inhibit NO production from eNOS [127]. Although findings are equivocal [146], the G894T polymorphism, alongside being a T allele carrier, has been associated with cardiovascular disease [147–149], of which a key risk factor is diminished NO bioavailability [150, 151]. Intriguingly, despite the small sample size ($n = 14$), Hobbs et al., [127] found that NO_3^- supplementation (beetroot bread) only reduced BP in patients who were both T allele carriers and had the G894T polymorphism in the eNOS gene. A more recent study examined the influence of the G894T polymorphism and NO_3^- therapy on mortality in chronic heart failure patients [146]. Somewhat at odds with the findings of Hobbs et al., [127], Azzam et al. [146] found that NO_3^- therapy (source not specified) increased the risk of mortality in patients with the G894T polymorphism, and to a greater extent in G allele carriers, suggesting that NO_3^- therapy might increase mortality in advanced heart failure. However, as this study was observational,

cause-effect relationships cannot be established. Moreover, the findings are at contrast to the beneficial effects of NO_3^- shown in most [152–155], but not all [156, 157], short term intervention trials which show that NO_3^- improves cardiac function and/or exercise capacity in heart failure patients. Clearly, more studies with larger cohorts are required to determine the extent to which genetic variation influences the responsiveness to NO_3^- supplementation, but the findings from these two studies raise the possibility that that genetic factors could contribute towards the inter-individual variability reported by many studies.

Smoking has been shown to increase plasma and salivary concentrations of thiocyanate [158], a compound which competitively inhibits uptake of NO_3^- into the salivary glands [159], potentially reducing the amount of 'substrate' available to the oral bacteria for reduction into NO_2^- . Consequently, it is possible that smokers will experience compromised NO_3^- metabolism and thus a diminished physiological response to NO_3^- supplementation versus non-smokers. Indeed, Bailey et al. [128] demonstrated a smaller increase in salivary NO_3^- , plasma NO_3^- and NO_2^- concentration, and an attenuated BP response, following a NO_3^- bolus (beetroot juice) in smokers compared to non-smoking controls.

It is possible that supplemental NO_3^- is ineffective at eliciting meaningful physiological changes in individuals habitually consuming a high NO_3^- diet. Nevertheless, as population intake of NO_3^- is typically low — Babateen et al. [93] reported a median intake of 108 mg/d in healthy individuals. With very few individuals regularly consuming NO_3^- levels to match those provided through supplementation [160], high habitual NO_3^- intake is unlikely to explain a lack of response to NO_3^- supplementation in most 'non-responders'. Alternatively, there is compelling evidence to suggest that consumption of other dietary compounds alongside NO_3^- may have the capacity to influence response to this compound, such that an individual's background diet could determine (at least transiently) their status as a NO_3^- 'responder' or 'non-responder'. For example, consumption of glucosinolate-rich vegetables, such as those from the *Brassica* family like broccoli, cauliflower, and cabbage, proximal to consumption of NO_3^- -rich vegetables was shown to blunt the BP lowering response of the latter [130]. Interestingly, this appears to be related to a similar mechanism to which smoking attenuates the effect of NO_3^- . Specifically, during processes that result in plant cell membrane damage such as mastication, glucosinolates are exposed to the enzyme myrosinase, which catalyses the hydrolysis of glucosinolates into thiocyanate [161]. Although consumption of thiocyanate-rich vegetables leads to lower salivary and plasma thiocyanate concentrations compared with smoking, Dewhurst-Trigg

et al. [130] showed that the BP-lowering effect of a NO_3^- -rich smoothie was attenuated by the presence of thiocyanate rich vegetables. In that study, thiocyanate did not seem to interfere with NO_3^- transport into the mouth (as evident by similar salivary NO_3^- concentrations when NO_3^- was consumed alongside vegetables that were both high and low in thiocyanate), suggesting that thiocyanate may influence other aspects of NO_3^- metabolism. Specifically, co-ingestion of thiocyanate synthesising vegetables and NO_3^- -rich vegetables lowered salivary NO_2^- concentration compared to ingestion of NO_3^- -rich vegetables alone. This suggests that some *Brassica* vegetables might transiently alter the oral microbiome, consistent with the antimicrobial effects of thiocyanate derivatives in the oral cavity [162].

A study by Hughan et al. [163] found that the co-ingestion of sodium NO_3^- alongside conjugated linoleic acid, an unsaturated fatty acid particularly abundant in dairy and meat products, attenuated the rise in plasma NO_3^- and NO_2^- concentrations and suppressed the BP-lowering and platelet-inhibiting effects that were apparent when supplements were administered in isolation. Mechanistically, co-consumption of conjugated linoleic acid altered the metabolic fate of ingested NO_3^- leading to the formation of conjugated linoleic acid nitration products, which do not appear to have the same vasodilatory and platelet inhibiting properties as NO_2^- and NO. Likewise, Bailey et al. [164] found that the ingestion of iodide, which is fortified in many foods [165] and known to compete for salivary NO_3^- uptake [159], lowered salivary NO_3^- concentration when co ingested with NO_3^- rich beetroot juice. However, the increase in salivary and plasma NO_2^- concentration, alongside the lowering of BP, were similar compared with NO_3^- alone. Finally, a possible interaction between dietary NO_3^- and sulphate was identified by Kuhnle et al. [105] who indicated that when estimated sulphate intake was low, higher dietary NO_3^- intake was associated with lower BP. Conversely, when sulphate intake was high, this association was reversed, such that greater NO_3^- intake was actually associated with higher BP. The mechanistic basis through which sulphate could modulate the BP lowering effects of dietary NO_3^- is presently unknown.

Collectively, the evidence presented above indicates that the response to NO_3^- is unlikely to be uniform between individuals, and could also potentially differ within individuals based around malleable lifestyle factors such as habitual diet. Better understanding the factors that influence responsiveness to NO_3^- is crucial to maximise the efficacy of NO_3^- -based interventions and will facilitate the development of targeted interventions for individuals most likely to benefit from consumption of this compound. Given many of the factors which appear to moderate the effectiveness of NO_3^- impact the

oral conversion of this compound into NO_2^- , future research could also explore the potential physiological effects of direct NO_2^- administration (for a recent example, see [166]), which does not require processing in the mouth and could theoretically elicit more consistent responses between individuals. Nevertheless, caution should be taken to ensure such a strategy does not increase formation of potentially carcinogenic nitrosamines [167].

Research focus 5: oral microbiota and oral health

Once in the oral cavity, NO_3^- is reduced to NO_2^- during the anaerobic respiration of facultative and obligate bacteria which are particularly abundant on the dorsal surface of the tongue [168]. The oral microbiome collectively comprises over 700 individual species or phylotypes of bacteria that are organised in a series of complex interdependent communities [169]. To date, 14 species of bacteria have been identified as NO_3^- reducers, the majority of which are from the genera *Veillonella*, *Prevotella*, *Neisseria*, and *Haemophilus* [170]. A greater relative abundance of these bacteria on the tongue has been shown to augment the rate and magnitude of salivary NO_2^- production following the ingestion of NO_3^- rich beetroot juice [171]. Conversely, disruption of the oral microbiome by antibacterial mouthwash causes a transient loss of viable NO_3^- -reducing bacteria [172] and severely blunts the generation of NO_2^- in the saliva [173]. Strong antibacterial mouthwash has also been shown to increase BP, likely due to suppression of NO production from the NO_3^- - NO_2^- -NO pathway [174–176]. These data confirm the essential role of the oral bacteria in NO homeostasis and support the hypothesis that oral and systemic health are inextricably linked [177].

The mouth is continually exposed to the external environment and is regularly subjected to brushing, flossing, and nutrient intake, all of which may influence the physiological conditions inside the oral cavity and alter the composition of the bacterial milieu [178]. Ageing is known to cause a reduction in salivary flow rate [179] and has been reported to alter the composition of the oral microbiome in some [180, 181] but not all [182] studies. Other factors may also be expected to influence the abundance and activity of oral bacteria, including exercise, diet, oral and systemic diseases, haemodialysis [183] and peritoneal dialysis [184] and medication (particularly antibiotics). In particular, the ingestion of NO_3^- -rich beetroot juice has been shown to increase salivary pH and cause meaningful alterations to the oral microbiome in favour of oral health [182, 185]. Given the multitude of potential modifiers, it is perhaps unsurprising that there is profound between-individual variation in the abundance of NO_3^- -reducing bacteria [121].

Of note, these authors also reported significant within-individual week-to-week variability in the abundance of these bacteria and the magnitude by which plasma NO_2^- increased following the ingestion of NO_3^- -rich beetroot juice. This was despite participants standardising their diet, physical activity, use of mouthwash, teeth brushing, and tongue cleaning between visits. The unpredictability in how different individuals respond to NO_3^- supplementation and how the same individual responds across repeated visits poses a particular challenge for researchers who wish to explore the therapeutic effects of this dietary intervention.

While recent advancements in genomic sequencing techniques have greatly enhanced our understanding of human bacterial interactions in the context of NO homeostasis, several important questions remain unanswered. To date, the majority of the research exploring links between the oral microbiome and health outcomes has only reported the relative abundance of phyla, genera, or species. Although this quantifies the proportional makeup of the community structure it does not reveal the metabolic activities of individual bacterial species [186] which may vary depending on substrate availability, metabolite expression from neighbouring microbes and host cells, and the impact of environmental conditions [187]. Future research should deploy meta-transcriptomic analysis to determine how factors such as diet, medication, physical activity, ageing, and disease influence NO_2^- and NO_3^- reductase gene expression of the oral bacteria. Furthermore, data from epidemiological studies and short-term intervention trials seem to support the notion that increasing habitual dietary intake of NO_3^- can improve markers of oral health and reduce the incidence of caries [185, 188, 189]. It remains to be established whether dietary NO_3^- supplementation may also be an effective treatment method for those already suffering from oral diseases such as chronic periodontitis.

Research focus 6: risks versus rewards

NO_3^- is increasingly recognised as a beneficial ion that protects against chronic disease, yet, as noted in Section 3 of this review, historically, it was considered a food contaminant with adverse health effects, particularly increased risk of certain cancers and methaemoglobinemia [1, 88]. While the aforementioned WHO ADI for NO_3^- of 3.7 mg/kg of body mass remain in place today, the discovery of multiple positive health effects of NO_3^- have prompted a re-examination of these claims.

In 2004 the WHO reaffirmed their restrictions on NO_3^- intake yet, in 2008, a panel of experts from the European Food Safety Authority, concluded that the epidemiological evidence did not support an association between NO_3^- and cancer risk [190]. Similarly, in 2010,

the International Agency for Research on Cancer confirmed that there was inadequate evidence to suggest NO_3^- from food or water was carcinogenic in humans [191]. Evidence that NO_3^- might cause infant methemoglobinemia, which was first mooted in the 1940s [192], has also been questioned. Indeed, an investigation conducted on behalf of the WHO in 2004 found no exposure-response relationship between dietary NO_3^- and methemoglobinemia in infants [193]. It is also worth noting that although some studies report mild adverse symptoms with high NO_3^- intake such as nausea and sickness, to the authors knowledge, no serious adverse events have ever been reported in clinical trials administering NO_3^- [1, 93].

Notwithstanding, the available evidence does not rule out the possibility that prolonged consumption of NO_3^- above the ADI could harm health. Currently, at least with short to medium term intakes, research suggests that doses exceeding the ADI are needed to optimise vascular health or exercise performance [17, 48]. Because most human trials have only examined the acute health effects (< 4 weeks) of increased NO_3^- intake, the long-term safety of consuming NO_3^- in amounts that exceed the ADI is not well understood. At present, epidemiological studies provide the strongest evidence that prolonged, high intakes of NO_3^- are safe. Indeed, these indicate that rather than being harmful, dietary NO_3^- intake is inversely associated with cardiovascular disease risk [102, 194] and certain cancers [117]. Furthermore, diets and dietary patterns high in fruits and vegetables are linked to greater longevity [195, 196], protection against type 2 diabetes [197] and chronic obstructive pulmonary disease [198], and improved cardiovascular [92, 199, 200] and cognitive health [201, 202]. This suggests that higher intake of dietary NO_3^- , at least through plants, is more likely to be associated with health benefits than adverse effects.

Some animal studies have explored the longer-term effects of high dietary NO_3^- intake on health. In a study in rats, 10 weeks of a low sodium NO_3^- dose (0.1 mmol/kg/d), which the authors suggest is equivalent to amounts achievable in the human diet, reduced BP, whereas a much higher dose (1 mmol/kg/d), elevated BP [203]. Interestingly, this study found that the high NO_3^- dose down-regulated eNOS activity, not only suggesting a crosstalk between the canonical and NO_3^- - NO_2^- -NO pathway, but also that any vascular benefits afforded by NO_3^- supplementation could wane over time. Nonetheless, these findings were not supported by a more recent animal study from the same group. Hezel and colleagues [204] fed mice the human equivalent of 350 mg/d or 26 mg/d of sodium NO_3^- . After 17 months, mice consuming the high NO_3^- diet did not have elevated BP or any other adverse health effects, despite the fact the dose

exceeded the WHO recommended ADI for an adult under ~ 95 kg. On the contrary, the high NO_3^- diet decreased plasma insulin and modulated inflammation, findings consistent with the metabolic benefits observed in acute human studies [205]. These effects need to be verified in humans but support the notion that prolonged increases in NO_3^- intake are not harmful to health.

It is important to note that any carcinogenic risk attributed to NO_3^- intake could be mitigated by the intake of antioxidants such as vitamin C or (poly) phenols, which are present in most fruits and vegetables. Studies have shown that vitamin C and E are effective inhibitors of nitrosamine formation [167]. In addition, (poly) phenols, which are abundant in commonly consumed NO_3^- sources such as spinach and beetroot [206], can also abrogate nitrosamine formation [73]. Thus, increasing NO_3^- intake through a greater vegetable intake may significantly lessen the risk of any NO_3^- induced nitrosamine formation. This could partly explain why diets high in vegetables are associated with a reduced and not heightened risk of cancer.

Health concerns have also been raised over the high oxalate content of NO_3^- -rich vegetables [207, 208]. Oxalates are present in several foods, but particularly high in spinach, beetroot, and rhubarb [208, 209]. Intake of these foods increases urinary oxalate excretion, a risk factor for renal stone formation [209–211], thus, it is currently recommended that foods rich in dietary oxalates are consumed in moderation [208, 210]. However, the link between dietary oxalates and kidney stone formation remains equivocal. Although consuming oxalate rich foods increases oxalate excretion, a large prospective study (> 190,000 participants) found only modest non-significant associations between dietary oxalate intake and kidney stone risk, concluding that dietary oxalate intake is not a major risk factor for the formation of kidney stones in younger or older adults [212]. Furthermore, the Dietary Approaches to Stop Hypertension (DASH) diet, which is high in oxalates and NO_3^- -rich vegetables [1], was recently shown to increase urinary oxalate excretion but reduce the risk of kidney stone formation in ~ 260 patients [213]. The authors attributed these findings to the high calcium and magnesium content of the diet limiting oxalate absorption. This is supported by previous research showing that oxalates from beetroot have low bioavailability (< 1%), owing to their high calcium content [209]. While more prospective human trials are needed, evidence that oxalate rich vegetables increase the risk of kidney stone formation is limited.

To summarise, claims that dietary NO_3^- promotes cancer or methemoglobinemia, or that dietary oxalates cause kidney stones are weak and unsubstantiated.

Rather, there is compelling evidence that dietary NO_3^- has salutary health effects and warrants consideration as a long-term therapeutic treatment strategy to manage vascular and metabolic health. Notwithstanding, longer-term studies in humans are lacking and thus it cannot be ruled out that a prolonged increase in NO_3^- intake, above the WHO recommended ADI, may have adverse effects for some individuals. Thus, it is incumbent that researchers examine the long-term safety of increasing dietary NO_3^- consumption in a range of contexts and populations. This research will be vital for convincing the public and regulators that NO_3^- consumption is safe and that current recommendations to limit dietary NO_3^- intake should be re-considered.

Conclusions

This article has briefly outlined the current state of knowledge around the potential health effects of dietary inorganic NO_3^- . Six key areas worthy of future research were identified to enhance understanding of the potential role of NO_3^- in improving population health. As such, it is hoped that this article will help direct researchers to further explore the role of NO_3^- as a potential health-promoting dietary component.

Abbreviations

ADI: Acceptable daily intake; BP: Blood pressure; CVD: Cardiovascular disease; CKD: Chronic kidney disease; DASH: Dietary approach to stop hypertension; eNOS: Endothelial nitric oxide synthase; NIH: National Institutes of Health; NO_3^- : Nitrate; NO: Nitric oxide; NO_2^- : Nitrite; $\dot{V}\text{O}_{2\text{peak}}$: Peak oxygen uptake; WHO: World Health Organisation

Acknowledgements

Not applicable.

Authors' contributions

This review was conceived by OMS and TC, and designed by OMS, CE, AIS, MS, SJB and TC. OMS, CE, AIS, MS, SJB and TC drafted and critically revised the manuscript. OMS created the schematic. All authors approved the final version of the manuscript prior to submission.

Funding

No specific funding was received to support the writing of this review.

Availability of data and materials

Not applicable.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

T.C is an Associate Editor for BMC Sport Science, Medicine & Rehabilitation. All other authors declare no conflict of interest.

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Received: 24 February 2021 Accepted: 26 May 2021

Published online: 07 June 2021

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