Nitrate, the oral microbiome, and cardiovascular health: a systematic literature review of human and animal studies

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ABSTRACT

Background: Dietary nitrate is an important source of nitric oxide (NO), a molecule critical for cardiovascular health. Nitrate is sequentially reduced to NO through an enterosalivary nitrate-nitrite-NO pathway that involves the oral microbiome. This pathway is considered an important adjunct pathway to the classical L-arginine–NO synthase pathway.

Objective: The objective of this study was to systematically assess the evidence for dietary nitrate intake and improved cardiovascular health from both human and animal studies.

Design: A systematic literature search was performed according to PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines by using key search terms in Medline and EMBASE databases and defined inclusion and exclusion criteria.

Results: Thirty-seven articles on humans and 14 articles on animals were included from 12,541 screened references. Data on the effects of dietary nitrate on blood pressure, endothelial function, ischemic reperfusion injury, arterial stiffness, platelet function, and cerebral blood flow in both human and animal models were identified. Beneficial effects of nitrate on vascular health have predominantly been observed in healthy human populations, whereas effects in populations at risk of cardiovascular disease are less clear. Few studies have investigated the long-term effects of dietary nitrate on cardiovascular disease clinical endpoints. In animal studies, there is evidence that nitrate improves blood pressure and endothelial function, particularly in animal models with reduced NO bioavailability. Nitrate dose seems to be a critical factor because there is evidence of cross-talk between the 2 pathways of NO production.

Conclusions: Evidence for a beneficial effect in humans at risk of cardiovascular disease is limited. Furthermore, there is a need to investigate the long-term effects of dietary nitrate on cardiovascular disease clinical endpoints. Further animal studies are required to elucidate the mechanisms behind the observed effects. *Am J Clin Nutr* 2018;107:504–522.

Keywords: vegetables, nitrate, nitric oxide, oral microbiome, cardiovascular diseases

INTRODUCTION

Cardiovascular disease is the number one cause of death globally and contributes a major burden to public health systems worldwide (1). Several observational cohort studies have found plant-based diets rich in vegetables to be associated with a lower incidence of cardiovascular disease clinical endpoints (2–4). Specific vegetable groups, such as green leafy vegetables, have been shown to be the most beneficial (5–9). There are many bioactive components in green leafy vegetables that may benefit cardiovascular health. One component that has gained research interest in the past decade is nitrate (10).

Nitrate is present in all vegetables at various concentrations; however, the richest sources of nitrate are beetroot and green leafy vegetables (11). Increasing nitrate intake through the diet is one potential strategy to increase nitric oxide (NO) bioavailability (12). NO plays an important role in vascular tone and

The salary of JMH was supported by a National Health and Medical Research Council of an Australia Senior Research Fellowship and a Royal Perth Hospital Medical Research Foundation Fellowship. The salary of JRL was supported by a National Health and Medical Research Council Career Development Fellowship.

LCB and NPB contributed equally to this work.

Supplemental Tables 1 and 2 and Supplemental Figures 1 and 2 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/ajcn/.

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Abbreviations used: AIx, augmentation index; BH_4, tetrahydrobiopterin; CKD, chronic kidney disease; DASH, Dietary Approaches to Stop Hypertension; DBP, diastolic blood pressure; eNOS, endothelial nitric oxide synthase; FMD, flow-mediated dilatation; NO, nitric oxide; NOS, nitric oxide synthase; PWV, pulse-wave velocity; SBP, systolic blood pressure.

Received September 20, 2017. Accepted for publication November 21, 2017.

First published online April 9, 2018; doi: https://doi.org/10.1093/ajcn/nqx046.

integrity and is a vital molecule for cardiovascular health (12). Reduced NO bioavailability has been observed in individuals with cardiovascular disease (13). Strategies to increase NO in healthy individuals and those at risk of cardiovascular disease may reduce cardiovascular-related events in the wider population.

Due to the increased research interest in the vascular benefits of dietary nitrate, the aim of this review was to provide an overview of dietary nitrate as a source of NO, the importance of the oral microbiome in the nitrate-nitrite-NO pathway, and dietary sources of nitrate. We also systematically compiled evidence to date on the effects of nitrate ingestion on blood pressure, arterial stiffness, endothelial function, platelet function, and cerebral blood flow in human and animal studies. This systematic literature search was conducted by using criteria outlined in the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) checklist. Key search terms used in Medline and EMBASE databases are outlined in **Supplemental Table 1**, and inclusion and exclusion criteria are included in **Supplemental Table 2**. The PRISMA flow charts for human studies can be found in **Supplemental Figure 1** and for animal studies can be found in **Supplemental Figure 2**. Articles were excluded if full texts could not be accessed or if the articles were not in English.

TWO PATHWAYS TO NO

NO is an important cell signalling molecule critical for vascular homoeostasis (13). A powerful vasodilator, NO relaxes smooth muscle tissue and increases regional blood flow (14). NO also inhibits platelet and leukocyte adhesion to the vessel wall, delaying the onset of atherogenesis (15). NO is generated through the L-arginine–NO synthase (–NOS) pathway and the recently described enterosalivary nitrate-nitrite-NO pathway.

L-ARGININE–NOS PATHWAY

NO is synthesized predominantly through the classical L-arginine–NOS pathway (16), which involves 3 types of NOS isoforms. These include neuronal NOS, cytokine-inducible NOS, and endothelial NOS (eNOS) (17). Due to the large mass of the endothelium within the body, eNOS is a major contributor to NO production. The regulation of eNOS activity is via intracellular calcium (Ca^{2+}) (18) and several signal transduction pathways, including phosphoinoside 3-kinase and adenylate cyclase (AC) pathways (19). An increase in shear stress, cyclic strain, or receptor activation of vascular endothelium by biochemical stimuli (bradykinin, acetylcholine, thrombin, adenosine diphosphate, and serotonin) causes a release of Ca^{2+} from intracellular stores, stimulating eNOS activity (17, 20). Phosphorylation of several residues on the eNOS dimer is also an important requirement for activation (19). Equimolar amounts of NO and L-citrulline are produced by using L-arginine and molecular oxygen together with tetrahydrobiopterin (BH_4) in a complex oxygen-dependent 5-electron-transfer reaction (18, 21).

NO synthesised from L-arginine in the endothelium diffuses across the cell membrane to nearby smooth muscle cells, stimulating soluble guanylate cyclase (18). This results in the synthesis of cyclic guanosine monophosphate from guanosine triphosphate, triggering the relaxation of smooth muscle cells (18). Uncoupling of eNOS by reduced bioavailability of BH_4 or the substrate L-arginine can lead to the production of superoxide or H_2O_2 (22). Furthermore, studies have shown that reduced tissue concentrations of BH_4 and increased superoxide generation are associated with risk factors for atherosclerosis (23–25).

NITRATE-NITRITE-NO PATHWAY

Historically, nitrate and nitrite have been considered to be environmental pollutants and potential carcinogenic residues in the food chain (26). Now, however, nitrate and nitrite are considered important molecules for cardiovascular health (27).

Vegetables are a major source of nitrate consumed in the human population (28). When nitrate is ingested, it is absorbed in the proximal area of the small intestine (12). Nitrate then enters the bloodstream and mixes with endogenous sources of nitrate (mainly derived from oxidation of NO through the L-arginine–NOS pathway). Approximately 75% of circulating nitrate is excreted by the kidneys. The rest (~25%) is actively taken up by the salivary glands, where nitrate is concentrated in saliva and secreted in the oral cavity (29, 30). Nitrate is then reduced to nitrite by facultative anaerobic bacteria found in the deep clefts on the dorsal surface of the tongue (31). The commensal bacteria in the oral cavity use nitrate as an alternative electron acceptor to oxygen during respiration, reducing nitrate to nitrite by nitrate reductases (32). Once swallowed, a proportion of nitrite is rapidly protonated, forming nitrous acid (HNO_2) in the acidic environment of the stomach (33). Nitrous acid decomposes further to form NO, having localized benefits (33). This nonenzymatic reduction of nitrite to NO is enhanced by vitamin C and polyphenols (34, 35). The remaining nitrate and nitrite in the stomach enter the small intestine and are absorbed into the bloodstream where they mix with endogenous forms of nitrate and nitrite (mainly derived from oxidation of NO through the L-arginine–NOS pathway).

The 1-electron reduction of nitrite to NO in the blood and tissues is catalyzed by both enzymatic and nonenzymatic pathways (10). Enzymatic pathways include a number of proteins and enzymes, including globins (e.g., hemoglobin, myoglobin, cytoglobin, and neuroglobin), xanthine oxidoreductase, cytochrome P450, mitochondrial proteins, carbonic anhydrase, aldehyde oxidase, and eNOS (10). Nonenzymatic pathways include protons, polyphenols, and vitamin C (10). Both enzymatic and nonenzymatic reductions of nitrite to NO are enhanced during hypoxia and at a low pH (10, 36). Recent evidence suggests that the acidic environment of the stomach plays an important role in the reduction of nitrite to NO (37).

The nitrate-nitrite-NO pathway and the L-arginine–NOS pathway are interconnected through the anions, nitrate and nitrite. Nitrate and nitrite are the oxidation end products of NO metabolism through the L-arginine–NOS pathway but can also be derived from the diet (32). Nitrate and nitrite, derived from the diet and derived as oxidation end products of NO metabolism, are both recycled through the nitrate-nitrite-NO pathway. Both pathways become a storage pool for NO production. Because the L-arginine–NOS pathway requires molecular oxygen to produce NO, nitrite reduction to NO via the nitrate-nitrite-NO pathway may form as a backup system for NO production during hypoxia. A crucial step in the nitrate-nitrite-NO pathway is nitrate-to-nitrite reduction by the oral microbiome.

THE ORAL MICROBIOME

The oral microbiome is the second most diverse microbial community in the human body, comprising 50–100 billion bacteria from >700 prokaryotic taxa, as well as a fungal and viral flora (38). Disturbances to the composition, and therefore function, of the oral microbiome are thought to play a role in a number of diseases, including cardiovascular disease (38). Whether this link is related in part to the nitrate-nitrite-NO pathway is garnering research interest. An important step in the nitrate-nitrite-NO pathway is the reduction of nitrate to nitrite by facultative anaerobic bacteria found in the oral cavity. Reduced oral bacterial nitrate-to-nitrite reduction, both in the presence and absence of dietary nitrate intake, could have detrimental effects on the circulating NO pool, with subsequent vascular effects. In the presence of nitrate intake, interrupting the nitrate-nitrite-NO pathway with an antibacterial mouthwash or the spitting out of saliva prevented the resultant increase in salivary and plasma nitrite and the associated decrease in blood pressure (39, 40). In the absence of dietary nitrate intake, increases in blood pressure with concomitant decreases in salivary and plasma nitrite were observed with daily chlorhexidine-based antibacterial mouthwash use in both healthy volunteers (41) and individuals with treated hypertension (42). This could be explained by the fact that nitrate and nitrite, produced as end-products of NO metabolism, are recycled through the nitrate-nitrite-NO pathway back into the circulating NO pool. Thus, nitrate-to-nitrite reduction by the oral microbiome could play a key role in blood pressure control. The influence on other measures of vascular health has yet to be determined.

The fundamental role of the oral microbiome in the nitrate-nitrite-NO pathway and possibly blood pressure control makes understanding all of the factors that influence oral nitrate-to-nitrite reduction an important research area. Indeed, there is evidence of a considerable variation between individuals in the nitrate-reducing capacity of the oral microbiome (43). The first set of factors to consider is the use of antibacterial mouthwashes, antibacterial toothpastes, and antibiotics. Given the results of the studies described above, the widespread use of daily mouthwash in the general population is of potential concern. The mouthwash used in these studies, however, contained chlorhexidine, a strong antibacterial agent. Different effects have been observed with other types and strengths of antibacterial mouthwashes (44). To date, only one study to our knowledge has examined the effect of antibacterial toothpaste, which contained triclosan, on oral nitrate-to-nitrite reduction (45), with no effect observed. These results need to be confirmed in additional studies examining the effect of mouthwash and toothpaste on oral nitrate reduction. Interestingly, epidemiologic studies show that regular tooth brushing and mouthwash use, indicative of good oral hygiene, are associated with a decreased risk of hypertension and cardiovascular disease (46, 47). The effect of antibiotic use on oral nitrate-to-nitrite reduction has yet to be ascertained.

Other important factors are those inherent to the complex oral microbial community, such as bacterial genetics, the presence and influence of other microorganisms, and environmental pressures. There are a number of potential nitrate-reducing taxa present in the oral microbiome. Doel et al. (48) identified *Veillonella* spp. as the most abundant nitrate-reducing genus followed by *Actinomyces, Rothia*, and *Staphylococcus* spp. (48). Hyde et al. (49) confirmed *Veillonella* spp. as the most abundant nitrate-reducing genus present but also detected *Prevotella, Neisseria*, and *Haemophilus* at a higher abundance than *Actinomyces* spp. Nitrate-to-nitrite reduction by these bacteria is highly variable both within and between bacterial species and needs to be examined in the context of the huge interdependent microbial network in which they exist. This network comprises a heterogeneous microbial community within a biofilm that communicates by using a process called quorum sensing. These communities are highly complex, with all members influencing their health and vitality. Interestingly, the presence of nitrite reducers may prevent the accumulation of nitrite in the saliva and, as such, have a negative influence on the nitrate-nitrite-NO pathway (49). Microbial nitrate metabolism can also be altered by environmental influences such as pH and oxygen tension. A low pH in an oral microenvironment together with increased nitrate and nitrite concentrations can select for nitrate-reducing bacteria (50). Nitrate-reducing bacteria are facultative anaerobes. A low- or no-oxygen environment will therefore result in the nitrate reductive pathway being utilized for respiration. Other potential factors influencing nitrate-to-nitrite reduction that require future investigation include host factors, such as age, diet, and oral health.

The evidence of the link between oral health and cardiovascular disease being related to the nitrate-nitrite-NO pathway is strongly suggestive. Future studies will need to examine this relation in the context of the large number of factors that could influence oral nitrate-to-nitrite reduction.

DIETARY SOURCES OF NITRATE AND NITRITE

Vegetables contribute ~80% of dietary nitrate intake in the human population (28, 51–54). Nitrate ingested in the diet can also be derived from other food sources, such as fruit, grains, and animal products, with the remainder coming from drinking water. Many countries have strict regulations to maintain low amounts of nitrate in drinking water due to underlying health concerns, such as methemoglobinemia (55). High amounts, however, have been detected in private wells in rural areas due to nitrogen-based fertilizer use in agricultural areas (56). Another controversial health concern is the addition of nitrate and nitrite to meat and their potential to form N-nitrosoamines, which are potential carcinogens (29). Compounds such as polyphenols, vitamins C and E, and other antioxidants inhibit the formation of N-nitrosoamines (56). These compounds are abundant in vegetables. A large number of countries have also set maximum levels for nitrate in vegetables, particularly for lettuce and spinach, which are known to accumulate high amounts of nitrate (57). These maximum amounts vary across harvest period, being higher in winter and if grown under cover, and lower in summer and if grown in open air (57).

Dietary nitrite, on the other hand, contributes only a small amount to human exposure and is mainly consumed from animal-based foods such as cured meats and bacon (52). Nitrite is added to these products as a preservative and to enhance taste and appearance (52). Although a small amount of nitrite is consumed from these food sources, the majority of nitrite exposure (70–90%) is derived from the in vivo conversion of nitrate to nitrite through endogenous pathways (58).

The nitrate content of vegetables depends on many different factors, including the biological properties of plants,

fertilizer use, soil conditions, sun exposure, and cooking and storage methods. The biological properties of plants can influence the amount of nitrate that accumulates in that plant. For example, nitrate accumulates in different parts of the plants, with the leaf and stem having the highest concentrations and the bulb and fruit having the lowest (28). In our recently developed reference database for assessing dietary nitrate in vegetables (11), leafy vegetables were found to have the highest nitrate content, with Chinese flat cabbage and arugula containing the highest concentrations of nitrate (3000 mg/kg fresh weight). Corn, mushrooms, and peas had the lowest nitrate content (<50 mg/kg fresh weight). The nitrate concentration in vegetables also differs between varieties. For example, Chinese lettuce has a 3-fold higher nitrate value than iceberg lettuce (11).

Nitrogen-based fertilizers enhance the growth of plants and thus have an impact on how much nitrate accumulates in vegetables. Nitrate located in the soil of growing vegetables is transported via the plant xylem system to the leaves of the vegetables (52). Because organic vegetables tend to be grown in fertilizers containing less nitrogen, by comparison, conventionally grown vegetables tend to accumulate higher nitrate concentrations (11, 59).

Other factors, such as handling, storage, and processing, as well as temperature and light intensity, can also influence the amount of nitrate in vegetables (52). Higher nitrate concentrations are observed in vegetables grown in winter than in those grown in summer, and vegetables grown under cover contain higher nitrate concentrations than do those grown outdoors in the same season and the same region (11, 52).

Storage in ambient temperature can also reduce the nitrate content of fresh vegetables. Under refrigerated and frozen storage conditions, nitrate concentrations appear to be unaffected (52). Endogenous nitrate reductase activity and the amount of bacterial contamination due to postharvest storage and wilting processes reduce nitrate and subsequently increase nitrite in fresh vegetables (52). Being water soluble, nitrate is also reduced with washing and cooking methods by ~10–15% and 50%, respectively (52). Because nitrate is also found in the skin of vegetables, peeling the skin can also reduce nitrate concentrations by ~20–34% (52).

NITRATE INGESTION AND ITS EFFECTS ON VASCULAR FUNCTION

Dietary nitrate is now considered an important alternative source of NO. Human and animal studies to date have focused on the effects of nitrate ingestion on blood pressure, arterial stiffness, endothelial function, platelet function, and cerebral blood flow, as discussed below. A summary of the beneficial effects of nitrate ingestion on these cardiovascular-related outcomes in human and animal studies is shown in **Figure 1**. Benefits of nitrate ingestion on exercise performance are not covered in this review.

BLOOD PRESSURE

Evidence that decreased NO production was associated with hypertension raised the possibility that nitrate, through the nitrate-nitrite-NO pathway, could partially account for the blood pressure–lowering effects of green leafy vegetables. Randomized controlled trials such as the Dietary Approaches to Stop Hypertension (DASH) trial have been shown to reduce blood pressure (60). It has been suggested that the high nitrate content of the DASH diet contributes to the blood pressure–lowering effects observed (28). The DASH diet has been estimated to include as much as 1222 mg (19.7 mmol) nitrate/d (28). This amount can, however, differ by as much as 700% due to the wide variation in nitrate in vegetables (28). An Acceptable Daily Intake of 3.7 mg nitrate/kg body weight was set by the Joint Food and Agricultural Organization and WHO (52). For an average person weighing 70 kg, this is calculated to be 259 mg nitrate. The DASH diet can provide ≤500% more nitrate than this Acceptable Daily Intake.

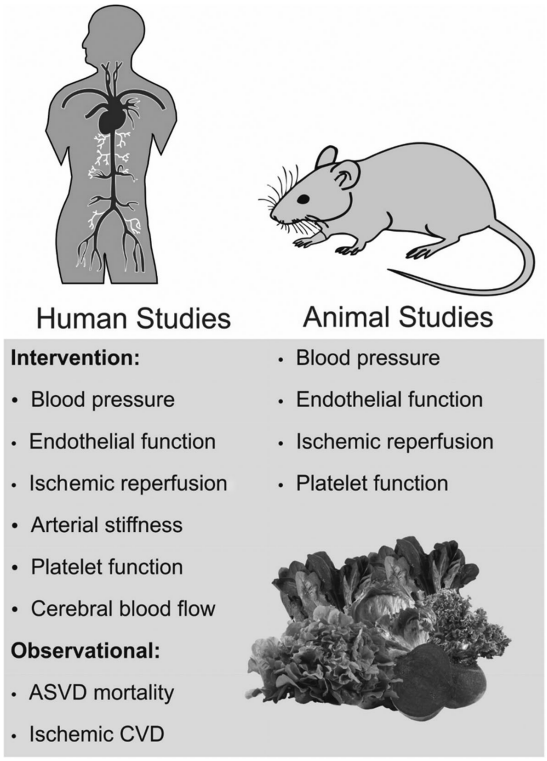

FIGURE 1 Observed beneficial effects of nitrate ingestion on cardiovascular-related health outcomes in human and animal studies. ASVD, atherosclerotic vascular disease; CVD, cardiovascular disease

The DASH diet is associated with reductions of 4.5 mm Hg in systolic blood pressure (SBP) (61). This blood pressure reduction is similar to that seen in a meta-analysis that showed that consumption of inorganic nitrate and nitrate-rich beetroot juice is associated with an SBP reduction of 4.4 mm Hg (62). There is now substantial evidence from human intervention trials to show blood pressure reductions with short-term intake of dietary nitrate in healthy populations (62). However, the effects of chronic nitrate intake on blood pressure in older populations and populations at risk of cardiovascular disease remain uncertain (50, 63–68).

Human studies

Our systematic literature search found 27 acute studies (≤24 h) (**Table 1**) (40, 50, 63, 69–85) and 15 chronic studies (>1 d) (**Table 2**) (50, 65–68, 87–94) in 32 publications investigating the effects of nitrate ingestion on blood pressure. Beetroot juice was

TABLE 1
Intervention studies investigating the acute effects of inorganic nitrate on BP in humans[1]

BP effect	Nitrate source	Nitrate dose	Duration, h	Subjects (mean ± SD age)	Screening/baseline BP	First author, year (ref)
↓ Clinic SBP ↓ Clinic DBP	Beetroot juice	583 mg (9.4 mmol)	3	Young: 10 M; 3 F (25 ± 4 y); old: 9 M; 3 F (64 ± 5 y); healthy	Optimal/normal	Hughes, 2016 (69)
↓ Clinic DBP	Sodium nitrate	800 mg (12.9 mmol)	5	11 M; 7 F (28 ± 1 y); healthy	Optimal/normal	Jonvik, 2016 (70)
↓ Clinic SBP ↓ Clinic DBP	Beetroot juice	800 mg (12.9 mmol)	5			
↓ Clinic SBP ↓ Clinic DBP	Rocket salad beverage	800 mg (12.9 mmol)	5			
↓ Clinic SBP ↓ Clinic DBP	Spinach beverage	800 mg (12.9 mmol)	5			
↓ Clinic SBP ↓ Clinic DBP	Beetroot juice	375 mg (6 mmol)	3	Nitrate: 12 M; 21 F (53 ± 10 y); placebo: 12 M; 22 F (53 ± 12 y); hypercholesterolemic	Normal	Velmurugan, 2016 (50)
↓ Clinic SBP	Spinach	220 mg (3.5 mmol)	3.5	6 M; 20 F (58.8 ± 7.6 y); healthy	Optimal	Liu, 2013 (71)
↓ Clinic DBP	Beetroot juice	500 mg (8.1 mmol)	2	20 M (61 ± 7 y); overweight	High-normal	Joris, 2013 (72)
↓ Clinic SBP	Spinach	182 mg (2.9 mmol)	3.3	6 M; 24 F (47 ± 14 y); healthy	Optimal	Bondonno, 2012 (73)
↓ Clinic SBP	Potassium nitrate	496 mg (8 mmol)	3	14 (28 ± 2 y); healthy	Optimal	Bahra, 2012 (74)
↓ Clinic SBP ↓ Clinic DBP	Potassium nitrate	1488 mg (24 mmol)	24	8 M; 12 F (23 ± 1 y); healthy	Optimal	Kapil, 2010 (75)
↓ Clinic SBP ↓ Clinic DBP	Potassium nitrate	248 mg, 744 mg (4 mmol, 12 mmol)	3	6 (29 ± 2 y); healthy	Optimal	
↓ Clinic SBP	Beetroot juice	341 mg (5.5 mmol)	3	9 (25 ± 1 y); healthy	Normal	
↓ Clinic SBP ↓ Clinic DBP	Beetroot juice	1395 mg (22.5 mmol)	24	9 M; 5 F (26 ± 5 y); healthy	Optimal	Webb, 2008 (40)
↓ Ambulatory DBP in T carriers only	Beetroot bread	68 mg (1.1 mmol)	6	14 M (34 ± 9 y); healthy	Normal	Hobbs, 2014 (76)
↓ Ambulatory DBP	Beetroot bread	68 mg (1.1 mmol)	6	23 M (31 ± 2 y); healthy	Normal	Hobbs, 2013 (77)
↓ Ambulatory SBP ↓ Ambulatory DBP	Beetroot juice	0–707 mg (0–11.4 mmol)	24	18 M (31 ± 3 y); healthy	High-normal	Hobbs, 2012 (78)
↓ Ambulatory SBP ↓ Ambulatory DBP	White beetroot-enriched bread	99 mg (1.6 mmol)	24	14 M (25 ± 1 y); healthy	High-normal	
↓ Ambulatory SBP ↓ Ambulatory DBP	Red beetroot-enriched bread	112 mg (1.8 mmol)				
↓ Ambulatory SBP (M only)	Beetroot juice	465 mg (7.5 mmol)	24	15 M; 15 F (43 ± 3 y); healthy	High-normal	Coles, 2012 (79)
No effect on clinic BP	Beetroot gel	391 mg (6.3 mmol)	3	4 M; 1 F (27 ± 2 y); healthy	Optimal	da Silva, 2016 (80)
No effect on clinic BP	Beetroot juice	341 mg (5.5 mmol)	2.5	5 M; 15 F (Nitrate: 21 ± 1 y); placebo: 7 M; 13 F (21 ± 1 y); healthy	Optimal	Wightman, 2015 (81)
No effect on clinic BP	Sodium nitrate	0.1–10 mg/kg body weight	4	15 M (25 ± 1 y); healthy	Optimal	Rodriguez-Mateos, 2015 (82)
No effect on clinic BP	Beetroot juice	694 mg (11.2 mmol)	2	5 M; 4 F (57 ± 10 y); heart failure	Optimal	Coggan, 2015 (63)
No effect on clinic BP	Beetroot juice	310 mg (5 mmol)	3	7 M; 4 F (25 ± 5 y); healthy	Optimal	Bakker, 2015 (83)

(*Continued*)

TABLE 1 (*Continued*)

BP effect	Nitrate source	Nitrate dose	Duration, h	Subjects (mean ± SD age)	Screening/baseline BP	First author, year (ref)
No effect on clinic BP	Beetroot juice	738 mg (11.9 mmol)	3	Young: 11 M; 5 F (27 ± 6 y); old: 8 M; 7 F (59 ± 6 y); healthy	Normal/high-normal	Shepherd, 2016 (84)
No effect on clinic BP	Beetroot juice	403–434 mg (6.5–7.0 mmol)	2	20 M (23 ± 3 y); healthy	Optimal	Lefferts, 2016 (85)

[1]Screening and baseline BP was based on criteria from the Australian guidelines for the diagnosis and management of hypertension in adults (86). BP, blood pressure; DBP, diastolic blood pressure; ref, reference; SBP, systolic blood pressure; ↓, reduction.

the most common nitrate source used in both acute and chronic studies. Twenty-four-hour ambulatory blood pressure, the preferred diagnostic method for assessing hypertension (95, 96), was used in 10 studies (65–68, 76–79, 88, 90). Clinic blood pressure was used in 34 studies (40, 50, 63, 65, 67, 69–75, 80–85, 87–94), and 4 studies used home blood pressure monitoring (66, 67, 88, 90).

Acute studies

The acute effects of nitrate ingestion on blood pressure were investigated between 2 and 24 h with nitrate doses ranging from 68 to 1488 mg (1.1 to 24 mmol) (Table 1). Five studies showed a significant reduction in SBP only (71, 73–75, 79), and 4 studies showed a significant reduction in only diastolic blood pressure (DBP) (70, 72, 76, 77). Eleven studies showed significant reductions in both SBP and DBP (40, 50, 69, 70, 75, 78). Acute reductions in SBP ranged from 2.7 to 22.2 mm Hg and from 2.6 to 23.6 mm Hg for DBP. Reductions in blood pressure were seen across the entire range of nitrate doses investigated and in subjects who were healthy (40, 69–71, 73–79), overweight (72), and hypercholesterolemic (50). Sample sizes of these populations ranged from 6 to 67 participants. Blood pressure reductions were not seen in 7 studies (63, 80–85). These populations consisted of subjects who were healthy (80–85) and subjects with heart failure (63). Sample sizes of these populations ranged from 5 to 40 participants.

Chronic studies

The chronic effects of nitrate ingestion on blood pressure were investigated in 15 studies from 3 to 42 d (6 wk) in duration, with nitrate doses ranging from 155 to 1104 mg/d (2.5 to 17.8 mmol/d) (Table 2). Three studies showed a significant reduction in SBP (89–91), and 3 other studies showed a significant reduction in DBP (87, 92, 93). Only one study showed a significant reduction in both SBP and DBP (88). In total, 7 studies showed a significant reduction in blood pressure. It is worth noting that the study conducted by Ashworth et al. (89) was not clear whether the significant reductions in blood pressure were acute or chronic because the subjects were advised to eat high-nitrate vegetables 2–3 h before blood pressure was taken on the final day. Reductions in SBP ranged from 4.0 to 8.1 mm Hg and reductions in DBP ranged from 2.4 to 12 mm Hg, with nitrate doses ranging from 165 to 1104 mg/d (2.7 to 17.8 mmol/d). Reductions in blood pressure were seen in one study that used 24-h ambulatory blood pressure monitoring (88), 2 studies that used home blood pressure (88, 90), and 6 studies that used clinic blood pressure (87–89, 91–93). Blood pressure reductions were seen in subjects who were healthy (87, 89, 92, 93), at moderate cardiovascular risk (91), and older and overweight (91), and in those who had grade 1 hypertension (treated and untreated) (88). These studies included a mix of young (mean age: <37 y) (87, 89, 92, 93) and older cohorts (mean age: >56 y) (88, 90, 91). Most studies that showed reductions in blood pressure had low sample sizes (n range: 6–25), except for Kapil et al. (88), which had a sample size of $n = 64$.

Blood pressure reductions were not seen in 8 studies (50, 65–68, 94). These populations consisted of subjects who were older (94), prehypertensive (67), treated hypertensive (66), overweight and obese (65), type 2 diabetic (68), and hypercholesterolemic (50). These populations were all older adult populations (mean age: >60 y) with larger sample sizes (n range: 27–67) (50, 65–68), apart from one study that had a sample size of $n = 8$ (94).

There is now clear and convincing evidence that nitrate reduces blood pressure within hours of ingestion. The evidence of chronic ingestion of nitrate on blood pressure is less clear. Studies suggest that chronic intake of nitrate lowers blood pressure in young healthy individuals; however, these blood pressure–lowering effects are not seen in older individuals and in individuals at risk of cardiovascular disease. Recent evidence suggests possible interactions between sulfate and nitrate, which may explain some of these inconsistencies (97). However, research is needed to further investigate this theory.

Animal studies

We identified 17 studies in 12 publications that assessed the effect of nitrate supplementation on blood pressure in an animal model (**Table 3**). Nitrate sources included $NaNO_3$- ($n = 10$), KNO_3- ($n = 1$), and $Mg(NO_3)_2$- ($n = 1$) supplemented drinking water. Nitrate doses ranged from 0.1 to 4.27 mmol · kg^{-1} · d^{-1} and treatment time ranged from 1 wk to 12 mo. The number of animals in each treatment group ranged from 5 to 23. Nine studies reported a decrease in blood pressure after nitrate supplementation and 5 studies reported no change in blood pressure. Only one study reported an increase in blood pressure; Carlström et al. (108) reported a significant increase in mean arterial pressure in healthy rats after 8 wk of nitrate supplementation (1 mM · kg^{-1} · d^{-1}). In the same study, a decrease in blood pressure was seen with a 0.1-mM dose of nitrate. In 2 studies in which high blood pressure was induced, either by the use of spontaneously hypertensive rats (101, 107) or by administration of a high-fructose diet (102), nitrate supplementation prevented the increase in blood pressure observed in the control group. In a study by Hezel et al. (104), a

TABLE 2
Intervention studies investigating the chronic effects of inorganic nitrate on BP in humans[1]

BP effect	Nitrate source	Nitrate dose	Duration, d	Subjects (mean ± SD age)	Screening/baseline BP	First author, year (ref)
↓ Clinic DBP	Beetroot juice	450 mg/d (7.3 mmol/d)	3	6 M (24 ± 1 y); healthy	Normal	Keen, 2015 (87)
↓ Clinic, home and ambulatory SBP ↓ Clinic, home and ambulatory DBP	Beetroot juice	398 mg/d (6.4 mmol/d)	28	$n = 64$ (26 M; 38 F); nitrate (58 ± 14 y) or placebo (56 ± 16 y); drug-naive and treated hypertensive	Grade 1 hypertension	Kapil, 2015 (88)
↓ Clinic SBP	High-nitrate vegetables	339 ± 133 mg/d (5.5 ± 2.1 mmol/d)	7	19 F (20 ± 2 y); healthy	Optimal	Ashworth, 2015 (89)
↓ Home SBP No effect on clinic and ambulatory BP	Beetroot juice	300–400 mg/d (4.8–6.4 mmol/d)	21	$n = 21$ (12 M; 9 F); beetroot (63 ± 2 y); placebo (61 ± 1 y); older overweight	Normal/high-normal	Jajja, 2014 (90)
↓ Clinic SBP	Sodium nitrate	9.3 mg · kg body weight^{-1} · d^{-1}	28	4 M; 7 F (63 ± 6 y); moderate cardiovascular risk	High-normal	Rammos, 2014 (91)
↓ Clinic DBP	Japanese traditional diet	18.8 mg · kg body weight^{-1} · d^{-1}	10	10 M; 15 F (36 ± 10 y); healthy	Optimal	Sobko, 2010 (92)
↓ Clinic DBP	Sodium nitrate	6.2 mg · kg body weight^{-1} · d^{-1}	3	15 M; 2 F (mean age: 24 y); healthy	Optimal	Larsen, 2006 (93)
No effect on clinic BP	Beetroot juice	375 mg/d (6 mmol/d)	42	Nitrate: 12 M; 21 F (53 ± 10 y); placebo: 12 M; 22 F (53 ± 12 y); hypercholesterolemic	Normal	Velmurugan, 2016 (50)
No effect on clinic and ambulatory BP	Beetroot juice	600 mg/d (9.7 mmol/d)	7	14 M; 16 F (62 ± 5 y); overweight and obese	Normal/high-normal	Lara, 2015 (65)
No effect on home and ambulatory BP	Beetroot juice	434 mg/d (7 mmol/d)	7	10 M; 17 F (63 ± 4 y); treated hypertensive	High-normal	Bondonno, 2015 (66)
No effect on clinic, home and ambulatory BP	Green leafy vegetables	300 mg/d (4.8 mmol/d)	7	12 M; 26 F (61 ± 7 y); prehypertensive	High-normal	Bondonno, 2014 (67)
No effect on ambulatory BP	Beetroot juice	465 mg/d (7.5 mmol/d)	14	18 M; 9 F (67 ± 5 y); T2D	Grade 1 hypertension	Gilchrist, 2013 (68)
No effect on clinic BP	High nitrate diet	155 mg/d (2.5 mmol/d)	3	3 M; 5 F (73 ± 5 y); older	High-normal	Miller, 2012 (94)
	Beetroot juice	527 mg/d (8.5 mmol/d)	3	3 M; 5 F (73 ± 5 y); older	Normal	
	Combination	682 mg/d (11 mmol/d)	3	3 M; 5 F (73 ± 5 y); older	High-normal	

[1]Screening and baseline BP was based on criteria from the Australian guidelines for the diagnosis and management of hypertension in adults (86). BP, blood pressure; DBP, diastolic blood pressure; ref, reference; SBP, systolic blood pressure; T2D, type 2 diabetes; ↓, reduction.

decrease in mean arterial pressure and SBP was only seen in old (22 mo) Sprague-Dawley rats and not in young (3 mo) rats. It is important to note that, although both groups were receiving the same concentration of nitrate in their drinking water, the younger rats were receiving a much higher dose of nitrate (776 μmol · kg^{-1} · d^{-1} compared with 290 μmol· kg^{-1} · d^{-1}), due to their higher water intake and lower body weight. In a study by Khalifi et al. (105), a decrease in SBP was only seen in diabetic Wistar rats and not their healthy counterparts. This may be due to positive effects of nitrate supplementation on NO status and oxidative stress, which would have been compromised in the diabetic rats but not in the healthy rats. Other studies have shown that higher doses of nitrate can reduce blood pressure in animal models that were shown to have reduced NO bioavailability (100, 102, 104).

ENDOTHELIAL FUNCTION

The endothelium lines the entire vascular system and plays an essential role in the maintenance of vascular homeostasis (112). Dysfunction of the endothelium has been identified in the development of atherosclerosis-related diseases (113). Flow-mediated dilatation (FMD) via noninvasive ultrasound measures the endothelial function of the brachial artery (114, 115). It is the gold-standard method for assessing conduit artery endothelial function (114) and is significantly associated with cardiovascular disease events (116, 117). It was previously shown from a meta-analysis of 14 prospective cohort studies that the risk of experiencing a cardiovascular event is reduced by 13% for every 1% increase in FMD (118). The degree of endothelial function

TABLE 3
Intervention studies investigating the effects of inorganic nitrate in animals[1]

Effect	Nitrate source	Background diet	Nitrate dose	Duration	Animals	First author, year (ref)
Blood pressure						
↓ MAP (6.7-mmol dose only)	KNO_3 in drinking water	Not described	2.5 or 6.7 mmol · kg^{-1} · d^{-1}	3 wk	Hypoxia WT male mice ($n \geq 8$)	Baliga, 2012 (98)
No change in MAP					Hypoxia eNOS KO male mice ($n \geq 8$)	
↓ MAP	$NaNO_3$ in drinking water	Not described	0.1 mmol · kg^{-1} · d^{-1}	8 wk	Rats ($5 \leq n \geq 15$)	Carlström, 2010 (99)
↓ MAP (1-mM dose only)	Supplemented with $NaNO_3$	High-salt diet	0.1 or 1 mmol · kg^{-1} · d^{-1}	8–11 wk	UNX male Sprague-Dawley rats	Carlström, 2011 (100)
Prevented ↑ in MAP	$NaNO_3$ in drinking water	Not described	1 mmol · kg^{-1} · d^{-1}	8 wk	Male SH rats ($n = 6$)	Chien, 2014 (101)
No change in MAP		Normotensive Wistar Kyoto rats ($n = 6$)				
↓ MAP	Supplemented with $NaNO_3$	High-fructose diet	1.8 mmol · kg^{-1} · d^{-1}	6 wk	Male Sprague-Dawley rats ($n = 8$)	Essawy, 2014 (102)
Prevented ↑ in MAP				10 wk from start	Male Sprague-Dawley rats ($n = 8$)	
↓ MAP	$NaNO_3$ in drinking water	Standard feed pellets	0.2 mmol · kg^{-1} · d^{-1}	1 wk	Male Sprague-Dawley rats ($n = 7$)	Petersson, 2009 (103)
↓ MAP and ↓ DBP				5 d	Male Sprague-Dawley rats	
No change in MAP or SBP	$NaNO_3$ in drinking water	Standard feed pellets	0.8 mmol · kg^{-1} · d^{-1}	2 wk	Young male Sprague-Dawley rats ($n = 8$)	Hezel, 2016 (104)
↓ MAP and ↓ SBP			0.3 mmol · kg^{-1} · d^{-1}		Old male Sprague-Dawley rats ($n = 5$)	
No change in SBP	$NaNO_3$ in drinking water	Standard feed pellets	0.1 g/L	8 wk	Male Wistar rats ($n = 8$)	Khalifi, 2015 (105)
↓ SBP					Diabetic male Wistar rats ($n = 8$)	
No change in BP	$NaNO_3$ in drinking water	Western-type diet	0.2 mmol/d	14 wk	LDL receptor KO mice ($n = 15$)	Marsch, 2016 (106)
Smaller increase in SBP	$Mg(NO_3)_2$ in drinking water	Not described	0.3 mmol · kg^{-1} · d^{-1}	4 wk	Male SH rats ($n = 7$)	Vilskersts, 2014 (107)
↓ MAP (0.1-mM dose only) ↑ MAP (1-mM dose only)	$NaNO_3$ in drinking water	Standard feed pellets	0.1 or 1 mmol · kg^{-1} · d^{-1}	8–10 wk	Male Sprague-Dawley rats ($n = 5$–12)	Carlström, 2015 (108)
Vascular function						
↓ Ach-mediated vasorelaxation (1-mM dose only)	$NaNO_3$ in drinking water	High-fat diet	0.1, 1 or 10 mmol · kg^{-1} · d^{-1}	2–4 wk	WT C57BL/6 mice ($n = 5$–12)	Bakker, 2016 (109)
No vasorelaxation				eNOS KO mice ($n = 5$–12)		
↑ Ach-mediated vessel relaxation (0.1- and 1-mmol dose only)		10 wk			Male *ApoE* KO mice ($n = 8$–12)	
Ischemic reperfusion						
↑ Perfusion recovery	$NaNO_3$ in drinking water	Not described	5.0 mmol · kg^{-1} · d^{-1}	2 wk	Male NMRI mice or C57BL/6 mice ($n = 21$–23)	Hendgen-Cotta, 2012 (110)
Platelet function						
↓ Collagen-induced platelet aggregation	$NaNO_3$ in drinking water	Standard feed pellets	1 g/L	1 wk	WT C57BL/6 mice ($n \geq 5$); eNOS KO mice ($n \geq 5$)	Park, 2013 (111)

[1] Ach, acetylcholine; DBP, diastolic blood pressure; eNOS, endothelial nitric oxide synthase; KO, knockout; MAP, mean arterial pressure; NMRI, Naval Medical Research Institute; NO, nitric oxide; ref, reference; SBP, systolic blood pressure; SH, spontaneously hypertensive; UNX, uninephrectomized; WT, wild-type; ↑, increase; ↓, decrease.

TABLE 4
Intervention studies investigating the acute effects of inorganic nitrate on endothelial function in humans[1]

FMD effect	Nitrate source	Nitrate dose	Duration, h	Subjects (mean ± SD age)	First author, year (ref)
↑ FMD	Beetroot juice	375 mg (6 mmol)	3	Nitrate: 12 M; 21 F (53 ± 10 y); placebo: 12 M; 22 F (53 ± 12 y); hypercholesterolemic	Velmurugan, 2016 (50)
↑ FMD	Beetroot juice	310 mg (5 mmol)	3	7 M; 4 F (25 ± 5 y); healthy	Bakker, 2015 (83)
↑ FMD	Sodium nitrate	0.1–10 mg/kg body weight	4	15 M (24 ± 1 y); healthy	Rodriguez-Mateos, 2015 (82)
↑ FMD	Beetroot juice	500 mg (8.1 mmol)	2	20 M (61 ± 7 y); overweight	Joris, 2013 (72)
↑ FMD	Sodium nitrate	9.3 mg/kg body weight	1.5	5 M; 5 F (26 ± 1 y); healthy	Heiss, 2012 (120)
↑ FMD	Spinach	182 mg (2.9 mmol)	4	6 M; 24 F (47 ± 14 y); healthy	Bondonno, 2012 (73)
No effect	Potassium nitrate	496 mg (8 mmol)	3	14 (28 ± 2 y); healthy	Bahra, 2012 (74)

[1]FMD, flow-mediated dilatation; ref, reference; ↑, improvement.

is determined by the change in brachial artery diameter before and after a shear stress stimulus, induced by reactive hyperemia (116). In the forearm vasculature, FMD provides a measure of endothelium-derived NO bioavailability (119).

Human studies

Our systematic literature search found 7 acute studies (≤24 h) (**Table 4**) (50, 72–74, 82, 83, 120) and 4 chronic studies (>1 d) (**Table 5**) (50, 68, 88, 91) in 10 publications investigating the effects of nitrate ingestion on FMD. Beetroot juice was the most common nitrate source used in both acute and chronic studies.

Acute studies

The acute effects of nitrate ingestion on FMD were investigated between 1.5 and 4 h with nitrate doses ranging from 6 to 772 mg (0.1 to 12.4 mmol) (Table 4). The lower nitrate dose in this range was estimated by using the global average body weight of 62 kg, because no average body weight was reported in this study (82). Six studies showed a significant improvement in FMD (50, 72, 73, 82, 83, 120), and one study showed no effect (74). Improvements in FMD ranging from 0.5% to 4.0% were seen across the entire range of nitrate doses investigated. Beetroot juice was also found to attenuate the postprandial impairment of FMD after a high-fat meal (72). Improvements in FMD were seen in mainly healthy populations (73, 82, 83, 120). Other populations in whom improvements in FMD were seen included hypercholesterolemic (50) and overweight (72) subjects. These healthy and at-risk populations consisted of 3 studies in younger cohorts (mean age: ≤27 y) (82, 83, 120) and 3 studies in older cohorts (mean age: >45 y) (50, 72, 73), with an overall sample size ranging from 5 to 67. No effects on FMD were observed in 1 healthy population of 14 participants aged 28 y (74).

Chronic studies

The chronic effects of nitrate ingestion on FMD were investigated in studies with durations that ranged from 14 to 42 d (2 to 6 wk) with nitrate doses ranging from 375 to 577 mg/d (6.0 to 9.3 mmol/d) (Table 5). The higher nitrate dose in this range was estimated by using the global average body weight of 62 kg, because no average body weight was reported in this study (91). Three studies showed a significant improvement in FMD (50, 88, 91), and 1 study had no effects (68). In particular, Rammos et al. (91) showed that dietary nitrate reversed vascular dysfunction in older adults with moderately increased cardiovascular risk. Improvements in FMD ranged from 0.5% to 1.1% and were seen across the entire range of nitrate doses investigated. Increases in FMD (∼1%) were seen in 2 studies (50, 88) by using similar nitrate doses from beetroot juice (375 and 398 mg/d). Ingestion of a slightly higher nitrate dose of 577 mg/d (9.3 mmol/d) with the use of sodium nitrate showed a 0.5% improvement (91). Improvements in FMD were seen in subjects with hypercholesterolemia

TABLE 5
Intervention studies investigating the chronic effects of inorganic nitrate on endothelial function in humans[1]

FMD effect	Nitrate source	Nitrate dose	Duration, d	Subjects (mean ± SD age)	First author, year (ref)
↑ FMD	Beetroot juice	375 mg/d (6 mmol/d)	42	Nitrate: 12 M; 21 F (53 ± 10 y); placebo: 12 M; 22 F (53 ± 12 y); hypercholesterolemic	Velmurugan, 2016 (50)
↑ FMD	Beetroot juice	398 mg/d (6.4 mmol)	28	$n = 64$ (26 M; 38 F); nitrate: (58 ± 14 y) or placebo (56 ± 16 y); drug-naive and treated hypertension	Kapil, 2015 (88)
↑ FMD	Sodium nitrate	9.3 mg · kg body weight^{-1} · d^{-1}	28	4 M; 7 F (63 ± 6 y); moderate cardiovascular risk	Rammos, 2014 (91)
No effect	Beetroot juice	465 mg/d (7.5 mmol/d)	14	18 M; 9 F (67 ± 5 y); T2D	Gilchrist, 2013 (68)

[1]FMD, flow-mediated dilatation; ref, reference; T2D, type 2 diabetes; ↑, improvement.

TABLE 6
Intervention studies investigating the acute effects of inorganic nitrate on IR in humans[1]

IR effect	Nitrate source	Nitrate dose	Duration, h	Subjects (mean ± SD age)	First author, year (ref)
Attenuated IR-induced endothelial dysfunction	Potassium nitrate	1488 mg (24 mmol)	3	12 (25 ± 1 y); healthy	Kapil, 2010 (75)
	Beetroot juice	341 mg (5.5 mmol)	3		
Attenuated IR-induced endothelial dysfunction	Beetroot juice	1395 mg (22.5 mmol)	2	4 M; 6 F (27 ± 7 y); healthy	Webb, 2008 (40)

[1]IR, ischemic reperfusion; ref, reference.

(50), treated and untreated hypertension (88), and moderate cardiovascular risk (91). All of the populations were older adult populations (mean age: >50 y) with large sample sizes ($n > 60$), except for 1 study that had a sample size of 11 (91). No effects on FMD were observed after 14 d of nitrate ingestion (beetroot juice) in 27 subjects with type 2 diabetes (68).

Animal studies

Numerous studies reported that blood vessels with a damaged endothelium have impaired vasorelaxation in response to acetylcholine (Table 3) (121, 122). We identified 3 animal studies, from 2 publications, investigating the effects of dietary nitrate supplementation on endothelial function (108, 109). Bakker et al. (109) showed that, although supplementation with very-high-dose nitrate (10 mmol · kg^{-1} · d^{-1}) had no effect on acetylcholine-mediated vessel relaxation in a mouse model of atherosclerosis, low- (0.1 mmol · kg^{-1} · d^{-1}) and moderate- (1 mmol · kg^{-1} · d^{-1}) dose nitrate supplementation significantly improved the endothelial dysfunction associated with this mouse model. In addition, Carlström et al. (108) reported that dietary supplementation with a high dose of nitrate (1 mmol · kg^{-1} · d^{-1}) was associated with attenuated acetylcholine-mediated vasorelaxation. These observations are in support of the theory proposed by Carlström et al. that there is cross-talk between the 2 pathways of NO production. They suggest that high doses of dietary nitrate may inhibit production of NO through the L-arginine–NOS pathway, leading to a net decrease in the amount of NO reaching the smooth muscle cells of the blood vessel (108). Although Bakker et al. showed improvements with a 1 mmol · kg^{-1} · d^{-1} dose of nitrate and Carlström et al. reported no improvements with the same dose, the animal model used is likely an important factor because the *ApoE* knockout mice used in the study by Bakker et al. (109) have reduced NO bioavailability.

ISCHEMIC REPERFUSION INJURY

Ischemic reperfusion injury is tissue damage caused by a period of ischemia or lack of oxygen. Lack of oxygen during an ischemic period results in inflammation and oxidative damage leading to microvascular dysfunction (123). Local and systemic tissue ischemia remains the major cause of death from cardiovascular disease (1). Because the nitrate-nitrite-NO pathway is enhanced in times of hypoxia, this pathway may provide a back-up to the classical L-arginine–NOS pathway.

Human studies

Our systematic literature search found 3 acute studies (2 publications) investigating the effects of nitrate ingestion on ischemic reperfusion injury (**Table 6**) (40, 75). Beetroot juice was the most common nitrate source used. The acute effects of nitrate ingestion on ischemic reperfusion injury were investigated between 2 and 3 h with nitrate doses ranging from 341 to 1488 mg (5.5 to 24 mmol) (Table 6). Benefits were also seen in all studies in which beetroot juice (40, 75) and potassium nitrate (75) attenuated ischemia reperfusion–induced endothelial dysfunction measured by using FMD. Improvements were seen in young (mean age: <28 y) healthy populations (40, 75), with an overall sample size ranging from 10 to 12.

Animal studies

We found only one study describing the effects of dietary nitrate supplementation on ischemia-induced revascularization in an animal model (Table 3). In a study by Hendgen-Cotta et al. (110), mice were treated with either nitrate (1 g/L $NaNO_3$ in drinking water) or NaCl (control) for 14 d. Perfusion recovery in the ischemic hind limb was significantly improved in mice treated with nitrate compared with controls via a significant increase in capillary density. These results suggest that dietary nitrate supplementation may represent a strategy to enhance ischemia-induced revascularization.

ARTERIAL STIFFNESS

Pulse-wave velocity (PWV) is a measure of aortic stiffness and is a strong predictor of cardiovascular events (124–126). PWV is recognized as the most simple, noninvasive, robust, and reproducible technique to determine arterial stiffness and is considered the gold-standard measurement of arterial stiffness (127). PWV measures arterial stiffness by dividing the estimated distance between the carotid and femoral arteries by the pulse transit time, the time delay between the carotid and femoral waveforms. A tonometer is used to capture the carotid waveform and a cuff is placed around the femoral artery to capture the femoral waveform. The augmentation index (AIx) is another measure of arterial stiffness, which provides a composite measure of elastic plus muscular artery stiffness and wave reflection. The AIx has also been shown to be an independent predictor of future cardiovascular disease events (128).

Human studies

Our systematic literature search found 7 acute studies (≤24 h) (50, 69, 71, 72, 74, 77, 85) and 5 chronic studies (>1 d) (50, 65, 67, 88, 91) in 10 publications investigating the effects of nitrate consumption on arterial stiffness (**Table 7**). Beetroot juice was the most common nitrate source used in both acute and chronic studies.

TABLE 7
Intervention studies investigating the effects of inorganic nitrate on arterial stiffness in humans[1]

Arterial stiffness effect	Nitrate source	Nitrate dose	Duration	Subjects (mean ± SD age)	First author, year (ref)
↓ AIx (young only)	Beetroot juice	583 mg (9.4 mmol)	Acute (3 h)	Young: 10 M; 3 F (25 ± 4 y); old: 9 M; 3 F (64 ± 5 y); healthy	Hughes, 2016 (69)
↓ PWV	Potassium nitrate	496 mg (8 mmol)	Acute (3 h)	14 (28 ± 2 y); healthy	Bahra, 2012 (74)
↓ PWV ↓ AIx	Beetroot juice	375 mg/d (6 mmol)	Acute (3 h)	Nitrate: 12 M; 21 F (53 ± 10 y); placebo:	Velmurugan, 2016 (50)
↓ PWV			Chronic (42 d)	12 M; 22 F (53 ± 12 y); hypercholesterolemic	
↓ PWV ↓ AIx	Beetroot juice	398 mg/d (6.4 mmol/d)	Chronic (28 d)	$n = 64$ (26 M; 38 F); nitrate (58 ± 14 y) or placebo (56 ± 16 y); drug-naive and treated hypertension	Kapil, 2015 (88)
↓ PWV ↓ AIx	Sodium nitrate	9.3 mg · kg body weight^{-1} · d^{-1}	Chronic (28 d)	4 M; 7 F (63 ± 6 y); moderate cardiovascular risk	Rammos, 2014 (91)
No effect on PWV and AIx	Beetroot juice	403–434 mg (6.5–7.0 mmol)	Acute (2 h)	20 M (23 ± 3 y); healthy	Lefferts, 2016 (85)
No effect on PWV and AIx	Beetroot bread	68 mg (1.1 mmol)	Acute (6 h)	23 M (31 ± 2 y); healthy	Hobbs, 2013 (77)
No effect on PWV and AIx	Beetroot juice	500 mg (8.1 mmol)	Acute (2 h)	20 M (61 ± 7 y); overweight	Joris, 2013 (72)
No effect on PWV and AIx	Spinach	220 mg (3.5 mmol)	Acute (3.5 h)	6 M; 20 F (59 ± 8 y); healthy	Liu, 2013 (71)
No effect on PWV	Beetroot juice	600 mg/d (9.7 mmol/d)	Chronic (7 d)	14 M; 16 F (62 ± 5 y); overweight and obese	Lara, 2015 (65)
No effect on PWV and AIx	Green leafy vegetables	300 mg/d (4.8 mmol/d)	Chronic (7 d)	12 M; 26 F (61 ± 7 y); prehypertensive	Bondonno, 2014 (67)

[1] AIx, augmentation index; PWV, pulse-wave velocity; ref, reference; ↓, reduction.

Acute studies

The acute effects of nitrate ingestion on arterial stiffness were investigated between 2 and 6 h, with nitrate doses ranging from 68 to 583 mg (1.1 to 9.4 mmol) (Table 6). Three studies showed a significant decrease in arterial stiffness (50, 69, 74), and 4 studies showed no effect (71, 72, 77, 85). A significant decrease of 0.3 m/s in PWV was observed in 2 studies (50, 74) with a nitrate dose of 375 mg (6 mmol) from beetroot juice (50) and 496 mg (8 mmol) from potassium nitrate (74). The study by Velmurugan et al. (50) consisted of a large sample size of 67 hypercholesterolemic men and women with a mean age of 53 y, whereas the study by Bahra et al. (74) consisted of a smaller sample of 14 healthy individuals with a mean age of 28 y. Hughes et al. (69) showed a reduced AIx in young, but not old, adults after a nitrate dose of 583 mg (9.4 mmol). No effect was seen in 4 studies with nitrate doses ranging from 68 to 500 mg (1.1 to 8.1 mmol) with the use of beetroot juice (72, 85), beetroot-enriched bread (77), and spinach (71). These studies consisted of healthy (71, 77, 85) and overweight (72) subjects.

Chronic studies

The chronic effects of nitrate ingestion on arterial stiffness were investigated from 7 to 42 d (1 to 6 wk) with nitrate doses ranging from 300 to 600 mg/d (4.8 to 9.7 mmol/d) (Table 7). Three studies showed a significant decrease in arterial stiffness after nitrate ingestion (50, 88, 91), and 2 studies showed no effect (65, 67). Studies found a significant decrease of 0.2–1.2 m/s in PWV with nitrate doses ranging from 375 to 577 mg/d (6 to 9.3 mmol/d) with the use of beetroot juice and sodium nitrate [577 mg/d was estimated by using the global average body weight of 62 kg because no average body weight was reported in this study (91)]. The populations in whom an effect was observed had moderate cardiovascular risk (91), untreated and treated hypertension (88), and hypercholesterolemia (50). No effect was seen in 2 studies with nitrate doses of 300 mg/d (4.8 mmol/d) from green leafy vegetables (67) and 600 mg/d (9.7 mmol/d) from beetroot juice (65) and in populations who were overweight and obese (65) and prehypertensive (67). It has been shown that for every 3.4-m/s increase in PWV, the risk of experiencing a cardiovascular event is increased by 17% (124). Therefore, a decrease of 0.2–1.2 m/s in PWV is likely to provide a small but significant reduction in the risk of experiencing a cardiovascular disease event.

Animal studies

Upon search of the literature, we found no animal studies investigating the effects of dietary nitrate supplementation on arterial stiffness.

PLATELET FUNCTION

Platelets play a major role in the acute complications of atherosclerosis in the late stages of the disease, which can subsequently lead to atherosclerosis-related events (129). NO has been shown to inhibit platelet aggregation and adhesion to the endothelial wall (130) and there is now evidence to suggest that dietary nitrate may repress platelet reactivity.

Human studies

Our systematic literature search identified 5 acute studies (≤24 h) (40, 131, 132) and 1 chronic study (>1 d) (50), in 4 publications, investigating the effects of nitrate intake on platelet function (**Table 8**). Potassium nitrate was the most common nitrate source used in acute studies, and beetroot juice was used in the chronic study.

Acute studies

The acute effects of nitrate ingestion on platelet function were investigated between 2.5 and 3 h with nitrate doses between 31 and 1054 mg (0.5 and 17 mmol) (Table 8). All 5 studies showed reductions in platelet aggregation and reactivity (40, 131, 132). Velmurugan et al. (131) showed that nitrate ingestion decreased platelet reactivity in healthy men but not in healthy women. This was observed with both beetroot juice (192 mg or 3.1 mmol) and potassium nitrate (496 mg or 8 mmol). Further studies that used beetroot juice (1054 mg or 17 mmol) (40) and potassium nitrate (31 and 124 mg or 0.5 and 2 mmol) (132) showed reductions in platelet aggregation. All of the cohorts consisted of young healthy populations and had small sample sizes ($n < 25$). Further acute studies are needed to replicate these findings in older adult populations at risk of developing cardiovascular disease.

Chronic studies

The chronic effects of nitrate ingestion on platelet function were investigated in only one study (Table 8) (50). Velmurugan et al. (50) showed a reduction in platelet-monocyte aggregates after 42 d of daily beetroot juice ingestion with a nitrate dose of 375 mg/d (6 mmol/d). This study had a large sample size ($n = 67$) of older men and women aged 53 y with hypercholesterolemia. There is a strong need for further chronic studies to investigate the effects of nitrate ingestion on platelet function in healthy populations and to replicate findings in older adult populations at risk of cardiovascular disease.

Animal studies

To our knowledge, only one animal study has been published investigating the effects of dietary nitrate supplementation on platelet function (Table 3). In this study, wild-type C57BL/6 mice were supplemented with 1 g $NaNO_3$/L in their drinking water for 1 wk, fed a low-nitrate diet, or continued on standard mouse feed pellets (control) (111). Platelet aggregation was significantly decreased in the group supplemented with nitrate and was significantly increased in the group fed the low-nitrate diet in comparison to the control group. These findings show that the manipulation of nitrate concentrations in blood, via supplementation or dietary restriction, could affect platelet function in mice, although further studies are required to corroborate this finding.

CEREBRAL BLOOD FLOW

The effect of dietary nitrate on cerebral blood flow has been investigated in several studies due to the observed effects of dietary nitrate on vasodilation and increases in blood flow. Diminished blood flow to the brain is likely to contribute to the pathophysiologic processes underlying vascular cognitive impairment (133).

Human studies

Our systematic literature search identified 1 acute study (≤24 h) (134) and 1 chronic study (>1 d) (135) in 2 publications investigating the effect of nitrate ingestion on cerebral blood flow (**Table 9**). Sodium nitrate and a high-nitrate diet were used as nitrate sources.

Acute studies

Presley et al. (134) showed that the consumption of a high-nitrate diet (769 mg or 12.4 mmol nitrate) over a 24-h period increased regional cerebral perfusion in frontal lobe white matter in older adults with a mean age of 75 y (Table 9). This was

TABLE 8
Intervention studies investigating the effects of inorganic nitrate on platelet function in humans[1]

Platelet effect	Nitrate source	Nitrate dose	Duration	Subjects (mean ± SD age)	First author, year (ref)
↓ Platelet reactivity in men but not women	Beetroot juice	192 mg (3.1 mmol)	Acute (3 h)	M: 12 (26 ± 1 y); F: 12 (24 ± 2 y); healthy	Velmurugan, 2013 (131)
↓ Platelet reactivity in men but not women	Potassium nitrate	496 mg (8 mmol)	Acute (3 h)	M: 12 (27 ± 1 y); F: 12 (29 ± 2 y); healthy	
↓ Platelet aggregation	Beetroot juice	1054 mg(17 mmol)	Acute (2.5 h)	5 M; 1 F (31 ± 2 y); healthy	Webb, 2008 (40)
↓ Platelet aggregation	Potassium nitrate	124 mg (2 mmol)	Acute (2.5 h)	4 M; 3 F (18–44 y)[2]	Richardson, 2002 (132)
↓ Platelet aggregation	Potassium nitrate	31 mg, 124 mg (0.5 mmol, 2 mmol)	Acute (2.5 h)	3 M; 3 F (18–44 y)	
↓ Platelet-monocyte aggregates	Beetroot juice	375 mg/d (6 mmol/d)	Chronic (42 d)	Nitrate: 12 M; 21 F (53 ± 10 y); placebo: 12 M; 22 F (53 ± 12 y); hypercholesterolemic	Velmurugan, 2016 (50)

[1] ref, reference; ↓, reduction.
[2] Range (all such values).

TABLE 9
Intervention studies investigating the effects of inorganic nitrate on cerebral blood flow in humans[1]

Cerebral blood flow effect	Nitrate source	Nitrate dose	Duration	Subjects (mean ± SD age)	First author, year (ref)
↑ Regional cerebral perfusion in frontal lobe white matter but no effect on global cerebral perfusion	High-nitrate diet	769 mg (12.4 mmol)	Acute (24 h)	14 (75 ± 7 y); older	Presley, 2011 (134)
No effect on cerebral blood flow	Sodium nitrate	6.2 mg · kg body weight^{-1} · d^{-1}	Chronic (3 d)	20 M (25 ± 1 y); healthy	Aamand, 2014 (135)

[1]NOS, nitric oxide synthase; ref, reference; ↑, increase.

particularly evident in the dorsolateral prefrontal cortex and anterior cingulate cortex. In the same study, however, the acute effects of a high-nitrate diet did not modify global cerebral perfusion.

Chronic studies

Aamand et al. (135) found no effects after 3 d of sodium nitrate ingestion (477 mg or 7.7 mmol nitrate/d, based on a study mean weight of 77 kg) on cerebral blood flow in 20 healthy men (Table 9).

Animal studies

No animal studies investigating the effects of dietary nitrate supplementation on blood flow were found.

SUMMARY: NITRATE INGESTION AND ITS EFFECTS ON VASCULAR FUNCTION

Human intervention studies have now shown that the ingestion of nitrate lowers blood pressure and improves endothelial function. These studies were predominantly in healthy populations and of short duration. It is yet to be established whether nitrate ingestion has the same effects in populations at higher risk of cardiovascular disease, because few studies have been conducted and findings are inconsistent. Further research is also needed to understand the long-term effects of nitrate intake on cardiovascular clinical endpoints.

EPIDEMIOLOGIC EVIDENCE

Epidemiologic studies have found that plant-based diets rich in vegetables are associated with lower rates of cardiovascular disease (2, 4, 136–141). In particular, cohort studies have shown specific vegetable groups that are high in nitrate, such as green leafy vegetables, to be most beneficial (6–9). The exact mechanisms for the protective effects shown in these studies are still unknown. The Mediterranean diet (3, 142), the DASH diet (60, 143), and a vegetarian diet (144, 145), all rich in vegetables, have been shown to be particularly beneficial for cardiovascular health. These diets are likely to contain substantially higher amounts of nitrate than the average Western diet. Thus, nitrate is one possible candidate for explaining cardiovascular health benefits seen with higher vegetable intakes (146).

There are very few observational epidemiologic studies investigating nitrate intake and cardiovascular-related health outcomes (**Table 10**). Although databases have been established to calculate the nitrate intake in observational epidemiologic studies (150–152), there was a strong need for a more comprehensive database with compiled up-to-date data. Our recently developed database on the nitrate content of vegetables (11) now gives researchers the opportunity to conduct more observational epidemiologic studies with an adequate assessment of nitrate intake.

To date, there have been 2 articles published that used the database on the nitrate content of vegetables (11). We have shown nitrate intake to be inversely associated with atherosclerotic vascular disease mortality in a cohort of older women (mean ± SD age: 75 ± 3 y) (53). In comparison to lower intakes of nitrate from vegetables of <53 mg/d (median: 39 mg/d), the inverse relation with atherosclerotic vascular disease mortality reached a plateau at intakes of 53–76 mg/d (median: 63 mg/d) (53). In the same cohort of older women, we also observed an inverse relation between nitrate intakes from vegetables and common carotid artery intima-media thickness, as well as ischemic cerebrovascular disease events (hospitalization or death) (54). The inverse relation with ischemic cerebrovascular disease events also reached a plateau at intakes of 53–76 mg/d (median: 63 mg/d) (54).

Before these studies were published, the Tehran Lipid and Glucose Study reported on the relation between the consumption of nitrate-containing vegetables and risk of hypertension (149) and chronic kidney disease (CKD) (147), both risk factors for cardiovascular disease. These studies investigated nitrate intake by assessing whole vegetables containing nitrate. The authors further categorized nitrate-containing vegetables into low-nitrate, medium-nitrate, and high-nitrate vegetables. It is worth noting that these studies essentially investigated whole vegetables and then different types of vegetables according to their nitrate contents and not nitrate as a separate entity. It is, however, difficult to separate nitrate intake from vegetable intake because the 2 can be highly correlated, as we have previously shown ($r = 0.75$, $P < 0.001$) (53). Golzarand et al. (149) found a significant inverse association between the intake of nitrate-containing vegetables and 3-y incidence of hypertension in the highest tertile compared with the lowest tertile of nitrate-containing vegetables. There were no significant associations observed between low-nitrate–, medium-nitrate–, and high-nitrate–containing vegetables and 3-y risk of hypertension. Because no associations were found between categories of nitrate-containing vegetables, it is difficult to determine whether the inverse association shown with total nitrate-containing vegetables is due to vegetable intake alone. This cohort consisted of 1546 Iranian men and women (57% women), aged 38 ± 12 y, without hypertension at baseline. In the same cohort, Mirmiran et al. (147) found that the highest

TABLE 10
Observational epidemiologic studies of dietary nitrate and cardiovascular-related health outcomes[1]

Study design and population	Nitrate intake assessment	Primary outcome	Adjusted variables	Results	First author, year (ref)
15-y follow-up study; $n = 1226$; Australian older women; diabetes and ASVD-free; aged 75.1 ± 2.7[2] y	FFQ	ASVD mortality	Model 1: unadjusted; model 2: age and energy; model 3: age, BMI, physical activity, alcohol intake, history of smoking, socioeconomic status, calcium supplementation group, organic nitrate medication, antihypertensive medication, statin medication, low-dose aspirin, renal function, and energy intake	↓ ASVD mortality	Blekkenhorst, 2017 (53)
15-y follow-up study; $n = 1226$; Australian older women; diabetes and ASVD-free; aged 75 ± 3 y	FFQ	Ischemic cerebrovascular disease hospitalization and death	Model 1: unadjusted; model 2: age and energy; model 3: age, BMI, energy intake, alcohol intake, energy expended in physical activity, antihypertensive medication, statin medication, low-dose aspirin medication, organic nitrate medication, history of smoking, and treatment	↓ Ischemic cerebrovascular disease hospitalization and death	Bondonno, 2017 (54)
Cross-sectional and 3-y follow-up study; $n = 1538$ cross-sectional and $n = 1229$ follow-up; Iranian men and women (57% women); aged 38.0 ± 12.0 y	FFQ	eGFR and CKD	Model 1: age, sex, and BMI; model 2: additional adjustment for smoking, education, physical activity, diabetes, and hypertension; model 3: additional adjustment for dietary intake of energy, fiber, and potassium	↓ eGFR, ↑ CKD (cross-sectional); no association for 3-y follow-up of CKD	Mirmiran, 2016 (147)
5.8-y follow-up study; $n = 2139$; Iranian men and women (54.6% men); T2D-free; aged 38.9 ± 12.6 y	FFQ	T2D	Model 1: diabetes risk score; model 2: additional adjustment for dietary total fat, fiber, and vitamin C	No association	Bahadoran, 2017 (148)
3-y follow-up study; Iranian men and women (57% women); aged 38 ± 12 y	FFQ	Hypertension	Model 1: adjusted for age and sex; model 2: additional adjustment for weight, 3-y weight change, smoking, education, physical activity, and baseline SBP and DBP; model 3: additional adjustment for dietary intake of energy, fiber, sodium, potassium, and processed meat	No association	Golzarand 2016 (149)

[1] ASVD, atherosclerotic vascular disease; CKD, chronic kidney disease; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; FFQ, food-frequency questionnaire; ref, reference; SBP, systolic blood pressure; T2D, type 2 diabetes; ↑, increase; ↓, decrease.
[2] Mean ± SD (all such values).

compared with the lowest tertile of intake of nitrate-containing vegetables was associated with a lower estimated glomerular filtration rate and a higher prevalence of CKD at baseline. This could be a demonstration of reverse causality bias in which the diagnosis of chronic disease has altered dietary intake. There was no association with the occurrence of CKD after 3 y of follow-up after excluding patients with CKD at baseline. Last, Bahadoran et al. (148) recently reported findings on the potential effects of dietary nitrate and nitrite on the occurrence of type 2 diabetes in the same cohort of Iranian men and women (Tehran Lipid and Glucose Study). The authors reported on 2139 adults who were free of type 2 diabetes at baseline, with a median follow-up of 5.8 y. Nitrate and nitrite values were determined from a recent survey conducted on frequently consumed food items among Iranians (153). Nitrate and nitrite concentrations of 87 foods were determined by using spectrophotometric methods. The authors found no associations between nitrate intake and the risk of developing type 2 diabetes. However, they showed an increased risk of type 2 diabetes among participants with higher intakes of total and animal-based nitrite in the presence of low vitamin C intake. The same was not observed in participants with high intakes of vitamin C (>108 mg/d) (148), suggesting that diets high in vitamin C may counteract the suggested adverse effects of nitrite on type 2 diabetes. However, higher intakes of total and animal-based nitrite in the presence of low vitamin C intake may be a marker of an unhealthy diet and lifestyle, which may also be associated with a higher prevalence of type 2 diabetes.

There is a lingering concern that nitrate and nitrite may form cancerous compounds, such as nitrosamines (10). The majority of epidemiologic studies to date have investigated relations between nitrate intake and cancer outcomes. A report compiled by the International Agency for Research on Carcinogenicity concluded, "Ingested nitrate or nitrite under conditions that

result in endogenous nitrosation is probably carcinogenic to humans (Group 2A)" (154). Conditions that increase endogenous nitrosation are complex but could involve interactions between the amount of nitrate and nitrite consumed, stomach acidity, smoking status, medical conditions, and low intakes of nutrients that are likely to decrease the potential for nitrosation such as polyphenols, vitamin C, and vitamin E (56).

Now that there is a comprehensive database on the nitrate content of vegetables available, researchers have the opportunity to further investigate the associations between chronic intake of nitrate and health outcomes. Further research is needed to elucidate the relations between different populations, including young and older age groups, low and higher background nitrate intakes, and healthy and at-risk populations.

CONCLUSIONS

There is now strong evidence to suggest that dietary nitrate derived from vegetables can reduce blood pressure and other markers of vascular function in healthy populations. There is a need for further research to investigate whether similar effects are observed in populations at risk of developing cardiovascular disease. Few studies have investigated the long-term effects of dietary nitrate on cardiovascular disease clinical endpoints; large observational follow-up studies are required to address this. Further animal studies are required to elucidate the mechanisms behind the observed beneficial effects. Increasing nitrate in the diet through the consumption of nitrate-rich vegetables may prove to be an achievable and cost-effective way to reduce the risk of cardiovascular disease.

The authors' responsibilities were as follows—LCB, NPB, AHL, and CPB: conducted the research; LCB, NPB, JMH, and CPB: wrote the manuscript; LCB, NPB, and CPB: had primary responsibility for the final content; and all authors: designed the research, critically revised the manuscript for important intellectual content, and read and approved the final manuscript. None of the authors had a potential conflict of interest to report.

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