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Role of Dietary Nitrate on Human Health: A Narrative Review

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Abstract

There has been a perspective shift regarding the role of inorganic nitrate consumption in human health. It was once thought to be a potentially harmful food additive, but more research has been conducted on the potential health benefits of dietary nitrate. This narrative review aimed to summarize important findings about the role of dietary nitrate in human health. Literature search was performed through PubMed and Google Scholar on the topics of dietary inorganic nitrate. This brief review was then synthesized summarizing the current evidence concerning its dietary source, safety concern, metabolism, and some potential health benefits. Sodium nitrate is a naturally occurring compound in many vegetables. It is absorbed in saliva and converted into nitrite, which can be converted into nitric oxide (NO), providing vasodilatory benefits. While concerns about nitrate safety are limited, it offers potential health benefits like improved cardiovascular health and biofilm dispersal. Dietary nitrate has also been recommended as a sports supplement because it has been shown to improve athletic performance. Nitrate consumption may also be beneficial in the treatment of several other medical conditions, including Coronavirus Disease 2019 (COVID-19). In general, dietary nitrate can boost production of nitric oxide which is beneficial for health. All current findings regarding the benefits of naturally occurring nitrate substantiate the recommendation of daily vegetable consumption.
Keywords: Norganic nitrate, Nitrite, Nitric oxide, Cardiovascular, Vegetables.

Introduction

In the human diet, there are various substances beyond what are known as macronutrients (carbohydrates, proteins, fats) and micronutrients (minerals and vitamins). Besides, the various metabolites with biological activities, plants can also contain several inorganic compounds that they absorb from their environment. This article will discuss one of these compounds, sodium nitrate ($NaNO_3$), which is subject to evolving perception regarding its health effects.

About half a century ago, sodium nitrate was known as a food additive with quite serious risks. It was said that nitrate could trigger methemoglobinemia and form carcinogenic compounds, although there were no convincing studies on this. However, in recent studies, it is stated that this compound is naturally present in many vegetables whose intake is known to be inversely related to the incidence of cancer[(1,2)]. The purpose of this narrative review is to summarize the updated findings on the impacts of dietary nitrate for human health.

Methods

On the topic of dietary inorganic nitrate, a literature search of published manuscripts was conducted through PubMed and Google Scholar search engine. The main search terms were "dietary nitrate", "inorganic nitrate", and "health". Only English articles from the most recent decade were used for the review. Verbatim key messages were then organized by dietary sources, metabolism (nitrate kinetics), safety considerations, and the potential health benefits.

Dietary Sources

Although not officially considered a nutrient, some foodstuffs contain nitrate (NO_3^-), which is usually in the form of sodium nitrate ($NaNO_3$). These inorganic compounds presents naturally in various plants, both terrestrial and aquatic plants. Plants, including the edible ones, need to absorb nitrate to produce amino acids and proteins, a process that involves reducing nitrate using energy from photosynthesis [(3)]. However, many people are concerned about the use of this compound as a food additive. Processed meat products generally use nitrates or nitrites as preservatives because of their antibacterial properties against the deadly pathogen *Clostridium botulinum*[(4)]. In addition, nitrate can also enter the food chain as an environmental contaminant in water due to its intensive use in agriculture, animal husbandry and waste treatment. From the United

States and Europe it was reported that nitrate levels in drinking water were very low [5]. Interestingly, vegetables turned out to be the dominant source of dietary nitrate. Approximately 80% of nitrate intake comes from vegetables, with minor contributions from drinking water, animal foods and grains [6]. Leafy green vegetables tend to contain higher levels of nitrates in their leaves. On the other hand, plants that grow below the soil surface can also have high nitrate content because this compound is stored in tubers without being converted into amino acids. Beetroot is known to store nitrate in its root tubers in high concentrations [7].

Nitrate levels from each vegetable vary, not only between vegetables but even within the same vegetable the nitrate content can vary depending on growing conditions. Apart from beetroot, vegetables that are categorized as high-nitrate vegetables with an average nitrate level exceeding 1 g/kg are generally leafy vegetables, such as spinach, lettuce, radish, chicory. Onions, tomatoes, and some fruits are among those that have low nitrate content with an average nitrate content not reaching 100 mg/kg. Meanwhile, many vegetables have an average nitrate level in the range of 100-1000 mg/kg, for example carrots, cucumbers, cabbage, green onions, and green beans [2].

Nitrate Kinetics:The Enterosalivary Circulation

The physiology of nitrate cannot be discussed apart from the physiology of nitrite, its metabolite. Nitrate is a stable compound with a high bioavailability. After being swallowed, the nitrate ion is rapidly absorbed from the proximal part of the gastrointestinal tract [8]. According to the proportion of nitrate that enters the digestive tract, the salivary glands take up about a quarter of the nitrate circulating in the circulatory system and concentrate it in saliva to levels 10-20 times higher than the levels of nitrate in blood plasma [9].Several species of the microbiome inhabiting the oral cavity mediate the denitrification process that converts nitrate in saliva to nitrite (NO_2^-). This reaction depends on a set of nitrate reductase enzymes produced by several types of bacteria, which are not present in the human genome [8]. Low oxygen pressure will activate the nitrate reductase enzyme which is owned by facultative anaerobic bacteria [10]. *Veillonella* is the most widely reported genus of bacteria involved in this process because of its ability to metabolize lactate [11]. When the oral microbiome population was intervened with antimicrobial administration, the amount of nitrate reduced to nitrite in saliva was significantly reduced [12].

As much as 70-80% of nitrite exposure in the human body comes from reduction of nitrate in the oral cavity, making this reduction process the main source of nitrite for humans [4,13].

In the acidic stomach environment, facilitated by the nitrite reductase enzyme from enteric bacteria, nitrite is then reduced to nitric oxide, otherwise known as NO [8]. NO formation also takes place in the stomach when nitrites react with vitamin C or other polyphenols [14,15]. Thus, NO formation is more likely to occur when nitrates are consumed as part of a polyphenol-rich diet, as are most vegetables. Unlike the stable nitrate, nitrite tend to be more reactive in their metabolism; either oxidized to nitrate in an oxygenated environment or reduced to NO. Most of the nitrates and nitrites are excreted through the kidney [6].

Safety Considerations

As a food additive, nitrate has raised concerns about its safety. Basically, nitrate itself is not dangerous, but other compounds that can form after being ingested can be worrisome. The maximum legal limit of nitrate as a food additive has been established to 50 mg/kg of product [16]. Even though as mentioned earlier, many vegetables naturally contain more nitrates than the mentioned limit. The entero-salivary circulation mechanism of nitrates and nitrites is actually an essential pathway for health [8].Nitrate metabolism also provides a mechanism that prevents excessive conversion of nitrates to nitrites, thus preventing the risk of toxicity [17].

Nitrite had been suspected of causing methemoglobinemia but this is an uncommon case to occur at reasonable levels of nitrate intake and without bacterial contamination. Hemoglobin does have a higher affinity for nitrites than oxygen, so that the presence of nitrite in the blood circulation system can expel oxygen from the form of oxyhemoglobin and then form methemoglobin [18]. However, extreme amounts of nitrate are required before clinically significant methemoglobinemia can develop. In fact, nitrate consumption of about 5 mmol daily for 4 weeks produced functional health benefits unrelated to methemoglobin formation [19].

Intake of nitrate or nitrite is classified under conditions that result in endogenous nitrosation as potentially carcinogenic agents to humans [20]. This does not mean that nitrates or nitrites themselves can automatically cause cancer. The main concern is when nitrite reacts with amino acids produced during protein digestion, as happens when the body digests processed meat products that contain nitrate or nitrite. There will be a reaction that produces a group of N-nitroso compounds (NOC), which have been clearly shown to be carcinogenic. However, a study by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) stated that there is no evidence that confirm the carcinogenicity of nitrate. If nitrite are indeed a carcinogen, then swallowing saliva should be prohibited because saliva contains about 50-100 mmol/mL nitrite [21].

In addition, when consumed as part of a normal diet containing vegetables, other bioactive compounds reduce the formation of nitrosamines by up to 50% [22].

What distinguishes processed meat from vegetables in the context of nitrate metabolism is that vegetables contain compounds that can prevent the formation of NOC in the digestive tract [(23)]. Such compounds include antioxidants such as vitamin C, vitamin E, and various polyphenols which are commonly found in various edible plants [(24)]. However, this does not mean that vegetables can prevent the harmful effects of consuming foods that already contain NOC, or what is called as preformed NOC. Based on studies, preformed NOC that presents in processed meat is more at risk of causing rectal cancer than the risk of NOC that is formed after the digestive process in the body [(25)]. Besides that, vegetables are not a source of preformed NOC because they contain much less protein fragments.

In 2002 JECFA reconfirmed that the Acceptable Daily Intake (ADI) of nitrate is 3.7 mg/kg body weight [(26)], equivalent to 222 mg nitrate per day for a 60 kg adult. Nitrate exposure obtained from eating 400 g of various vegetables daily with nitrate content around the median level is generally estimated at 157 mg/day [(27)]. Thus, the ADI limit of nitrate will not be exceeded by the daily consumption of vegetables in the recommended portion. Thoughts have also developed regarding the possibility that inorganic nitrate content may contribute to the healthful properties of a diet rich in vegetables. Analysis of nitrate levels from the Dietary Approaches to Stop Hypertension (DASH) diet indicated that the recommended diet for hypertensive patients may contain nitrates at levels that even exceed the ADI [(28)].

Potential Health Benefits from Dietary Nitrate

The potential health benefits from naturally occurring nitrate along with the supporting references are summarized in Table 1. Although some of the literature only mention about nitrite or NO, they are also considered to substantiate the potential benefits of nitrate because as mentioned earlier, nitrate consumption leads to the increased of nitrite and NO in human body.

Table 1. Potential health benefits of dietary nitrate

Health area	Finding	Reference
Cardiovascular health	Cardioprotective effect from nitrate supplementation	29
	Reduced blood pressure by either inorganic nitrate salt, Japanese diet, certain vegetables, or beetroot juice	17,30
	Reduced blood pressure and increased plasma nitrite by beetroot juice	19
Athletic performance	Enhanced exercise performance by beetroot juice as a source of nitrate	31–34
	Nitrate supplementation up to ~4 weeks reduce oxygen cost	35
	Nitrate improves skeletal muscle function by increasing efficiency of energy metabolism of muscle	36
	Beetroot juice prolongs time to exhaustion by reducing muscle fatigue and perceptual response	37
Biofilm dispersal	NO as a signaling molecule impacting biofilm	38–40
	Green vegetables consumption may increase number of nitrate-reducing bacteria in the mouth	41
	Nitrate may act as prebiotic to treat oral dysbiosis	42
	Nitrate may kill *P. aeruginosa*, either in planktonic or in biofilm state	43,44
Other medical conditions	Protection of various tissues by nitrate from ischemia reperfusion injury	45,46
	Nitrite may inhibit growth of *H. pylori*	47
	NO potential in prevention and early treatment of COVID-19	48–51

Cardiovascular Health

The underlying concept behind many of nitrate's potential benefits is its role in compensating for reduced NO production, a common occurrence in the aging process. A decrease in NOS enzyme activity has an impact on increasing blood pressure and other cardiovascular problems [(52)].

Cardiovascular disease is still a major cause of morbidity and mortality in society. By far, the strongest risk factor for cardiovascular disease is aging, caused by vascular dysfunction, including hardening of the arteries (aorta and carotid arteries) and endothelial dysfunction. Hardening of the arteries increases systolic blood pressure, pulse pressure, blood flow and pressure which damages the microvascular network. This damage results in pathophysiological remodeling of the heart, including left ventricular hypertrophy and increased risk of heart failure; as well as a major risk factor for cognitive dysfunction, dementia, and chronic kidney disease [(53)].

Conditions of decreased cardiovascular function due to decreased activity of the NOS enzyme can be compensated for by administration of nitrate so that NO can be formed without relying on the NOS enzyme. Nitrate supplementation has been shown to have the same cardio protective effect as the formation of NO from arginine using the NOS enzyme [(29)].

One study found that intake of nitrates from spinach reduced arterial stiffness and lowered blood pressure at 180 minutes after one week of supplementation. This shows the potential of using vegetables as a source of natural nitrate as an effective way to maintain cardiovascular health [30]. Previously, the effect of nitrate intake on human blood pressure was summarized by Lidder and Webb, who showed that a reduction in systolic and diastolic blood pressure could be obtained from consuming nitrate, both in the form of salt, the traditional Japanese diet, certain vegetables, and beetroot juice [(17)].

Clinical studies on hypertensive patients who were given beetroot juice containing nitrate of 250 ml/day increased plasma nitrite 5 times and significantly lowered blood pressure compared to placebo [(19)]. Vascular endothelial function mediated by dilation of the brachial artery flow was also improved in the beetroot juice-intervened group. Improvements in endothelial function and arterial stiffness have also been observed in patients with hypercholesterolemia and healthy older adults after supplementation with beetroot juice for more than 4 weeks. In heart failure patients with ejection fraction, beetroot juice supplementation has been shown to reduce systemic vascular resistance and increase cardiac output and exercise capacity [(54)]. Although beneficial effects have not been observed in all studies, most data have suggested dietary regulation as an approach to improve circulating nitrite and NO bioavailability as well as to enhance cardiovascular health [(53)].

There are several biochemical mechanisms capable of reducing nitrite to NO and all of them occur more readily under hypoxic conditions; different from NO synthesis using the NOS enzyme from L-arginine which requires molecular oxygen [(7)]. In general, the vasodilatory effect that occurs after ingestion of nitrates is mediated by the sequential reduction of nitrate to NO. The effect of relaxing blood vessels is strengthened by the presence of ascorbic acid, also known as vitamin C, as a reducing agent that facilitates the conversion of nitrate [(55)]. The vasodilatory effect of inorganic nitrate also needs to be differentiated from similar effects caused by organic nitrates, which are commonly used as pharmacological therapy in the form of nitroglycerin for people with angina. Although organic nitrate are also metabolized in the body into NO and nitrites, this biotransformation requires certain enzymes whose processes are prone to triggering tolerance conditions [(56)].

A diet rich in natural nitrate benefits people with cardiovascular disease not only because of their effect on blood vessels. Nitrate consumption was also found to significantly inhibit platelet activity compared to the control group. Platelet adhesion and aggregation are themselves important mechanisms in the pathogenesis of stroke and acute coronary syndromes [(55)].

Athletic Performance

With NOS enzymes, NO can be synthesized through two amino acid pathways, namely: arginine and citrulline as well as consumption of inorganic nitrate sources. Arginine in the form of L-arginine which is naturally found in food sources of protein, is an essential amino acid that plays a role in several metabolic pathways including the synthesis of NO [(57)]. On the other hand, the effects of L-arginine on blood flow and exercise performance in young adults are equivocal. Some studies report benefits of L-arginine on exercise performance, but other studies report no effect on muscle endurance or exercise performance [(34)].

Several studies have investigated the effects of diets rich in inorganic nitrate, which function as NO substrates via the nitrate-nitrite-NO pathway, on NO metabolism and exercise performance. Inorganic nitrates have become an increasingly popular NO precursor supplement for those looking to improve muscle performance and blood flow. Nitrate are naturally found especially in green leafy vegetables and root vegetables like beetroot [(57)]. Consumption of beetroot juice has been shown to consistently increase NO bioavailability in adults [(58)].

The effects of dietary nitrate supplementation on isokinetic, isometric, and resistance training have also been investigated. Consumption of beetroot juice containing 11.2 mmol nitrate in 12 healthy adults was shown to significantly increase peak power at the highest angular velocity (±4%) during isometric exercise. Additionally, administration of 12.9 mmol nitrate in the form of beetroot juice to adolescent males was shown to result in greater peak leg extension strength than placebo [(31)]. Other studies examining the effects of nitrates on exercise performance have mostly used beetroot juice as a natural inorganic nitrate provider and have yielded positive results for various types of exercise [(32–34)].

Intervention studies show that nitrate supplementation before exercise is beneficial to athletic performance because it reduces oxygen costs [(35)], increases muscle efficiency [(36)], and prolongs the time to exhaustion [(37)]. Increased exercise capacity as a benefit of nitrate supplementation is not only seen in healthy people, but also in patients who have decreased NOS enzyme activity due to certain diseases. For example in patients with chronic obstructive respiratory disease [(59)] and patients with chronic renal failure [(60)].

Biofilm Dispersal

Bacteria are more resistant to antimicrobial therapy when they coaggregate to form a biofilm compared to when they are in planktonic conditions separated between their cells, because biofilms represent microbial communities that have their own defense and communication systems [61]. The transition of an infectious disease from acute to chronic is often associated with the formation of a biofilm layer. Therefore, targeting biofilms is a promising strategy in the treatment of infectious diseases where biofilms form. In industrial abiotic environments, the addition of nitrate to anaerobic biofilms led to a 65% decrease in the number of bacterial species, thereby shifting the bacterial composition to a community with lower species diversity [62].

Nitric oxide acts as a signaling molecule having an effect on biofilm [38–40]. Since nitrate intake can increase NO production in the body, a diet rich in nitrates can be useful in treating infectious diseases where biofilms form, as is common in dental and oral diseases [63]. Dental plaque is a biofilm with an unhealthy composition due to dysbiosis, where pathogenic bacteria have dominated the composition of the microbiome. Increasing nitrate intake through consumption of green vegetables is thought to be important for increasing the number of nitrate-reducing bacteria in the oral cavity [41]. This is intended to suppress the growth of acid-triggering bacteria thereby protecting teeth from caries [42]. Inorganic nitrates have also been proposed as potential prebiotics to treat dysbiosis in the oral microbiome, especially in type 2 DM patients [42].

There is also a potential anti-biofilm effect of nitrate and nitrite outside of the mouth. For example, nitrates can kill *Pseudomonas aeruginosa* biofilms that cause cystic fibrosis [43]. The presence of nitrate and nitrite can reduce cell aggregation and reduce biofilm formation. Nitrate-mediated inhibition of *Staphylococcus aureus* biofilm formation was inhibited by the addition of an NO scavenger, indicating NO involvement in this process [64]. It has also been shown that sodium nitrate has an antimicrobial effect on planktonic cultures as well as on *Pseudomonas aeruginosa* biofilms [44].

Other Medical Conditions

The reduction of nitrite to NO is triggered under conditions of hypoxia and ischemic stress, so it can play a therapeutic role in these conditions. Ischemia is a condition in which there is an obstruction or reduced blood flow along with oxygen to a part of the body. Paradoxically, reperfusion or restoration of blood flow after ischemia injures the tissue [65]. In animal models, nitrite is highly cytoprotective and able to protect tissues-including those in the heart, brain, kidney, and liver-from ischemia reperfusion injury. Therefore, nitrite-based therapy, which can be supplied from nitrate intake, has the opportunity to treat medical conditions related to ischemia reperfusion, such as myocardial infarction, stroke, organ transplantation, heart failure, and sickle cell anemia [45]. Pre-clinical trials have shown that dietary nitrite intake can alter susceptibility to cardiac and hepatic ischemia reperfusion injury [66]. In this context, nitrate can be considered as a precursor that provides nitrite to the systemic circulation following enterosalivary circulation. Studies in humans show that consumption of beetroot juice as a source of nitrate has an impact on reducing infarct volume [46].

Nitrite that are formed endogenously after nitrate ingestion also plays a role in inhibiting the growth of *Helicobacter pylori* [47], a pathogenic bacterium that triggers stomach ulcers [67]. There are also studies that try to look at the benefits of nitrate intake in metabolic syndrome and type 2 diabetes mellitus (DM). However, as has been studied, the data is still inconsistent, so further research is needed [68]. Other studies suspect that differences or abnormalities in vitamin C metabolism play a role in the absence of metabolic benefits from consuming nitrates in type 2 DM patients [69].

Another medical condition in which nitrate intake can provide benefits is infection with COVID-19. The disease that causes a global pandemic accelerates endothelial damage and NO deficiency [70]. One of the characteristic symptoms is a decrease in oxygen saturation due to respiratory disorders [71]. This condition can improve by regulating nutritional intake which also provides adequate nitrates [48]. Increasing body NO levels with intake of nitrates from food can also be considered for the prevention and treatment of the early stages of COVID-19 disease, as well as for preventing cardiovascular complications [49,50]. In addition, the number of viruses can also be reduced because NO is involved in the interaction between the SARS-CoV-2 virus and its host receptors [51].

Conclusion

The potential health benefits of ingesting inorganic nitrates are principally mediated by the nitrate-nitrite-NO metabolic pathway which involves the enterosalivary circulation. Reduction of nitrate to nitrite is mediated by certain oral bacteria, while reduction of nitrite to NO is triggered under acidic and hypoxic conditions. With an understanding of the kinetics of inorganic nitrate after it is digested in the body, it has been revealed that natural nitrate intake is a complementary source of NO synthesis in the body, without relying on the NO synthase enzyme.

Improving cardiovascular health and increasing physical exercise capacity are the themes that have so far been the most researched about the benefits of consuming nitrates. Several of the other potential benefits of nitrates have only been tested at the pre-clinical level. So far, the understanding of the health benefits of inorganic nitrates has strengthened the recommendation of regular vegetable consumption on daily basis. Other physiological functions derived from inorganic nitrate intake may be unveiled by future studies.

With current knowledge of the potential efficacy of nitrate intake, concerns about its potential toxicity seem to be exaggerated. It is also understood that when consumed as part of vegetables, other phytochemical compounds will reduce the formation of carcinogenic compounds. Therefore, this article does not focus on supporting the use of nitrate as a food additive, but rather on supporting the consumption of vegetables as natural nitrate providers.

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Conflicts of Interest

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Author Contribution

The authors confirm contribution to the paper as follows: study conception and design: ARF; data collection: ARF & MIS; analysis and interpretation of results: ARF & MIS. Author; draft manuscript preparation: ARF & MIS. All authors reviewed the results and approved the final version of the manuscript.

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