Derleme
Review

Adnan Menderes Üniversitesi Sağlık Bilimleri Fakültesi Dergisi 2019: 3(2); 120-130
Journal of Adnan Menderes University Health Sciences Faculty

Her Yönüyle Nitrat: Metabolik Yolları, Kaynakları ve İnsan Sağlığına Etkileri

Nitrate in All Respects: Metabolic Pathways, Sources, and Human Health

Mohamed Ghellam[1], İlkay Koca[1]

[1]Ondokuz Mayis University, Faculty of Engineering, Food Engineering Department

ÖZ

Nitrat, doğal olarak meydana gelen azot formudur. Hava, toprak, su ve bitkilerde bulunur. Aynı zamanda küredilmiş et ürünleri ve peynir gibi gıdalarda gıda katkı maddesi olarak da kullanılır. Eskiden beri, methamoglobinemia ve kanser gibi potansiyel zararlı etkilere sahip, gıda ve su için toksik kirletici olarak kabul edilmiştir. Son zamanlarda, nitratın insan vücudu için potansiyel faydaları olduğu bildirilmektedir. Bu derlemede, nitrat kaynakları ve akümülasyonuna etki eden faktörler, insan vücudunda metabolizması yanı sıra sağlık üzerine yararlı etkileri tartışılmaktadır.

Anahtar Kelimeler: Nitrat, nitrit, nitrikoksit, sağlık

ABSTRACT

Nitrate is a naturally occurring form of nitrogen. It exists in the air, soil, water, and plants. It is also used as a food additive in foodstuffs such as cured meats and some cheese types. Ever since the nitrate was considered as a toxic contaminant of food and water with potentially harmful effects such as methemoglobinemia and cancer. More recently, it is reported that dietary nitrate has beneficial effects for the human body. In this review, it is discussed the dietary sources of nitrate and their affecting factors, and metabolism of nitrate in the human body, as well as its benefits on human health.

Key Words: Nitrate, nitrite, nitric oxide, health

1. INTRODUCTION

The biological cycle of nitrogen contains nitric oxide (NO) and anions like nitrate and nitrite. The obtaining of these chemical elements can naturally start with the fixation of atmospheric nitrogen thanks to symbiotic microorganisms existing in some plant's roots and lightning in atmospheric events, or it can be industrially occurred by specific process of conversion even more by fossil fuel combustion (1). After that fixation, in plants and in animals, a number of conversions undergo to produce in vivo the bioactive NO and their intermediates; nitrate, and nitrite.

Consumption of high nitrite and nitrate containing foods and beverages have been associated with some harmful effects like methemoglobinemia, common in infant, and the possible production of N-nitrosamines responsible for the carcinogenic activity (2). In human body, the conversion of nitrate to nitrite is linked to the generation of nitrosamines, where under gastric acidic conditions the nitrite interact with secondary amino compounds to form the carcinogenic nitrosamine, which is implicated in the induction of certain cancer such as liver,

Sorumlu Yazar: Mohamed Ghellam
Ondokuz Mayis University, Faculty of Engineering, Food Engineering Department, Samsun, TURKEY
16210571@stu.omu.edu.tr
Geliş Tarihi: 05.11.2018 – Kabul Tarihi: 24.01.2019

kidney, pancreas, colon, and bladder cancers (3). Nevertheless, the presence of L-ascorbic acid, polyphenols, and other antioxidants in foods, could be potent inhibitors for nitrosamines generation (2). While it is suggested that converted nitrite are 10 times toxic than nitrate, the rate of conversion is known to be higher in babies and patients with gastrointestinal illnesses due to the lower acidity of the stomach (3).

Nitrate is relatively non-toxic, in contrast, its digestion and the metabolic reaction products such as nitrite, nitric oxide are implicated in the oxidation of oxyhemoglobin (containing Fe +2 -heme) to form nitrate and methemoglobin (containing Fe+3-heme) (4,5). Consequently, methemoglobin becomes unable to transport oxygen, Though, in adults, methemoglobin reductase enzyme reconverts it again to oxyhemoglobin, which is not the case of babies and other group of people who are lacking of this enzyme leading to cyanosis or what is called "blue baby syndrome" (5,3).

For more than four decades, nitrate and nitrite ingestion has been caused a debate at many levels about their implication on human health. This situation has led the scientific researches to investigate further to understand the mechanisms and the main interaction of nitrite and nitrate within the human body and their safety (6). Recently, interesting researches and studies are obtained, which inorganic and organic nitrite/nitrate have shown many potential properties that have favorable effects on human health. The nitrite and nitrate could promote health and prevent from some serious diseases (7,8). The resulting NO is believed to reduce the heart failure, cardiovascular diseases and to improve vascular function (9). Moreover, it may be a promising management means for diabetes and obesity (10). Considering, it has the ability to enhance life quality, it is also suggested that the daily intake of nitrate is needed to be increased (11).

Daily acceptable intake (ADI) for both nitrate and nitrite, have been determined, in which that of nitrate was set at 3.7 mg/kg of body weight (222 mg/day for a 60 kg adult), and that of nitrite was at 0.07 mg/kg (4.2 mg/day for a 60 kg adult) (12).

We aimed in this review to highlight the main metabolic pathways for the different nitrate and nitrites sources in the human body and to show the essential dietary sources and the major affecting factors that determine the nitrate/nitrite concentration's variation. Furthermore, we mentioned some important and recent experimental and clinical studies, also, their intriguing obtained results in which in general exhibit beneficial effects on human health.

2. NITRIC OXIDE AND METABOLIC PATHWAYS

Organic and inorganic nitrates, in spite of having some similar therapeutics effects by means of their conversion into NO, the structure of compounds and their pharmacodynamic properties are different each other's (13). Organic nitrates are complex synthesized compounds used medicinally as vasodilators (14). Nitroglycerine and isosorbide mononitrate are well known organic nitrates, which are administrated under various forms (13). They are readily absorbed via buccal, rectal, transdermal routes and also inhalation (14). However, inorganic nitrates anions are obtained by dietary sources and endogenously by the oxidation of NO (15). The main difference between organic nitrate/nitrite and inorganic nitrate/nitrite is that chronic therapy by organic nitrates results in the development of nitrate tolerance and endothelial dysfunction (9).

Unlike inorganic nitrates/nitrites, organic nitrates/nitrites undergo the first-pass metabolism with different oral bioavailability. They are rapidly distributed in the systemic circulation and subsequently in the body with a quick onset of action (1-3 min). Again, they are subjected a rapid metabolic reaction by several enzymes (14). In contrast, they are bioactivated by some common enzymes such as xanthine oxidase, mitochondrial aldehyde dehydrogenase and P450 enzymes likewise with the inorganic group responsible for nitrate bioactivation (9). Similarly, the metabolism of organic nitrate/nitrite is very quick, leading to a rapid offset (15-30 min) (14).

2.1. L-arginine nitric oxide synthase (NOS) pathway

The classical known synthesis pathway of NO is carried out by NOS which requires L-arginine and molecular oxygen. Many synthetase isoforms are involved in the endogenous reaction of NO production. In fact, each isoform like neuronal NOS, endothelial NOS, and inducible NOS, is characterized by the site of synthesis and dependence of cofactors such as nicotinamide adenine dinucleotide phosphate (NADPH), flavin mononucleotide (FMN), flavin adenine dinucleotide (FAD) and the others (16). Whereby, the action generated NO is dependent on its site and its concentration (17). As endothelial NOS is the dominant enzyme, which is affected by age and under some physiological conditions, the NO production declines to become one of the causes of endothelial dysfunction and the etiology of cardiovascular-related symptoms (18).

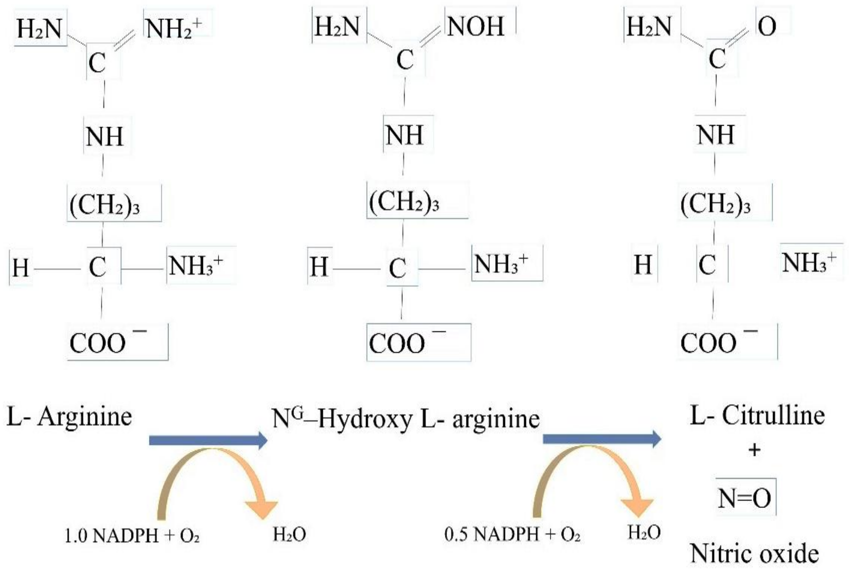

Figure 1: Mechanism of NO synthesis from L-arginine by nitric oxide synthase pathway, adapted from [19].

Cardiovascular diseases are among risk factors which have been associated in the dysfunction of endothelium and the impairment of nitric oxide synthase (NOS) pathways, for instance; diabetes mellitus, hypercholesterolemia, obesity, smoking, and aging is another factor that affects the function of this pathway. A healthy endothelium is recognized to play a crucial role in a cardiovascular system whereby it could function properly (17,20). On the other hand, endothelial dysfunction is known to be one of the major precursors of cardiovascular diseases (14). In addition, the dysfunction of the endothelium cannot be only over the relaxation of vessels but also, could affect the vascular homeostasis, and increase the vascular inflammation and the progression of atherosclerosis (20).

2.2. Nitrate-nitrite-NO pathway

The end-products of L-arginine NOS pathway and the diet are the main sources of nitrate and nitrite in the body (17). Recently, an alternative pathway for the nitrate-nitrite-NO pathway, which is different from the classical one, was discovered, and also responsible for the production of the bioactive NO (18). After consuming a nitrate-containing meal, the dietary nitrate absorbed at upper gastrointestinal tract, are mixed with that resulted from the oxidation of endogenous NO, thereby, the plasma nitrate levels increase and remain at a half-life of 5 to 6 h (18).

High extent of nitrate is lost in urine, and approximately 25% of nitrate is concentrated in salivary glands, wherein levels could reach 20-folds higher than plasma (22). Again, while this salivary nitrate is secreted in saliva, the existing commensal bacteria on the dorsal surface of the tongue will reduce it to nitrite (23).Nitrite is considered as a by-product produced by these microorganisms since they use nitrate as a terminal electron acceptor (17). Indeed, as it is demonstrated by (24) and in literature, those oral bacteria, which play a potential role in this pathway, could be affected by the several mouthwash solutions (23).

When nitrite reaches the acidic environment of the stomach, it's protonated to nitrous acid to form further the NO. The reduction from nitrite can be enhanced by reducing agents like polyphenols and L-ascorbic acid (22).On the other hand, the remaining of nitrite and nitrate are absorbed into systemic circulation (25). Also, nitrate can be reduced to NO in blood and other tissues by enzymatic and non-enzymatic routes (18). Therefore, it is viewed that nitrate-nitrite-NO pathway needs to be considered as storage pools for NO, specifically, when the NO generation by NOS enzymes are not sufficient such as during hypoxia conditions and uncoupling of NOS (21).

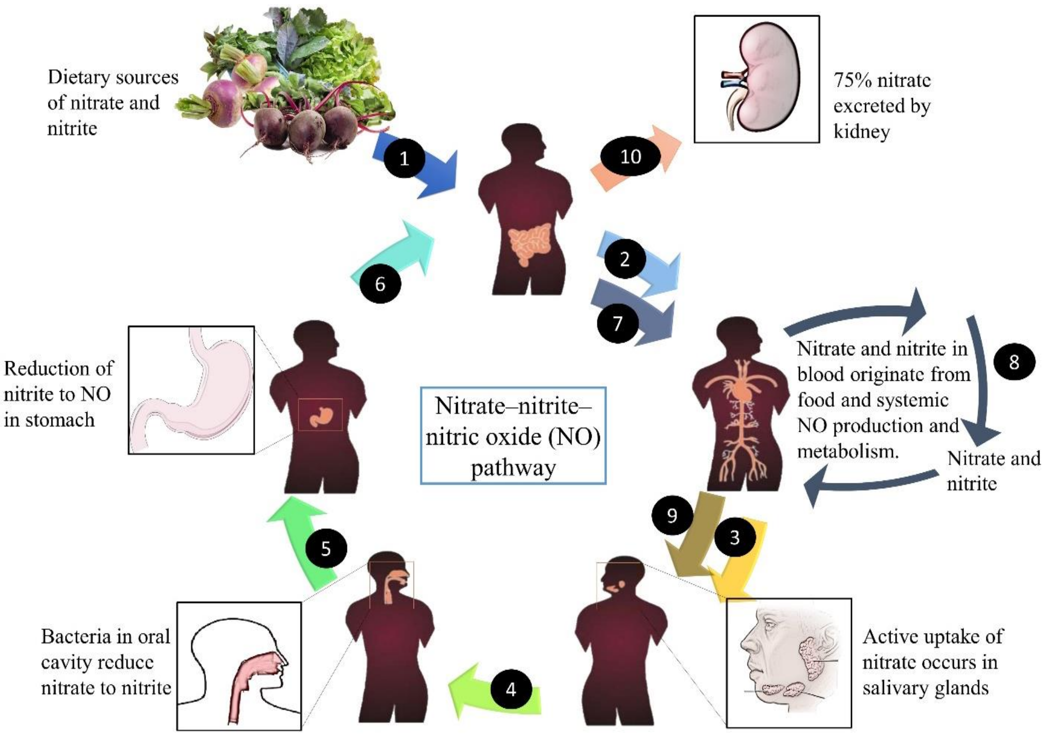

Figure 2: The nitrate-nitrite-nitric oxide pathway. After ingestion nitrate-rich food, is absorbed in the small intestine (1) from where it enters the circulation (2). There is an active uptake of the remaining nitrate by the salivary glands (3). In the oral cavity, the nitrate is reduced to nitrite by nitrate reducing bacteria found predominantly on the dorsal surface of the tongue (4). Some of this nitrite is reduced to NO in the acidic environment of the stomach (5). The remaining nitrite is absorbed in the small intestine (6) from where it enters the circulation (7). In the circulation, nitrite can be reduced to NO (8). Nitrate and nitrite can be formed by systemic NO metabolism. Nitrate thus formed could enter the cycle together with ingested nitrate (9). Approximately 75% of nitrate is excreted via the kidneys (10) adapted from [(17).

3. NITRATE SOURCES

Recent years, throughout researches and new findings regarding nitrate on human health, some biological effects have been attributed to nitrate, nitrite and NO. For this reason, it became more evident to mention the three molecules when the subject is only one of them.

Nitrate and nitrite can be obtained from dietary sources (exogenous) or by series of endogenous reactions like oxidation of NO. Or, an opposite reaction can occur by reduction reaction, when nitrate and nitrite will be substrates for the production of NO. These reactions take place in some organs and tissues depending on several factors; lifestyle, food type, commensal bacteria, physical activity and other factors which could influence those conversions (26).

Dietary sources of nitrate and nitrite are various. While 80% of nitrates are provided by vegetables, water and some foodstuffs show less providing extent in the diet (26). Nitrate as a food additive is used in cured meats, cheese products, fish products and some beverages (27).

In food processing and especially in curing meats, nitrate salts (potassium and sodium nitrates) are frequent additives. The reduction of nitrate to nitrite is known to contribute to the flavor and color of cured meats and to inhibit lipid oxidation. They also play a role against certain pathogens growth and spores such as Clostridium botulinum (22, 8).

Drinking water naturally contain low levels of nitrate, generally less than 10mg/L, but due to the overuse of nitrogen fertilizers in agriculture have changed the rule and many contaminations have been seen in water sources likewise wells, rivers and lakes. Thereafter, many countries legislations have strictly limited the levels of nitrate in drinking waters (8, 28).

Last decades, nitrosamine and its carcinogenicity caught the attention of scientific researchers. which led many studies to analyze the content of nitrate and nitrite in foodstuff, vegetables, and fruits. Most of the analysis have determined the existence of factors that govern the variation in the level of nitrate and nitrite. Table 1 listed the amount of nitrate and nitrite of some vegetables.

Table 1. The nitrate and nitrite amount of some vegetables in literature

	Nitrate, mg/kg	Nitrite, mg/kg	References
Beets	1680-3590	2.1-29.8	[30]
Broccoli	395; 29-1140	0.07; 0.01-9.5	[26,30]
Cabbage	41-939; 37-1831	0.9-30; 0.01-0.4	[31,30]
Carrot	100	-	[32]
Celery	20-4269; 1390	0.02-0.5	[30,32]
Chard	2360	-	[32]
Cucumber	130	-	[32]
Kale	41-1319	1.2-4.4	[31]
Lettuce	1156; 1870	2.6	[31,32]

Parsley	9-2441	1.3-13.4	[31]
Pear	60.56-178.43	-	[33]

Table 1. The nitrate and nitrite amount of some vegetables in literature (continued)

Rhubarb	55-6500	-	[48]
Spinach	7410;797-1427; 65-8000; 1890	0.3; 0-137.2; 5.2-13.8	[26,31,30,32]
Tomato	392	0.3	[26]
Turnip	23.4-654; 10-4800	1.1-1.4	[31, 48]
Turnip sprouts	54-1447	1.1- 57.0	[31]

Fresh, frozen and processed vegetables, have been purchased at retail in US markets, in order to study their contents of nitrate and nitrite (29).As result, the content of nitrate and nitrite for canned vegetables were in general lower than that of the frozen vegetables. Generally, nitrite levels in processed vegetables were lower than 1 ppm except for turnip 4.4 ppm, and spinach 6.1 ppm. After the obtained data, it is said that no need for concern about the content of nitrite for the fresh and the processed vegetables. But unsuitable conditions and prolonged storage could lead to conversion of nitrate to nitrite.

It had been found that pickled fruits and vegetables have lower nitrate contents average compared to that of corresponding raw materials in literature. As long as the brining process has a potential to dilute nitrate and therefore reduce it for about 30 to 70%. The pickled products after being acidified, brined and pasteurized, thus the reduction of nitrate content for vegetables would have to be expected (2).Generally, the processing of vegetables like peeling, washing, and cooking or another type of thermal treatment have shown an action to reduce the amount of nitrate. In addition, storage conditions [time, temperature] can help change the amounts of nitrate and nitrite in vegetables (23).

After revealing the concentration of nitrate for fresh vegetables based on the consumed edible portion, (33) reported that leafy vegetables [spinach, rocket, celery, parsley] have higher levels of nitrate and their contents in organs can be listed in decreasing order as follows: Petiole >leaf >root >stem >inflorescence >tuber >bulb.

When it comes to harvesting date effects, this factor might be one of the important factors to determine the content of nitrate or nitrite in vegetables. As it is demonstrated by the significant effect of harvest date presented by some leafy vegetables [spinach, cabbage] grown in open fields (35). Also, spinach has the higher content of nitrate in autumn and winter than it's in spring (34). Leafy vegetables harvested in the early morning, are known to have a high concentration of nitrate, compared to that harvested at midday. For this reason, recommendations are given to farmers to follow a harvest during midday to reduce the nitrate accumulation which is relatively affected by light (36). Another factor to consider in harvesting, even the blade/petiole ratio in spinach define the content of nitrate (34).

A study on a group of vegetables has been carried to investigate the effect of cultivar and harvest date on nitrate and nitrite, under open field and greenhouse conditions (35). For both growing field types, they indicated that the cultivar of vegetables did not have a significant effect. But not the case for squash and tomatoes, grown in greenhouse, they had a significant effect on the level of nitrate. Whereas, it was reported that spinach plant presented a genotypic variation to accumulate nitrate and in the activity of nitrate reductase (36).

In general, as vegetables are the main source of nitrate, the concentration of the latter is influenced by agricultural and environmental factors such as soil moisture, type of soil, fertilizers, light intensity (day), temperature, variety, vegetation period, harvesting time and crop protection strategies. Above all of that, levels of nitrate in food can be influenced by processing and the conditions of storage (31, 37).

4. BENEFICIAL HEALTH EFFECTS

During the last years, many experimental and clinical studies have been done on nitrate, nitrite and NO and their effects on human and animal's health. Most of them, and generally after trials by the ingestion of inorganic nitrate or nitrate-rich food (e.g. spinach, beetroot...) under many forms (meal, juice...) during short-term, lasted from some days to several weeks. Their results were interesting and showed many beneficial actions which could be attributed to HNDs (high nitrate diet).

For 30 healthy volunteers, a study has been carried out to examine the pharmacokinetics (saliva, plasma, urine) of dietary nitrate, and to determine the formation of protein-bound 3-nitrotyrosine and platelet function for 30 healthy volunteers (38). It was found out that HND does not cause a significant acute change in platelet function or in plasma concentrations of 3-nitrotyrosine, which is implicated in many inflammatory conditions, neurodegenerative diseases, and cancer. Hence, and regarding this finding, dietary nitrate does not show carcinogenic activity and could be not harmful for human health. Furthermore, after HNDs and reductive polyphenols (such as epicatechin and quercetin), NO exerts gastroprotective effects by boosting blood flow and mucus production in the gastric mucosa, which could have the potential to amplify the vascular-protective benefits of low-dose aspirin, while diminishing its pro-ulcerative risk (39).

Throughout the reviewed studies investigating the effect of nitrate/nitrite administration on various aspects of T2DM (type 2 diabetes mellitus) by their conversion to NO, it was reported that those administration could have an ability to enhance the insulin signaling pathway, glucose uptake and attenuate insulin resistance and diabetes complications (40). As well as, a favorable effect on lipid and lipoprotein metabolism and reducing the accumulation of adipose fat and hence, a decrease in weight gain. Besides, the increasing production of NO, the reducing of oxidative stress by nitrate and nitrite, the increase of adipose tissue browning, and the increase of insulin secretion from the pancreas; all of these effects are potentially promising for the management of obesity and T2DM (10).

Recent studies of nitrate supplementation, particularly in the form of vegetables and their juices, exhibited a potential to reduce the O2 cost of submaximal exercise, increase muscle contractility and improve exercise tolerance. Moreover, numerous investigations assume that HNDs may improve aspects of cognitive performance both at rest and during exercise (13, 11). When in a study over normotensive older participants during 3days, the effects of control and high nitrate diets examined. It is observed that an HND at breakfast elevated plasma nitrate and nitrite levels throughout the day which may have practical utility for the timing of physical activity and a high-nitrate supplement for older adults with vascular dysfunction (41).

HNDs and its possible implications on cardiovascular system health have been carried out in previous studies and HNDs generally had numerous effects on this system like BP (blood pressure), for both SBP (systolic blood pressures) and DBP (diastolic blood pressures), and platelet function.

During 10 days, a study had examined the effect of Japan traditional diet (JTD), which is originally an HND, on blood pressure of 25 healthy volunteers (men and women) (42). It found out that nitrate naturally provided by the JTD was 18.8 mg/kg/bw/day, exceeding the ADI by five times (ADI, 3.7 mg/kg/bw). For both plasma and salivary levels of nitrate and nitrite, JTD demonstrated high concentration compared with control diets. Also, DBP was lower 4.5 mm Hg compared with non-JTD. In addition, a study has assessed the acute effects of nitrate, which was in HND (220 mg of nitrate derived from spinach), on the BP and arterial stiffness in 26 healthy participants (men and women) compared with control diet with low nitrate content (43). The spinach consuming group resulted in; 7-fold and 8-fold increase in nitrite and nitrate, respectively, in salivary concentrations from pre-meal to 120 min post meal, also resulted in higher large artery elasticity index, and lower pulse pressure and SBP. Even, significant reductions in SBP and DBP measures have been reported between the pre-breakfast and the post meal assessments for all treatments by beetroot juice (41).

Another, after studying the effects of a 6 weeks daily intake of HND (beetroot juice) compared with placebo intake [nitrate-depleted beetroot juice] on vascular and platelet function in untreated hypercholesterolemics. It is found that, dietary nitrate resulted in an absolute increase in the flow-mediated dilatation and small improvement in the aortic pulse wave velocity. Even more, a small but significant reduction (7.6%) in platelet-monocyte aggregates compared with an increase of 10.1% in the placebo group (43). Previously, another study had systematically assessed the effects of inorganic nitrate and beetroot supplementation on BP for sixteen trials conducted between 2006 and 2012 (45). It revealed that this kind of short-term supplementation exert a significant decline in SBP. It is suggested that fruits and vegetables provide a substrate for reduction of nitrate to nitrite and NO, which leads to vasodilatation and a decrease in BP. Other studies have shown the production of NO has the ability to inhibit endothelial inflammatory cell recruitment, platelet aggregation, protect heart from ischemia (18, 46). Likewise, it was mentioned that inorganic nitrate may play a major role in the cardiovascular health benefits, presumably through enhancing NO bioavailability in the vasculature (16).

The composition of the salivary microbiome was altered after the nitrate treatment but not after the placebo treatment (44). A previous study revealed that nitrite in saliva had significant antimicrobial benefits when it was swallowed and converted to nitrous acid and other nitrogen oxides in the intestinal tract. And, the bactericidal effects of gastric fluids are significantly enhanced by the presence of ingested nitrite. This has been demonstrated for known foodborne pathogens such as Escherichia coli 0157:H7 and Salmonella (47).

Collectively, and regarding the recent studies and the obtained results, nitrate-rich dietary.

or different supplementation of nitrate, particularly that of some vegetables [such as spinach, beetroot], they generally had beneficial effects in lowering the blood pressure, reduction in platelet aggregation, obesity, and T2DM, in bactericidal effect and anticarcinogenic activity. Hence, it is suggested that this safe, well-tolerated, low-cost supplementation could be a potential cardio-protective element for prevention and treatment of cardiovascular diseases and decrease the risk of death by cardiovascular-related causes, even increase the longevity index (13,44,11).

4. CONCLUSION

Throughout this review, we can see that the consumption of dietary nitrate by healthy individuals, especially that of vegetables that contain high content should not be avoidable sources since they have positive effects to promote health and reduce the risks of occurrence of some diseases. Nitrate could be a potential fighter against cardiovascular diseases which are globally considered among the first causes of death, therefore it could be an opportunity to increase well-being and longevity. However, children and people who have gastrointestinal problems, they need to keep away from those nitrate-rich foodstuffs due to their undesirable consequences that could be manifested after their consumptions.

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