An updated review on nitrate exposure from drinking water and dietary sources and effects on human health

Georgia Vogiatzi,[1] **Michael Liontos**,[2] **Marios Kostakis**,[3] **Niki Maragou**,[3,4] **Nikolaos S. Thomaidis**[3,*]

1. 3rd Cardiology Department, Thorax Diseases Hospital "Sotiria", School of Medicine, National and Kapodistrian University of Athens

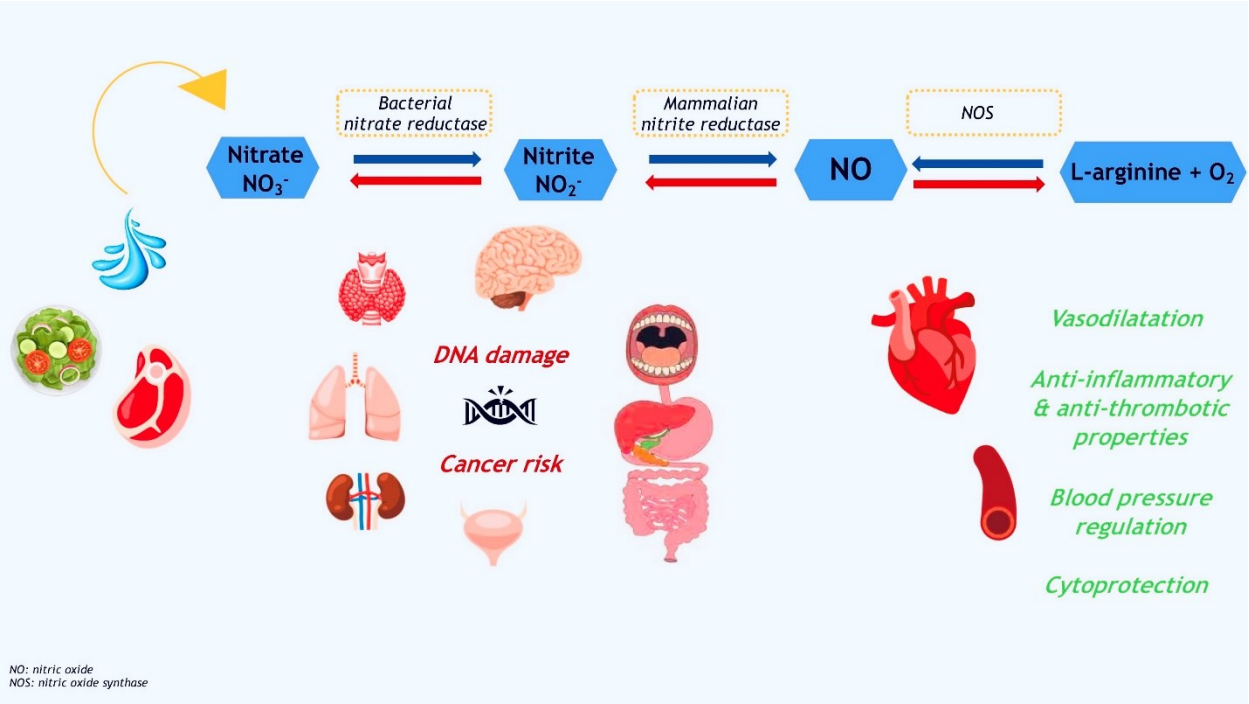
2. Department of Clinical Therapeutics, Alexandra Hospital, National and Kapodistrian University of Athens

3. Laboratory of Analytical Chemistry, Department of Chemistry, National and Kapodistrian University of Athens

4. Department of Food Science and Technology, University of West Attica

Address for correspondence:
Laboratory of Analytical Chemistry, Department of Chemistry,
National and Kapodistrian University of Athens
Panepistimiopolis Zografou, 157 71 Athens, Greece
Tel: +30 210 727 4317 –Fax: +30 210 727 4750
E-mail: ntho@chem.uoa.gr

GRAPHICAL ABSTRACT



Abstract

Nitrates are ubiquitous in the environment and key intermediates of various biochemical reactions in the nitrogen cycle, along with nitrites. A considerable source of nitrates is drinking water. There is substantial disagreement among scientists over the current limits for nitrate concentration in drinking water. Most adverse health effects related to drinking water nitrate are likely due to a combination of high nitrate ingestion and factors that increase endogenous nitrosation. The two main health issues are the association between nitrate and (i) cardiovascular diseases and (ii) the incidence of cancer. On one hand, there is evidence for nitrate as a possible cause of serious diseases which remains controversial and on the other hand there is emerging evidence of benefits of nitrate in cardiovascular health. Subgroups within a population may be more susceptible than others to the adverse health effects of nitrate. Here, we discuss the physiologic roles of nitrate and nitrite and their potential effects in order to derive rational bases for dietary recommendations.

Introduction

Nitrates are ubiquitous in the environment[1] and key intermediates of various biochemical reactions in the nitrogen cycle, along with nitrites[2]. There are two sources of nitrates in the human body: exogenous (external) and endogenous (internal). The endogenous sources of nitrates are derived from the oxidation of nitric oxide[2,3]. However, human exposure to nitrates is mainly from exogenous sources, notably through the diet. The dominant source of dietary nitrate is vegetables, as they contribute to 60–80 % of the total nitrate intake. Another considerable source of nitrates is drinking water. Nitrates in drinking water are usually the result of groundwater contamination by fertilizers and animal or human waste. Moreover, sodium and potassium nitrates, together with the respective nitrite salts, are added as food additives in meat products to develop their characteristic flavor and stabilize the red color, to inhibit the growth of the bacterium *Clostridium botulinum*, as well as to retard the lipid oxidation. Their addition not only improves the quality characteristics of meat products but also has an antimicrobial effect[3,4,5,6,7]. Except for meat products, nitrates can be found in dairy products[5,8], fish and fish products[5], fruits[1,9] and beverages[1,5].

Nitrates constitute a debatable subject, as the assertion that their presence is a threat to human health comes into conflict with growing evidence showing their significant health benefits. On the one hand, nitrates are considered indispensable dietary components to maintain nitric oxide homeostasis, in cooperation with nitrites[10]. Additionally, according to studies, the dietary nitrate exhibits gastroprotective properties, protects against cardiovascular diseases and has also anti-inflammatory action[7].

However, several studies have correlated health problems such as ovarian and bladder cancer, stomach cancer, miscarriage, and adverse effect on the nervous system, with increased nitrate consumption[11]. Moreover, high intake of nitrates can cause abdominal symptoms such as ache,

diarrhea, vomiting[12], alterations in vitamin levels and disturbance in thyroxin production[13]. Nitrates are not dangerous on their own[1], but they can become when converted to nitrites[5]. Specifically, nitrates are converted to nitrites by the enzyme nitrate reductase. This enzyme is present mainly in the saliva, as well as in the stomach and everywhere in the human body where pH is low. Approximately 5 % of the ingested nitrate in water and food is converted to nitrite by bacteria in the saliva[4]. Nitrites react with hemoglobin, which is responsible for the transportation of oxygen at the cellular level, and form methemoglobin[1,3,13]. This disorder, called methemoglobinemia, is characterized by reduced ability of the blood to carry oxygen that can lead to cyanosis and coma, and even death[13]. New-borns and children under one year of age are very sensitive to methemoglobinemia, also known as "blue baby syndrome"[5,6]. Another concern for human health relating to the metabolism of dietary nitrate is the potential formation of N-nitroso compounds (nitrosamines and nitrosamides) from nitrites[3,4]. Nitrites react with secondary amines and amides in the acidic conditions of the stomach and lead to the formation of nitrosamines and nitrosamides, respectively[14,15]. Many of these N-nitroso compounds have been found to be carcinogenic, teratogenic and mutagenic[9,12]. Therefore, taking into account the wide presence of nitrates in human life and the fact that high intakes of them could be detrimental to public health, the European Union (EU) has set legal limits to prevent potential adverse impacts of high nitrate concentrations in food and drinking water. The Regulation (EC) No 1881/2006 has established maximum levels for nitrates in vegetables, in particular green leafy vegetables such as spinach (2000–3500 mg/kg), lettuce (2000–5000 mg/kg) and rucola (6000–7000 mg/kg). This Regulation has also a limit of 200 mg/kg for nitrates in processed cereal-based foods and baby foods for infants and young children[16]. According to the Regulation (EC) No 1333/2008, the permitted food additives sodium nitrate (E 251) and potassium nitrate (E 252) have also maximum levels.

Specifically, a maximum level of 150 mg/kg has been set for cheese products. Moreover, this legislation has set maximum concentrations for nitrates (E 251, E 252) in traditionally cured meat products ranging from 10 to 300 mg/kg. As for processed fishes, an upper limit of 500 mg/kg exists in the case of pickled herring and sprat[17]. In the case of drinking water, the primary concern in legislation regarding nitrates is the protection against methemoglobinemia in infants [11]. For this reason, the Directive 98/83/EC, which was revised by the latest Directive (EU) 2020/2184, sets a maximum level of 50 mg/L for nitrates in drinking water. In addition, according to the Directive, the condition ([nitrate](mg/L))/50+([nitrite](mg/L))/3 should not exceed 1 mg/L[18]. Furthermore, authorities such as the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the European Food Safety Authority (EFSA) have recommended an Acceptable Daily Intake (ADI) of 3.7 mg/kg bw/day for nitrates in adults[7],[10]. A few years later, EFSA concluded that this ADI is also safe for infants and younger children in leafy vegetables[19]. Recently, EFSA reassessed the ADI for nitrates, based on new data in literature, and concluded that there is no need to revise the current ADI[20]. Finally, in another recent scientific opinion of EFSA about the risk assessment of nitrates in feed, EFSA proposed that the transfer of nitrates from feed to food product of animal origin is negligible, although it was pointed out that the scientific data are limited[21].

Taken into consideration the above, the aim of the current study is to critically review current evidence regarding the role of nitrates in cancer and cardiovascular disease development.

Effects of drinking and dietary nitrates in tumorgenesis

As referred above, human uptakes nitrates through drinking water and food consumption. Several lines of evidence indicate that nitrates could be associated with cancer through their metabolism in an acidic environment to N-nitroso compounds. The latter are considered as potential

carcinogenic as shown in animal studies[22]. Under this perspective, several studies have evaluated nitrate ingestion though the diet and/or water consumption and its possible association with several types of cancer. In 2010, the International Agency for Research on Cancer (IARC) of the World Health Organization (WHO) published a monograph summarizing the evidence of studies published up to 2006[23]. Authors concluded that there was inadequate evidence in humans for the carcinogenicity of nitrate in food or drinking water. In the following paragraphs, all major contemporary studies are reviewed according to cancer type.

1. Bladder cancer

There is a biological rationale for the association of nitrate uptake and bladder cancer risk. Almost 70% of ingested nitrate is excreted in the urine and formation of N-nitroso compounds could also occur in the bladder. In addition, nitrosation products with carcinogenic potential are also excreted in the urine[24]. Several case-control studies and prospective cohort studies have evaluated the association of nitrate uptake and bladder cancer with mixed results. More specifically, the Netherlands cohort study evaluated 120,852 men and women, 55-69 years of age at entry[25]. Nitrate uptake was estimated via a food frequency questionnaire and by linking the postal code of individual residence at baseline to water company data. After 9.3 years of follow-up 889 bladder cancer cases were identified but no significant association with nitrate exposure was found. Two additional population-based case-control studies conducted in the United States focused on the association of nitrate intake through processed and cooked meat consumption and the bladder cancer risk [26,27]. Both studies indicated a positive association between processed meat intake and bladder carcinogenesis but there was no direct link with nitrates. However, authors concluded that the effect could be greater in the presence of high nitrate intake. This hypothesis was analyzed in

the New England Bladder Cancer Study (NEBCS) that evaluated nitrate intake from water and diet as well as possible interaction with factors affecting endogenous nitrosation, including smoking and vitamin C and processed meat consumption[28]. An association between bladder cancer risk and increased (>95th percentile) average daily drinking water nitrate intake was noted. A positive interaction between average daily drinking water nitrate intake and processed red meat consumption was also found confirming previous hypothesis. Analogously, the Iowa cohort study in postmenopausal women confirmed the association between long term exposure to high nitrate concentration in drinking water and bladder cancer, while dietary nitrate intake had no effect[29].

2. Breast cancer

Breast cancer is the most common malignancy among women and several dietary components have been investigated as possibly modifiable risk factors of the disease[30]. Given the potentially carcinogenic effects of N-nitroso compounds, a case control study investigated the risk of breast cancer in relation to nitrate and antioxidant vitamins intak[31]. The primary hypothesis of the study was that nitrates and vitamins are naturally found in the same dietary sources (green leafy vegetables) and antioxidants decrease endogenous nitrosation counteracting potential carcinogenic effects of the nitrates. As a consequence, the study concluded that women with higher intake of nitrate to folate ration doubled the risk for breast cancer development. This association was further investigated in The Iowa Women's Health Study, a large prospective cohort study that enrolled almost 100,000 postmenopausal women aged 55 to 69[32]. The study evaluated dietary intake of nitrate and folate as well as nitrate intake from drinking water. No association of breast cancer risk with dietary or water nitrate was found and the effect of the nitrate to folate ratio was not confirmed.

3. Colorectal cancer

Red and processed meat are considered as carcinogenic for humans and have been directly linked with colorectal cancer development[33]. However, the exact mechanism of this association remains elusive since several components of meat e.g., heme, polycyclic aromatic hydrocarbons and heterocyclic amines could promote cancer. Nitrates are used as preservatives in processed meat and a constitute source of N-nitroso compounds that are also possible carcinogens.

Several cohort and case control studies have evaluated colorectal cancer risk in relation to nitrate intake from three major sources: processed meat, diet in general and drinking water. Two major cohort studies enrolling more than half a million adults investigated the role of several compounds of red and processed meat, including nitrates, in colorectal cancer development[34,35]. Data from both studies indicate that increased red and processed meat consumption is related to colorectal cancer and the risk is higher for rectal and distal colon carcinomas. Nitrates along with heme iron, nitrite, heterocyclic amines and aromatic hydrocarbons are implicated in the mechanism of colon carcinogenesis[34],[35]. It is of interest that patients in the highest quartile of nitrate intake have also greater risk to develop colon adenomas, a precancerous lesion diagnosed by colonoscopy or sigmoidoscopy[36]. The latter could serve to distinguish a high-risk population for colorectal cancer screening.

In contrast, the analysis of nitrates from all dietary sources and drinking water revealed mixed results[37,38,39,40]. A recent metanalysis of fifteen cohort and case control studies though showed a marginal association of dietary nitrate intake and colorectal cancer risk but no association for nitrates in drinking water[41].

4. Gastric cancer

Gastric cancer is the sixth most common neoplasia and its frequency is relatively higher in Asian population[30]. Epidemiological data clearly demonstrate the association of gastric carcinomas with dietary habits. However, the potential risk from nitrate intake for gastric cancer development remains controversial. Older case control studies indicated that high nitrate intake almost doubled the risk for gastric cancer[41,42]. However, these data were limited by the retrospective nature. Large prospective cohort trials investigated the role of nitrates from food and drinking water and no association with gastric cancer risk was found[43],[44],[45]. Furthermore, two meta-analyses that were recently published concluded that high or moderate dietary nitrate intake may protect from gastric cancer[44,46.] In both meta-analyses there is significant heterogeneity among studies due to differences in the study design, the percentage of men enrolled, the different sources of the nitrates and different cut-off values used. Regarding the later, high intake of nitrates through consumption of green leafy vegetables is considered protective for gastric cancer, since antioxidants included in vegetables decrease endogenous nitrosation. In contrast, increased intake through drinking water may be hazardous and further studies are needed to investigate this association[47,48].

5. Gliomas

Studies in animals provided evidence that early life exposures could influence the risk for adult glioma development. Therefore, dietary nitrate intake through endogenous N-nitroso compounds formation could affect brain carcinogenesis. Initial case-control studies were contradictory, but these studies are limited by their retrospective nature and possible recall bias [49,50,51.] The hypothesis that nitrates could be implicated in gliomas was prospectively assessed in combined data from 3 prospective cohort studies, but no association was found[52]. These results were further confirmed

by a large prospective study evaluating not only the role of dietary nitrate intake but also possible modifications from antioxidants or nitrosation promoters[53]. The study suggested that nitrate intake do not increase glioma risk and the result was independent from meat and/or vegetables consumption. Overall data are against the hypothesis that high nitrate intake during early life could modify adult glioma risk.

6. Ovarian cancer

Epithelial ovarian cancer is the gynecological malignancy with the highest mortality. Genetic factors that determine disease predisposition are well characterized, mainly for high grade serous histological type but epidemiological studies indicate that environmental factors should also determine the risk for disease development[54]. Almost a hundred case control and cohort studies have evaluated the role of several food components in ovarian cancer risk[55]. Two cohort studies examined nitrate intake from drinking water[56],[57] and one dietary intake[58]. All three studies detected a positive association between higher nitrate levels in drinking water and public wells as well the highest intake quintile of dietary nitrate and ovarian cancer risk. The same conclusion reached by a recent meta-analysis of these studies[55]. No association with a specific histological type was indicated.

7. Pancreatic cancer

N-nitroso compounds induce pancreatic tumors in animals[59]. N-nitroso compounds are formed with nitrosation from nitrates in drinking water and dietary sources. The results from prospective cohort and population-based case-control studies conducted to investigate the association of nitrates with pancreatic cancer risk were inconclusive[59,60,61]. However, these studies evaluated total

nitrates and not the amount of specific N-nitroso compounds that are considered potentially carcinogenic. Another case-control study evaluated pancreatic cancer risk in relation to the estimated concentration of specific N-Nitroso compounds[62]. Again, no association was found with total nitrates but there was a significant positive association with the estimated levels of N-nitrosodiethylamine (NDEA) and N-nitrosodimethylamine (NDMA) that warrants further investigation.

8. Renal cancer

The role of nitrates as precursor of potential carcinogens in kidney cancer risk has been evaluated as part of large prospective cohort and case control trials[63],[64],[65]. No specific association was noted in these studies regarding nitrate intake. However, when nitrate exposure from drinking water was evaluated in subgroups with above median red meat consumption (nitrosation enhancer) or below medial vitamin C intake (nitrosation inhibitor) an increased risk for renal cell carcinoma was revealed[65].

9. Thyroid cancer

Several dietary and lifestyle factors may play a role in thyroid cancer Etiology[66]. Two large studies conducted in United States have studied the possible role of nitrates in thyroid cancer risk[67,68]. Increased nitrate concentration in water was associated with thyroid cancer risk especially in subjects that received vitamin C below median levels[67]. Analogously, in the NIH-AARP Diet and Health study, that followed-up almost half a million men and women for an average of 7 years, increased nitrate intake was associated with almost doubled risk for thyroid cancer in men but not women[68]. The risk was independent of the histological subtype. These results were not confirmed

in a large cohort study conducted in China[69]. Several factors including different definitions of exposure, wide variations in nitrate exposure and different iodine status are responsible for this heterogeneity and further research is needed to clarify possible associations. The results of the studies are summarized in **Table 1**.

Effects of drinking and dietary nitrates in cardiovascular diseases

To date, firm conclusions about the impact of dietary nitrates cannot be drawn as a small number of studies have contributed additional mixed results. As it is above-mentioned nitrates intake from drinking water or diet are transformed in nitrite which can be protonated to nitrous acid (HNO2), and subsequently in dinitrogen trioxide (N2O3), nitrogen dioxide (NO2), and nitric oxide (NO). Hence, the main pathway for NO synthesis is from L-arginine, by specific NO synthases[70],[71]. When this pathway is dysfunctional, the nitrate-nitrite-NO pathway acts as a backup system to ensure > 50% of NO produced in the human body[10,72]. NO has been proved to participate in a wide range of physiological functions in cardiovascular system, such as the maintenance and regulation of vascular tone [73], the regulation of blood pressure[74,75], the inhibition of platelet adhesion and aggregation [76,77,] and other immunoregulatory effects[78].

It is known that NO can be subjected to oxidation by oxygen or reactive oxygen species (ROS) and generate reactive nitrogen species and can also rapidly react with other radicals to form S-nitrosothiols (R-SNOs), leading to damage of DNA and proteins rapidly react with other radicals to form S-nitrosothiols (R-SNOs)[79,80]. However, the major NO signaling pathway in cardiovascular system, responsible for the vasodilatory properties of NO is the formation cyclic GMP (cGMP) through the activation of soluble guanylyl cyclase[81,82].

Since the first report on NO effects in the vasculature[83], numerous experimental and human studies have taken place examing the potential beneficial effects of exogenous nitrate and nitrite on different areas related to cardiovascular system, such as blood pressure, ischemia/reperfusion and heart failure[7,84].

1. Blood Pressure (BP)

The BP-lowering effects of oral nitrites, have been demonstrated in experimental studies since 1995[85,86], and about 15 years later the effects of nitrate administration were studied. It was found that low dose nitrates administration reduced mean arterial BP[87] and this impact follows a dose-dependent pattern[88]. In addition, nitrate administration reduced the levels of oxidative stress markers in plasma and urine and attenuated cardiac hypertrophy and fibrosis. In an experimental study of Carlström et al. using rats, it was shown that treatment with high doses of dietary nitrate for a long period resulted in a paradoxical BP increase, coupled to inhibition of vascular eNOS activity. Those results lead to the speculation that the favorable effects of nitrate on cardiovascular function are more pronounced when endogenous NO synthesis from NOS is compromised[89].

The effects of dietary nitrate on BP in healthy subjects have been largely investigated[80]. Dietary supplementation with sodium nitrate in non-hypertensive volunteers resulted in a significant reduction in diastolic and systolic BP[90]. Interestingly, the decrease in systolic BP correlated with the increase of nitrite in plasma, and interruption of the enterosalivary circuit (by spitting) resulted in the abolishment of these BP-lowering effects. Based on these results, the endogenously production of nitrate, generated by the NOSs, was investigated in healthy subjects with a low-dose nitrate administration, where the recycling of nitrate to nitrite, normally performed by oral bacteria, was inhibited. BP was increased, while the levels of plasma nitrites were risen[91]. After several

studies in non-hypertensive volunteers, the effect of dietary nitrate was examined in a phase II study in 68 hypertensive subjects[92]. The observed lowering of systolic BP by a mean of 8 mmHg was persistent throughout a 4-week period. Despite these promising findings, in a similar study, also among hypertensive subjects, no decrease in BP was observed, although the nitrite concentration in plasma was increase[93]. Period of treatment, categories of anti-hypertensive medications and different measurements methods of BP pressure, may constitute possible explanatory factors.

2. Ischaemia–reperfusion injury and myocardial infarction

The fact that the increased bioavailability of NO in ischaemia–reperfusion injury is definitely associated with protective effects[94] led to studies regarding the effects of nitrate and nitrite in animal models with cardiac ischaemia. In 2004, in an ex-vivo animal model of myocardial infarction it was found that nitrite substantially reduced infarct[95]. The same protective effects were showing the following years, in an in-vivo models[96,97,98]. Nitrite also preserved cardiac function in a model of global ischaemia after cardiac arrest and resuscitation[99]. The underlying mechanism through which nitrates exert cytoprotective effects involves the interaction of nitrite reaction products with mitochondria.

In the clinical setting, in one study with mild ischaemic insult in the forearm which significantly reduced flow mediated dilatation (FMD) by ~60%, supplementation with dietary nitrate prevented endothelial dysfunction, and restored FMD to normal level[90]. In addition, in a small study involving patients with peripheral artery disease, dietary nitrate improved exercise capacity in terms of longer walking distance and peak walking time[100]. Interestingly, two phase II studies on the effects of nitrite therapy after acute myocardial infarction showed contradictory results.

NIAMI study showed that, in 229 patients presenting with acute ST-segment elevation myocardial infarction (STEMI), randomized to receive either an intravenous infusion of sodium nitrite or matching placebo immediately before primary percutaneous intervention there was no reduction in infarct size[101]. Hence, in another study, with this time intracoronary nitrite administration prior to balloon dilatation in patients with STEMI, there was a reduction in major adverse cardiac events (MACEs) at 1 year in the nitrite group. Maybe, these discrepancies could be attributed to the mode of administration, the dose of nitrite or the selected group of subjects[102].

3. Heart failure (HF)

Mechanistically, nitrate reaction products including NO affect mitochondrial efficiency through downregulation of proteins involved in proton leak (ANT and UCP-3). Few studies have investigated the direct effects of nitrate or nitrite on cardiac function, and the data have been inconsistent. An ex-vivo experiment in muscle from mice revealed that nitrate increased force muscle contraction, through the modulation of calcium-handling proteins[103]. In a rat heart model, Pellegrino et al. found that nitrite modulates cardiac contractility through cGMP/protein kinase G-dependent signalling, shown as a decrease in left ventricular pressure and relaxation[104]. Furthermore, in the hearts of rats exposed to chronic hypoxia, dietary nitrate supplementation markedly elevated cardiac L-arginine concentrations and prevented hypoxia-induced changes in cardiac mitochondrial function, suggesting improved tissue oxygenation[105].

It has been shown that nitrate reaction products, including NO, affect mitochondrial efficiency in humans[106]. Zamani et al proved that dietary nitrate increased exercise vasodilatory and cardiac output reserves in patients with heart failure with preserved ejection fraction (HFpEF), resulting in better exercise capacity, and reduced arterial wave reflections[107]. A randomized, placebo-

controlled, study showed that dietary nitrate supplementation (beetroot juice) increases exercise performance in people with HF with reduced ejection fraction (HFrEF)[108]. Two large epidemiological studies, the Reasons for Geographic and Racial Differences in Stroke (REGARDS) Study[109] and the National Health and Nutrition Examination Surveys (NHANES)[110] may be useful to better assess the relation between dietary nitrate and nitrite and cardiovascular disease. However, the lack of information of food nitrate and nitrite concentrations, is another obstacle to the development of a solid epidemiologic basis for quantifying cardiovascular and other health benefits of dietary nitrates and nitrites in human populations. The most important studies are summarized in **Table 2**.

Discussion

Data presented in this review indicate differential effects of dietary nitrates in the risk of cancer and cardiovascular disease development. This is most probably the result of the different products of nitrates metabolism in humans that affect either cancer cells or the cardiovascular system.

Regarding cancer, nitrate intake through food or water consumption has been investigated as a risk factor in several neoplasms. Since nitrates and their metabolites are absorbed through the gastrointestinal tract and excreted mainly through urine, it is anticipated that the vast majority of studies focused on gastrointestinal and genitourinary malignancies. However, the role of nitrates in carcinogenesis has also been investigated in gynecological, lung, thyroid and brain malignancies, as well as lymphomas. The results of these studies are not consistent regarding the cancer risk imposed by nitrate consumption. However, there are several factors that should be taken into consideration. First of all, different sources of dietary nitrate intake may differentially affect carcinogenesis. Vegetables and animal products are both dietary sources of nitrates, but

increased nitrate intake due to higher consumption of green vegetables is also associated with higher antioxidants levels. The latter reduce nitrosation counteracting potential carcinogenic effects of the nitrates overall. Therefore, negative results in studies evaluating total nitrates intake from dietary sources and cancer may hinder a possible link between nitrates from animal sources and carcinogenesis.

The role of nitrates in relation to molecular alterations of neoplasms also deserves further investigation. N-nitroso compounds produced as a result of endogenous nitrosation of nitrates are linked to the occurrence of KRAS gene mutations[111]. KRAS mutations are detected in almost half of colorectal carcinomas and considered driver mutations for the pathogenesis of the disease. Also, KRAS mutations are more frequently encountered in right-sided colon carcinomas and associated with worse prognosis. Current studies evaluating the role of nitrates in colorectal carcinomas have not taken into consideration the molecular characteristics of the disease. It should be noted though that there have been shown no relation between the sidedness of the disease and the nitrates intake up to now. As the molecular characterization of several neoplasms generalizes, future studies could evaluate possible mechanistic link between nitrates consumption and pathogenesis of specific molecular subtypes of neoplasms.

As for cardiovascular diseases, accumulating data showing protective effects of dietary nitrate, particularly under ischemic conditions, have led to the thinking that these compounds could be considered nutrients. Although, these particular anions for more than three decades have been considered as an unwanted diet constituent, it is striking that blood pressure reduction, platelet function inhibition and improved endothelial function have been observed with mild dietary nitrate intake. The amounts of these anions needed for the effects on the cardiovascular system, are easily achieved *via* a rich intake of vegetables and fruits. However, the DASH diet that lowers BP could

be moderately estimated to contain 5–10 mmol nitrate, exceeding the recommended daily intake (set at 3.7mg/kg daily). In many countries, the levels of nitrates consumption are strictly controlled and there are recommendations based upon two main concerns; methaemoglobinaemia and carcinogenesis. Moreover, despite the encouraging results of exogenously delivered inorganic nitrate, the physiological relevance of endogenously generated nitrate and nitrite is not fully cleared. The salutary effects of this inorganic molecules should not be confused with organic nitrates (nitroglycerine, isosorbide mononitrate, isosorbide dinitrate) used as therapeutic agents. Both generate NO, but they have different pharmacokinetic profiles, with the vasodilatory effect of the inorganic nitrate been much lower. Therefore, further research is needed before any promising findings can be translated into effective and therapeutic strategies in clinical cardiovascular practice.

Conclusions

Given that nitrate is an everyday dietary constituent, this research area is particularly intriguing. To date, the number of well-designed studies of individual health outcomes is still too few to draw firm conclusions about risk from drinking water nitrate ingestion. Controlled human feeding studies and additional research are much needed to elucidate the unequivocal findings reported from some clinical studies. In addition, we should not forget today's strict regulations of nitrate levels in food and drinking water. We have to change our current thinking that inorganic nitrate may be a threat to human health. There is an urgent need to consider the possibility that populations' subgroups respond differently in theses anions. This requires greater collaboration between experts who hold opposing views over the interpretation of the available data. It is time to end the many years of uncertainty and move toward science-based standards.

Table 1. Studies summarizing the impact of nitrates and nitrite in cancer risk

Author	Type of study	Type of Cancer	Population	Parameters analyzed	HR/OR (95%CI)
Zeegers MP, et al[25]	Cohort study	Bladder	120,852 men and women, 55-69 years of age	Highest quintile of nitrate intake from 1. food, 2. drinking water, 3. total	1. RR=1.06 (0.81-1.31) 2. RR=1.06 (0.82-1.37) 3. RR=1.09 (0.84-1.42)
Ferrucci LM, et al[26]	Cohort study	Bladder	300,933 men and women, aged 50 to 71 years	Highest quintile of dietary nitrate intake	HR= 0.80 (0.58-1.10)
Catsburg CE, et al[27]	Case control study	Bladder	1,660 bladder cancer cases/1,586 contorls	Highest quintile of dietary nitrate intake	OR= 0.9 (0.60-1.35)
Barry KH, et al[28]	Case control study	Bladder	987 bladder cancer cases/ 1080 controls	1. Drinking water nitrate concentration >95th percentile. 2. Highest quintile of dietary nitrate intakes from processed meat	1. OR= 1.5 (0.97 -2.3) 2. OR= 1.4 (1.0-2.0)
Jones RR, et al[29]	Cohort study	Bladder	34,708 postmenopausal women	Highest quartile of drinking water concentration	HR= 1.48 (0.92-2.40)
Inoue-Choi M, et al[32]	Cohort study	Breast	34,388 postmeno-pausal women	Highest quartile of dietary nitrate intake	HR= 0.86 (0.74 – 1.01)
Etemadi A, et al[34]	Cohort study	Colorectal	407,720 participants	Highest quintile of dietary nitrate intake	HR= 1.18; 95%CI: 1.08-1.28
Cross AJ, et al[35]	Cohort study	Colorectal	300,948 participants	Nitrate intake from processed meat	HR, 1.16; 95% CI,

					1.02-1.32; P(trend) = 0.001
Ward MH, et al[36]	Case-control study	Colorectal	146 cases/ 228 controls	Highest quartile of dietary nitrate intake	OR= 2.0; 95% CI = 1.0-3.9
Fathmawati J , et al[37]	Case control study	Colorectal	75 colorectal cancer cases/75 contorls	NR	NR
Knekt P, et al[38]	Cohort study	Colorectal	9985 adults	Highest quartile of nitrate intake	RR= 1.04; 95% CI 0.54-2.02
Dellavalle CT, et al[39]	Cohort study	Colorectal	73,118 women, 40-70 years old	1. Nitrate uptake 2. Highest quintile of nitrate intake among women with vitamin C intake below the median	1. HR = 1.08; 95% CI: 0.73-1.59 2. HR = 2.45; 95% CI: 1.15-5.18; p= 0.02
Jones RR, et al[40]	Cohort study	Colorectal	15,910 women	Highest quintile of nitrate concentration in drinking water	1. HR= 0.97 (0.75, 1.26) Colon 2. HR= 0.64 (0.38, 1.07) Rectum
Hernández-Ramírez RU, et al[42]	Case-control study	Gastric	275 cases/ 478 controls	Highest tertile of nitrate intake from animal sources	OR= 1.92; 95% CI 1.23–3.02
van Loon AJ, et al[43]	Cohort study	Gastric	120,852 men and women aged 55-69 years	1. Highest quintile of dietary nitrate intake .2 Highest quintile of nitrate intake from water supply	1. RR= 0.80, 95% CI 0.47-1.37, trend-P = 0.18, 2. RR= 0.88, 95% CI 0.59-1.32,

					trend-P = 0.39
Taneja P, et al[48]	Case-control study	Gastrointestinal cancers	78 cases/ 156 controls	Nitrate in drinking water	OR= 1.20; 95% CI 1.04–1.34
Ward MH, et al[47]	Case-control study	Gastric	163 cases/ 321 controls	Highest quartile of drinking water concentration	OR=1.2, 95% CI 0.5-2.7 stomach; OR=1.3, 95% CI 0.6-3.1 esophagus
Mueller B, et al[49]	Case-control study	Gliomas	836 cases/ 1485 controls	Highest quartile of dietary nitrate intake	No association
Chen H, et al[50]	Case-control study	Gliomas	236 cases/ 449 controls	Highest quintile of dietary nitrate intake	No association
Ward MH, et al[51]	Case-control study	Gliomas	215 cases/ 498 controls	Water supply with ≥10mg/L nitrate	OR= 1.1; 95% CI 0.6-2.0
Michaud DS, et al[52]	Cohort study	Gliomas	335 glioma cases	Highest dietary nitrate intake	RR= 1.02; 95% CI 0.66 - 1.58
Dubrow R, et al[53]	Case-control study	Gliomas	545,770 participants	Highest quartile of dietary nitrate intake	No association
Inoue-Choi M, et al[56]	Cohort study	Ovarian	28,555 postmenopausal women	Highest quintile of dietary nitrate intake	HR=0.61, 95% CI 0.40–0.95; p-trend=0.02
Aschebrook-Kilfoy B, et al[58]	Cohort study	Ovarian	151 316 women aged 50-71 years	Highest quintile of dietary nitrate intake	HR= 1.31; 95% CI: 1.01-1.68
Aschebrook-Kilfoy B, et al[60]	Cohort study	Pancreas	492,226 participants	Highest quintile of dietary nitrate intake	HR= 1.01; 95% CI 0.85-1.20

Quist A, et al[61]	Cohort study	Pancreas	41,836 women, aged 55-69	1. 95th percentile of average water suplly nitrate intake 2. Dietary nitrate intake	1. HR= 1.18, 95% CI 0.52 - 2.67 2. HR= 1.02, 95% CI 0.92 - 1.14
Zheng J, et al[62]	Case-control study	Pancreas	957 cases / 938 controls	Dietary nitrate intake	No association
Ward MH, et al[65]	Case-control study	Renal	201 cases/ 1,244 controls	Dietary nitrate intake	OR= 1.03, 95% CI 0.66 - 1.60
Dellavalle CT, et al[63]	Cohort study	Renal	491841 participants	Dietary nitrate intake	HR = 1.00, 95% CI 1.00-1.00
Jones RR, et al[64]	Cohort study	Renal	15577 women	1. 95th percentile of average water suplly nitrate intake 2. Dietary nitrate intake	1. HR=2.3,95 %CI:1.2–4.3;p-trend=0.33. 2. HR=1.1, 95%CI 0.71–1.60; p-trend=0.80
Aschebrook-Kilfoy B, et al[69]	Case-control study	Thyroid	490,194 adults, aged 50-71 years old	Highest quintile of dietary nitrate intake	RR= 2.28, 95% CI: 1.29-4.041; p-trend <0.001

Table 2. Most important studies about the impact of nitrates and nitrite in cardiovascular diseases

Author	Type of study	Disease	Population	Results	HR/OR (95%CI)
Kanematsu Y, et al[86]	Animal study	BP	25 hypertensive rats	Chronic treatment with oral nitrite tended to lower SBP, but only HDN treatment lowered SBP at 8 wk	NS
Petersson J, et al[87]	Animal study	BP	rats	MAP was significantly lower in animals fed with nitrate in the drinking water for 1 week	P<0.05
Carlström M, et al[88]	Animal study	BP	rats	LDN was as protective as the HDN with the exception of BP which was significantly reduced only in the HDN group	P<0.05
Carlström M, et al[89]	Animal study	BP	rats	LDN reduced BP HDN was associated with a paradoxical elevation	P <0.05
Ashmore T, et al[105]	Animal study	Cardiac mitochondrial function and energetics	40 male Wistar rats given water supplemented with 0.7 mmol l−1 NaCl (as control) or 0.7 mmol l−1 NaNO3, elevating plasma nitrate levels by	Complex I respiration rates and protein levels were 33% lower in hypoxic/NaCl rats compared with normoxic/NaCl controls ATP levels were 62% lower	$P \leq 0.05$ for protein P ≤ 0.001) for ATP levels

			80%, and were exposed to 13% O2 (hypoxia) or normoxia (n = 10 per group) for 14 days		
Larsen FA, et al[83]	Randomized, double-blind, crossover study	BP	3-day supplementation with sodium nitrate (0.1 mmol/kg/day) or PL (sodium chloride, 0.1 mmol/kg/day) in 17 healthy subjects	DBP was 3.7 mmHg lower after nitrate supple- mentation MAP was 3.2 mmHg lower	P<0.02 for DBP P<0.03 for MAP
Webb JA, et al[90]	Open-label cross-over study	BP	14 healthy subjects	2.5h post ingestion reduced SBP 10.4±3.0 mmHg 3h post ingestion reduced DBP 8.1±2.1 mmHg and MAP 8.0±2.1 mmHg	P<0.01 for SBP, DBP and MAP
Kapil V, et al[92]	Prospective single-center, double-blind, randomized, placebo-controlled trial	BP	34 drug-naive and 34 treated patients with HTN randomized to receive either 4 wk daily supplementa-tion with	SBP and DBP decreased compared with baseline by 7.7 mmHg and 2.4 mmHg, respectively	95% CI, 3.5–11.8; P<0.001 for SBP 95% CI, 0.0–4.9; P=0.050 for DBP

			dietary nitrate or PL		
Bondonno CP, et al[93]	Randomized, placebo-controlled, double-blind crossover trial	BP	27 subjects taking 1-3 antihypertensive medications	no differences in home BP and 24-h AMBP with 1-wk intake of nitrate-rich beetroot juice compared to PL	NS
Kenjale AA, et al[100]	Randomized, open-label, crossover study	PAD	8 subjects with PAD underwent resting blood draws, followed by consumption of 500 ml BR or PL and subsequent blood draws prior to, during, and following a maximal CPX test	BR increased plasma [NO_2^-] after 3 h Subjects walked 18% longer before the onset of claudication pain 17% longer peak walking time DBP was lower in the BR group at rest and during CPX testing	$P \leq 0.01$ for [NO_2^-] $P \leq 0.01$ for pain $P \leq 0.05$ for peak walking time $P \leq 0.05$ for DBP
Zamani P, et al[107]	Randomized, double-blind, crossover study	HFpEF	17 subjects comparing a single dose of NO3-rich beetroot juice (NO3(-), 12.9 mmol) with an identical nitrate-depleted PL	NO3(-) led to increased peak VO2 (12.6±3.7 vs. 11.6±3.1 mL $O_2 \cdot min(^{-1}) \cdot kg(^{-1})$ and total work performed (55.6±35.3 vs. 49.2±28.9 kJ), greater reductions in SVR (-42.4±16.6% vs. -31.8±20.3%), increased CO (121.2±59.9% vs. 88.7±53.3%) and reduced aortic AI	P=0.005 for peak VO2 P=0.04 for total work performed P=0.03 for SVR P=0.006 for CO P=0.03 for aortic AI

				(132.2±16.7% vs. 141.4±21.9%)	
Coggan AR, et al[108]	Randomized, double-blind, placebo-controlled, crossover study	HFrEF	10 subjects with mild-to-moderate nonischemic HFrEF were tested 2 h after ingesting 140 mL of a concentrated BR supplement containing 11.2 mmol of NO3-	NO_3^- ingestion increased (VO_2 peak by 8 ± 2% (ie, from 21.4 ± 2.1 to 23.0 ± 2.3 $mL \cdot min^{-1} \cdot kg^{-1}$) and improved time to fatigue (from 582 ± 84 to 612 ± 81 seconds)	P < 0.05 for VO2 peak P < .05 for time to fatigue

Abbreviations: BP: blood pressure; SBP: systolic blood pressure; DBP: diastolic blood pressure; HDN: high dose nitratres, wk: week; MAP: mean arterial pressure, LDN: low dose nitrates, HTN: hypertension; AMBP: ambulatory blood pressure; BR: beetroot; PAD: peripheral arterial disease; PL: placebo: HFpEF: heart failure with preserved ejection fraction; vs.: versus; SVR: systemic vascular resistance; CO: cardiac output; HFrEF: heart failure with reduced ejection fraction.

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