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Nitrate: The Dr. Jekyll and Mr. Hyde of human health?

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ABSTRACT

Background: Dietary nitrate has a controversial role in human health. For over half a century, the nitrate content of the three major dietary sources – vegetables, meat, and water – has been legislated, regulated, and monitored due to public health concerns over cancer risk. In contrast, a growing and compelling body of evidence indicates that dietary nitrate, particularly from vegetables, protects against cardiovascular disease and other chronic diseases. This evidence for the protective effect of nitrate is overshadowed by the potential for nitrate to form carcinogenic *N*-nitrosamines.

Scope and approach: The nitrate content, regulations and estimated intake from vegetables, meat and water are described. The evidence that nitrate, through its effects on nitric oxide, improves cardiovascular disease outcomes, cognitive health, musculoskeletal health, and exercise performance as well as the potential to protect against other debilitating health outcomes (nitrate as Dr Jekyll) is discussed. The underlying assumption that all nitrate, irrespective of source, leads to the formation of carcinogenic *N*-nitrosamines and the evidence of an association between the different sources of nitrate and cancer (nitrate as Mr Hyde) is examined.

Key findings and conclusions: The current theory that nitrate, is a carcinogenic contaminant in meat, water, and vegetables is not fully supported by available evidence. Definitive studies examining the beneficial or harmful effects of source-dependent nitrate have yet to be performed. Studies with improved exposure assessment and accurate characterization of factors that affect endogenous nitrosation are also needed to draw conclusions about risk of cancer from dietary nitrate intake.

1. Introduction

Confusion in the scientific literature and fear in public perception currently hinders the understanding of dietary nitrate's role in human health. While there is now robust evidence for the benefit of nitrate-rich green leafy vegetables on cardiovascular health, a recent review stated, “excessive accumulation of nitrates in vegetables is a common issue that poses a potential threat to human health” (Bian et al., 2020). A headline in the Daily Mail, February 17, 2020, also stated: “Cancer alert over rocket: trendy salad leaves exceed safe levels of carcinogenic nitrates in

one in every ten samples”. Nitrate's reputation as a threat to human health has persisted since 1970, when two studies showed that nitrate can form *N*-nitrosamines, which are highly carcinogenic in laboratory animals (Spiegelhalter et al., 1976; Tannenbaum et al., 1976). The three major dietary sources of nitrate are vegetables, meat, and water. Whether different sources of nitrate provide equal benefit or harm is unclear. For example, unlike meat and water-derived nitrate, vegetables contain high levels of vitamin C and/or polyphenols that may inhibit the production of *N*-nitrosamines (Ahluwalia et al., 2016). Following several landmark clinical trials showing nitrate intake improves markers

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of vascular health, there is now good evidence that vegetables high in nitrate play an important role in preventing cardiovascular disease (CVD) (Ahluwalia et al., 2016; Blekkenhorst, Bondonno, et al., 2017; Bondonno et al., 2017; Bondonno et al., 2021). Therefore, nitrate’s role as friend or foe in human health must be fully understood if its potential benefits are to be harnessed. This is crucial given that dietary nitrate intake has been shown to increase nitric oxide (NO) (Bondonno et al., 2012), a signalling molecule known to be a key player in cardiovascular health (Lundberg & Weitzberg, 2010) by which nitrate-rich vegetables are hypothesized to prevent CVD. As NO has wide-ranging physiological

effects – there is hardly a lifestyle-linked disease where altered NO is not implicated in some way (Pacher et al., 2007) – the observed benefits of nitrate could extend to other health conditions. For example, there is evidence for benefits on cognitive function, eye-health, muscle function as well as improved physical performance. This review will discuss the major dietary sources of nitrate and the evidence for beneficial or detrimental effects with nitrate intake from these sources, as well as examine the underlying assumption that all nitrate, irrespective of its source, leads to the formation of *N*-nitrosamines which are deemed carcinogenic and are linked to cancer. This is the first review to provide

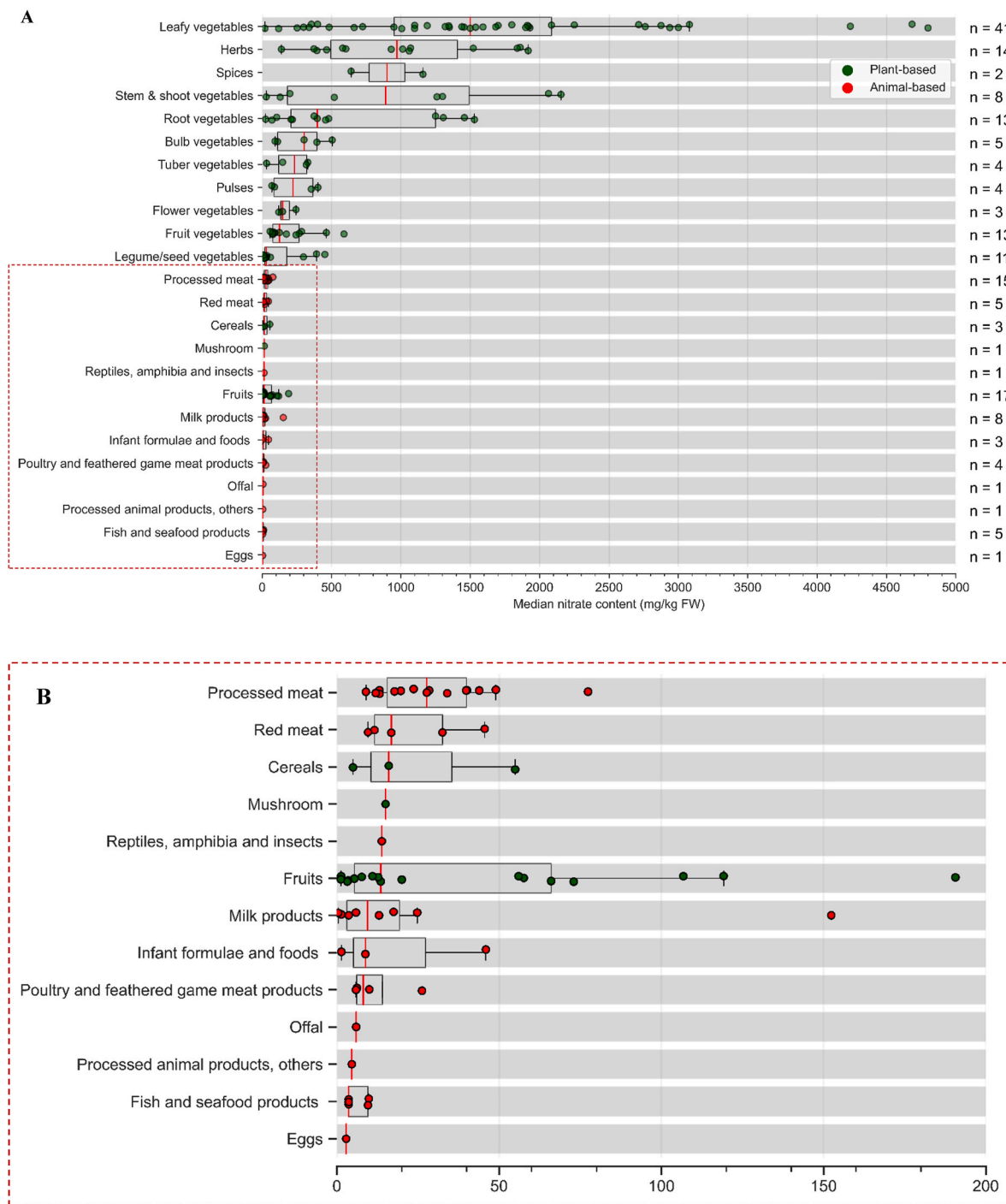


Fig. 1. Nitrate content (mg/kg fresh weight (FW)) of dietary sources by food group. Each dot represents the median nitrate content of the food (only those with >2 references included) within the food group, with n being the total number of foods included. The red line is the median nitrate content of the food group. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

a comprehensive and critical analysis of the health implications of nitrate in the context of nitrate source.

2. Sources, guidelines, and intake of dietary nitrate

Plants (primarily vegetables), meat, and water are the major sources of exogenous nitrate exposure. The nitrate content of these sources varies considerably (Fig. 1), is regulated in most countries, and a wide range of estimated intakes have been reported.

2.1. Plant foods

Nitrate is part of the nitrogen cycle, playing an important role in the nutrition and function of plants. The variation in nitrate content of plant foods is influenced by genetic, environmental and cultivation factors (Santamaria et al., 2001) as well as postharvest conditions including storage and processing methods (Bian et al., 2020).

The genetic influences not only include differences between species but within species, varying according to cultivar and genotype. A nitrate content of plant food products database reports levels ranging from 4 mg/kg fresh weight (FW) (apricot) to 4500 mg/kg FW (arugula) (Zhong, Blekkenhorst, et al., 2022). In general, the nitrate content of plant foods can be listed in descending order (most to least) as leafy vegetables > stem and shoot vegetables > herbs > spices > root vegetables > pulses > flower vegetables > bulb vegetables > tuber vegetables > nuts > fruit vegetables > legumes/seeds > cereals > fruits. Of all the plant foods, those with very high nitrate concentrations (>2500 mg/kg FW) include arugula (rocket), butterhead lettuce, Chinese flat cabbage, Chinese spinach, Chinese white cabbage, mustard, and purslane. Those with high nitrate concentrations (>1000 mg/kg FW) include spinach, most lettuce varieties, Swiss chard, celery, watercress, garden cress, radish, swede, beetroot, and herbs such as garlic chives, cilantro, fennel, and basil. Fruit has the lowest nitrate concentration with most fruits (apart from banana and strawberry) having concentrations <25 mg/kg FW (Zhong, Blekkenhorst, et al., 2022).

There is a complex interaction between environmental and cultivation factors which include temperature, humidity, photoperiod and light intensity, soil conditions, and fertilisation. Most studies have been performed with vegetables which differ according to geographical location; month, season, and year of cultivation; whether grown open air versus undercover; and organic versus conventional cultivation (Blekkerhorst, Prince, et al., 2017). Postharvest conditions also impact the nitrate content of vegetables. These include storage time and conditions and food processing. There are mixed reports on the effects of storage temperature (ambient, refrigeration and freezing) on levels of nitrate in vegetables (Kmiciek & Lisiewska, 1999; Pinto et al., 2010). Bacterial contamination and endogenous nitrate reductase activity during storage and wilting processes, including fermentation, can reduce the nitrate content of vegetables. Food processing methods (washing, peeling, and cooking) all decrease the nitrate content of vegetables (Alexander et al., 2008).

Due to potential implications for public health, the nitrate content of fresh spinach, preserved, deep-frozen or frozen spinach, fresh lettuce and Iceberg lettuce has been legislated by the Commission of the European Union for all its member states since 1997 with the latest regulation amended in 2011 (European Union Commission, 2011). Due to the variation in environmental and cultivation conditions described above, maximum levels are set depending on season and whether grown open air or undercover (Table 1). While not all other countries have regulations, most countries monitor nitrate levels in these vegetables due to potential health concerns with high intakes (Kalaycıoğlu & Erim, 2019).

2.2. Meat

Nitrate is found in most animal-based food products, both naturally

Table 1

COMMISSION REGULATION (EU) No 1258/2011 of 2 December 2011 amending Regulation (EC) No 1881/2006 as regards maximum levels for nitrates in vegetables.

Vegetable	Maximum permitted level of nitrate (mg/kg)
Fresh spinach	3500
Preserved, deep-frozen or frozen spinach	2000
Fresh lettuce	Harvested 1 October to 31 March
	Grown undercover
	Grown in open air
	Harvested 1 April to 30 September
	Grown undercover
	Grown in open air
Ice-burg type lettuce	Grown undercover
	Grown in open air
Arugula (rocket)	Harvested 1 October to 31 March
	Harvested 1 April to 30 September

occurring and as a permitted food additive. A nitrate content of animal-based food products database has reported levels ranging from 0.5 mg/kg (skimmed milk) to 101 mg/kg (chorizo) (Zhong, Liu, et al., 2022). Most animal-based food products have nitrate levels below 50 mg/kg (red meat, most processed meat products, poultry, most dairy products, most fish products, eggs, offal, and infant formula).

Specific processed meat products have higher amounts of nitrate as sodium and potassium nitrate are permitted food additives (E251, E252). As nitrate is reduced to nitrite through the action of bacterial nitrate reductases, sodium and potassium nitrate are used as a source of nitrite in products which have a lengthy ripening or drying process for example, chorizo and dry cured ham (Zhong, Blekkerhorst, et al., 2022). Nitrite controls the growth of pathogenic bacteria, in particular *Clostridium botulinum*, prevents rancidity, and contributes to the characteristic bright red colour and smoky flavour (Bedale et al., 2016). The use of sodium and potassium nitrate, while authorised for cured meat products, is heavily regulated in most countries (Table 2). Recently there has been a shift by some countries to use vegetable extracts as a natural source of nitrate and nitrite. These sources include celery, beetroot, spinach, leek, and Swiss chard. While accepted in the USA and Canada, this is not permitted by the European Union (Flores & Toldrá, 2021).

Most meat products have low nitrate levels compared to vegetables, notable exceptions are chorizo (median: 101 mg/kg, IQR: 60–106), fresh sausage (median: 77 mg/kg, IQR: 40–160), cows milk powder (median: 52 mg/kg, IQR: 9–152) and canned fish (median: 54 mg/kg, range: 18–77) (Zhong, Liu, et al., 2022). It is worth noting that nitrate is not a permitted additive for fresh sausage, milk powder and canned fish. Naturally occurring nitrate in raw meat, from nitrogen metabolism of the animal and in the animal feed, and non-meat ingredients, for example, soy concentrate, herbs, and spices, could contribute to the nitrate content of fresh sausage. With regards to milk powder, it is speculated that the nitrate content is due to prolonged heat, fouling, and contamination (Ministry for Primary Industries, 2020). The higher nitrate content of canned fish is of concern. The European Commission has discovered the widespread use of nitrite to enhance fish colour, specifically tuna, a practice which is banned (European Commission, 2021a).

2.3. Water

Nitrate is a naturally occurring compound in both surface and groundwater. The presence of nitrate in water is due to drainage from soil. In soil, nitrate is an important part of the nitrogen cycle and plays an essential role in plant growth and development. Nitrogen and ammonia are sources of nitrate (which is produced by nitrifying bacteria, *Nitrosomonas* and *Nitrobacter*). With the increased use of ammonia-

Table 2

Maximum permitted levels of nitrate and nitrite (mg/kg) in animal food products for the European Union (EU), USA, Australia and New Zealand, China, Japan, and Canada.

Animal food product	Maximum permitted level of nitrate and nitrite (mg/kg)											
	EU		USA		Australia, New Zealand		China		Japan		Canada	
	NO ₃	NO ₂	NaNO ₃	NaNO ₂	NO ₃	NO ₂	NO ₃	NO ₂	NO ₃	NO ₂	NO ₃	NO ₂
Non-heat-treated processed meat		150	500	200		125	500	150	70	70	200	200
Fermented, uncooked processed comminuted meat products					500							
Non-sterilised heat-treated processed meat		150				125	500	150	70	70	200	200
Sterilised heat-treated processed meat		100	500	200		125	500	150		70		
Commercially sterile canned cured meat			500	200		50						
Traditionally immersion cured meat products with specific provisions	50–300	10–175	500	200			500	150	70	70	200	200
Traditional Dry cured products	250	50–175	500	200			500	150	70	70	200	200
Side bacon											120	
Slow dried cured meat			500	200	500	125						
Fish and seafood products (including molluscs and crustaceans)											200	
Only pickled herring and sprat	500											
Smoked, cured tunafish				10								
Smoked, cured sablefish, salmon, and shad			500	200								
Cod roe			200									
Fish ham										50		
Fish sausage										50		
IKURA (salted/processed salmon roes)										5		
SUJIKO (salted salmon roes)										5		
TARAKO										5		
Whale meat bacon									70	70		
Cheese and cheese products	150				50						200 (residue 50)	
Cheese										200		
SAKE										100		
References	European Union Commission (2011)		Code of Federal Regulations (2020)		Australia New Zealand Food Authority (2013)		(National Health and Family Planning Commission of the PRC, 2014)		The Japan Food Chemical Research Foundation (2021)		Department of Justice Canada (2021)	

rich fertilizers and manures, wastewater treatment, use of septic tanks, planting of nitrogen-fixing crops, and fossil fuel combustion, the rate at which both nitrate and its sources is deposited in land has doubled leading to increased nitrate concentrations in water (WHO, 2003). In the European Union, agriculture is considered responsible for over 50% of nitrogen drainage into surface water (European Commission, 1991; 2021b). Nitrate is now considered a water contaminant due to these higher levels and is regulated and monitored by most countries.

Aligning with the WHO recommendation that drinking water nitrate be below 50 mg/L, the Nitrates Directive was adopted by the European Commission in 1991 to protect water quality across all its 27 member states (European Commission, 1991). Measures implemented include: (1) surface and groundwater monitoring at over 50 000 sites with the limit being 50 mg/L of nitrate; (2) identification and designation of areas as “Nitrate Vulnerable Zones” (NVZs) which are areas of land that drain into nitrate polluted water or waters at risk of pollution; (3); development of voluntary Codes of Good Agricultural Practice which includes time periods and ground conditions for fertilizer use; (4) implementation of compulsory action programmes for farmers within NVZs; (5) a limit to the amount of nitrogen that can be applied annually of 170 kg/ha; and (6) national monitoring and reporting every 4 years by each member state (European Commission, 1991). In the latest report (2021), over 14% of groundwater monitoring sites had levels above 50 mg/L with the majority <25 mg/L (European Commission, 2021b).

In the United States (US), the maximum level of nitrate in drinking water of 10 mg/L nitrate-nitrogen (NO₃-N), which is approximately 44 mg/L nitrate, is regulated by the US Environmental Protection Agency

(EPA). Monitoring over a 22-year period (1988–2010), ~70% of the 56 monitored networks showed no change in groundwater nitrate concentration (Lindsey & Rupert, 2012). It has been estimated that 0.42% of public water supplies exceed the maximum level of nitrate in drinking water (Pennino et al., 2017). Most surface and groundwater have nitrate levels below 4 mg/L (WHO, 2003).

In Australia, guidelines published in 2011 specifies that levels less than 50 mg/L are safe to drink, levels of 50–100 mg/L are safe to drink for all adults including pregnant women but not infants less than 3 months of age, drinking water with nitrate concentrations greater than 100 mg/L is not recommended (NHMRC/NRMMC, 2011). In Australian water supplies, supplying most of the major urban centres, typical concentrations are usually less than 0.15 mg/L, ranging up to 51 mg/L. However, concentrations up to 1300 mg/L have been recorded in groundwater supplies in rural areas (NHMRC/NRMMC, 2011).

In other regions of the world, levels of nitrate reported in groundwater for Africa range from 0 to 359 mg/L (Morocco, Niger, Nigeria, and Dakar); Asia range from 0 to 487 mg/L (India, Japan, Lebanon, and Turkey); South America range from 0.9 to 42 mg/L (Venezuela); and New Zealand range from 0.09 to 111 mg/L (IARC, 2010).

2.4. Guidelines and intake

The Acceptable Daily Intake (ADI) of nitrate of 0–3.7 mg/kg body weight (~260 mg/70 kg adult) (WHO, 2014, pp. 1–14) was established by the Scientific Committee for Food (SCF) in 1997 and the Joint Food and Agriculture Organization/World Health Organization (WHO)

Expert Committee on Food Additives (JECFA) in 2003. These values are based on a chronic feeding study in rats published in 1958 citing unpublished data. The ADI has been reviewed on several occasions by both organisations taking into account toxicity and epidemiological data and was endorsed by the European Food Safety Authority after conducting a risk assessment of nitrate consumption in 2008 and reviewed in 2015 (EFSA, 2017a). These current ADI guidelines do not differentiate between source of nitrate intake. It is also a dose that can be exceeded by consuming a serve of nitrate-rich vegetables for example, one serve of rocket (80 g) contains ~360 mg nitrate (Zhong, Blekkenhorst, et al., 2022). The upper limit ~260 mg/d for a 70 kg adult corresponds to the concentration of nitrate with observed beneficial effects on blood pressure and blood vessel function in clinical trials (Blekkenhorst, Bondonno, et al., 2018).

Falling within the ADI guidelines, the global total dietary nitrate intake has been estimated to range from 58 to 218 mg/d by the International Agency for Research on Cancer (IARC) Monograph on the Evaluation of Carcinogenic Risks to Humans report (IARC, 2010). A systematic review, conducted in 2018 of 55 observational studies that calculated daily nitrate intake in adults, reported a median [IQR] intake of 108 [87–145] mg/d in healthy individuals and 110 [89–153] mg/d from studies that included disease cases from case-control studies or individuals who developed diseases during follow-up (Babateen et al., 2018). A study investigating the nitrate content of American, Japanese, Chinese and Indian meal patterns report an estimated intake of 110 mg/d, 219 mg/d, 231 mg/d and 101 mg/d, respectively (Keller et al., 2020). Aligning closely with these estimates we have reported a median [IQR] of 67 mg [36–105] for participants of the Danish Diet Cancer and Health Study (Bondonno et al., 2021); mean (range) of 79 (12–231) mg/day for participants of the Perth Longitudinal Study of Aging in Women (Bondonno et al., 2017); mean (SD) of 129 (62) mg/d for participants of the Blue Mountains Eye Study (Liu et al., 2018); and 65 (95% CI: 65–66) mg/d in Australian Longitudinal Study on Women's Health (Jackson et al., 2019). However, these estimates come from diet questionnaires and thus it cannot be assumed that these are the levels actually consumed.

There is however potential for considerable variability within individuals and populations. For example, individuals who follow the Dietary Approaches to Stop Hypertension (DASH) diet may consume as much as 1000 mg/d (Hord et al., 2009). Modelling of an American diet and DASH diet with added spinach and/or nitrate supplements has demonstrated that dietary nitrate intake can range from 50% up to 250% of the ADI (Keller et al., 2020). High nitrate intakes have been reported in China 486 mg/adult/day (Zhong et al., 2002); and in Japan >1100 mg/adult/day (Sobko et al., 2010).

Vegetables are the main contributors to dietary nitrate intake accounting for ~80% of intake, followed by drinking water (~1–20%) and meat (~5–10%) (EFSA, 2008; Food Standards Australia New Zealand, 2013). There are, however, several inherent problems with estimating nitrate exposure. Firstly, the nitrate content of food, particularly plant foods, varies considerably according to environmental factors, geographic location, food processing and storage conditions as described in section 2.1. Secondly, most studies utilise a food frequency questionnaire (FFQ) to estimate intakes. FFQs lack granularity in that vegetables with different nitrate profiles may be grouped together; specific foods may be missing from the FFQ which is crucial when the food is both commonly consumed by the population and is an important dietary source of nitrate; detailed information about food preparation, growing and/or storage, processing and preparation is lacking. Unless the FFQ has been validated for intakes of the major dietary sources of nitrate, it should only be used to rank individuals and not assumed to provide information about absolute levels of intake. Thirdly, there are several physiological and lifestyle factors that influence the bioavailability of nitrate namely, age, fitness and smoking status, nitrate supplement and medication use, the composition of the oral microbiome, gastric acidity, and disease status (with certain diseases altering NO

homeostasis). Conclusions, therefore, cannot be drawn about absolute nitrate exposures.

3. Nitrate as Dr Jekyll

The discovery that nitrate is an exogenous source of NO (Lundberg & Govoni, 2004) has garnered intense research interest as NO is a key signalling molecule in the cardiovascular system, central nervous system (CNS), and immune system (Aliev et al., 2009). There is now a body of evidence that nitrate, through its effects on NO, improves CVD outcomes, musculoskeletal health, and exercise performance as well as has the potential to protect against other debilitating health outcomes.

3.1. Nitrate as an exogenous source of nitric oxide (NO)

There are now two recognised pathways to NO production, the endogenous L-arginine-NO synthase (NOS) pathway, and the exogenous nitrate-nitrite-NO pathway (Fig. 2).

3.1.1. The L-arginine-NOS pathway

Originally thought to be sole source of NO, it is now known that the L-arginine-NOS pathway yields approximately 70% of NO in the human body. Through this pathway, NO is produced from a five electron, multi-step oxidation of L-arginine to L-citrulline with molecular oxygen and nicotinamide adenine dinucleotide phosphate (NADPH) as co-substrates. This highly synchronised reaction is catalysed by a group of enzymes, the NO synthases (NOS). There are three isoforms of NOS namely NOS1, referred to as neuronal NOS (nNOS), NOS2, referred to as inducible NOS (iNOS), and NOS3 referred to as endothelial NOS (eNOS) (Förstermann U et al., 1994). This pathway is well defined and has been extensively reviewed (Bondonno et al., 2016).

3.1.2. The nitrate-nitrite-NO pathway

The discovery that nitrate and nitrite, oxidative end-products of endogenous NO metabolism through the L-arginine NOS pathway, are recycled back into NO and other bioactive nitrogen oxides, through the enterosalivary nitrate-nitrite-NO pathway, raised the possibility that dietary nitrate could also be an important source of NO (Lundberg & Govoni, 2004). It is now also established that through this pathway (described in Fig. 2), dietary nitrate (NO_3^-) is reduced to nitrite (NO_2^-) and subsequently to NO \cdot and is a nitric oxide synthase (NOS) - and oxygen - independent source of NO (Pettersson et al., 2009; Webb et al., 2008).

3.2. Nitrate and cardiovascular health

Since the discovery of the nitrate-nitrite-NO pathway, a growing and compelling body of evidence has developed showing that dietary nitrate improves cardiovascular function and health and lowers long-term risk of CVD. Several comprehensive reviews on the role of nitrate in cardiovascular health have been published and only a brief overview will be given here (Blekkenhorst, Bondonno, et al., 2018; Bondonno et al., 2018; Jackson et al., 2018; Raubenheimer et al., 2019). Most studies have investigated effects of nitrate intake from plant sources with very few investigating nitrate from animal or water sources.

3.2.1. Nitrate from plant sources

Over 60 randomized controlled trials (RCTs) investigating the effects of dietary nitrate from plant sources on markers of cardiovascular health (blood pressure, endothelial function, arterial stiffness, platelet reactivity and platelet aggregation) have been conducted (Benjamin et al., 2022; Blekkenhorst, Bondonno, et al., 2018; He et al., 2021; Jackson et al., 2018; Li et al., 2020). Plant sources of nitrate have included spinach, spinach juice, beetroot juice, arugula juice, and beetroot blends with interventions ranging from 2 h to 42 days. Most studies have included blood pressure as the primary outcome of interest and have

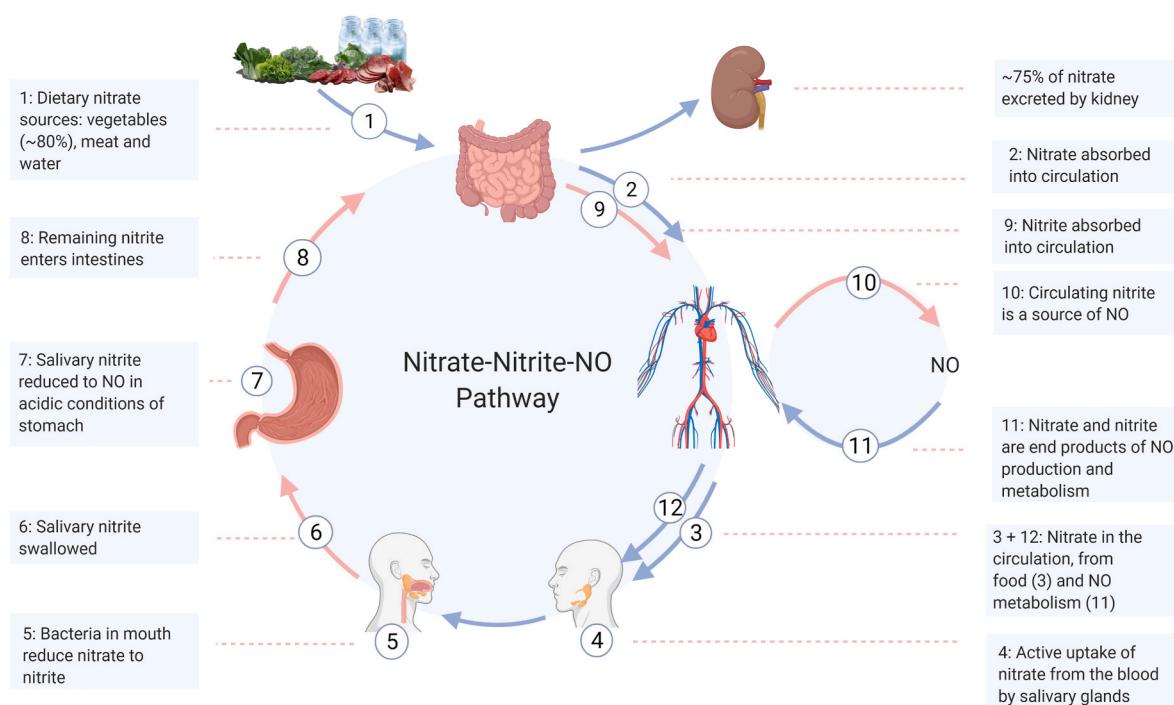


Fig. 2. The nitrate-nitrite-NO pathway.

demonstrated clinically meaningful decreases in blood pressure. This evidence is strongest in studies that have used nitrate-rich beetroot juice and is generally consistent across individuals with normal to slightly elevated blood pressure. However, conflicting evidence exists for the effects of nitrate from leafy green vegetables and in individuals at risk of CVD (e.g., hypertensive; type 2 diabetes, T2DM; overweight/obese) (Li et al., 2020). These trials included leafy green vegetables with similar nitrate doses as those administered with beetroot juice. The largest RCT to date (n = 243 participants) has not shown any significant effects of nitrate-rich leafy green vegetables consumed daily for 5 weeks, on blood pressure (Sundqvist et al., 2020). Similar results have been reported in several smaller RCTs (Blekkenhorst, Lewis, et al., 2018; Bondonno et al., 2014; Bondonno et al., 2015). A meta-analysis of clinical trials conducted prior to 2018 reported significant reductions in blood pressure, endothelial function (as measured by flow mediated dilatation), arterial stiffness and platelet aggregation (Jackson et al., 2018). Other recent meta-analyses have reported significant reductions in blood pressure in clinical trials of more than 3 days duration (Li et al., 2020), in older adults (He et al., 2021), and those with arterial hypertension (Benjamim et al., 2022).

Observational studies investigating the association between habitual nitrate intake (assessed using an FFQ, a self-reported measure of usual intake) from plant sources and cardiovascular health outcomes have only been conducted over the last 5 years. To the authors knowledge, only five observational studies have been conducted (Blekkenhorst, Bondonno, et al., 2017; Bondonno et al., 2017; Bondonno et al., 2021; Jackson et al., 2019; Liu et al., 2018) with significant inverse associations observed for risk of stroke (Bondonno et al., 2017), self-reported cardiovascular-related complications (Jackson et al., 2019), incident CVD (i.e., hospitalisations related to ischaemic heart disease, heart failure, ischemic stroke and peripheral artery disease) (Bondonno et al., 2021) and atherosclerotic CVD mortality (Blekkenhorst, Bondonno, et al., 2017). Most studies have investigated the association with CVD endpoints demonstrating an inverse association that plateaus around 60 mg/day (~1 cup of leafy green vegetables). Two studies have investigated the association between nitrate intake from plant sources and blood pressure with one study demonstrating an inverse association

(higher vegetable nitrate was associated with a lower blood pressure compared to individuals with lower nitrate intakes) (Bondonno et al., 2021) and the other, no association (McGrattan et al., 2022). However, the latter only considered dietary nitrate from all sources.

3.2.2. Nitrate from animal sources

To the authors' knowledge, there have been no RCTs with the specific aim of investigating the potential health effects of nitrate-rich animal products, such as processed meats, on markers of cardiovascular health. Meta-analyses have been conducted investigating the effects of red meat on CVD risk factors (O'Connor et al., 2017), as well as comparing meat sources (e.g., beef versus poultry and/or fish) (Maki et al., 2012) and whether the relation of red meat on CVD risk factors depend on the composition of comparison diets (Guasch-Ferré et al., 2019). All meta-analyses demonstrated no detrimental effects of red meat on cardiovascular health. It is worth noting that most studies in these meta-analyses examined minimally processed lean red meats and did not specifically aim to address meats with added versus naturally occurring nitrate.

There is an overwhelming amount of observational epidemiological research investigating the associations of processed and unprocessed meat intake with a range of health outcomes including cardiovascular disease. Meta-analyses of large cohort studies have found that dietary patterns with a moderate reduction in processed and red meat consumption are associated with lower CVD mortality, stroke, myocardial infarction, and T2DM (Johnston et al., 2019; Zeraatkar et al., 2019). Observational studies investigating the relation between nitrate intake from animal sources and CVD risk are limited. Thus, the link, if any, between the nitrate/nitrite content of meat and cardiovascular health outcomes is unclear. A recent food composition database has now been developed to enable researchers and scientists to estimate nitrate intake from animal sources and investigate associations with CVD risk (Zhong, Liu, et al., 2022).

3.2.3. Nitrate from other sources

Other sources of nitrate in the form of nitrate salts (e.g., sodium nitrate, potassium nitrate) have been investigated with markers of

cardiovascular health in RCTs. Studies conducted have included outcomes such as blood pressure, endothelial function, and arterial stiffness measures, and have generally reported improvements with both sodium and potassium nitrate with varying doses (Blekkenhorst, Bondonno, et al., 2018). However, most studies have been conducted in healthy individuals with very few studies conducted in those at risk of CVD.

Observational studies investigating the associations of other sources of nitrate, including nitrate in drinking water, with CVD endpoints are surprisingly scarce. To the authors knowledge, only one study has been published which aimed to explore the association of nitrate in drinking water (as a marker of drinking water quality) with causes of death related to CVDs (Houthuijs et al., 2022). However, no results were reported for the association between nitrate in drinking water and cardiovascular-related mortality due to the association being considered irrelevant because it was not observed after adjustment for spatial heterogeneity in regional mortality.

3.3. Nitrate and cognitive health

There is now well-established evidence that dietary nitrate intake is associated with a reduction of cardiovascular risk factors through effects on NO. NO is a regulatory molecule in both the cardiovascular system and the central nervous system (Aliev et al., 2009). Cognition and brain function rely on the cardiovascular system for blood supply (Kulshreshtha et al., 2019). NO is fundamental in the maintenance of cerebral basal vascular tone regulating cerebrovascular haemodynamics (Toda et al., 2009). Furthermore, endothelial derived NO has a significant role in the inhibition of tau phosphorylation. Tau, a protein with six isoforms, plays a key role in stabilizing microtubules in nerve cells. In Alzheimer's Disease (AD), tau proteins are abnormally folded compared to normal tau. Hyperphosphorylation of tau is a hallmark of AD pathology (Faraco et al., 2019), and neuronal-derived NO has an important function in neuronal and neurovascular communication (Du et al., 2015; Mapelli et al., 2017). The Alzheimer's Disease Neuroimaging Initiative (ADNI) cohort data-driven analyses has identified that vascular dysfunction in the brain occurs before other hallmarks of AD pathology such as β -amyloid ($A\beta$) deposits and hyperphosphorylated tau accumulation (Iturria-Molina et al., 2016). In line with this, there is accumulating evidence for a strong link between mid-life vascular risk factors and later life brain health, with most AD cases having a mixed pathology of both vascular dysfunction and phosphorylated tau. Furthermore, lower cerebral blood flow has been associated with impairment in several cognitive domains in observational/cohort studies (Kapasi et al., 2018; Lane et al., 2019; Leeuwis et al., 2017). The 2017 Lancet Commission on 'Dementia prevention, intervention, and care' has identified mid-life hypertension as an important modifiable risk factor for dementia (Livingston et al., 2017).

Nitrate and cognitive health have only been investigated using nitrate from plant sources. To our knowledge, no study has investigated nitrate intake from other sources and cognitive health. Although, there is evidence of beneficial effects of dietary nitrate on cerebrovascular homeostasis, the effect of nitrate intake on cognitive function from clinical trials varies. Seven out of twelve clinical trials observed that intake of dietary nitrate (from beetroot juice) was associated with improved cognitive function and cerebral blood flow. (Bond et al., 2013; Gilchrist et al., 2014; Presley et al., 2011; Thompson et al., 2015; Thompson et al., 2016; Vanhatalo et al., 2021; Wightman et al., 2015). On the other hand, four clinical trials have shown no effect of dietary nitrate (from beetroot juice) on cognitive function (Kelly et al., 2013; Lefferts WK et al., 2016; Shannon et al., 2017; Thompson et al., 2014). The difference in the effect of dietary nitrate on cognitive function in these clinical trials could be attributed to several factors including nitrate dose, age of the participants (three studies were conducted in older population), background diet, sample size, duration of the clinical trial and timing and sensitivity of cognitive function measures used. The intervention length in acute studies was a single dose with nitrate

ranging from 310 mg to 775 mg and the intervention length in chronic studies varied from two days to two weeks with nitrate dose ranging from 397 mg to 794 mg/day. The sample size ranged from 10 to 24 participants (mean age <30 years) in acute studies and from 12 to 36 participants (mean age >60 years in three out of five studies). So far, no comprehensive cognitive battery has been utilised to measure a range of cognitive domains in clinical trials of dietary nitrate intake.

It is important to acknowledge that any short-term effects of increased nitrate intake on measures of cognitive function may be distinct from long-term effects on cognitive decline. Exploration of the long-term impact of higher nitrate intakes on cognitive decline and dementia require evidence from long-term prospective cohort studies. Due to the long pre-clinical phase of dementia, the long-term association of dietary nitrate and cognition can only be reasonably examined using prospective observational studies. However, to date, there are no prospective observational studies investigating long-term relationship of habitual dietary nitrate intake with cognition, cognitive decline, and dementia.

3.4. Nitrate, musculoskeletal function, and human performance

Nitrate supplements were first reported in 2007 to improve oxygen cost during a submaximal cycling task (Larsen et al., 2007). Since then, a plethora of research has been undertaken in the area, including numerous meta-analysis and systematic reviews. Over time, the performance science literature has started to recognise nitrate supplements (e.g. beetroot juice, sodium nitrate) as an ergogenic aid (Peeling et al., 2018). In older and clinical populations dietary nitrate, coming predominantly from vegetables, has also been linked with better muscle function, comprising muscle strength as well as physical function (Coggan, Leibowitz, Spearie, et al., 2015; Sim, Lewis, et al., 2019; Sim et al., 2021). Such findings could have major clinical implications, as better muscle function is essential to prevent injurious falls, a major cause of fracture (Sim, Prince, et al., 2019). To gain an appreciation for the benefits of nitrate on human performance, it is important to acknowledge a range of potential mechanisms.

At present the exact mechanism responsible for these benefits remain elusive. Potential mechanisms through which dietary nitrate enhances physical performance include increases in skeletal muscle perfusion and oxygenation, contractile efficiency and mitochondrial function (Jones et al., 2018). Specifically, during intense exercise, higher circulating NO may increase oxygen transport and uptake by the muscle. It is beyond the scope of this section to provide a detailed review into potential mechanism (see (Jones et al., 2018) for review), instead we focus on the evidence for dietary nitrate on human performance.

When considering older or clinical populations, nitrate has been studied for its potential to support muscle function, comprising muscle strength and physical function. For example, acute nitrate supplementation (~600–700 mg) is reported to enhance skeletal muscle contractile properties in healthy middle-aged individuals and those with heart failure (Coggan, Leibowitz, Kadkhodayan, et al., 2015; Coggan, Leibowitz, Spearie, et al., 2015; Haider & Folland, 2014). Here, a small randomized controlled trial (n = 9, mean age 57 years) reported acute beetroot juice supplementation (containing ~700 mg of nitrate) increased peak knee extensor power (~10%) and velocity of knee extension (~12%) (Coggan, Leibowitz, Spearie, et al., 2015). Using an identical nitrate dosage, similar results in 12 healthy middle-aged adults were also reported for lower limb function, as indicated by faster knee extension velocity (11%) and power (6%) (Coggan, Leibowitz, Kadkhodayan, et al., 2015). A detailed review has eloquently summarised the potential benefits (or lack thereof) for nitrate supplements on the vasculature and physical performance in those with heart failure. This work concluded that acute nitrate supplements (e.g. a single dose or up to 9 days) may not consistently improve a range of physical performance outcomes in such populations (Ferguson et al., 2021).

Recent epidemiological data suggests that higher habitual dietary

nitrate intake is beneficial for muscle function. We have reported that habitual daily intake of ~100 mg/d of nitrate, primarily from green leafy vegetables, was associated with better muscle strength and physical function (Sim, Lewis, et al., 2019). In this cross-sectional study of 1420 community-dwelling older women (≥ 70 years), compared to individuals in the lowest tertile of nitrate intake (< 65 mg/d), women in the highest nitrate intake tertile (> 84 mg/d) had 35% and 28% lower odds for weak grip strength and slow timed-up-and-go (TUG), respectively. Noteworthy, when habitual nitrate intake was assessed longitudinally over 12 years in 3759 healthy adults (age 25–85 years, 56% female) results remained similar (Sim et al., 2021). Specifically, individuals in the highest tertile of nitrate intake (median intake: 91 mg/d) had 2.6 kg stronger (11%) and 0.24 s faster 8 ft-TUG (4%) compared with individuals in the lowest tertile of nitrate intake (median intake: 47 mg/d). These results were not influenced by physical activity levels.

Findings are supported by a systematic review and individual participant data meta-analysis comprising 268 participants (19 studies, 81.3% male) reporting that either acute or chronic nitrate intake was able to increase maximal muscle power by ~5% (Coggan et al., 2021). Age, sex, or test modality (i.e. small vs. large muscle mass dependent exercises) did not appear to influence results. Another meta-analysis examining the effect of nitrate on contractile properties of skeletal muscle suggested benefits for maximum voluntary contraction ($n = 11$) and peak power output ($n = 8$) (Esen et al., 2022). However, the aforementioned meta-analysis comprised of RCTs that typically adopted large doses of dietary nitrate, often with the performance task undertaken by younger more physically active participants. From a public health perspective, current epidemiological evidence suggests including one to two daily serves of nitrate-rich green leafy vegetables as part of overall vegetable intake to support/optimize muscle function (Sim, Lewis, et al., 2019; Sim et al., 2021). Improved muscle function could have substantial long-term benefits for reducing the risk of musculoskeletal disease.

From an athletic performance perspective, the capacity of nitrate to improve performance has been well researched. However, when assessing the sports science literature, it is important to acknowledge that athletic performance is multi-faceted comprising of a range of activities with very different physiological demands. For example, a physical performance task may be highly anaerobic or aerobic (e.g. 100 m sprint vs. 42.2 km marathon), and the mechanisms by which nitrate impacts performance may differ. A meta-analysis from 2021 of 73 studies comprising 1061 adults examined the effect of nitrate supplementation on endurance sports performance (Gao et al., 2021). They reported improved exercise time to exhaustion (mean difference [MD] 25.3 s), power output (MD 4.6 W) and distance travelled (MD 163.7 m) but no differences for perceived exertion, time-trial performance or work done. Such findings may be attributed to improved metabolic efficiency with a significant decrease in oxygen uptake (MD -0.04 L/min) recorded during exercise. Others have also reported that the effect size of nitrate supplementation on endurance performance is small ($d = 0.17$) and could have limited utility as an ergogenic aid in participants with excellent aerobic fitness (maximal oxygen uptake ≥ 65 ml/kg/min) and other optimised training parameters (Senefeld et al., 2020). A systematic review from 2021 concluded that nitrate supplementation could result in improvements (1.2–5.4%) in fatigue resistance during short repeated sprint efforts that are highly applicable to team sport athletes (Rojas-Valverde et al., 2021). Finally, in 2022, an Expert Consensus derived via the Modified Delphi Technique (12 panel members) was undertaken to examine the efficacy of dietary nitrate as an ergogenic aid (Shannon et al., 2022). Key findings include (i) benefits of dietary nitrate on performance may be diminished in individuals with a higher aerobic fitness (peak oxygen consumption > 60 ml/kg/min); (ii) nitrate can be safely consumed acutely (8–16 mmol; 496–992 mg) or chronically (4–16 mmol/d; 248–992 mg/d) to maximise ergogenic effects. Of importance, vegetables and their juices were promoted as the favourable

method to increase daily nitrate intake. To this end, current evidence would suggest that dietary nitrate has the capacity to improve human performance in a diverse range of populations, with benefits likely to be observed with higher habitual intakes from vegetables as well as acutely from beetroot juice supplements.

3.5. Nitrate and other health outcomes

3.5.1. Nitrate and diabetes

Epidemiological studies have observed that higher intakes of green leafy vegetables are associated with a lower risk of T2DM (Pokharel et al., 2022; Villegas et al., 2008). Dietary nitrate, abundant in green leafy vegetables (Hord et al., 2009), is yet to be explored in observational cohort studies for its association with T2DM.

To date, only two RCTs have explored the effect of dietary nitrate in people with T2DM. These provided beetroot as a source of dietary nitrate to experimental group participants for 2 and 24 weeks, respectively, and observed a clear increase in circulating nitrate, but no significant effect was observed on either glucose or insulin parameters (Bahadoran et al., 2021; Gilchrist et al., 2013). It is speculated that the absence of effect among people with T2DM could be due to disruption in the enterosalivary nitrate-nitrite metabolism or due to both metformin and nitrate competing for some mechanisms including activation of AMP-activated protein kinase (Cordero-Herrera et al., 2020).

Animal studies contradict findings from human studies and indicate dietary nitrate as a potent bioactive for better metabolic health (Lundberg et al., 2018). Animals treated with dietary nitrate/nitrite have shown improved insulin sensitivity, glucose tolerance and elevated insulin secretion compared to controls (Gheibi et al., 2018; Li et al., 2016). Furthermore, with weight gain being linked with incident T2DM among adults (Kodama et al., 2014), a 2020 meta-analysis reported lower weight gain in the experimental group treated with inorganic nitrate via drinking water compared to control animals (Bahadoran et al., 2020). Current evidence suggests future clinical trials are essential to replicate these findings among humans.

3.5.2. Nitrate and eye health

Nitric oxide is involved in ocular blood flow regulation (Grunwald et al., 1999) as well as the maintenance of endothelial cell function and vascular tone (Palmer et al., 1987). Studies suggest that NO can exert both protective and toxic effects on eye health as per its concentration and situation (Erdinest et al., 2021; Tummanapalli et al., 2021). Its controlled production is essential for functions such as tear production, corneal wound healing, while its overproduction is associated with dry eye, conjunctivitis, pterygium and microbial keratitis. After the discovery of the nitrate-nitrite-NO pathway, it was hypothesized that a similar pathway may also exist in the eye and contribute to NO-mediated physiological mechanisms (Park et al., 2020). Recent FDA approval of a NO donating prostaglandin analogue to reduce intraocular pressure among POAG patients (Kaufman, 2018) signifies the importance of NO signalling in the eye. It is assumed that both bacteria and mammalian enzymes mediate the nitrate reduction pathway in the eye (Park et al., 2020) but subsequent mechanistic and intervention studies are essential to understand the metabolism pathway.

Ocular diseases namely, primary open-angle glaucoma (POAG), manifested by an increase in intraocular pressure, and age-related macular degeneration (AMD), due to endothelial dysfunction, have been investigated in relation to dietary nitrate intake. Prospective observational studies from the Netherlands and the US observed a lower POAG risk [HR and 95% CI_{Q5vs.Q1}: 0.79 (0.66–0.93) for pooled Nurses' Health Study and Health Professionals Follow-up Study and 0.38 (0.20–0.72) for Rotterdam study] with higher dietary nitrate intake (Kang et al., 2016; Vergoesen et al., 2022). Additionally, an *in vivo* study showed reduced reactivity to NOS inhibition indicating abnormality in NO signalling among patients with POAG compared to healthy controls (Polak et al., 2007). Furthermore, higher nitrate was associated

with lower risk of incident early AMD [OR and 95% CI_{Q3vsQ1}: 0.61 (0.41–0.90) and 0.65 (0.44–0.96) for total dietary nitrate and vegetable nitrate, respectively] using data from Blue Mountains Eye Study (Gopinath et al., 2018). However, a threshold effect was observed for AMD with no further risk reduction for higher quartiles of total dietary nitrate or vegetable nitrate intake. In a cross-sectional analysis of the same study, higher total dietary nitrate and vegetable nitrate intake were associated with larger retinal arteriolar caliber and smaller retinal venular caliber suggesting favourable changes to the retinal microvasculature (Gopinath et al., 2020).

3.5.3. Anti-inflammatory effects of nitrate

Cell culture, animal and human studies have emerged indicating that the modulation of inflammatory processes and the immune system is an important mechanism through which dietary nitrate exerts its cardiovascular benefits (Carlstrom et al., 2018; Raubenheimer et al., 2019). NO signalling is critically involved in modulating inflammation and immune cell function (Bogdan, 2001), and anti-inflammatory effects of nitrate and nitrite have been shown in pre-clinical and clinical settings (Carlstrom et al., 2018; Raubenheimer et al., 2019). Cell culture and animal studies have suggested a role of the dietary nitrate–nitrite–NO pathway in attenuating vascular inflammation through beneficially modulating soluble inflammatory mediators (e.g., interleukin (IL)-1 β, IL-6, tumor necrosis factor) (Sindler et al., 2011; Stokes et al., 2009; Yang et al., 2017), leukocyte phenotypes and functions, and counteracting unwanted leukocyte interactions with the vasculature (Jadert et al., 2012; Stokes et al., 2009). Experimental studies in humans that have investigated the effects of an increased dietary nitrate intake on soluble inflammatory markers in the blood have produced mixed results, with either positive (Ashor et al., 2016; Rammos et al., 2014) or no effects (Raubenheimer et al., 2022; Velmurugan et al., 2016). Some evidence suggests that there may be an inflammatory ‘threshold’ above which dietary nitrate might exert more pronounced or consistent effects, and that individuals with elevated levels of inflammatory markers such as CRP may benefit the most (Raubenheimer et al., 2019). Recent human intervention studies have also examined whether the positive effects of nitrate on leukocyte-vasculature interactions and the functional and phenotypic status of circulating leukocytes, as observed in cell culture animal models, translates into benefits in humans (Raubenheimer et al., 2019). These studies have shown somewhat more consistent benefits of an increased nitrate consumption on blood cellular markers of inflammation and leukocyte-platelet interactions in various human populations (Raubenheimer et al., 2017; Velmurugan et al., 2016). In one of the few randomized clinical studies in this context, the consumption of nitrate-rich beetroot juice over six weeks reduced monocyte-platelet aggregation (which plays an important role in the development of inflammation in vascular diseases) in hypercholesterolaemic patients (Velmurugan et al., 2016). Chronic low-grade inflammation contributes to the pathology of several age-related conditions and chronic diseases (Minihane et al., 2015), in particular, the development and aggravation of cardiovascular diseases including atherosclerosis and hypertension (Libby & Hansson, 2015). Further long-term, randomized, placebo-controlled clinical studies measuring a combination of multiple inflammation markers in addition to clinical outcomes are required to determine the efficacy of dietary nitrate to prevent or treat disorders and diseases associated with chronic inflammation.

4. Nitrate as Mr Hyde

Nitrate and nitrite have the potential to form *N*-Nitroso compounds (NOCs; R¹R²NN=O) which are highly carcinogenic in laboratory animals (Gangolli et al., 1994) and are associated with cancers in humans. The International Agency for Research on Cancer has summarised that: “Ingested nitrate or nitrite under conditions that result in endogenous nitrosation is probably carcinogenic to humans (group 2A)” (IARC, 2010).

4.1. Nitrate and *N*-nitrosamine formation

There are two NOCs subgroups, *N*-nitrosamines (R¹R² are alkyl, aryl groups or cyclic ring) and *N*-nitrosamides (one of R¹R² is acyl group) (EPA, 2016). Humans are exposed to both *N*-nitrosamines and *N*-nitrosamides due to their widespread environmental occurrence being found in drinking water, food products, tobacco products, personal care products, medicinal products and rubber-based products (including food packaging) (EMA, 2020). As only *N*-nitrosamines have been detected in food, they are the focus of this discussion. *N*-nitrosamines can also be formed endogenously (in the gastrointestinal tract, cell-mediated nitrosation, bacterial nitrosation) and it is estimated that this endogenous formation accounts for up to 97% of *N*-nitrosamines exposure (Gushgari et al., 2018; Jakszyn, 2006). The formation of stable *N*-nitrosamines requires secondary or tertiary amines and nitrosating compounds. In this context, nitrate and nitrite ions are poor nitrosating agents. However, they can act as precursors of nitrous anhydride (N₂O₃), both exogenously and endogenously, which can further activate the nitrosation reaction (Fig. 3). There is a positive, although nonlinear, correlation between *N*-nitrosamines formation and level of added nitrite (Herrmann, Granby, & Duedahl-Olesen, 2015). This link between *N*-nitrosamines ingestion and endogenous formation and cancers has led to strict regulations on the use and level of nitrate and nitrite in food products worldwide, particularly processed meat, and water.

4.1.1. Occurrence, formation, and dietary exposure of *N*-nitrosamines in nitrate and nitrite containing foods

The total *N*-nitrosamine (TNA) content in food products is generally lower than 10 μg/kg, which is much lower than those in tobacco products (>16 000 μg/kg) and personal care products (>1500 μg/kg) (Gushgari et al., 2018; Lee, 2019). The *N*-nitrosamines detected in food, their common sources, and their IARC carcinogenic risk classification are detailed in Table 3. Food sources of *N*-nitrosamines include fats, oils, and sweets; meat products; fish products; canned vegetables; pickled, fermented plant-based food products; beverages including alcoholic beverages; grains; dairy products; fruit; rice and tofu (Table 4). Relatively high concentrations of *N*-nitrosamines are found in cooked, cured and smoked, nitrate and nitrite containing meat products (e.g., ham, bacon, sausages), processed fish products, pickled and fermented vegetables (e.g., kimchi). High *N*-nitrosamines were previously found in some improperly processed malt products (such as roasted malt, beer and other malt beverages), but this has been mitigated through processing optimisations and levels have now reduced by ~96% (Gushgari et al., 2018). It should be noted that *N*-nitrosamines in food products can be grouped into volatile and non-volatile *N*-nitrosamines, the analysis of which requires different extraction and analytical methods (Bian et al., 2021) (Table 3). Most studies, have focused on volatile *N*-nitrosamines, but much higher amounts of non-volatile *N*-nitrosamines were recently detected in processed meat products (Herrmann, Granby, & Duedahl-Olesen, 2015). Approximately 20 different volatile *N*-nitrosamines have been found in human foods, primarily in processed meat products (Niklas et al., 2022). *N*-nitrosodimethylamine (NDMA) and

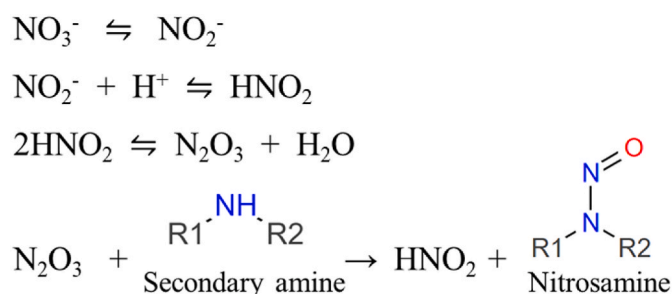


Fig. 3. The formation of *N*-nitrosamines.

Table 3
N-nitrosamines found in human food products and drinking water (EPA, 2016).

N-Nitrosamines	Common sources (regulatory limits and/or guidelines)	IARC group
Volatile		
N-nitrosodimethylamine (NDMA)	Drinking water (WHO, German and Australia: 100 ng/L; Canada: 40 ng/L; EU: no standard; US: No federal standard; US California and Massachusetts: 10 ng/L; Canada Ontario: 9 ng/L) Processed meat products (USDA: 10 mg/kg as total volatile NAs) Beer (US and Japan: 5 µg/L; Canada: 1.5 µg/L; Italy, Switzerland, Germany, Netherlands and Belgium: 0.5 µg/L) Fish products (USDA: 4–7 mg/kg)	2A
N-nitrosodie-thylamine (NDEA)	Drinking water; Meat products; Packaging	2A
N-nitrosodibutylamine (NDBA)	Packaging	2B
N-nitrosopiperidine (NPIP)	Piperidine containing spices like black pepper	2B
N-nitrosopyrrolidine (NPYR)	Drinking water; Processed fish products; Fermented vegetable products; Pyrrolidine ring containing compounds	2B
N-nitrosomorpholine (NMOR)	Packaging	2B
N-Nitrosodipropylamine (NDPA)	Drinking water	–
N-Nitrosomethylethylamine (NMEA)	Drinking water	2B
N-nitrosodiphenylamine (NDPhA)		3
Non-volatile		
N-nitrosoproline (NPRO)		3
N-nitrososarcosine (NSAR)		2B
N-nitrosohydroxyproline (NHPRO)		–
N-nitroso-thiazolidine-4-carboxylic acid (NTCA)		–
N-nitroso-2-hydroxymethyl-thiazolidine-4-carboxylic acid (NMTCA)		–
N-nitrosopipecolic acid (NPIC)		–

2A (probably carcinogenic to humans).

2B (possibly carcinogenic to humans).

3 Not Classifiable as to its Carcinogenicity to Humans.

-, unknown or no data.

N-nitrosodiethylamine (NDEA) are the most frequently monitored N-nitrosamines, as they are potent carcinogens (Table 3).

The N-nitrosamines formation in food also depends on the food matrix, presence of amines, co-occurring nitrosation catalysts and inhibitors, storage and processing conditions (Gushgari et al., 2018). Increased cooking temperature during frying, grilling, and roasting can promote the formation of various N-nitrosamines, NDMA, N-nitrosopiperidine (NPIP), and nitrosopyrrolidine (NPYR) in particular (De Mey et al., 2017). For example, much higher N-nitrosamines levels are reported in high-heat processed meat products like fried bacon (35.6 µg/kg versus 10.8 µg/kg in raw bacon) (Herrmann, Duedahl-Olesen, & Granby, 2015; Lee, 2019). Gaseous nitrogen oxides in smoke also can elevate N-nitrosamine formation in smoked meat and fish products (De Mey et al., 2017). Curing accelerators (such as ascorbic acid, sodium ascorbate, erythorbic acid and sodium erythorbate) inhibit nitrosation reactions but also shorten the curing period (De Mey et al., 2017; EFSA, 2017b). Accordingly, the USDA requires the addition of curing accelerators along with nitrate and nitrite in curing process (Code of Federal Regulations, 2020).

European Food Safety Authority (EFSA) has estimated that the average exogenous exposure to NDMA and NDEA through processed meat products are 0.2–2.6 and 0.03–0.4 ng/kg body weight (bw)/day, respectively (EFSA, 2017b). The NDMA is the main contributor,

Table 4
Total N-nitrosamine levels (TNA) reported in food.

Food	TNA range (mean; µg/kg)	References
Fats, oils and sweets (21 studies)	0–44 (8.9)	Gushgari and Halden (2018)
Meat (118 studies)	0.1–121 (8.1)	Gushgari and Halden (2018)
Bacon (uncooked)	0	Lee (2019)
Bacon (smoked, cured, fried, cooked)	7.5–35.6 (NAs in fat part of fried bacon is three times higher than those in lean part)	Lee (2019), Park et al. (2015)
Ham (smoked, cured, cooked)	3.6–6.4	Lee (2019)
Red meat and uncooked poultry	0	Lee (2019)
Pork (smoked, cured, cooked, fried, grilled, canned)	4.3–9.3 (fat part of fried pork: 25.9)	Lee (2019)
Processed meat products (cooked, fried, grilled)	2.2–11.0	Lee (2019)
Poultry (smoked, cured, fried, cooked, grilled)	1.6–22.4	Lee (2019)
Fish products (59 studies)	0–43.9 (5.6)	Gushgari and Halden (2018)
Salted fish	0.12–373.19	Park et al. (2015)
Canned vegetables (21 studies)	0.02–40.5 (5.4)	Gushgari and Halden (2018)
Kimchi	0.13–6.9	Park et al. (2015)
Beverages (13 studies)	0.2–45.7 (5.0)	Gushgari and Halden (2018)
Alcoholic beverages (41 studies)	10.2–45.7 (2.0)	Gushgari and Halden (2018)
Grains (8 studies)	0.2–4.6 (2.1)	Gushgari and Halden (2018)
Dairy (8 studies)	0–1.6 (0.5)	Gushgari and Halden (2018)

comprising approximately 90% of the total volatile N-nitrosamines exposure. In contrast, Herrmann et al. suggested that NDMA only contributes to about 40%, with NPYR being another 50% of the total volatile N-nitrosamines exposure (Herrmann, Duedahl-Olesen, et al., 2015). Moreover, Herrmann et al. summarised that exposure to volatile N-nitrosamines through nitrite containing processed meat products is low as compared to the non-volatile N-nitrosamines, 0.34 ng/kg bw/day versus 33 ng/kg bw/day for adults (15–75 years old) (Herrmann, Duedahl-Olesen, et al., 2015). In addition, N-nitroso-thiazolidine-4-carboxylic acid (NTCA) is reported to be the main non-volatile nitrosamine, accounting for 90%, followed by N-nitroso-2-methyl-thiazolidine-4-carboxylic acid (NMTCA) (5%) and N-nitrosoproline (NPRO) (2%). Nonetheless, as mentioned previously, exogenous N-nitrosamines exposure is believed to account for only a small proportion of the total N-nitrosamines exposure, with the remaining being from endogenous formation (Gushgari et al., 2018; Jakszyn, 2006; WHO, 2016).

A recent report by the French Agency for Food, Environmental and Occupational Health & Safety (ANSES) confirmed the link between nitrate and nitrite exposure and the risk of colorectal cancer, through consumption of nitrate and nitrite containing processed meat and drinking water (ANSES, 2022; IARC, 2015). In contrast, vegetables, many of which can accumulate high levels of nitrate, contribute to over 80% dietary nitrate intake but are extensively demonstrated to have multiple health benefits. This inconsistent associations highlight the potential different endogenous formation of N-nitrosamines formation after consumption of the different sources of nitrate and nitrite (Table 5) (Van Breda et al., 2019). Heme iron in meat products can promote N-nitrosamine formation (IARC, 2015), whereas reducing components (e.g., polyphenols, vitamin C and vitamin E) in leafy vegetables are suggested to inhibit the endogenous formation of N-nitrosamines (Ahluwalia et al., 2016).

Table 5
Studies investigating *N*-nitrosamine formation.

Food groups (n, number of participants)	Impacts on endogenous NA formation	Reference
High-nitrate water + red meat (n = 10)	Urinary nitrate excretion ↑↑ Apparent total <i>N</i> -nitroso compounds (ATNC) in faecal water ↑↑	Van Breda et al. (2019)
High-nitrate water + white meat (n = 10)	Urinary nitrate excretion ↑ ATNC in faecal water ↑	
High red meat (n = 6)	Faecal ATNC ↑ (3-fold) Faecal nitrite level ↑	Bingham et al. (1996)
White meat/fish (n = 6)	Faecal ATNC and nitrite – no effect	
High red meat (n = 12)	Faecal ATNC ↑ Faecal nitrite level ↑ as compared with low red meat diet (n = 12) and vegetarian diet (n = 12)	Cross et al. (2003)
The European Prospective Investigation into Cancer and Nutrition (EPIC-EURGAST) study	High correlation between intake of iron from meat and faecal ATNC (r = 0.948)	Jakszyn et al. (2006)
High nitrate + Fish meal (high amines) (n = 25)	Urinary nitrate excretion ↑ Urinary NDMA ↑ Urinary NPIP – no effect	Vermeer et al. (1998)

4.1.2. Occurrence, formation, and exposure of *N*-nitrosamines in water

Foodstuffs, including food and alcohol, are the main exogenous *N*-nitrosamines source for non-smokers (Gushgari et al., 2018; Jakszyn, 2006). However, *N*-nitrosamines (e.g., NDMA, NDEA, NDBA and NPYR) are widely detected in drinking water, with NDMA being the most commonly detected *N*-nitrosamine. The mechanism for *N*-nitrosamine formation in drinking water and related determinants were summarised by WHO (WHO, 2016) and Krasner et al. (2013). Generally, the TNA in water is < 50 ng/L and NDMA is < 20 ng/L. Although NDMA, NDEA, NDPA, NPYR and NDPhA have been listed on the third Contaminant Candidate List (CCL3, unregulated contaminants that are known to or anticipated to occur in public water systems and may require regulation under the Safe Drinking Water Act) by US EPA, no maximum contaminant levels have been established (EPA, 2014). However, institutions and local governments have established guidelines on NDMA in drinking water, albeit at different levels (Table 3). US EPA assessed that 0.7 ng/L NDMA in water represents 1 in 10⁻⁶ lifetime cancer risk. However, NDMA exposure through drinking water is demonstrated to be low, being <2.8% of total exogenous NDMA intake and <0.02% of estimated endogenous NDMA (Fristachi & Rice, 2007; Hrudey et al., 2013).

Drinking water also contains nitrate at varying concentrations as discussed in section 2.3. The WHO, IARC, health and environmental authorities (e.g., US EPA, European Environment Agency, Health Canada) and researchers have been regularly summarizing and updating the nitrate exposure through drinking water, and its health implications (See section 2.3 and 3.3). However, only few studies have examined the endogenous formation of *N*-nitrosamines in the human body after intake of nitrate from drinking water, most of which indicated an elevated *N*-nitrosamines formation (Van Breda et al., 2019).

4.2. Nitrate and cancer

4.2.1. Nitrate from dietary sources

To a far greater extent than with water, nitrate (and nitrite) from foods are consumed within the milieu of compounds contributing to the whole food matrix. In the WHO/IARC report of the 2006 assessment of the carcinogenicity of ingested nitrate and nitrite, the panel concluded there was “limited evidence of carcinogenicity” for nitrite in food, and “inadequate evidence of carcinogenicity” for nitrate in food and nitrate/nitrite in drinking-water (IARC, 2010). The latter was attributed to the high concentration of vitamin C and other inhibitors of nitrosation in vegetables, the primary dietary source of nitrate. Since 2010, more

studies on nitrite and cancer have been conducted but these are outside the scope of this review.

4.2.1.1. Combined dietary sources. For colorectal cancer, Hosseini et al. found, in their meta-analysis, that total dietary nitrate was associated with an increased risk (HR: 1.13; 95%CI: 1.04–1.23) (Hosseini et al., 2021). An earlier meta-analysis found no association between total dietary nitrate and colorectal cancer (Xie et al., 2016). For gastric cancer, Zhang et al. reported that high dietary nitrate intakes were associated with a lower risk of gastric cancer (OR: 0.81; 95%CI: 0.68–0.97) (Zhang et al., 2019). Two earlier meta-analyses found a similar direction of association and effect size between dietary nitrate and gastric cancer (Song et al., 2015; Xie et al., 2016). The results of the meta-analyses need to be interpreted with caution, due to factors such as critical heterogeneity between the studies, particularly relating to differences in the estimation of dietary nitrate intake (Song et al., 2015; Zhang et al., 2019), and, as for water, the inability to take into account factors which influence endogenous nitrosation, as well as the small number of studies included in some of the analyses (Xie et al., 2016).

In recent examinations of the IWHS, the NEBCS, and the Netherlands Cohort Study, authors have reported associations between total dietary nitrate intake (with approximately 97% of nitrate coming from vegetables (Jones et al., 2016)), and have found no association for bladder cancer (Barry et al., 2020; Jones et al., 2016; Zeegers et al., 2006), renal cancer (Jones et al., 2017), pancreatic cancer (Quist et al., 2018), colon and rectal cancer (Jones et al., 2019), nor with any other cancers of the digestive system (Buller et al., 2021).

4.2.1.2. Animal-derived sources. The intake of nitrate from processed meat has been associated with higher risks of, bladder (Barry et al., 2020), colorectal (ANSES, 2022; Etemadi et al., 2018), breast (Chazelas et al., 2022; Inoue-Choi et al., 2016), and prostate cancer (Sinha et al., 2009). Other studies have not identified an association (Jones et al., 2016; Jones et al., 2019). Much less evidence is available for the association between nitrate/nitrite in unprocessed red meat and cancer. Interestingly, in a study conducted in three US-based cohorts, the higher risk of colorectal cancer seen among participants with the highest, compared to the lowest, intakes of dietary nitrate (HR: 1.18; 95%CI: 1.08–1.28) from meat did not differ across anatomical subsites (i.e., proximal colon, distal colon and rectum) (Etemadi et al., 2018).

4.2.1.3. Plant-derived sources. No associations between plant-derived nitrate and bladder (Barry et al., 2020; Jones et al., 2016), renal (Jones et al., 2017), pancreatic (Quist et al., 2018), colon or rectal (Chazelas et al., 2022; Jones et al., 2019), breast (Chazelas et al., 2022), or prostate cancer (Chazelas et al., 2022) have been observed in the IWHS, NEBCS or NNS, while an inverse association was seen for gallbladder cancer in IWHS (Buller et al., 2021).

4.2.2. Nitrate from drinking water

The contamination of drinking water by nitrate and its potential link with cancer has been a public health concern for the last four decades (Hill et al., 1973), yet our understanding of this risk is far from complete. Early investigations were mostly ecological in design, focused on gastric cancer (Cantor, 1997), concluding that epidemiological evidence was uncertain. In 2005, Ward et al. recommended that additional studies were required to follow-up on the emerging evidence linking nitrate in drinking water to increased risk of cancer, and that these should take into account concomitant factors that either promote or inhibit nitrosation (Ward et al., 2005). In 2006, a panel of 19 experts assessed the carcinogenicity of ingested nitrate and nitrite and concluded that, for nitrate or nitrite in drinking-water, there was “inadequate evidence of carcinogenicity”, but that epidemiological studies were limited, exposure levels were low, and factors affecting endogenous nitrosation had rarely been considered (IARC, 2010). Since then, an increasing number

of observational studies on nitrate in drinking water and risk of cancer have been conducted, primarily in Europe and in the US, and with many investigating cancers of the digestive system. In recent years, several meta-analyses have been published with the latest and most comprehensive published in 2022 (Picetti et al., 2022). Picetti et al. included 60 observational studies (38 case-control studies; 12 cohort studies; 10 other study designs) published between 1990 and 2021, of which 59 studies investigated nitrate exposure and 4 studies investigated nitrite exposure from drinking water. Colorectal and gastric cancers were the most reported outcome in the included studies. The meta-analysis identified an association of nitrate in drinking water with gastric cancer (OR: 1.91; 95%CI: 1.09–3.33, including only case-control studies) but not with any other cancer (Picetti et al., 2022). Likewise, Hosseini et al. in their meta-analysis also found no evidence of an association between drinking water nitrate and colorectal cancer (HR: 1.04; 95%CI: 0.92–1.23) (Hosseini et al., 2021). Contrary, however, Essien et al. found an association between drinking water nitrate and increased risk of colon cancer (OR: 1.14; 95%CI: 1.04–1.23) but no association for any other cancers (Essien et al., 2020). There are several potential explanations for this discrepancy, including differences in studies included, as well as methodological differences, and the authors generally conclude that despite the increasing pool of evidence, the number of studies for any outcome is still too few to draw a definite conclusion on the association between nitrate in drinking water and risk of cancer. Additionally, a critical limitation of existing meta-analyses is the fact that factors which influence endogenous nitrosation, such as dietary patterns and smoking, were largely not considered. Therefore, the results of primary studies are also important to consider in understanding the extent of available evidence and the direction of identified associations. For example, 8 out of 15 included studies in the review by Picetti et al. reported an increased risk of colorectal cancer in the highest nitrate drinking water quantile analysed and, for all cancers investigated except brain cancer, every identified association was in the direction of an increased risk of disease (Picetti et al., 2022). A recent systematic review of studies from 2015 to 2022 found ample evidence to conclude that higher nitrates intake from drinking water was associated with higher risk of colorectal cancer (ANSES, 2022).

Examining factors that potentially influence nitrosation, ovarian cancer risk in the Iowa Women's Health Study (IWHS) was twice as high for women in the highest quartile (2.98 mg/L) compared with the lowest quartile (0.47 mg/L) of $\text{NO}_3\text{-N}$ (HR: 2.03; 95%CI: 1.22–3.38), with stronger associations when vitamin C intake was below the median intake (Inoue-Choi et al., 2015). Further, an association between drinking water nitrate levels >2.07 mg/L and increased risk of bladder cancer has also been reported among participants in the New England Bladder Cancer Study (NEBCS), and with evidence of interaction between participants consuming ≥ 31 g/day of red meat (OR: 2.6; 95% CI: 1.3–5.1) or ≥ 6.5 g/day of processed red meat (OR: 3.5; 95%CI: 1.8–6.9) (Barry et al., 2020). It is crucial that future studies investigate potential effect modifying factors where possible as it is plausible that dietary nitrate only forms *N*-nitrosamines under certain conditions.

Most published studies have reported the level of nitrate from municipal water sources to be below the WHO recommended limit of 50 mg/L (equivalent to 11.3 mg/L $\text{NO}_3\text{-N}$). Associations identified in both meta-analyses and primary studies are often found at a level below the current regulatory limits, calling into question the need for such limits to be revised. Additionally, most studies on nitrate from drinking water and risk of cancer have been conducted in high-income countries using public well data. There is a paucity of evidence from countries outside of Europe and the US, and for studies investigating the association using nitrate exposures in private wells (Ward et al., 2018). Areas supplied by private wells are often rural, may have higher levels of nitrate due to higher use of nitrogen fertilizers in crop culture and manure from animal feeding operations, and may be subject to less rigorous monitoring of contaminant levels than public wells managed by municipalities (WHO, 2016). Estimating exposure for private well users is important because it

allows assessment of risk over a greater range of nitrate concentrations (Ward et al., 2018), but a challenge to this is the limited measurement data available.

In summary, recent meta-analyses have identified associations between drinking water nitrate and increased risk of gastric and colorectal cancer, but also evidence from individual studies is suggestive of an association between drinking water nitrate and other cancer types. However, there is still a need for studies to investigate the association by factors known to influence endogenous nitrosation, such as diet, and for studies to be conducted using private well data and in areas where the nitrate concentration may exceed regulatory limits.

5. Perspectives and conclusion

Discovery of the multiple roles that NO plays in cardiovascular homeostasis was a Nobel Prize-winning paradigm shift in medicine. The subsequent discovery of a unique endogenous pathway by which dietary nitrate is a source of NO changed the view of nitrate from a nutritional perspective. This has led to a large body of research conducted over the past 15 years providing evidence for the cardiovascular health benefits of vegetable-derived nitrate, including benefits on blood pressure, other vascular measures, and a reduction in long term cardiovascular disease risk. As NO has wide-ranging physiological effects, it is now emerging that the observed benefits of nitrate could extend to other debilitating diseases. There is a growing body of evidence linking low nitrate intakes with diabetes, inflammation, eye disease and poor muscle function, a major risk factor for falls and fracture. Additionally, nitrate is a recognised sports ergogenic aid and the potential for nitrate to improve cognitive function and reduce the risk of dementia has also been postulated.

The discovery, over 50 years ago, that nitrate has the potential to form carcinogenic *N*-nitrosamine compounds resulted in an assumed harm of high nitrate intakes from any source. Given the evidence of a benefit of vegetable nitrate on CVD risk, nitrate consumption from vegetables is unlikely to be problematic, but convincing evidence is needed to override cancer concerns. Understanding the relationship of the different sources of nitrate to cancer is of critical importance because: (i) guidelines on nitrate intake do not differentiate between dietary sources, which could be a crucial factor determining whether consumption is linked with beneficial versus harmful effects; (ii) there is controversy in the literature with some researchers calling for a change in the guidelines on nitrate intake while others are actively promoting the negative effects of nitrate intake; (iii) the public are unlikely to listen to messages to increase intake of nitrate-rich vegetables if they are concerned about a link between nitrate intake and cancer; (iv) nitrate-rich vegetable intake should not be promoted if there is an increased risk of cancer with higher intake; (v) high dosage nitrate supplements are used as an ergogenic aid in sport; and (vi) vegetable nitrate extracts are being added to cured meat products with a “clean label” claim.

In summary, the assumption that all sources of nitrate are equal in benefit or harm is theoretical and needs to be explored. Future studies should consider dietary source when examining the beneficial or harmful effects of nitrate intake. Studies with improved exposure assessment and accurate characterization of individual factors that affect endogenous nitrosation are urgently needed to draw firm conclusions about risk of cancer from drinking water and dietary sources of nitrate/nitrite. As there are a large number of benefits associated with nitrate consumption, it is crucial that its link with cancer is thoroughly understood.

Declarations of competing interest

None.

Author contributions

Catherine P Bondonno: Conceptualization, Writing - Original Draft, Writing - Review and Editing, Supervision. **Liezhou Zhong:** Writing - Original Draft, Writing - Review and Editing. **Nicola P Bondonno:** Writing - Original Draft, Writing - Review and Editing. **Marc Sim:** Writing - Original Draft, Writing - Review and Editing. **Lauren C Blekkenhorst:** Writing - Original Draft, Writing - Review and Editing. **Alex Liu:** Writing - Review and Editing. **Anjana Rajendra:** Writing - Original Draft, Writing - Review and Editing. **Pratik Pokharel:** Writing - Original Draft, Writing - Review and Editing. **Dorit W Erichsen:** Writing - Original Draft, Writing - Review and Editing. **Oliver Neubauer:** Writing - Original Draft, Writing - Review and Editing. **Kevin D Croft:** Writing - Review and Editing. **Jonathan M Hodgson:** Writing - Review and Editing.

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No data was used for the research described in the article.

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