Ingestão de alimentos ricos em nitratos (folhas verdes e beterraba) : efeitos na pressão arterial

Vascular effects of dietary nitrate (as found in green leafy vegetables and beetroot) via the nitrate-nitrite-nitric oxide pathway

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Nitrate 'Veg-Table' 'Nitrate Units'

Introduction

The discovery that dietary (inorganic) nitrate has important vascular effects came from the relatively recent realization of the 'nitrate-nitrite-nitric oxide (NO) pathway'. Dietary nitrate has been demonstrated to have a range of beneficial vascular effects, **including reducing blood pressure, inhibiting platelet aggregation, preserving or improving endothelial dysfunction, enhancing exercise performance in healthy individuals and patients with peripheral arterial disease.** Pre-clinical studies with nitrate or nitrite also show the potential to protect against ischaemia-reperfusion injury and reduce arterial stiffness, inflammation and intimal thickness. However, there is a need for good evidence for hard endpoints beyond epidemiological studies. Whilst these suggest reduction in cardiovascular risk with diets high in nitrate-rich vegetables (such as a Mediterranean diet), others have suggested possible small positive and negative associations with dietary nitrate and cancer, but these remain unproven. Interactions with other nutrients, such as vitamin C, polyphenols and fatty acids may enhance or inhibit these effects. In order to provide simple guidance on nitrate intake from different

vegetables, we have developed the Nitrate 'Veg-Table' with 'Nitrate Units' [each unit being 1 mmol of nitrate (62 mg)] to achieve a nitrate intake that is likely to be sufficient to derive benefit, but also to minimize the risk of potential side effects from excessive ingestion, given the current available evidence. The lack of data concerning the long term effects of dietary nitrate is a limitation, and this will need to be addressed in future trials.

Effects of vegetables on blood pressure and cardiovascular disease events

Until recently dietary nitrate (NO_3^-) and nitrite (NO_2^-) were considered to lack any useful physiological activity in the circulation [1], despite these inorganic anions also being derived from nitric oxide (NO, produced endogenously by the action of the nitric oxide synthases (NOS), on the substrate amino acid, L-arginine [2]), a reactive free radical and potent vasodilator [3]. Rather, there have been longstanding major concerns regarding their toxicity, particularly potential carcinogenicity. However, a definite link still remains to be established. From 2001, nitrite was discovered to provide an important alternative source of nitric oxide, particularly when oxygen levels are reduced [4, 5], such as in the microcirculation, causing vasodilatation [6] and in 2004 in ischaemic hearts, with protective effects [7]. Even then, dietary nitrate was still thought to lack any effect in the circulation as it was not thought to increase circulating nitrite concentrations [8]. However, over the last 5 years or so there has been increasing evidence of physiological effects of nitrate, particularly on the cardiovascular system and the realization of the 'nitrate-nitrite-NO pathway'. This review will describe some of the key background to the field, provide an update on the latest cardiovascular studies, review the current situation with some of the potential carcinogenic effects and introduce a Nitrate 'Veg-Table' and nitrate units to guide patients and health professionals on what may be effective and safe amounts of nitrate to ingest.

Mediterranean and Japanese traditional diets are generally regarded as healthy. Both are associated with a low incidence of cardiovascular disease and longevity [9-13]. These diets share several similarities in that both are high in fruit and vegetables, fish (and olive oil – Mediterranean), and low in red meat. **A key beneficial common component may be the high dietary nitrate content, particularly of green leafy vegetables such as lettuce, spinach and rocket in the Mediterranean diet and ta cai, garland chrisantemum, laver, spinach and chin gin c. in the Japanese traditional diet [14]**. Indeed, vegetarian diets are associated with lower blood pressure [15-18] and green leafy vegetables have been associated with a reduced incidence of non-fatal myocardial infarction or fatal coronary heart disease and stroke [19, 20]. However, given the observational nature of these studies, they should be interpreted with caution [21]. More definitive evidence of an effect (albeit short term) on the surrogate outcome of blood pressure reduction is available from prospective controlled intervention trials, such as the DASH study [22-24].

Typically, around 85% of dietary nitrate (the inorganic nitrate anion, NO_3^-) is derived from vegetables [25-27], with most of the remainder from drinking water, though the concentration of this may vary considerably [28]. Dietary nitrite (NO_2^-) is mainly derived from cured meats, where it is added to prevent the development of botulinum toxin [29] and is reviewed elsewhere [30-33]. Inorganic nitrate has also been used for meat curing for centuries, and as the major component of gun powder since medieval times [1], and has several similarities, but

also marked differences from the organic nitrates, such as nitroglycerin which also has an important history as an explosive and medicinal product. These differences are reviewed elsewhere [34]. Vegetables can be categorized according to their nitrate content (see Table 1, Nitrate 'Veg-Table'): high nitrate content vegetables (>1000 mg kg−1) belong to the following families: Brassicaceae (rocket, the highest nitrate accumulating vegetable), Chenopodiaceae (beetroot, spinach), Asteraceae (lettuce) and Apiaceae (celery) [35-37]. Nitrate content also varies across the plant: leaf > stem > root [37-39]. Most common vegetables are in the medium range for nitrate content (100–1000 mg kg−1), including peppers, garlic, potatoes and carrots at the lower end, and green beans, cabbage and turnip at the upper end. Vegetables notable for their low nitrate content (<100 mg kg−1) are onions and tomatoes.

Table 1. The Nitrate 'Veg-Table': vegetables, ranked from highest to lowest according to mean nitrate content [range] expressed in mg kg−1, mmol per UK portion (80 g) and as a guide as the approximate number of nitrate units per portion (1 nitrate unit = 1 mmol) to facilitate estimation of nitrate intake or to modify intake as desired. Also included is tap water and bottled water for comparison. Nitrate content sourced from the following references [35, 37, 40, 54-62]:

Vegetables	Nitrate content Mean [range] (mg kg^{-1})	Nitrate content mean [range] [mmol per UK portion (80 g)]	Approximate nitrate content per UK portion (80 g) 1 nitrate unit = 1 mmol (62 mg
High	**1890** [1213–2650]	**2.44** [1.57–3.42]	
Rocket	2597 [2597]	3.35 [3.35–3.35]	
Spinach	2137 [965–4259]	2.76 [1.24–5.50]	**2**
Lettuce	1893 [970–2782]	2.44 [1.26–3.60]	
Radish	1868 [1060–2600]	2.41 [1.37–3.35]	
Beetroot	1459 [644–1800]	1.88 [0.84–2.32]	
Chinese cabbage	1388 [1040–1859]	1.79 [1.34–2.40]	
Medium	**316** [168–518]	**0.41** [0.22–0.67]	
Turnip	624 [307–908]	0.80 [0.40–1.18]	
Cabbage	513 [333–725]	0.66 [0.44–0.94]	
Green beans	496 [449–585]	0.64 [0.58–0.76]	
Leek	398 [56–841]	0.51 [0.06–1.08]	
Spring onion	353 [145–477]	0.46 [0.19–0.61]	
Cucumber	240 [151–384]	0.31 [0.19–0.50]	$^1/_2$
Carrot	222 [121–316]	0.29 [0.16–0.40]	
Potato	220 [81–713]	0.28 [0.10–0.92]	
Garlic	183 [34–455]	0.24 [0.05–0.58]	
Sweet pepper	117 [93–140]	0.15 [0.11–0.18]	
Green pepper	111 [76–159]	0.14 [0.10–0.21]	
Low	**78** [25–203]	**0.10** [0.03–0.27]	
Onion	87 [23–235]	0.11 [0.03–0.31]	**1/10**
Tomato	69 [27–170]	0.09 [0.03–0.23]	
Water	(mg l^{-1})	(250 ml glass)	(250 ml glass)
Tap	**26** [22.8–30.3]	**0.10** [0.09–0.12]	**1/10**
Mineral	**2.6** [<0.1–6.3]	**0.01** [<0.0004–0.025]	**1/100**

Nitrate content is influenced by environmental, agricultural and genetic factors [28, 36]. The main environmental factors are humidity, temperature, water content and exposure to sunlight. For example, summer grown lettuce (longer sunshine hours and less rainfall) has lower mean nitrate concentration than winter grown [40]. Agricultural factors such as nitrogen fertilization and concomitant use of herbicides also play their part in varying degrees to influence the nitrate content of vegetables [38, 41, 42]. Agricultural factors include nitrogen fertilization, degree of nitrogen fixation of atmospheric nitrogen by symbiotic bacteria in non-leguminous plants in addition to leguminous plants [43] and the nitrate reductase activity in

the root, genetically deficient in the lettuce, but very active in peas which contain low nitrate concentrations [44-46].

Juicing vegetables is a popular and convenient way of increasing vegetable intake and a range of commercially produced juices are available. While the nitrite content of freshly prepared homemade juice is negligible, after 2 days at room temperature the nitrite concentration of beetroot juice increases dramatically to almost 600 mg l−1, though remains low if kept refrigerated below 4°C [47]. The conversion is due to bacterial nitrate reductases which are less of a problem with industrially-produced raw juices which are lightly pasteurized.

The Acceptable Daily Intake (ADI) set by the European Food Safety Authority for nitrate is 3.7 mg kg−1 (0.06 mmol kg−1). This equates to ∼260 mg day–1 for a 70 kg adult (∼4.2 mmol). The World Health Organization (WHO) first set an upper limit for nitrate in food in 1962 derived from studies showing that daily intakes of ∼500 mg of sodium nitrate kg−1 body weight had been found to be harmless to rats and dogs. This figure was divided by 100 to yield an ADI for humans of 5 mg sodium nitrate (=3.7 mg nitrate kg−1), a figure that has stood ever since [48].

The vegetarian diet has been demonstrated to contain ∼4.3 mmol nitrate day−1, close to the ADI, almost four times greater than a 'normal' diet, which contains ∼1.2 mmol nitrate [49]. A study of 3 days' supplementation with ∼120 g of round green lettuce demonstrated that ∼70% of the nitrate load was excreted and the total amount of urinary nitrate excretion in a 24 h period increased from 53 mg (∼0.9 mmol) on the normal diet to 223, 241 and 243 mg on days 2, 3 and 4 respectively; equivalent to ∼4 mmol nitrate [8]. It should be remembered that nitrate is also produced endogenously. This was discovered in 1916 by Mitchell et al. [50] and confirmed by Green et al. [51] and Leaf et al. [52], who found that [15N]-L-arginine was the source of [15N]-nitrate. Indeed, the main source of nitrite in plasma was thought to be from the L-arginine-NO pathway [53] with no contribution from dietary nitrate [8].

The Nitrate 'Veg-Table' (Table 1) provides a guide to the nitrate content of high, medium and low nitrate-containing vegetables. Nitrate content was obtained from a range of sources [35, 37, 40, 54-62]. The average nitrate content is also expressed per UK portion of 80 g. Also, for ease of use for patients and health professionals, we propose that 'nitrate units' are used, 1 nitrate unit being equivalent to 1 mmol nitrate. This is a new concept that we put forward, analagous to the use of existing potassium points [63], primarily used as a guide to avoid excessive potassium intake in patients at risk of hyperkalaemia. Thus 'nitrate units' may be used as a guide to avoid excessive nitrate ingestion, or to avoid exceeding the ADI, if this is desired, or indeed to increase nitrate intake for functional benefit, such as blood pressure lowering or enhancement of exercise performance. The ADIs of nitrate for a range of given body weights is presented in Table 2. A UK portion (80 g) of a high nitrate-containing vegetable, such as beetroot or a green leafy vegetable, will therefore contain ∼2 mmol (or 2 units) of nitrate, about half the current ADI in a 70 kg person, which is 4.2 mmol. A portion of a medium nitrate-containing vegetable will provide ∼0.5 mmol nitrate, whereas low nitrate containing vegetables (onions and tomatoes) would provide just 0.1 mmol nitrate. A non-vegetarian 'normal' diet, containing ∼1 mmol nitrate day−1 [8, 49], therefore has the equivalent of ∼2 UK portions of medium nitrate containing vegetables/ day. It is easy to

underestimate how much should be ingested for a UK portion, particularly for green leafy salad leaves: 80 g would fill a fairly large serving bowl.

Table 2. Weight-based guide to the 'acceptable daily intake' (ADI) of dietary nitrate in mg, mmol and 'nitrate units' (1 Nitrate unit = 1 mmol). ADI = 3.7 mg kg−1, and 62 mg nitrate = 1 mmol

Weight (kg)	Acceptable Daily Intake, ADI (mg)	Acceptable Daily Intake, ADI in mmol or 'Nitrate units'
50	185	3.0
60	222	3.6
70	259	4.2
80	296	4.8
90	333	5.4
100	370	6.0

The limits for drinking water stem from the association between infantile methaemoglobinaemia, also known as 'blue baby syndrome' and high nitrate concentrations in well water, which was established in the 1940s [64]. Since nitrate concentrations below 44 mg l−1 were rarely associated with methaemoglobinaemia [65], this resulted in the imposition of the limits for drinking water of 45 and 50 mg l−1 in the USA and Europe respectively, even though methaemoglobinaemia is highly unlikely in the absence of the contaminating bacteria found in wells, which are required to generate high concentrations of nitrite, via reduction of nitrate [66].

Potassium nitrate had been used as a diuretic for oedema since the 16th century [67] and different nitrate salts were in use until the mid-1930s when alternative diuretics became available [68, 69]. While small intravenous doses of potassium nitrite in cats (e.g. 0.08 g) and dogs (0.2 g) had been found to reduce arterial blood pressure by Reichert & Mitchell in 1880 [70], nitrite concentrations were not thought to increase after nitrate ingestion [8] and accounts of syncope and hypotension due to over-indulgence in sausages [71], are more likely to have been a result of nitrite than the suggested nitrate. While high concentrations of acidified nitrite had been shown to relax rabbit aorta strips in 1953 [72], nitrite was thought to lack any physiological effect until the demonstration of arterial-venous plasma nitrite gradients in 2000 [73], dilatation of arterial rings [4, 5], and in 2003 the dilatation of forearm resistance vessels with a concomitant drop in MABP of ∼7 mmHg [6]. The effect of nitrite appeared to be due to its conversion to NO by deoxyhaemoglobin [6, 74]. The dilatory effect of nitrite has been shown to be greatly enhanced during hypoxia in humans in both arteries and veins [75]

and in tissues, oxygen is a potent regulator of the rate and products of tissue nitrite metabolism. At low oxygen concentrations nitrite reduction to NO predominates, whereas at normal to high oxygen levels oxidation of nitrite to nitrate predominates [76]. Other 'nitrite reductases' have been demonstrated and are reviewed elsewhere [34] and are summarized in Table 3.

Table 3. Kinetic processes in the handling of dietary nitrate and inorganic nitrite derived from nitrate. XOR (xanthine oxidoreductase), AO (aldehyde oxidase), ALDH2 (aldehyde dehydrogenase type 2)

Kinetics	Dietary nitrate	Nitrite (derived from nitrate)
Absorption	Readily absorbed across upper gastrointestinal tract [110]. Do not undergo first pass metabolism [112]. Only a small fraction reaches the large bowel: <1% ingested nitrate excreted in faeces [113]; <2% is present in ileostomy fluid in patients with a total colectomy [113, 114], with negligible nitrite concentrations.	
	Bioavailability of nitrate from cooked spinach, raw lettuce and cooked beetroot ~100% [111]. Plasma tmax of 1.5–1.8 h [111].	Bioavailability of nitrite ~95–98% [112]. Plasma tmax ~3 h, when derived from oral nitrate [82], and 15–45 min following oral nitrite administration [112].
Distribution	Volume of distribution moderate ~0.3 l kg−1 [111], compared with that of water (~0.6 l kg−1). Plasma half-life of 5–8 h [77, 79, 111, 141, 142]. Concentrated in the salivary glands: 20–28% of a nitrate load is secreted in saliva [107, 113, 115, 116]. Entero-salivary circulation [1].	Volume of distribution of nitrite at steady-state is similar to that of nitrate, ~0.35 l kg−1 [86]. Rapid uptake into most tissues [108]. Half-life of 1–5 min ex vivo [77, 142] to ~20–45 min in vivo [86, 112].
Metabolism	Symbiotic bacteria on the posterior third of the tongue which contain nitrate reductases, predominantly Veillonella species, as well as Actinomyces, Rothia and Staphylococcus epidermidis	In the stomach, nitrite reacts with the acidic gastric environment producing nitrous acid which decomposes to form nitric oxide as well as other reactive nitrogen oxides [95]. Vitamin C and polyphenols have been

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	convert nitrate into nitrite which is then swallowed [117, 118]. May be inhibited by mouthwash [80]. Minimal or no conversion of nitrate to nitrite in the circulation in humans (though demonstrated in germ-free mice).	shown to reduce nitrite to NO [188]. In the circulation and tissues, nitrite is metabolized to nitric oxide by: deoxyhaemoglobin [6] [119-121], deoxymyoglobin [122], cytoglobin and neuroglobin [123, 124], XOR [7, 125], aldehyde oxidase [126], ALDH2 [127], carbonic anhydrase [137], eNOS [125, 128-130], cytochrome P450 [131-134]. Additional pathways: formation of nitro-fatty acids [139], nitrosothiols [138].
Excretion	∼65–75% of absorbed nitrate is renally excreted [110].	Renally excreted, with renal carbonic anhydrases involved in nitrite reabsorption [140].

Therefore, while nitrite now had an obvious potential to reduce BP, dietary nitrate was not thought to result in an increase in circulating nitrosothiols or nitrite [8], and therefore was thought to lack any effect on the vasculature. However, in 2004, Lundberg & Govoni detected an increase in plasma [nitrite] after ingestion of sodium nitrate [77] and in 2006 Larsen et al. demonstrated that diastolic BP was reduced by 3.7 mmHg following 3 days' dietary supplementation with sodium nitrate (0.1 mmol kg−1 day−1) compared with placebo sodium chloride in 17 healthy volunteers (randomized, double-blind, crossover design) [78]. Using a single ingestion of 500 ml beetroot juice [22.5 mmol (∼0.35 mmol kg−1)], Webb et al. demonstrated a more acute and more profound fall in both systolic BP (∼10.4 mmHg, baseline of ∼108 mmHg) and diastolic BP (∼8 mmHg) compared with placebo (500 ml water, crossover study) [79]. Importantly, the beetroot juice did not contain any detectable nitrite, and whilst plasma nitrate levels were already increasing by 30 min, the BP did not start to fall until the plasma nitrite concentration started to rise, with maximum changes in both occurring at ∼2.5–3 h, reflecting the time to produce nitrite from nitrate and for it to accumulate via the enterosalivary circulation (described below). Indeed, interruption of this process by asking volunteers to spit out all their saliva for 3 h immediately following beetroot juice ingestion completely blocked the rise in plasma nitrite and the reduction BP. This increase in plasma nitrite is also inhibited by the use of an antibacterial mouthwash just prior to nitrate ingestion in humans [80] and also blocks the 5 mmHg blood pressure reduction consequent to nitrate

supplemented drinking water in Sprague-Dawley rats [81], in addition to attenuating the gastric mucus thickness with loss of gastroprotective effects against ulcerogenic insults.

These studies therefore provided evidence for a 'nitrate-nitrite-NO' pathway. In order to provide further evidence that this effect was due to nitrite, Kapil et al., used potassium nitrate capsules, and demonstrated a dose dependent reduction in BP (with 4, 12 and 24 mmol nitrate) equivalent to beetroot juice [nitrate] with no effect seen with potassium chloride as control [82], suggesting a BP lowering effect of nitrate rich vegetables independent of any potential effect due to their potassium content [83, 84]. This study also demonstrated that the peak increase in plasma nitrite at ∼3 h was associated with a significant increase in cGMP, the most sensitive indicator of NO bioactivity [85], thus providing evidence of bioactive NO generation from nitrite.

The Kapil et al. study also provided a clue to the heterogeneity of blood pressure responses to dietary nitrate. Post hoc analysis revealed that nitrate reduced blood pressure in males – from a higher baseline (associated with a lower baseline plasma nitrite concentration) compared with BP in females, who had no response to nitrate (possibly a result of their higher baseline plasma nitrite concentrations). Whether there is a sex difference per se, or whether the BP response to dietary nitrate is dependent on baseline plasma nitrite/BP remains to be determined. However, strong inverse correlations were demonstrated between the peak decrease in BP and the baseline BP (both systolic and diastolic). This therefore holds promise that dietary nitrate will be more effective in reducing blood pressure when it is needed, i.e. in people with high normal BP or hypertension, and results of dietary nitrate in hypertensives are awaited. Similarly, dietary nitrate would appear not to induce unwanted hypotension in people with low normal BP. The enterosalivary circulation also provides an inherent limiting mechanism to prevent excessive conversion of nitrate to nitrite, avoiding the risk of nitrite toxicity.

An important potential advantage of inorganic nitrate/nitrite is the apparent lack of tolerance induction [86], which commonly limits the therapeutic use of organic nitrates [87]. This was supported by the dietary intervention study of Sobko et al. which showed a sustained reduction in diastolic BP of ∼4.5 mmHg over 10 days, when subjects took a Japanese traditional diet, rich in dietary nitrate, compared with a low nitrate control diet [9], and by the study of Vanhatalo et al., which showed a reduction in BP of ∼7/5 mmHg following 15 days' supplementation with 500 ml beetroot juice [88]. Studies showing effects of dietary nitrate, including other forms such as spinach [89], and very recently with red and white beetroot-enriched bread [90] on BP are summarized in Table 4.

Table 4. Effect of dietary nitrate on BP in human studies, showing duration, amount of nitrate given in mmol (*0.1 mmol kg−1 day−1 for 3 days = 7 mmol day−1 in 70 kg person) peak systolic and diastolic BP effect (SBP and DBP respectively) and timing of peak effect following ingestion

Study	Source	Nitrate (mmol)	Peak ΔSBP (mmHg) (time)	Peak ΔDBP (mmHg) (time)
Larsen et al. (2006) [78]	Sodium nitrate 3 days	∼7*	–	−3.7
Webb et al. (2008) [79]	Beetroot juice 500 ml × 1	22.5	−10.4 (2.5–3 h)	−8
		24	−9.4 (6 h)	−6.0 (2.75 h)
Kapil et al. (2010) [82]	Potassium nitrate × 1	12	−6 (2.25 h)	−4.5 (3 h)
		4	−2.5 (1.75 h)	−4.5 (2.25 h)
Kapil et al. (2010) [82]	Beetroot juice 250 ml × 1	5.5	−5.4	–
Sobko et al. (2010) [9]	Japanese Traditional Diet 10 days	∼18	–	−4.5
Vanhatalo et al. (2010) [88]	Beetroot juice (500 ml) 15 days	5.2	−7	−5
Bahra et al. (2012) [148]	Potassium nitrate × 1	8	−5	–
Bondonno et al. (2012) [89]	Spinach (200 g) × 1	3	−2.7	–
		2.3	−13.1 (2–3 h)	−16.6 (2–3 h)
Hobbs et al. (2012) [90]	Beetroot juice (100, 250, 500 g)	5.7	−20.5 (2–3 h)	−14.6 (2–3 h)
		11.4	−22.2 (2–3 h)	−18.3 (2–3 h)

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Study	Source	Nitrate (mmol)	Peak ΔSBP (mmHg) (time)	Peak ΔDBP (mmHg) (time)
Hobbs et al. (2012) [90]	Beetroot-enriched bread (100 g)	1.8	−16.5 (2–3 h)	−23.2 (2–3 h)
	with white beetroot	1.6	−19.3 (2–3 h)	−23.6 (2–3 h)

Platelet function

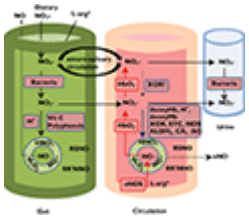
Platelets influence occlusive vascular disease through their interaction with the vessel wall [91], and play a significant part in the pathogenesis of acute coronary syndromes [92]. Nitric oxide inhibits platelet adhesion to the endothelium [93], and platelet aggregation [94]. Following the discovery of stomach NO synthesis from dietary nitrate, with potentially important protective effects against pathogens [95], and to maintain the integrity of the gastric mucosa [96], studies in healthy volunteers showed that oral potassium nitrate (2 mmol: equivalent to half a British flat lettuce [28]) inhibited platelet aggregation [97]. This was thought to be through the formation of nitrosothiols, which were detected in gastric juice, but not in the systemic or portal circulation [97, 98]. Nitrite was not a likely candidate as it was thought to be inert [99, 100], or possibly damaging [101], requiring hypoxic/ischaemic conditions, and millimolar nitrite concentrations [102-105] to generate NO, and not to increase in plasma following oral nitrate intake anyway [8, 106, 107]. Even if it did, Bryan et al. demonstrated that nitrite had no effect on platelet aggregation in rats [108]. While the inhibition of platelet aggregation by beetroot juice (500 ml, 22.5 mmol nitrate) [79], could have been anticipated from the previous studies [97, 109], the demonstration that this was due to nitrite, by asking the volunteers to spit out all their nitrite-containing saliva for 3 h immediately following beetroot juice ingestion, in order to interrupt the enterosalivary circulation and thus prevent absorption of nitrite, was surprising.

Kinetics of the nitrate-nitrite-NO pathway

With the realization of the nitrate-nitrite-NO pathway, the kinetics of dietary nitrate and nitrite (i.e. that derived from nitrate) are summarized in Table 3. Some of the key reactions of nitrate and nitrite in the upper gastrointestinal tract and the circulation are shown in the Figure 1.

Figure 1. Kinetic processes in the handling of dietary nitrate (NO3-) and inorganic nitrite (NO2-) derived from nitrate, from the gut to the circulation to the urinary tract. HNO2 (nitrous acid), N2O3 (dinitrogen trioxide), NO+ (nitrosonium ion), RSNO (nitrosothiol), RR'NNO (N-nitrosamine), eNO (exhaled NO), Vit C (vitamin C), deoxyHb (deoxyhaemoglobin), deoxyMb (deoxymyoglobin), XOR (xanthine oxidoreductase), AO (aldehyde oxidase), ETC (electron transport chain in mitochondria), CA (carbonic anhydrase), ALDH2 (aldehyde dehydrogenase

type 2), NOS/eNOS (endothelial nitric oxide synthase), L-arg* (L-arginine), *non-essential amino acid, but also derived from the diet. Nitrate reductase-possessing bacteria on the posterior third of the tongue and in some urinary tract infections convert nitrate to nitrite. Red and blue arrows represent pathways that are favoured under oxygenated and deoxygenated conditions, respectively



Absorption

Dietary nitrate is exceptionally well absorbed in the upper gastrointestinal tract [110], with the bioavailability of nitrate from cooked spinach, raw lettuce and cooked beetroot being ~100%, with plasma concentrations of nitrate peaking after 1 h [79], (tmax of 1.5–1.8 h) [111], and the bioavailability of nitrite, following ingestion of large amounts, being ~95–98% [112]. This is despite numerous reactions in the acidic environment of the stomach (see below). Indeed, nitrite is rapidly absorbed, with a plasma tmax of 15–45 min following oral nitrite administration [112], but this increases to ~3 h, when nitrite is derived from oral nitrate ingestion via the enterosalivary circulation [82]. Only a small fraction of nitrate reaches the large bowel, as <1% of ingested nitrate is excreted in the faeces [113] and <2% is present in ileostomy fluid in patients with a total colectomy [113, 114], with negligible nitrite concentrations. Nitrate and nitrite do not undergo significant first pass metabolism [112].

Distribution

The volumes of distribution of nitrate (~0.3 l kg−1) [111] and nitrite (~0.35 l kg−1) [86], are similar and about half that of water (~0.6 l kg−1). Nitrite is taken up rapidly into most tissues [108]. One of the curiosities of nitrate handling is that such a large proportion is concentrated in the salivary glands. Most estimates suggest that 20–28% of a nitrate load is secreted in saliva [107, 113, 115, 116].

Metabolism

The site of nitrate reduction to nitrite in humans is almost exclusively on the posterior third of the tongue, by nitrate reductase-containing symbiotic bacteria, predominantly Veillonella species, as well as Actinomyces, Rothia and Staphylococcus epidermidis [117, 118]. Whilst some conversion of nitrate to nitrite occurs on the ‘first pass’ of nitrate-containing food over the surface of the tongue before it is initially swallowed, the majority of nitrate reduction to nitrite occurs over the subsequent few hours, via the entero-salivary circulation [1], following concentration of nitrate in the salivary glands, and secretion in saliva, as described above [107, 113, 115, 116]. When nitrite reaches the stomach, some of it reacts with the acidic gastric environment producing nitrous acid which decomposes to form nitric oxide as well as other reactive nitrogen oxides [95] (see Figure 1 and Equations (1)-(3)):

$NO_2^- + H^+ \rightarrow HNO_2$ (1)

$2\,HNO_2 \rightarrow N_2O_3$ (2)

$N_2O_3 \rightarrow NO + NO_2$ (3)

Additional reactions between nitrite and vitamin C or polyphenols in the stomach also generate NO. Despite these numerous reactions in the stomach, the bioavailability of nitrite, when administered in large doses, is normally high (95–98%) [112]. Nitrite appears to mediate the majority of its effects through bioactivation to NO. This occurs mainly via nitrite reductases, which have selective activity under oxygen/hypoxic/ischaemic conditions; these include the deoxygenated globins (haemoglobin [6, 119-121], myoglobin [122], cytoglobin and neuroglobin [123, 124]), the molybdoflavoproteins, which have similar structures to some bacterial nitrite reductases (xanthine oxidoreductase [7, 125], aldehyde oxidase [126]), aldehyde dehydrogenase type 2, ALDH2 [127], eNOS [125, 128-130], cytochrome P450 [131-134] and the mitochondrial electron transport chain [135, 136]. In addition, carbonic anhydrase appears to possess a nitrite anhydrase function [137]. Alternative pathways of nitrite bioactivation include the formation of nitrosothiols [138] and the formation of nitro-fatty acids, such as nitro-oleic acid [139]. It is likely that a varying spectrum of bioactivation pathways operate, dependent on the tissue involved and the prevailing conditions, with different enzymes becoming more active under different conditions.

Excretion

The majority of an ingested load of nitrate, ~65–75% is renally excreted [110]. Small amounts of nitrite are excreted in the urine, with renal carbonic anhydrases being involved in nitrite reabsorption [140]. Certain bacteria that possess nitrate reductases and are associated with urinary tract infections increase urinary nitrite concentrations, the basis of the nitrite test on the urine dip stick. The half-life of nitrate is 5–8 h [77, 79, 111, 141, 142], which is much longer than that of nitrite, which has been reported as being 1–5 min ex vivo [77, 142] and ~20–45 min in vivo [86, 112]. This is a reflection of nitrate's stability in the circulation, and nitrite's propensity to be rapidly metabolized via oxidation to nitrate in oxygenated conditions and reduction to NO, as described above.

Arterial ageing and atherosclerosis

The risk factors for atherosclerosis also contribute to several important surrogate markers. The potential impact of dietary nitrate on each of these is considered below.

Endothelial dysfunction

Hypertension, diabetes, dyslipidemia, smoking and ageing are strong risk factors contributing towards atherogenesis and are associated with impaired endothelial function [143], a key step in the pathogenesis of atherosclerosis and a surrogate marker. Such endothelial dysfunction usually results, at least in part, from decreased NO production from endothelial NOS and/or reduced NO bioavailability, which both result in, and are further diminished by inflammation. Plasma nitrite concentration has been shown to reflect constitutive NOS activity in mammals

[144] and is inversely correlated with the number of cardiovascular risk factors, and positively correlated with endothelial function as assessed by flow mediated dilatation (FMD) in humans [145]. Also, changes in nitrite (nitrite reserve) with reactive hyperaemia during FMD also reflected endothelial function [146]. Hence, it may be anticipated that provision of dietary nitrate/nitrite may enhance endothelial function and suppress microvascular inflammation. Indeed, this was demonstrated in a hypercholesterolaemia model by Stokes et al. who fed mice a cholesterol-enriched diet for 3 weeks, resulting in impaired endothelium-dependent arteriolar vasodilator responses to acetylcholine which were restored to normal by administration of nitrite in the drinking water [147]. Similarly, nitrite inhibited the elevated leucocyte adhesion to, and emigration through, the venular endothelium resulting from the high cholesterol diet and also reversed the concomitantly elevated CRP to normal concentrations. Whilst inorganic nitrate/nitrite has the potential to improve endothelial dysfunction in patients, Bahra et al. found no effect in healthy volunteers with normal endothelial function. FMD was not altered at 3 h following the ingestion of dietary nitrate (8 mmol). However, a reduction in pulse wave velocity of ∼0.3 m s−1 accompanied the reduction in SBP of ∼5 mmHg [148]. Bondonno et al., (2012) found a small increase in mean FMD over 4 min of ∼0.5% in 30 healthy volunteers following ingestion of 200 mg spinach, but this was not as great as flavanoid-rich apple (∼1.1%) or apple and spinach (∼0.9%) suggesting a potentially greater effect of flavanoids on eNOS function than nitrate in the context of normal endothelial function in healthy volunteers [89]. Whilst many nutraceuticals and drugs have been tested for their capacity to enhance endothelium dependent dilatation via NO production, some have also been demonstrated to enhance endothelium-independent dilatation with nitrovasodilators, suggesting improved NO bioavailability [149]. It is likely that provision of nitrate/nitrite enhances NO bioavailability predominantly by inhibiting inflammation and inactivating reactive oxygen species (ROS), in addition to providing a source of NO per se, rather than by enhancing eNOS.

Arterial stiffness

In addition to finding decreased plasma nitrite concentrations in old (26–28 months) male C57BL6 control mice, Sindler et al. also detected depleted concentrations in the arteries and heart, which were restored to youthful levels (4–6 month controls) by 3 weeks' supplementation with sodium nitrite (50 mg l−1 in the drinking water) [150]. Nitrite also restored the impaired acetylcholine-dependent carotid dilatation and reduced markers of oxidative stress, including nitrotyrosine. Ageing [151-153], hypertension [154], endothelial dysfunction [155], inflammation [156] (and possibly ROS [157]) are also associated with stiffening of large arteries, which when assessed by pulse wave velocity is a strong independent predictor of cardiovascular events [158]. Thus, Sindler et al. found that nitrite reduced the elevated pulse wave velocity and levels of inflammatory cytokines IL-1b, IL6, INFγ and TNFα [150]. However, to what extent the reduction in PWV may have been due to a reduction in BP is not clear as the BP was not reported in this study.

Intimal hyperplasia

Intimal hyperplasia is also a pathological process in the development of atherosclerosis, associated with reduced NO bioavailability [159]. In a compelling series of experiments, Alef

et al. demonstrated that, following balloon injury of the rat carotid artery, sodium nitrite (whether through 24 h oral supplementation, or a single intraperitoneal injection, or via inhalation) markedly limited the development of intimal hyperplasia and inhibited smooth muscle cell proliferation, whereas a low nitrate/nitrite diet increased intimal hyperplasia. Even the late introduction of nitrite was able to reverse intimal hyperplasia [160]. Furthermore, the process was demonstrated to be dependent on xanthine oxidoreductase (XOR), which has previously been shown to reduce nitrite to NO in human vessels [125] in addition to animal vessels where it was upregulated following vascular injury. Here, XOR was protective in the presence of nitrite, through the generation of NO species as evidenced by S-nitrosothiols in the vessel wall. Chronic inhibition of XOR with allopurinol or 3 weeks of tungsten-rich diet increased intimal hyperplasia.

Ischaemia-reperfusion injury

Ischaemia-reperfusion injury is damage caused to tissues when blood supply returns or is restored after a period of ischaemia, which deprives the tissue of oxygen and nutrients. The restoring blood causes oxidative damage to the affected organ. Webb et al. demonstrated that infusions of sodium nitrite (10 and 100 μm) before and during an ischaemic insult in the isolated perfused rat heart (Langendorff preparation) resulted in the generation of nitric oxide with involvement of XOR and preserved left ventricular function and reduced infarct size compared with control saline [7]. This protective effect of nitrite in ischaemia-reperfusion injury has now been replicated by many other in vivo and in vitro studies showing protection in most organs tested such as the liver [161, 162], brain [163], heart [164] and kidneys [165] and reviewed by Dezfulian C et al. [166] and Webb & Ahluwalia [167]. Webb et al. translated this into a forearm model of ischaemia-reperfusion and demonstrated that nitrate supplementation in the form of beetroot juice attenuated endothelial function impairment following ischaemia-reperfusion injury during FMD of the brachial artery in healthy human subjects [79]. A similar effect was achieved with potassium nitrate [82].

Improving blood flow in hypoxic and ischaemic tissues

Given the capacity of dietary nitrate to result in vasodilatation, particularly of hypoxic or ischaemic vascular beds, Presley et al. studied the effect of a nitrate-rich diet on cerebral perfusion in older adults, aged ∼75 years using arterial spin labelling magnetic resonance imaging [168]. While there was no global effect, regional cerebral perfusion to the frontal white matter was enhanced, suggesting dietary nitrate may have the potential to enhance executive function and combat cognitive decline.

Plasma nitrite concentration has been shown to increase following exercise in young healthy individuals, but not in older individuals with impaired endothelial function due to impaired production and bioactivity of NO [169]. In patients with peripheral arterial disease (PAD), plasma nitrite falls during exercise. Therefore provision of dietary nitrate may be useful to supplement such deficiency in nitrite flux. Indeed, Kenjale et al. demonstrated that a single dose of 500 ml beetroot juice (9 mmol nitrate) increased both the time before onset of claudication by 18% and the total walking time by 17% in patients with PAD whilst maintaining elevated plasma nitrite concentrations, associated with a reduction in fractional O2 extraction by the gastrocnemius [170].

Exercise performance

A fundamental principle of exercise physiology was that during exercise, the oxygen cost is predictable for a given submaximal work rate [171-173]. However, when Larsen et al. tested the effects of 3 days of supplementation of sodium nitrate (0.1 mmol kg−1), as a potential source of NO, on exercise performance they surprisingly found that it resulted in reduction in oxygen consumption (of ∼0.16 l min−1) during submaximal exercise (between 45–80% of peak exercise) [174] with no effect on plasma lactate concentration, suggesting that exercise had become more efficient and the oxygen cost had been reduced. In a subsequent study, Larsen et al. found that nitrate supplementation (0.033 mmol kg−1 three times daily for 2 days) also reduced maximal oxygen consumption (VO2 max) by ∼0.10 l min−1 [175]. Bailey et al. found similar results with beetroot juice (∼11 mmol nitrate for 6 days), which reduced the increase in pulmonary oxygen uptake during moderate exercise by ∼19%, reduced the slow component of O2 uptake during severe exercise, and increased time to exhaustion [176].

Using 31P-MRS, Bailey et al. subsequently demonstrated that the ∼20% reduction in oxygen cost due to beetroot juice ingestion (5.1 mmol day−1 for 6 days) was due to a reduction in ATP cost of muscle force production (during knee-extension exercises) at both low and high intensity exercise [177]. There was also a 16% improvement in the time to task failure during severe exercise. Vanhatalo et al. extended the duration of observed beneficial effects of beetroot juice (nitrate 5.2 mmol day−1) on exercise performance and blood pressure lowering from acute effects at 2.5 h, to sustained effects over 15 days [88]. Using a nitrate-depleted beetroot juice as placebo, Lansley et al. demonstrated that it was the nitrate content of the active juice, rather than other components such as betaine or polyphenols, that was responsible for the improvements in the O2 cost of walking and moderate and severe intensity running and a 15% increase in time-to-exhaustion [178]. Other studies have demonstrated acute improvements in power output with nitrate during cycling (4 and 16.1 km) [179]. Also during exercise in hypoxia (with subjects breathing 14.5% O2), ingestion of 750 ml of beetroot juice over the preceding 24 h (9.3 mmol nitrate), resulted in reduced muscle metabolic perturbation and remarkably restored exercise tolerance and oxidative function to values observed in normoxia [180]. Most recently, supplementation with beetroot juice (∼8 mmol day−1) for 6 days has been found to reduce the time for trained cyclists to complete a 10 km time trial by ∼12 s, in a double-blind study compared with placebo (nitrate-depleted) beetroot juice [181]. The effects of dietary nitrate on exercise performance in human studies are summarized in Table 5.

Table 5. Effects of dietary nitrate on exercise performance in human studies, showing duration, amount of nitrate given in mmol (*0.1 mmol kg−1 day−1 or *0.033 mmol kg−1 3 times day−1 = ∼7 mmol day−1 in 70 kg person) and summary of effect

Study	Source	Nitrate (mmol d−1)	Duration	Effect on exercise
Larsen et al.	Sodium nitrate	∼7*	3 days	Reduction in O2 consumption (of ∼0.16 l min−1) during

Table 5. Effects of dietary nitrate on exercise performance in human studies, showing duration, amount of nitrate given in mmol (*0.1 mmol kg−1 day−1 or *0.033 mmol kg−1 3 times day−1 = ∼7 mmol day−1 in 70 kg person) and summary of effect

Study	Source	Nitrate (mmol d−1)	Duration	Effect on exercise
(2007) [174]	*0.1 mmol kg−1 day−1			submaximal exercise (between 45–80% of peak exercise) with no effect on plasma lactate concentration, suggesting that exercise had become more efficient and the O2 cost had been reduced
Bailey et al. (2009) [176]	Beetroot juice 500 ml day−1	∼11	6 days	Reduced the increase in pulmonary O2 uptake during moderate exercise by ∼19%, as well as reducing the slow component of O2 uptake during severe exercise, and increased time to exhaustion by ∼16%
Bailey et al. (2010) [177]	Beetroot juice 500 ml day−1	5.1	6 days	∼20% reduction in O2 cost due to a reduction in ATP cost of muscle force production (during knee-extension exercises) at both low and high intensity exercise, as well as ∼16% improvement in time to task failure during severe exercise
Larsen et al. (2010) [175]	Sodium nitrate *0.033 mmol kg−1 three times day−1	∼7*	2 days	Reduced maximal O2 consumption (VO2 max) by ∼0.10 l min−1
Vanhatalo et al.	Beetroot juice 500 ml day−1	5.2	15 days	Acute effects at 2.5 h, and sustained effects over 15 days observed with continued

Table 5. Effects of dietary nitrate on exercise performance in human studies, showing duration, amount of nitrate given in mmol (*0.1 mmol kg−1 day−1 or *0.033 mmol kg−1 3 times day−1 = ∼7 mmol day−1 in 70 kg person) and summary of effect

Study	Source	Nitrate (mmol d−1)	Duration	Effect on exercise
(2010) [88]				supplementation, demonstrating a reduction in steady state VO2 by ∼4% with elevations in peak power and work rate
Lansley et al. (2011) [178]	Beetroot juice 500 ml day−1	∼6.2	6 days	Improvements in the O2 cost of walking and moderate and severe intensity running and a 15% increase in time-to-exhaustion
Lansley et al. (2011) [179]	Beetroot juice 500 ml	∼6.2	Single dose	Acute improvements in power output have been demonstrated by ∼2.8% during cycling (4 and 16.1 km distances) for the same VO2
Vanhatalo et al. (2011) [180]	Beetroot juice 750 ml 24 h−1	9.3	250 ml: 24 h, 12 h & 2.5 h pre-	Exercise in hypoxia (subjects breathing 14.5% O2) restored exercise tolerance and oxidative function to values observed in normoxia
Kenjale et al. (2011) [170]	Beetroot juice 500 ml x1	∼9	Single dose	Increased time before onset of claudication by 18% and the total walking time by 17% in patients with peripheral arterial disease
Cermak et al. (2012) [181]	Beetroot juice shots (140 ml day−1)	∼8	6 days	Reduced time for trained cyclists to complete 10 km time trial by ∼12 s

A major advance in the mechanistic understanding of the reduced oxygen and ATP cost, came when Larsen et al. harvested skeletal muscle mitochondria and found that prior dietary nitrate supplementation improved oxidative phosphorylation efficiency (increased P : O ratio) indicating diversion of membrane potential away from uncoupling actions such as proton leak towards ATP synthesis [182]. Nitrate achieved this by reducing the expression of adenine nucleotide translocase (ANT), a site of proton leak. Additional effects include the nitrosation (-SNO) of complex I by nitrite and competition between NO and O2 with complex IV. Preservation of mitochondrial complex I activity, oxidative phosphorylation and attenuation of hydrogen peroxide generation and tissue lipid peroxidation may also be mechanisms by which dietary nitrate supplementation prevents doxorubicin-induced impairment of ventricular contractility and cell death, as recently demonstrated by Zhu et al. in a mouse model of doxorubicin-induced cardiomyopathy [183].

Pulmonary circulation

Nitrite also leads to dilatation in pulmonary vascular beds reducing pulmonary arterial pressure in humans and animals [184-186], and therefore has the potential to ameliorate pulmonary hypertension. Indeed, this is supported by a very recent study demonstrating a reduction in right ventricular pressure and hypertrophy, and pulmonary vascular remodelling with dietary nitrate treatment in mice exposed to 3 weeks of hypoxia, conditions associated with preferential reduction (of nitrite) to NO [187].

Interaction of nitrate-nitrite with other nutrients

The effects of dietary nitrate may be considerably enhanced or altered through interactions with other nutrients. For example, in addition to polyphenols in fruit and vegetables, Gago et al. found that red wine polyphenols [anthocyanin fraction and catechol (caffeic acid)] are very effective at converting nitrite to NO in vitro and in the human stomach [188]. Indeed, nitrite reductase activity has been associated with a broad range of dietary phenols (greatest to least activity): epicatechin-3-O-gallate, quercetin, procyanidin B8 dimer, oleuropein, procyanidin B2 dimer, chlorogenic acid, epicatechin, catechin, procyanidin B5 dimer [189]. Hawthorn berry extract has been found to have extremely potent nitrite reductase activity, in addition to containing polyphenols (∼5%) and Zand et al. recently performed a placebo-controlled study of 30 days twice daily supplementation with a formulation containing sodium nitrite, hawthorn berry extract, vitamin C, beetroot powder, vitamin B12 and L-citrulline in patients with three or more cardiovascular risk factors and found that it reduced triglycerides in patients with elevated triglycerides (>150 mg dl–1) at baseline. No significant reduction in blood pressure was seen however [190]. In addition, in wine, ethanol is nitrosated resulting in the formation of ethylnitrite, an organic nitrite, which has been shown to be a potent vasodilator [191]. Indeed, Moya et al. have previously demonstrated that inhaled ethyl nitrite results in sustained improvements in arterial oxygenation and haemodynamics in persistent pulmonary hypertension of the newborn [192].

Unsaturated fatty acids in the diet, such as linoleic and oleic acid are nitrated by nitrous acid, derived from nitrite in the acid environment of the stomach, forming nitro-fatty acids (NO2-FAs) [193]. NO2-FAs have several anti-inflammatory actions, inhibiting neutrophils, platelets and macrophages. Signalling pathways are mediated through S-alkylation of, for example,

nuclear factor κ B (resulting in inhibition of macrophage cytokine and iNOS expression) [194] and peroxisome proliferator-activated receptor-γ (PPAR-γ) [195]. Rudolph et al. demonstrated that subcutaneous injection of nitro-oleic acid markedly reduced atherosclerotic lesion formation in apoliproprotein E-deficient mice associated with a variety of anti-inflammatory effects including reduction in foam cell formation through attenuation of oxidized LDL-induced phosphorylation of signal transducer and activator of transcription-1 (STAT-1) [139]. The oral administration of nitro-oleic acid has also been shown to be effective in suppressing inflammation in experimental inflammatory bowel disease [196]. Also, Kelley et al., have found that nitro-oleic acid inhibits XOR, and is surprisingly more potent than allopurinol in terms of inhibition of superoxide production [197] and may be part of the mechanism by which nitro-oleic acid confers protection in a mouse model of renal ischaemia-reperfusion [198].

The study by Bondonno et al. examined the interaction of apple skin rich in flavonoids, quercetin and (−)-epicatechin and spinach as a source of dietary nitrate. Whilst apple and spinach reduced SBP (by ∼−3.3 mmHg and ∼−2.7 mmHg after 2 h respectively) compared with control, the combination of apple and spinach had no effect and resulted in an intermediate plasma nitrite concentration suggesting possible extravascular (e.g. stomach) reduction of nitrite by the apple flavonoids and ascorbic acid, as intravascular reduction might be expected to enhance BP reduction [89].

Cancer risk

Nitrate and nitrite have been used for curing meat for centuries, and remain the most effective method to reduce bacterial growth and kill botulinum spores. Major concern emerged in the 1960s [199], with the demonstration of carcinogenic dimethylnitrosamine formation (known to disrupt nucleic acids in the rat and cause liver tumours [200]) from sodium nitrite [201, 202]. However, chronic feeding of nitrite to rats, even when diethylamine was given at the same time, did not induce tumours [203]. Concern for dietary nitrate arose in 1976, when Spiegelhalder et al. [116] and Tannenbaum et al. [115], both independently suggested that conversion to nitrite in the enterosalivary circulation, could result in the formation of N-nitrosoamines. Whilst N-nitroso compounds have repeatedly been shown to be carcinogenic in animals [200, 204], reviews such as the 2003 Joint FAO/WHO Expert Committee on Food Additives (JEFCA) of the epidemiological and toxicological studies in humans have failed to establish a definite link between nitrate intake and risk of developing cancer [205-207]. However, as with most substances, some groups of individuals are likely to be more susceptible than others. For example, work from McColl's group has elegantly demonstrated that patients with Barrett's oesophagus have increased nitrosation at the gastro-oesophageal junction, the major site of adenocarcinoma of the human upper gastrointestinal tract [208].

The World Cancer Research Fund/American Institute of Cancer Research, Second Report 2007, did not show any increased risk of cancers, such as stomach or ovarian, with green leafy vegetables, and even showed trends towards beneficial effects [209]. Indeed, the recommendation from the report was to eat at least five portions/servings (at least 400 g) of a variety of non-starchy vegetables and fruits every day, specifically including green, leafy vegetables. Some epidemiological studies have shown that fruit and vegetables have protective effects against certain cancers [210]. Indeed, there may be other components in

fruit and vegetables which protect against damaging effects of nitrite such as ascorbate [211-213], vitamin E, phenolic compounds and fruit and vegetable juices [204, 214-217], although some of these interactions appear complex [212, 218, 219].

The National Institutes of Health-American Association of Retired Persons (NIH-AARP) Diet and Health Study prospectively studied large cohorts and have recently identified some potential associations between high nitrate intake, assessed using a 124-item food frequency questionnaire, and epithelial ovarian cancer [220] and thyroid cancer (nitrate competitively inhibits iodide uptake) [221]. However, no associations have been demonstrated between total nitrate or nitrite intake (processed meat) and pancreatic cancer [222], adenocarcinoma of the stomach and oesophagus [223], non-Hodgkin lymphoma risk overall [224], renal cell carcinoma [225] or bladder cancer. However, most of the associations are small, do not prove causality and should be interpreted with extreme caution. The limitations of extrapolating from epidemiological studies has been recently reviewed by Milkowski et al. [32]. Thus, the lack of data concerning the long term effects of dietary nitrate is a limitation, and this will need to be addressed in future trials.

Potential anti-cancer effects of beetroot

Quite independently of the nitrate/nitrite field, several in vitro and in vivo studies have demonstrated that red beet/beetroot extract has protective effects in various cancer cell lines, such as prostate and breast, liver, lung, oesophagus and skin [226-229]. These effects of beetroot (juice) have generally been ascribed to betanin, the major betacyanin constituent, which has strong antioxidant activity, and is particularly high in betalain extracts obtained from hairy root cultures of the red beetroot B. vulgaris [230]. Beetroot may represent a particularly safe source of dietary nitrate, with the potential to reduce, rather than increase cancer risk. Indeed, beetroot juice has even achieved a considerable degree of acceptance as an alternative medicine for cancer patients [231, 232]. However, the mechanisms require further clarification.

Dietary nitrate has important vascular effects mediated via the nitrate-nitrite-NO pathway. Whilst these effects are promising and suggestive that dietary nitrate underlies the apparent beneficial effects of vegetable rich diets, such as the Mediterranean and Japanese diets, they represent surrogate endpoints and further long term studies with hard endpoints will eventually be required to substantiate the beneficial effects and demonstrate that they outweigh any risk of cancer. Until such data are available, the quantity of nitrate consumed is largely the decision of the individual, depending on their requirement (which may be different in patients with hypertension, healthy individuals or athletes). Since health professionals may be called upon to give advice, and to guide individuals, we provide practical advice in the form of summary tables of nitrate content, ADIs for given weights, comparisons of the body's handling of nitrate and nitrite, and studies showing efficacy in exercise, and on blood pressure in healthy individuals. The results of studies in patients with hypertension are awaited.

All authors have completed the Unified Competing Interest form at http://www.icmje.org/coi_disclosure.pdf Vascular effects of dietary nitrate (available on request from the corresponding author) and declare no support from any organization for the

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