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NO-synthase independent NO generation in mammals

Jon O. Lundberg *, Eddie Weitzberg

Department of Physiology and Pharmacology, Karolinska Institutet, S-171 77 Stockholm, Sweden

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ABSTRACT

Inorganic nitrate (NO_3^-) and nitrite (NO_2^-) are part of the nitrogen cycle in nature. To the general public these anions are generally known as undesired residues in the food chain with potentially carcinogenic effects. Among biologists, these inorganic anions have merely been viewed as inert oxidative end products of endogenous nitric oxide (NO) metabolism. However, recent studies surprisingly show that nitrate and nitrite can be metabolized in vivo to form nitric oxide (NO) and other bioactive nitrogen oxides. This represents an important alternative source of NO especially during hypoxia when the oxygen-dependent L-arginine–NO pathway can be altered. A picture is now emerging suggesting important biological functions of the nitrate–nitrite–NO pathway with profound implications in relation to the diet and cardiovascular homeostasis. Moreover, an increasing number of studies suggest a therapeutic potential for nitrate and nitrite in diseases such as myocardial infarction, stroke, hypertension, renal failure and gastric ulcers.

1. Introduction

Nitrogen (N_2) is the most abundant element in the atmosphere. In the Nitrogen cycle atmospheric nitrogen is fixed into forms usable by living organisms. Bacteria oxidize the ammonia (NH_4^+) formed by N_2 fixation to nitrite (NO_2^-) and nitrate (NO_3^-), in a process termed nitrification. The nitrogen cycle is completed by the denitrification process, wherein nitrate is converted back to N_2 in a series of reductions catalyzed by anaerobic bacteria. In this pathway nitric oxide (NO) is an intermediate [1].

While bacteria generate NO by reduction under anaerobic conditions, mammals instead utilize oxidation reactions to produce this gas. Mammalian NO-synthases catalyze an oxygen-dependent five-electron oxidation of the amino acid L-arginine to form NO and L-citrulline. Extensive research during the past two decades has established NO as a critical regulator of vascular homeostasis, neurotransmission, redox signaling, cell respiration, and host defence [2].

Until recently, it was thought that the NO-synthases were exclusively responsible for the formation of NO in mammals. This view has now changed.

Here we discuss a previously unknown mammalian nitrogen cycle, in which the inorganic anions nitrate and nitrite are converted back to NO and other bioactive nitrogen oxides in blood and tissues. Interestingly, commensal bacteria play a central role in the first reductive step converting nitrate to nitrite, and then a number of mammalian enzymes and proteins participate in the formation of NO from nitrite. This nitrate–nitrite–NO pathway has attracted substantial scientific interest and its role in physiological processes, therapy and nutrition is currently under investigation.

2. Sources of nitrate and nitrite

The nitrate and nitrite that we are exposed to derive from endogenous as well as dietary sources (Fig. 1). The main dietary source of nitrate is vegetables, which typically account for up to 90% of the daily nitrate intake. Green leafy vegetables such as lettuce and spinach are particularly rich in nitrate. Nitrite is also found in some foodstuffs — for example, it is used as a food additive in meat to prevent the growth of *Clostridium botulinus* and to enhance its taste and appearance. The principal endogenous source of nitrate and nitrite in mammals is the L-arginine–NO pathway, which is constitutively active in numerous cell types throughout the body. NO is rapidly oxidized in blood and tissues to form nitrate and nitrite [2]. In blood the reaction of NO with oxyhemoglobin produces mainly nitrate and methemoglobin [2].

The levels of nitrate in plasma are typically in the 20–40 μM range while nitrite levels are substantially lower (50–300 nM) [3–6]. Regular exercise increases eNOS expression and activity [7] which results in higher circulating levels of nitrate [7–9]. In systemic inflammatory disorders such as sepsis and severe gastroenteritis, the levels of nitrate and nitrite are greatly increased due to massive iNOS induction [2,10,11]. In contrast, in diseases with

* Corresponding author. Fax: +46 8332278.
E-mail addresses: jon.lundberg@ki.se (J.O. Lundberg), eddie.weitzberg@ki.se (E. Weitzberg).

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endothelial dysfunction and reduced eNOS activity, as well as in eNOS deficient mice, plasma levels of nitrate and nitrite are typically lower [12,13].

The dietary contribution to systemic nitrate levels can be profound. As an example, a serving of a green leafy vegetable contains more nitrate [1] than what is formed endogenously over a day by all three NO-synthase isoforms [14].

3. The enterosalivary circulation of nitrate

After ingestion, nitrate is rapidly and effectively absorbed proximally from the gastrointestinal tract into the bloodstream, where it mixes with endogenously synthesized nitrate. Peak plasma concentrations are seen within 60 min of nitrate ingestion and the half-life of nitrate in plasma is 5–6 h. For as-yet-unknown reasons, the concentrations of nitrate excreted in saliva are exceptionally high; up to 25% of plasma nitrate is actively taken up by the salivary glands and secreted with saliva [15], and the resulting salivary nitrate concentrations are 10–20 times higher than the levels in plasma [6]. In the oral cavity commensal facultative bacteria convert nitrate to nitrite using specific nitrate reductase enzymes. These bacteria use nitrate as the terminal electron acceptor for ATP generation when oxygen supply is scarce. Bacterial nitrate reduction is very effective and salivary nitrite commonly exceeds 1 mM after a nitrate rich meal, which is more than four orders of magnitude higher than the levels found in plasma. Swallowed salivary nitrite then undergoes a variety of biologically relevant reactions in the acidic stomach as explained below.

4. NO-synthase independent generation of nitric oxide

NO-synthase independent generation of nitric oxide was first described in the stomach [16,17]. In 1994, two independent groups could show that NO and other reactive nitrogen oxides were generated non-enzymatically in large amounts following protonation of nitrite in swallowed saliva. Human stomach NO production was highly pH-dependent and could be almost abolished by proton pump inhibitors. The levels of NO in the stomach were in the range 10–100 ppm, i.e., several orders of magnitude higher than those required for vasodilation. At these high concentrations, NO and its reaction products are toxic to a variety of microorganisms which suggested a role for gastric NO in host defence. Benjamin and colleagues exposed enteropathogens to different combinations of acid and nitrite and *Escherichia coli* and *Candida albicans* species were remarkably resistant when exposed to acid alone but were killed if nitrite was added [17]. Subsequent studies have shown that acidified nitrite, as well as a combination of authentic human gastric juice and saliva, inhibit the growth of a variety of enteropathogens including *Salmonella*, *Shigella*, and *Helicobacter pylori* [18–20]. Besides NO, a variety of other reactive nitrogen intermediates (RNIs) are generated from nitrite under acidic conditions (e.g., HNO_2, N_2O_3, and NO_2) [1], and it is likely that several of these contribute to the antibacterial effects. The chemistry of acidified nitrite is complex and the amount of NO generated is dependent not only on pH and nitrite concentrations but also on the presence of other reducing agents (e.g., vitamin C, thiocyanate, polyphenols), proximity to heme groups, proteins, thiols, and the oxygen tension [21]. In aggregate, there are solid indications for a role of salivary nitrite in primary host defence against swallowed pathogens.

With very high levels of NO generated in the gastric lumen, it is plausible that NO can also affect the host mucosa. Using the rat gastric mucosa as a bioassay, Björne and colleagues studied the effects of human nitrite-containing saliva on gastric mucosal blood flow and mucus generation in vivo [22]. When human fasting saliva (low in nitrite) was placed onto the mucosa, no changes in blood flow

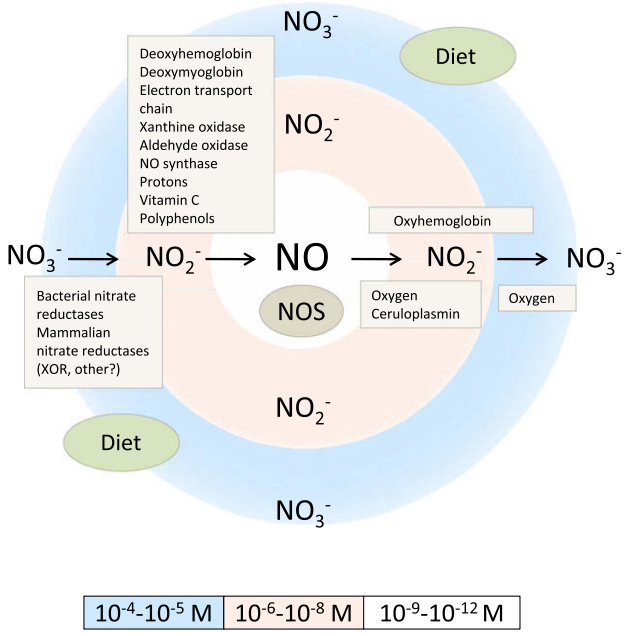

Fig. 1. Nitric oxide (NO) is generated by NO-synthases (NOS) and exerts various physiological functions throughout the body. The half-life of NO in biological fluids is extremely short due to rapid oxidation to nitrite (NO_2^-) and nitrate (NO_3^-). In our bodies nitrate can undergo reduction back to nitrite mainly by commensal bacteria in the oral cavity and to a lesser extent also by mammalian enzymes. In blood and tissues several pathways exist with the capacity to further metabolize nitrite to NO and other biologically active nitrogen oxides. Most of these pathways are greatly accelerated under hypoxic conditions. Nitrite reduction represents an alternative to the classical NOS pathway for the generation of NO in blood and tissues. Our diet (mainly green leafy vegetables) is a major contributor to the body pool of nitrate and ingestion of nitrate may fuel the nitrate–nitrite–NO pathway. Approximate concentration ranges of nitrate, nitrite, and NO in blood and tissues are presented.

and mucus generation were noted. In contrast, blood flow and mucus increased greatly when using nitrite-rich saliva, collected after oral ingestion of nitrate. Also, feeding rats with nitrate in the drinking water for one week, leads to a sustained increase in gastric mucosal blood flow and a thicker mucus layer [23]. These effects of nitrite are cGMP dependent and associated with formation of NO gas. They are also independent of NO-synthase and cyclooxygenase activity as evident from experiments using L-NAME and indomethazine [22]. Because an adequate blood flow and mucus generation is essential for maintaining gastric integrity, it is of interest to study effects of nitrate and nitrite in models of gastric injury. Indeed, several studies have found a potent protective effect of dietary nitrate in animal models of gastric ulcers [24–26]. Jansson and colleagues found that a 7-day pre-treatment with nitrate in the drinking water protected rats against ulcers induced by a non-steroidal anti-inflammatory drug (NSAID) [24]. Sobko and colleagues showed that in contrast to normal conventional rats, gastric NO levels are extremely low in germ-free rats and do not increase after ingestion of nitrate [27]. This demonstrates the central role of bacteria in bioactivation of salivary nitrate to nitrite.

In critically ill patients, endotracheal intubation and sedation interrupt the enterosalivary nitrate cycle which results in depleted gastric NO, nitrite, and *S*-nitrosothiol levels [28]. It has been hypothesized that the insufficient levels of gastric NO contribute to the gastric lesions and bacterial overgrowth commonly found in these patients [28].

In aggregate, when nitrite-rich saliva meets gastric acid a variety of biologically active nitrogen oxides are rapidly formed. The enterosalivary circulation of nitrate and its reduction to nitrite in the mouth by commensal bacteria seems to represent a beautiful

example of symbiosis. From the host the oral bacteria receive nitrate, an electron acceptor necessary for their respiration, and in return they supply the host with nitrite, a substrate needed for the generation of gastroprotective nitrogen oxides.

5. Systemic NO generation from nitrite

Soon after the discovery of gastric NOS-independent NO generation, Zweier et al. demonstrated profound NO generation in rat ischemic heart muscle which could not be effectively blocked by pharmacological NOS inhibitors [29]. They then demonstrated that N^{15}-labeled nitrite was reduced to NO. During global cardiac ischemia tissue pH fell below 6 and under these conditions reduction of nitrite to NO was greatly enhanced. Subsequent studies have demonstrated numerous pathways for nitrite reduction in vivo, many of which involve enzymes and heme-proteins (see below). Almost without exception nitrite reduction is greatly accelerated under hypoxic conditions, a situation when the oxygen-dependent NOS pathway may be malfunctioning [30]. Thus, the nitrite–NO pathway might be viewed as a backup system, to ensure sufficient NO generation along the entire physiological oxygen gradient. The exact mechanism and location for nitrite reduction in blood and tissues and its physiological role in regulation of cellular processes have not yet been pinpointed, but it is clear from animal experiments that administration of nitrite (or its precursor nitrate) has a therapeutic potential, especially in ischemia–reperfusion injury (Table 1).

6. Mechanisms of nitrite reduction

Clearly, a variety of proteins and enzymes can catalyze nitrite reduction to NO in blood and tissues (Fig. 1). In addition to this, nitrite reduction may be further enhanced by reducing agents such as vitamin C [31], polyphenols [32], and thiocyanate [33]. In blood deoxyhemoglobin has been shown to be an allosterically regulated nitrite reductase [10,34,35] and in cardiac muscle deoxymyoglobin can act as a nitrite reductase [36,37]. Xanthine oxidase (XO) is structurally related to bacterial nitrate and nitrite reductases and early studies have indeed shown that this enzyme can reduce both nitrate and nitrite [38]. More recently, it has been shown that NO is formed by XO-catalyzed reduction of nitrite [39,40–42]. Recently, it was shown that another mammalian molybdenum-containing enzyme, aldehyde oxidase is capable of reducing nitrite to NO [43]. Components of the mitochondrial respiratory chain in mitochondria are theoretically well suited for NO generation from nitrite as they could act as electron carriers when nitrite is reduced. Indeed, several studies have now convincingly demonstrated that respiring mitochondria in mammalian cells [44–46] and in plants [47] can generate NO from nitrite. The magnitude of NO generated from nitrite by mitochondrial enzymes in vivo and its physiological or pathophysiological role needs to be clarified. The high affinity of NO to the heme–iron of cytochrome oxidase might suggest a detrimental role since NO has been shown to impede the energy-linked respiration and to trigger mitochondrial generation of superoxide radicals [45,48–51]. On the other hand beneficial effects of NO on mitochondrial function have also been described. For example, NO stimulates mitochondrial biogenesis, both in vitro and in vivo, which results in increased mitochondrial function and enhanced ATP formation [50–52]. In addition, several of the tissue-protecting effects of nitrite in IR injury seem to be mitochondria targeted.

7. Vasodilatory effects of nitrite

The vasodilating properties of inorganic nitrite have been known for long. Sodium and potassium nitrite were used in the beginning of the 20th century as antihypertensives and Robert Furchgott used acidified sodium nitrite to relax preconstricted rabbit aortic rings as early as 1953 [53]. However in these early experiments the nitrite concentrations and the acidity used were far outside physiological levels and the mechanism of dilatation (NO generation) was unknown. More recently, Modin et al. showed that physiological levels of nitrite relaxed rat aortic rings when the acidity of the buffer solution was adjusted to just below pH 7, which is commonly seen in tissues during ischemia [54]. This relaxation was blocked by an inhibitor of soluble guanylyl cyclase (ODQ) and paralleled by NO generation in head space gas supporting that NO was mediating the effects. Interestingly, NO generation from nitrite and relaxation were further increased by vitamin C [54]. At the same time Gladwin et al. had noted arterio-venous gradients of nitrite in blood, indicating nitrite consumption along the vascular tree [5]. They then suggested an active role for nitrite in vasoregulation and later they went onto show that a low dose of nitrite dilated the human circulation when infused intra-arterially [34]. The mechanism for nitrite reduction in blood has been the matter of extensive research and is discussed in detail in several recent reviews [30,55–58].

8. Therapeutic effects of nitrite in ischemia–reperfusion injury

As discussed above the pathways for systemic nitrite reduction are greatly enhanced during hypoxic/ischemic conditions. Several studies in animal models of ischemia and reperfusion indicate that nitrite and nitrate can modulate hypoxic signaling. Administration of nitrite as well as nitrate protect against ischemia–reperfusion injury in liver [59,60], heart [59,61–63], brain [64], kidney [65], and chronic hind-limb ischemia [66] (Table 1).

The mechanism of nitrite-mediated cytoprotection is not fully elucidated but appears to be NO dependent and at least partly mitochondria targeted. A loss of cytoprotection has been demonstrated in most studies when treating the animals with the NO scavenger carboxy-PTIO [59,60,62,64] supporting a role of NO in the protective effects. Cytoprotection is not prevented by NO-synthase inhibition [59,62] or affected in eNOS knockout mice [59], demonstrating the independence of NO-synthases. Several different pathways may be responsible for NO formation from nitrite during hypoxia, probably depending on which tissue is involved and the severity of hypoxia. The involvement of xanthine oxidase in the reduction of nitrite to NO in the heart has been suggested on the basis of reduced efficacy after treatment with allopurinol [62,63,65]. Nitrite remains protective in isolated buffer perfused organs suggesting that the hemoglobin pathway is not necessary for this function. In the heart, deoxymyoglobin can serve as a nitrite reductase and it was recently shown that the protective effect of nitrite in a myocardial infarction model was lost in myoglobin knockout mice [36,67]. These findings suggest a therapeutic potential for nitrite and nitrate in human conditions associated with ischemia–reperfusion, such as myocardial infarction, stroke, and solid organ transplantation. Interestingly, the cytoprotective effects of nitrate and nitrite are accompanied by very modest increases in plasma nitrite. Similar or even greater increases in plasma nitrite levels are seen after ingesting a portion of a green leafy vegetable [6]; this evokes provocative questions about a putative role of nitrate as an active ingredient of the cardioprotective Mediterranean diet [1,68–70].

The vasodilatory effects of nitrite-derived NO may be partly responsible for the protective effects in conditions of ischemia–reperfusion [54,71] but more attention has been given to the mitochondria. It was recently demonstrated that nitrite can nitrosate complex I during ischemia and reperfusion [67]. This modification limits complex I-dependent reactive oxygen species (ROS) forma-

Table 1
The therapeutic effects of inorganic nitrite and nitrate have been evaluated in a number of disease models.

Intervention	Organ	Disease model	Species	Effect
Sodium nitrite	Heart	I/R Injury	Rat, mouse, dog	Infarct size ↓
	Heart	Transplantation	Rat	Allograft rejection ↓
	Heart	Cardiac arrest	Mouse	Cardiac function ↑ Neurological function ↑ Survival ↑
	Lungs	Pulmonary hypertension	Rat, mouse lamb	Pulmonary artery pressure ↓
	Liver	I/R Injury	Mouse	Hepatocellular injury ↓ and apoptosis ↓
	Kidney	I/R Injury	Rat, mouse	Renal dysfunction ↓ and renal injury ↓
	Kidney	Chronic NOS inhibition	Rat	Kidney damage ↓ Hypertension ↓
	Brain	I/R Injury	Rat	Infarct size ↓→
	Brain	Subarachnoid hemorrhage	Monkey	Cerebral vasospasm ↓
	Vasculature	Atherosclerosis	Mouse	Microvascular inflammation ↓ Endothelial dysfunction ↓
	Vasculature	Chronic limb ischemia	Mouse	Blood flow, angiogenesis, arteriogenesis↑
Sodium nitrate	Heart	I/R Injury	Mouse	Infarct size ↓
	Stomach	Gastric ulcer	Rat	Ulcer formation ↓
	Vasculature	Aortic clamping	Rat	Post-ischemic blood flow ↑

tion during reperfusion as well as activation of the mitochondrial permeability transition pore and cytochrome *C* release. As mentioned above, the mitochondrial respiratory chain enzymes can reduce nitrite to NO [44,72,73] which may contribute to the cytoprotective effects. In aggregate, the interaction of NO and nitrite with mitochondrial respiration and production of ROS is complex and further studies will clarify these processes in conditions of ischemia–reperfusion.

A number of studies indicate a protective effect of NO gas inhalation in conditions of ischemia–reperfusion both in animal models and in humans [74–78]. This suggests a transformation of NO into a more long-lived bioactive NO-species that can be transported in blood to extrapulmonary targets. The effects of inhaled NO were associated with significant increases in circulating nitrite which was suggested to be one responsible mediator.

In summary, the promising animal data discussed here indicate that nitrite possesses the characteristics of a useful adjunctive therapy for acute myocardial infarction, stroke and other conditions of ischemia–reperfusion injury.

9. Bioactivation of inorganic nitrate

As discussed above there are several pathways for the systemic conversion of nitrite to NO and other bioactive nitrogen oxides. While nitrite circulates at nanomolar levels, the levels of nitrate are about 20–40 μM, i.e., 100 times higher [79]. So in theory nitrate would be an even greater circulating pool of potential NO bioactivity than nitrite, provided that mechanisms existed for its reduction. The existence of an in vivo reduction of nitrate is clear from a study by Lundberg and Govoni [6] in which a fourfold sustained increase in plasma nitrite in healthy volunteers was demonstrated after ingestion of nitrate. This increase required enterosalivary circulation of nitrate since it could be completely prevented if the test subjects avoided swallowing after ingestion of the nitrate. Thus, commensal nitrate-reducing bacteria in the oral cavity contribute not only to gastric NO generation but also to the systemic delivery of nitrite. Interestingly, even without any ingestion of nitrate or nitrite whatsoever, the contribution from saliva to systemic levels of nitrite will still be considerable since nitrate is constantly generated endogenously from the L-arginine–NO pathway.

Clearly bacterial enzymes are highly effective in catalyzing nitrate reduction. Although there are anecdotal early reports on some nitrate reduction also by mammalian cells [80], the universal belief has been that mammalian enzymes cannot metabolize this stable anion. However, a recent study by Jansson et al. refutes this notion [81]. They noted substantial nitrate reduction in germ-free mice that were given nitrate systemically. In vitro experiments revealed that xanthine oxidase catalyzed nitrate reduction in various rodent and human tissues but the existence of alternative yet uncharacterized pathways were also suggested. Some nitrate reduction from purified XOR had been described earlier under strict anaerobic conditions [38,41,82], but the present study showed that this occurred in vivo and also under normoxia [81]. The existence of a mammalian nitrate reductase is very interesting although its role in normal regulation of nitrite and NO homeostasis remains to be elucidated. At present it seems as if most nitrite generated acutely in humans after a nitrate load, is derived from the bacterial pathway described above. Thus, a marked attenuation of the nitrate-induced plasma nitrite peak was recently noted in subjects that rinsed their mouth with an antibacterial mouthwash immediately prior to nitrate ingestion [83]. In addition, in a recent study in rats it was shown that the acute gastroprotective and blood pressure-lowering effects (see below) of dietary nitrate were abolished, if the animals were treated with an antibacterial mouthwash [84].

10. Inorganic nitrate and the cardiovascular system

Larsen and colleagues recently demonstrated a reduction in blood pressure in healthy volunteers after three days treatment with inorganic nitrate [85], and the year after they showed that dietary nitrate decreases whole body oxygen consumption in humans during submaximal exercise [86]. This latter finding was recently confirmed [87,88] but the mechanism is still to be elucidated. In a recent study, Webb and colleagues showed that blood pressure decreases if healthy volunteers ingest a natural nitrate source (beetroot juice). They could convincingly demonstrate that it was the nitrate in the juice that had the effect and it occurred via reduction to nitrite [89]. In addition, the dietary nitrate load also prevented endothelial dysfunction induced by an acute ischemic insult in the human forearm and significantly attenuated ex vivo platelet aggregation in response to collagen and ADP. In addition to this, Bryan et al. could recently show that dietary supplementation with nitrate also protects against myocardial infarction in a mouse model [90]. Kanematsu and colleagues studied chronic effects of dietary nitrite supplementation in rats treated with the NOS-inhibitor L-NAME [91]. Remarkably, a low dose of nitrite attenuated the L-NAME-induced increase in blood pressure and completely prevented the renal damage caused by chronic NOS inhibition. Thus it is clear that dietary nitrate is bioactivated

in vivo to form a compound with NO-like bioactivity and it seems as if the nitrate–nitrite–NO pathway can partly compensate for losses in endogenous NO generation by the NOSs. Although nitrite seems to be an obligate intermediate in bioactivation of inorganic nitrate, the exact mechanism needs to be settled. We still do not know exactly where and how nitrite is converted to vasodilatory NO, and if this occurs via intermediate formation of *S*-nitrosothiols or other nitrogen oxides.

11. Nutritional aspects of inorganic nitrate

Hypertension affects approximately 1 billion individuals worldwide and remains the most common risk factor for cardiovascular morbidity and mortality [92]. A diet rich in fruits and vegetables is associated with a lower blood pressure and reduced risk of cardiovascular events. Yet, despite extensive research the active ingredient(s) responsible for these effects has not been pinpointed and trials with single nutrients have been largely unsuccessful. Remarkably, in the aforementioned study by Larsen and colleagues [85] the blood pressure-lowering effect of dietary nitrate supplementation was similar to that seen in the healthy control group in the DASH project, a classical vegetable/fruit diet trial [93], indicating that nitrate could be an important active ingredient of this diet. It should be noted that the dose of nitrate used in this study (0.1 mmol/kg/day) is readily achievable through a diet rich in vegetables. Moreover, in the Webb study mentioned above, 0.5 l of fresh beetroot juice decreased systolic blood pressure as much as 10 mm Hg, and blood pressure was still significantly reduced when measured 24 h later [89]. If these results can be reproduced in clinical trials in hypertensives, and if we can show that the effect is sustained over time, it will open the door to entirely new strategies for prevention and treatment of this disorder. It is not unlikely that dietary nitrate may affect also other cardiovascular disorders, including atherosclerosis and ischemic heart disease, conditions in which a decreased NO bioavailability is thought to be central.

In summary, it is ironic that of all the natural constituents of vegetables, it is only one that is believed to have detrimental health effects – the nitrate anion. Yet, this particular nutrient is now emerging as a prime candidate for mediating cardioprotective effects of a vegetable rich diet.

12. Conclusions

In recent years, we have witnessed a substantial increase in our understanding of the mammalian nitrate–nitrite–NO pathway. This pathway may now be considered as an important alternative provider of NO besides the "classical" L-arginine–NO pathway. An important feature of the nitrate–nitrite–NO pathway is the augmentation by hypoxia which ensures NO at a wide range of oxygen levels. The redundancy in pathways that convert nitrite to NO indicates its importance in regulating physiological processes. Nevertheless, there are a number of outstanding issues that need to be resolved by future research. The true physiological role of nitrate and nitrite clearly needs to be determined. This is however a challenging task, since unlike the NOS-dependent physiology, which has been largely explored by the use of NOS inhibitors, there are no nitrite reductase inhibitors available. Moreover, the dual dietary and endogenous origin of nitrate and nitrite creates a substantial problem in experimental design. Another important area of research is to translate the promising therapeutic effects of nitrate and nitrite demonstrated in animal models into the clinical arena. Finally, the novel nutritional aspects of nitrate and nitrite need to be considered and taken into account when discussing safe levels of these anions in our diet.

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