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# Nitrate-Nitrite-Nitric Oxide Pathway

## Implications for Anesthesiology and Intensive Care

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### ABSTRACT

The gaseous radical nitric oxide is involved in numerous physiologic and pathophysiological events important in anesthesiology and intensive care. Nitric oxide is endogenously generated from the amino acid L-arginine and molecular oxygen in reactions catalyzed by complex nitric oxide synthases. Recently, an alternative pathway for nitric oxide generation was discovered, wherein the inorganic anions nitrate ( $\text{NO}_3^-$ ) and nitrite ( $\text{NO}_2^-$ ), most often considered inert end products from nitric oxide generation, can be reduced back to nitric oxide and other bioactive nitrogen oxide species. This nitrate-nitrite-nitric oxide pathway is regulated differently than the classic L-arginine-nitric oxide synthase nitric oxide pathway, and it is greatly enhanced during hypoxia and acidosis. Several lines of research now indicate that the nitrate-nitrite-nitric oxide pathway is involved in regulation of blood flow, cell metabolism, and signaling, as well as in tissue protection during hypoxia. The fact that nitrate is abundant in our diet gives rise to interesting nutritional as-

pects in health and disease. In this article, we present an overview of this field of research with emphasis on relevance in anesthesiology and intensive care.

NITROGEN (N) is a key component of DNA, RNA, and proteins, which makes it essential for all living organisms. In the form of nitrogen gas ( $\text{N}_2$ ), it is the most abundant element in the atmosphere, and thereby the largest pool of nitrogen on Earth. However, atmospheric nitrogen would be of no biologic use if not for the nitrogen cycle. As a first step in this cycle, atmospheric nitrogen undergoes fixation, a process in which nitrogen gas is converted to ammonium ( $\text{NH}_4^+$ ). Ammonium can then be oxidized to a variety of nitrogen oxides, including nitrite ( $\text{NO}_2^-$ ) and nitrate ( $\text{NO}_3^-$ ). The cycle is completed by the denitrification process where nitrate is serially reduced to nitrite, nitric oxide, nitrous oxide, and, finally, nitrogen gas ( $\text{N}_2$ ), which diffuses back into the atmosphere. Bacteria play an essential role in the nitrogen cycle because they are equipped with metabolic machineries suitable for catalyzing its different steps. In the anaerobic denitrification part of the nitrogen cycle, nitrate, nitrite, and nitric oxide are substrates for specific bacterial reductases, and the bacteria use these nitrogen oxides as terminal electron acceptors for respiration or for incorporation in biomass. The description of the nitrogen cycle serves as a relevant prologue to this review because some steps in this cycle also occur in mammals, where again, bacteria play a crucial role.

The formation of nitrogen oxides by prokaryotes has been known for more than a century, but it is only during the last decades that it has become clear that generation and metabolism of nitrogen oxides also occur in eukaryotic cells. In 1916, Mitchell *et al.* observed that humans excrete more nitrate than they ingest, but at that time, they could only speculate on the mechanisms.<sup>1</sup> In 1981, Green *et al.* used completely germ-free animals to demonstrate a net production of nitrate, independent of bacteria, as solid evidence of mammalian nitrate biosynthesis.<sup>2</sup> At approximately the same time, a series of seminal studies were published, eventually leading to the identification of nitric oxide as a major secre-

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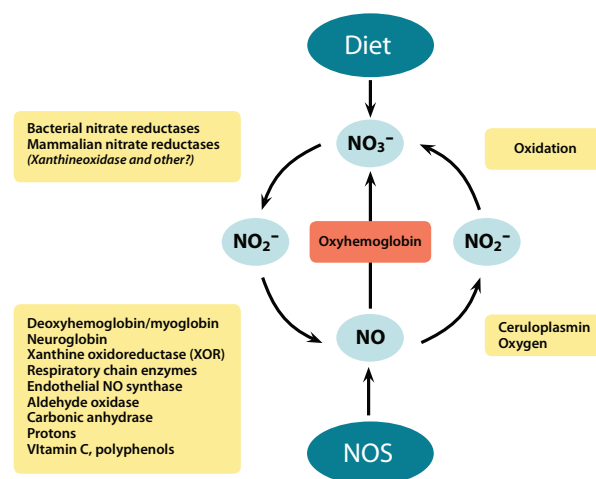
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tory product of mammalian cells. In 1980, Furchgott and Zawadzki identified an endothelium-derived relaxing factor that was later recognized as nitric oxide.<sup>3–5</sup> Specific nitric oxide synthases (NOSs) were described that use the *N*-guanidino nitrogen of L-arginine with molecular oxygen in a complex five-step oxidation process to generate nitric oxide.<sup>6</sup> Previously, Ferid Murad *et al.* described that organic nitrates, such as nitroglycerine, induce vasodilation by release of nitric oxide, activating soluble guanylyl cyclase and subsequent cyclic guanosine monophosphate formation.<sup>7</sup> These discoveries rendered Robert Furchgott, Louis Ignarro, and Ferid Murad the Nobel Prize in Physiology or Medicine in 1998. It has now been established that nitric oxide regulates a vast number of physiologic processes ranging from vasodilation to memory.<sup>6</sup> Nitric oxide signaling is partly regulated by the short half-life (milliseconds) in biologic systems because it is rapidly oxidized to nitrite and nitrate. For this reason, these inorganic anions have been considered merely as stable-end metabolites from nitric oxide production, and the scientific interest in these anions has primarily been as markers of NOS activity.

In 1994, two independent groups demonstrated formation of nitric oxide that was independent of NOS.<sup>8,9</sup> Nitric oxide gas was generated in the human stomach at high concentrations, and this production was dependent on gastric acidity and involved reduction of salivary-derived nitrite. Furthermore, nitric oxide generation was greatly enhanced after intake of nitrate.<sup>8</sup> An enterosalivary circulation of ingested nitrate exists in which, after absorption in the gastrointestinal tract, circulating nitrate is actively secreted in saliva and oral commensal bacteria reduced nitrate to nitrite.<sup>10</sup> A year later, Zweier *et al.* demonstrated NOS-independent nitric oxide generation from nitrite in the ischemic heart, thereby extending the occurrence of nitrite reduction outside the gastrointestinal tract.<sup>11</sup> From that time, a nitrate-nitrite-nitric oxide pathway has been established in which serial reduction of nitrate and nitrite generates nitric oxide and other bioactive nitrogen oxides throughout the body (fig. 1).<sup>12–14</sup> This pathway is suggested to be involved in many important biologic processes, including regulation of blood flow, cell signaling, and energetic, as well as tissue, responses to hypoxia.<sup>13</sup> In contrast to the classic L-arginine-NOS-nitric oxide pathway, the nitrate-nitrite-nitric oxide pathway is greatly enhanced by hypoxia and acidosis and may serve as a backup system to ensure nitric oxide generation during ischemic/hypoxic conditions when the oxygen-dependent NOSs may be malfunctioning.<sup>14,15</sup> The fact that this pathway can be fueled by exogenous nitrate and nitrite leads to interesting therapeutic and nutritional implications.<sup>16</sup> Our diet is a main provider of exogenous nitrate, and vegetables are especially rich in this anion. This has prompted several researchers to investigate the possibility that nitrate may be involved in the well-established beneficial effects of a diet rich in vegetables on cardiovascular disease.<sup>17</sup>

Many of the vast functions of nitric oxide are highly relevant in everyday anesthesiologic and intensive care practice,



**Fig. 1.** A schematic presentation of a mammalian nitrogen cycle. Nitric oxide (NO) is generated by nitric oxide synthases (NOS) in most cells of the body and participates in regulation of numerous physiologic functions. The bioactivity of nitric oxide is partly regulated by its rapid oxidation to nitrite ( $\text{NO}_2^-$ ) or, in the presence of oxyhemoglobin, to nitrate ( $\text{NO}_3^-$ ). Nitrate is the predominant nitric oxide oxidation product in the circulation. In our bodies, nitrate can undergo reduction to nitrite, and this process is strongly dependent on oral commensal bacteria but also to some extent by xanthine oxidoreductase and possibly other enzymes in tissues. In blood and tissues, nitrite can be further reduced to nitric oxide and other bioactive nitrogen oxides. There are several enzymatic and nonenzymatic routes that can catalyze this reduction, most of which are greatly enhanced under hypoxic conditions. This mammalian nitrogen cycle can be fueled by the diet because vegetables contain high amounts of inorganic nitrate. Modified with permission from reference 15.

including regulation of blood flow,<sup>18</sup> platelet function,<sup>19</sup> pulmonary function,<sup>20</sup> nerve transmission,<sup>21</sup> host defense,<sup>22</sup> metabolic control,<sup>23</sup> anesthetic action,<sup>24</sup> and pain.<sup>25</sup> This review will describe the current knowledge of the nitrate-nitrite-nitric oxide pathway with special focus on relevance to the anesthesiologist and intensive care physician.

## The Classic L-Arginine-NOS-Nitric Oxide Pathway

The discovery of nitric oxide as a signaling molecule in mammals triggered an enormous scientific interest, and to date, more than 100,000 articles have been published, within almost every field of medical science. Nitric oxide is produced endogenously in humans from the amino acid L-arginine by a family of enzymes known as NOSs. The genes for the three different NOS isoforms—endothelial NOS (eNOS), neuronal NOS, and inducible NOS—are located on different chromosomes.<sup>6</sup> eNOS, also known as NOS3, was first discovered in the vascular endothelium and plays an important part in regulating vascular tone. Neuronal NOS, also known as NOS1, was discovered in the brain and participates in central and peripheral neuronal physiology. Both eNOS and neuronal NOS are constitutively expressed, and their activa-

tion is calcium- and calmodulin-dependent. Inducible NOS, also known as NOS2, was first identified in macrophages and is important for fighting off infection. As implied by its name, transcription of inducible NOS is induced by agents involved in inflammation and infection, such as cytokines and lipopolysaccharides.<sup>6</sup> The different NOSs are not only located where they were first described but may appear in almost any cell type.

Nitric oxide production by the NOSs is a complex reaction that entails five electron transfers and requires the presence of several cofactors and substrates, including L-arginine, oxygen, tetrahydrobiopterin, and reduced nicotinamide adenine dinucleotide phosphate.<sup>6</sup> Nitric oxide is a reactive gas molecule with one unpaired electron, and these properties are important for its signaling and its ability to undergo many different reactions. Nitric oxide acts mainly in an auto/paracrine fashion, and signaling is limited by its rapid oxidation, especially in the presence of heme-containing proteins such as circulating hemoglobin. Nitric oxide binds rapidly to oxy-hemoglobin, which yields nitrate and methemoglobin. Of great interest is the ability of nitric oxide and other nitrogen oxide species to form adducts with proteins. By nitro(syl)ation and nitration, nitric oxide and other nitrogen oxides can modify and regulate protein function.<sup>26</sup> S-Nitros(yl)ated proteins serve to transmit nitric oxide bioactivity and to regulate protein function through mechanisms analogous to phosphorylation.<sup>27</sup> Circulating S-nitros(yl)ated proteins are able to convey nitric oxide-like bioactivity in an endocrine fashion.<sup>28</sup> Nitric oxide can initiate cellular signaling through activation of soluble guanylate cyclase after a secondary increase in cyclic guanosine monophosphate formation.<sup>7</sup> In addition, nitric oxide can act independently of cyclic guanosine monophosphate by the above mentioned protein interactions or by direct radical action on proteins and DNA.<sup>29</sup>

Nitric oxide is involved in a multitude of physiologic and pathophysiological processes with great relevance in anesthesiology and intensive care. A detailed description of these is outside the scope of this review, but it is clear that this molecule is involved in vasoregulation, nerve transmission, pain signaling, immune defense, metabolism, and mitochondrial function. Decreased nitric oxide bioavailability is considered a central event in several cardiovascular diseases<sup>30</sup> and in the metabolic syndrome,<sup>31</sup> and excess nitric oxide has been claimed to be responsible for the hypotension seen in septic shock.<sup>32</sup> Because direct measurement of nitric oxide is very difficult *in vivo*, investigators have instead used nitrate and nitrite as markers of nitric oxide production.<sup>33</sup>

## Sources of Nitrate and Nitrite

There are two major sources of nitrate and nitrite in the body. As mentioned above, the L-arginine-NOS pathway is a major source by the rapid oxidation of nitric oxide to nitrite and nitrate. In the circulation, nitric oxide oxidation is enhanced by the multicopper oxidase, ceruloplasmin.<sup>34</sup> However, nitrate is the dominating final oxidation product in

plasma with concentrations (micromolar) normally at least 2 orders of magnitude higher than nitrite (nanomolar).<sup>35</sup> The half-lives of nitrate and nitrite in the circulation are approximately 5–6 h and 20 min, respectively.<sup>36</sup> Nitrate is continuously excreted *via* the kidney, and measurement of urine concentrations can be used in conditions related to altered nitric oxide production.<sup>37,38</sup> In eNOS knockout mice, plasma concentrations of nitrite are reduced by up to 70%.<sup>39</sup> Plasma concentrations of nitrate and nitrite are increased by exercise<sup>40</sup> as a result of circulatory shear stress, which stimulates nitric oxide generation from eNOS. In systemic inflammatory disorders, such as sepsis<sup>41</sup> and severe gastroenteritis,<sup>42</sup> nitrate and nitrite concentrations are markedly increased because of massive iNOS induction. In contrast, patients with endothelial dysfunction, often as a result of hypertension, diabetes mellitus, or atherosclerosis, low plasma concentrations of nitrate and nitrite have been reported.<sup>43</sup>

The other major source of nitrate and, to a lesser extent, nitrite is our everyday diet. Vegetables are without question the dominant dietary source of nitrate (80%), and cured meat contains some nitrite used as a preservative against bacterial contamination as well as a color enhancer.<sup>44</sup> Green leafy vegetables, such as spinach, lettuce, and beetroot, are particularly high in nitrate, and ingestion is followed by a marked increase in systemic concentrations of nitrate and nitrite.<sup>45</sup> One serving of such a vegetable contains more nitrate than what is endogenously formed by the all three NOS isoforms combined during a day.<sup>15</sup> Drinking water, especially in rural areas, can contain considerable amounts of nitrate, although in most countries, the concentrations are strictly regulated.<sup>46</sup> The reason for this regulation is that nitrate has a bad reputation as being responsible for gastric cancer (through formation of *N*-nitrosamines) and blue baby syndrome (severe methemoglobinemia in infants).<sup>47,48</sup> There is, however, weak scientific evidence for any relationship between high nitrate intake and gastric cancer in humans.<sup>49</sup>

## Enterosalivary Circulation of Nitrate

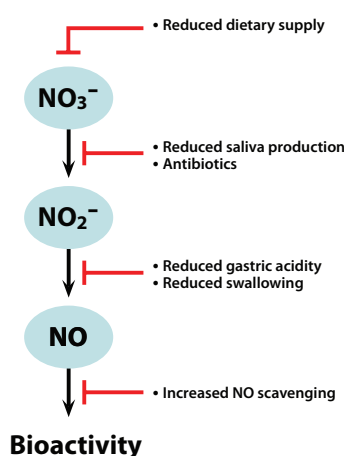
Through early cancer research, it was known that up to 25% of circulating nitrate is actively taken up by the salivary glands and concentrated 10- to 20-fold in saliva, but the reason and mechanism for this were unknown, other than its proposed pathologic role in formation of carcinogenic nitrosamines.<sup>10</sup> After ingestion of nitrate and effective absorption in the upper gastrointestinal tract, salivary concentrations of nitrate become very high (millimolar).<sup>45,50</sup> In the oral cavity, commensal facultative anaerobic bacteria, located in the deep crypts of the posterior part of the tongue, reduce nitrate to nitrite by action of nitrate reductase enzymes.<sup>51,52</sup> These bacteria use nitrate as an alternative terminal electron acceptor during respiration to gain adenosine-5'-triphosphate in the absence of oxygen. When swallowed saliva meets the acidic gastric milieu, part of the nitrite is immediately protonated to form nitrous acid (HNO<sub>2</sub>), which then decomposes to nitric oxide and other nitrogen oxides.<sup>8–9</sup> This re-

action is enhanced by low pH and by reducing compounds, such as ascorbic acid and polyphenols.<sup>53,54</sup> Concentrations of nitric oxide gas in the stomach can be substantial (more than 100 ppm) and sometimes beyond what is considered safe as a working environment by the authorities. Most of the salivary nitrite escapes the gastric conversion to nitric oxide and enters the systemic circulation.<sup>45</sup> Human nitrate reduction is highly dependent on the oral commensal bacteria because our cells do not convert nitrate to nitrite to a high degree. This is evident by studies where the biologic effects of ingested nitrate, as well as the concomitant increase in plasma nitrite, are abolished after avoiding swallowing of saliva<sup>45,55</sup> or by the use of an antibacterial mouthwash.<sup>50,56</sup> Moreover, germ-free mice have virtually no gastric nitric oxide, even after a nitrate load.<sup>57</sup> Several pathways have now been shown to reduce systemic nitrite to nitric oxide and other bioactive nitrogen oxides (see Systemic Nitrite Bioactivation section), which completes the mammalian nitrate-nitrite-nitric oxide pathway.<sup>13</sup>

## Stomach Nitric Oxide

With respect to the known pluripotency of nitric oxide, the high concentrations of nitric oxide normally found in the gastric lumen could be of physiologic importance. High concentrations of nitric oxide are known to be bactericidal,<sup>58</sup> and gastric nitric oxide could be a first-line defense against swallowed pathogens. Indeed, *in vitro* studies have shown that gastric juice and nitrite have markedly better antimicrobial effects on known enteropathogens compared with gastric juice alone.<sup>9,59–62</sup> Another proposed role for gastric nitric oxide is in the regulation of mucosal blood flow and mucus production, two important protective mechanisms for gastric mucosal integrity. Application of human saliva rich in nitrite onto rat gastric mucosa *ex vivo* increases mucosal blood flow and mucus production.<sup>63,64</sup> Furthermore, dietary nitrate supplementation in rodents protects the gastric mucosa against ulcerations induced by stress or a nonsteroidal antiinflammatory drug.<sup>65,66</sup> Taken together, these findings suggest that nitric oxide and other reactive nitrogen oxides generated from swallowed saliva have several important protective functions to uphold gastric mucosal integrity and to provide a first-line defense against bacterial infection.

In this respect, it is highly interesting that sedated and intubated intensive care patients, with poor salivary production and reduced swallowing of saliva and who are often treated with broad-spectrum antibiotics, have virtually abolished gastric nitric oxide (fig. 2).<sup>53,67</sup> This nitric oxide can be replenished by gastric administration of nitrite,<sup>53</sup> and additional nitrite also increases the circulating concentrations of nitrite in these patients.<sup>53</sup> Gastric lesions and bacterial colonization of the gastric lumen is common in the intensive care unit (ICU). In addition, it has been advocated that gastric bacterial colonization could function as a reservoir and later promote ventilator-associated pneumonia. With respect to



**Fig. 2.** Factors in the intensive care setting that may obstruct the nitrate-nitrite-nitric oxide pathway. Several important steps in nitrate-nitrite-nitric oxide (NO) pathway may be negatively affected in patients treated in the intensive care unit (ICU). The normal dietary intake of nitrate ( $\text{NO}_3^-$ , mostly from vegetables) will be almost abolished because both enteral and parenteral feeding formulas contain extremely low concentrations of nitrate and nitrite ( $\text{NO}_2^-$ ). A patient on full enteral or parenteral feeding is subjected to nitrate/nitrite starvation. Nitrate reduction to nitrite in the oral cavity depends on saliva production and active oral commensal bacteria. Intubated and sedated patients have poor saliva production and are often treated with broad-spectrum antibiotics, which will inhibit this part of the pathway. Normally, swallowed salivary nitrite will immediately be reduced to nitric oxide in the acidic stomach, and this nitric oxide may be involved in host defense and upholding gastric mucosal integrity. ICU patients often have problems swallowing saliva due to sedation and intubation, and their gastric pH is often increased, sometimes pharmacologically, to prevent gastric ulceration. This may partly explain high incidence of gastric ulceration and bacterial colonization found in ICU patients. Finally, many conditions in the ICU are associated with increased oxidative stress in which reactive oxygen species can scavenge nitric oxide, thereby reducing nitric oxide bioactivity.

gastric nitric oxide, the widespread use of  $\text{H}_2$  blockers or proton-pump inhibitors to prevent gastric lesions in the ICU will increase gastric pH, subsequently decreasing stomach nitrite reduction.<sup>67</sup> It is tempting to speculate that lack of gastric nitric oxide could partly explain the frequent occurrence of gastric lesions and pneumonia in the ICU. Future studies will reveal whether gastric supplementation with nitrite could have preventive effects in these patients.

## Systemic Nitrite Bioactivation

In addition to the simple protonation of nitrite in the stomach, there are several enzymatic pathways for conversion of systemic nitrite to nitric oxide and other bioactive nitrogen species. Hemoglobin, myoglobin, neuroglobin, xanthine oxidoreductase, aldehyde oxidase, carbonic anhydrase, eNOS, and mitochondrial enzymes have all been identified with having a role in nitrite bioactivation (fig. 1).<sup>14,15</sup> The relative

contribution from these pathways varies between tissues and is dependent on several factors, including local pH, oxygen tension, and redox status. In addition, reducing agents, such as vitamin C and polyphenols, catalyze non-enzymatic reduction of nitrite.<sup>54,68</sup>

Although the role of hemoglobin and myoglobin in the handling of bodily oxygen has long been studied, they have more recently been identified to interact with nitrogen oxide species. Early *in vitro* experiments postulated reactions between nitrite and hemoglobin, leading to nitrosyl-hemoglobin and nitric oxide, although there were differences between theoretical calculations and actual results.<sup>69,70</sup> Gladwin *et al.* recently resolved this discrepancy by showing that hemoglobin conformation and oxygen binding status affect its ability to reduce nitrite.<sup>71–73</sup> They showed that nitrite bioactivation is most prevalent during rapid deoxygenation, reaching a maximum conversion and nitric oxide-mediated effects at 50% oxygen bound hemoglobin.<sup>73,74</sup> They propose that this allosterically regulated control of nitrite bioactivation gives a sensing capacity to the erythrocyte to regulate microvascular blood flow by releasing nitric oxide-like bioactivity with vasodilatation in areas of poor oxygenation. Furthermore, they suggest that this mechanism could, at least partly, be responsible for physiologic hypoxic vasodilation.<sup>71</sup> Previously, another allosterically regulated mechanism for the erythrocyte to deliver nitric oxide-like bioactivity had been proposed by Stamler *et al.* nitric oxide binds to a cysteine thiol group on hemoglobin, creating circulating S-nitroso hemoglobin, which at distal parts of the circulation during deoxygenation releases nitric oxide to regulate microvascular blood flow.<sup>75</sup> Interestingly, this group recently showed that physiologic amounts of nitrite were able to promote generation of S-nitroso hemoglobin.<sup>76</sup> However, the exact role of the erythrocyte in physiologic regulation of blood flow is still not settled and has been under vivid scientific debate.<sup>77,78</sup>

Myoglobin has also been identified to have a role in nitrite bioactivation, specifically in myocardial ischemia-reperfusion (I/R) injury, much the same way as hemoglobin has been described to bioactivate nitrite.<sup>79</sup> Myoglobin is less complex than hemoglobin because of its monomeric structure and requires less than 50% oxygenation for nitrite reduction.<sup>80</sup> Research has shown that nitrite through reduction by myoglobin has a cardioprotective effect, which is lost in myoglobin-null mice.<sup>81</sup> Myoglobin has also been identified as involved in scavenging nitric oxide, thereby preventing excess nitric oxide from disrupting mitochondria function under normoxic conditions.<sup>82</sup> In addition, neuroglobin, a monomeric globin with unknown function that is present mostly in nervous and endocrine tissues, has recently been shown to have nitrite reductive properties.<sup>83</sup>

In addition to its role in purine catabolism and in reduction of molecular oxygen to superoxide, xanthine oxidoreductase (XOR) has been identified to reduce inorganic nitrate and nitrite under low oxygen tension as it occurs in ischemia.<sup>84–86</sup> XOR activity is up-regulated under ischemic and inflammatory conditions<sup>87</sup> and exists in two forms, as

xanthine oxidase or xanthine dehydrogenase, both of which consume oxygen and reduce nitrite to nitric oxide.<sup>85</sup> Our laboratory has identified XOR as a functional nitrate reductase under normal physiologic conditions,<sup>88</sup> and this process is enhanced under germ-free conditions,<sup>89</sup> with the latter possibly being a compensation for the lack of bacterial reduction of these anions.

Several mitochondrial proteins are capable of nitrite bioactivation. Complex III has been shown to reduce nitrite to nitric oxide under anoxic conditions.<sup>90</sup> In addition, complex IV<sup>91</sup> and ubiquinone/cytochrome *b<sub>c</sub>1*<sup>92</sup> can reduce nitrite to nitric oxide but at nonphysiological concentrations of nitrite. Interestingly, nitric oxide has been shown to bind to the complexes of the respiratory chain thereby inhibiting respiration.<sup>93–95</sup> This added function has been suggested to spare the tissue from oxidative stress during reperfusion (see I/R Injury). However, a pathologic role of nitric oxide interaction with cytochrome *c* oxidase with increased reactive oxygen species generation has been proposed.<sup>96</sup>

Mitochondrial aldehyde oxidase is another enzyme that has been shown to reduce nitrite to nitric oxide in rats, leading to vasodepressor activity.<sup>97,98</sup> Interestingly, aldehyde oxidase has also been identified in the activation of nitroglycerine.<sup>99</sup>

Mammalian cytochrome P450 enzymes are a family of enzymes involved with drug and dietary metabolism and that recently was shown to bioactivate nitrite to nitric oxide.<sup>100</sup> Nitric oxide can also reversibly bind and inhibit the catalytic activity of cytochrome P450.<sup>101</sup> Like most enzymatic nitrite bioactivation studies, these experiments occurred under anoxia, and the role of cytochrome P450 enzymes under normoxic conditions remains to be elucidated.<sup>14</sup>

eNOS can also bioactivate nitrite under anoxic and/or acidic conditions.<sup>102,103</sup> Webb *et al.* recently found eNOS, located on erythrocyte membranes, with the ability for nitrite bioactivation. Nitrite reduction was absent under normally oxygenated conditions.<sup>104,105</sup>

To summarize, there are several routes by which nitrite can be bioactivated to nitric oxide and other nitrogen oxides. In contrast to NOS-dependent nitric oxide production, the above-mentioned pathways are greatly enhanced during hypoxia and low pH. They may jointly be considered as a backup system to ensure bioactive nitric oxide under conditions where the NOSs may be dysfunctional.

## Nitrate and Nitrite in the Cardiovascular System

The vasodilatory action of pharmacological doses of inorganic nitrite has been known for almost a century.<sup>106</sup> However, recent studies have shown that much lower doses, near-physiologic concentrations of circulating nitrite, also have vasodilatory effects in several species,<sup>68,107–109</sup> including humans.<sup>110–113</sup> The potency of inorganic nitrite is much lower than the organic nitrates used in the clinical setting, (*e.g.*, nitroglycerine). However, the vasodilatory potency of nitrite

increases during hypoxia and acidosis probably because of enhanced reduction to bioactive nitric oxide.<sup>111,112,114</sup> This preference to vasodilate in areas of hypoxia and acidosis could be of future substantial clinical benefit and may partly explain some of the beneficial effects of nitrite in ischemia reperfusion situations as described below in the section on I/R injury. Moreover, the doses needed to protect against I/R injury will have very little effect on general blood pressure, which could be advantageous from a clinical perspective.

The nitrate-nitrite-nitric oxide pathway is boosted by dietary intake of nitrate.<sup>55</sup> It is well established that diets rich in fruit and vegetables (*e.g.*, the Mediterranean diet) protect against development of cardiovascular disease.<sup>115–118</sup> Because vegetables are naturally rich in nitrate, it seems reasonable to investigate if inorganic sodium nitrate alone, corresponding to the amount present in 100–300 g of a nitrate-rich vegetable, could affect blood pressure in healthy subjects. In a double-blind, placebo-controlled, cross-over designed study, sodium nitrate ( $0.1 \text{ mmol nitrate} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ ) was administered to healthy volunteers for 3 days after which blood pressure was measured.<sup>119</sup> Indeed, diastolic blood pressure was reduced by 4 mmHg after nitrate supplementation compared with placebo (NaCl), which suggests formation of vasodilatory nitric oxide. In a subsequent study, with a greater number of subjects, a similar effect was observed also on systolic pressure.<sup>120</sup> Webb *et al.* used beetroot juice as a natural source of nitrate to study the effect on blood pressure in healthy volunteers.<sup>55</sup> Subjects drank 500 ml of either the juice ( $0.3 \text{ mmol nitrate/kg}$ ) or water, and blood pressure was measured repeatedly over a 24-h period. A reduction in both systolic (10 mmHg) and diastolic blood pressure (8 mmHg) was noted within 3 h of ingestion, and the effect was still present 24 h after a single administration. Maximal effect on blood pressure coincided with peak increases in plasma nitrite concentrations. To demonstrate the central role of enterosalivary circulation in bioactivation of nitrate, the subjects avoided swallowing for a period after drinking the juice, and this procedure completely blocked the blood pressure-lowering effects of nitrate supplementation. In the same study, beetroot juice prevented ischemia-induced endothelial dysfunction, inhibited *ex vivo* platelet aggregation, the latter previously shown also to be achievable with oral intake of potassium nitrate.<sup>121</sup> The same group could recently show blood pressure-reducing effects, also with a considerably lower dose of beetroot juice, and effects were similar to those observed with equimolar amounts of potassium nitrate salt.<sup>122</sup> This suggests that the active ingredient in the juice is nitrate. Together, these studies show acute effects of inorganic nitrate on blood pressure related to elevation in systemic nitrite and concomitant indications of nitric oxide formation. Traditional organic nitrates, such as nitroglycerine, are classically associated with development of tolerance after repeated administration. In contrast, effects on blood pressure by nitrate and nitrite do not show any signs of tolerance. Rats treated with dietary nitrate for up to 5 days still have decreased blood pressure compared with controls.<sup>56</sup>

Similar observations have been reported in nonhuman primates with repeated administration of nitrite.<sup>113</sup>

It is reasonable to assume that nitrate would have even stronger effects in subjects with hypertension or other forms of cardiovascular disease because nitric oxide deficiency underlie these conditions. To date, no clinical trials have been performed in hypertensive patients, but in a recent study, we tested this hypothesis by investigating the effects of dietary nitrate in a rat model of renal cardiovascular disease, including hypertension induced by early unilateral nephrectomy in combination with a chronic high-salt diet for 10 weeks (unpublished data, Mattias Carlström Ph.D., Postdoctoral Researcher, Department of Physiology and Pharmacology, Karolinska Institute, Stockholm, Sweden, August 2010). Placebo rats developed renal and cardiovascular dysfunction, including hypertension, cardiac hypertrophy and fibrosis, proteinuria, and histologic, as well as biochemical signs of renal damage and oxidative stress. Blood pressure was dose-dependently lowered by nitrate. In addition, proteinuria and histologic signs of renal injury were almost completely prevented. Dietary nitrate increased tissue concentrations of bioactive nitrogen oxides and reduced the concentrations of oxidative stress markers in plasma and urine. In a different model of hypertension and kidney damage, induced by chronic blockade of NOS with *N*<sup>ω</sup>-nitro-L-arginine methyl ester, Kanematsu *et al.* demonstrated that chronic nitrite supplementation (100 mg/l drinking water) attenuated hypertension and that a very low dose of oral nitrite (1 mg/l) protected against *N*<sup>ω</sup>-nitro-L-arginine methyl ester-induced kidney injuries without significant changes in blood pressure.<sup>123</sup>

Table 1 summarizes the results from studies where the therapeutic effects of nitrate administration in various animal models and in humans have been investigated. Together, these studies show that nitrate may provide nitric oxide-like bioactivity that could partly compensate for disturbances in endogenous nitric oxide generation from NOS. The underlying mechanisms for nitrate-mediated antihypertensive effects and renal and cardiac protection require further investigations, but a reduction in oxidative stress is an interesting hypothesis supported by the data from Carlström *et al.*

## Mitochondria and Oxygen Consumption

Recent data suggest that many of the biologic effects of nitrite involve interaction with mitochondria.<sup>124,125</sup> In the last two decades, it has been established that the mitochondrion is a physiologic target for nitric oxide.<sup>126</sup> In competition with oxygen, nitric oxide binds to cytochrome *c* oxidase in the mitochondrial electron transport chain, which leads to inhibition of mitochondrial respiration.<sup>93–95</sup> It has been suggested that this reversible and partial inhibition of respiration would allow for better oxygen diffusion to more distant parts of a tissue.<sup>127</sup> This might not affect adenosine-5'-triphosphate production because there is normally excess mitochondrial capacity. These nitric oxide-elicited events also act as

**Table 1.** Therapeutic Effects of Inorganic Nitrate or Natural Sources Containing Nitrate

Organ System	Nitrate Source	Species	Effect	Ref.
Cardiac I/R	Sodium nitrate	Mouse	↓ Infarct size	148
Cardiovascular Renal I/R	Sodium nitrate	Rat	↑ MAP, ↑ Post-ischemic blood flow	88
Cardiovascular Stomach	Sodium nitrate	Rat	↓ MAP, ↓ DBP, ↑ Intra gastric NO formation, ↑ Mucus layer	56
Stomach	Potassium nitrate	Rat	↓ Ulcer formation, ↑ Intra gastric NO formation, ↑ Gastric blood flow	65
Stomach	Sodium nitrate	Rat	↓ Ulcer Formation, ↑ Mucus formation	66
Cardiovascular	Potassium nitrate	Human	↓ Platelet aggregation	121
Cardiovascular	Sodium nitrate	Human	↓ DBP, ↓ MAP	119
Cardiovascular	Beetroot juice	Human	↓ DBP, ↓ SBP, ↓ MAP, ↓ Platelet aggregation, ↓ Endothelial dysfunction	55
Cardiovascular	Potassium nitrate Beetroot juice	Human	↓ DBP, ↓ SBP	122
Cardiovascular	Trad. Japanese diet	Human	↓ DBP	174
Cardiovascular Musculoskeletal	Sodium nitrate	Human	↓ DBP, ↓ SBP, ↓ Moderate exercise oxygen consumption	124
Cardiovascular Musculoskeletal	Beetroot juice	Human	↓ SBP, ↓ Moderate exercise oxygen consumption, ↑ Exercise time to exhaustion	129
Cardiovascular Musculoskeletal	Beetroot juice	Human	↓ SBP, ↓ DBP, ↓ Low intensity exercise oxygen consumption, ↓ Muscle phosphocreatine, ↑ Work efficiency	131
Cardiovascular Musculoskeletal	Beetroot juice	Human	↓ SBP, ↓ DBP, ↓ MAP, ↓ Exercise oxygen consumption	130
Musculoskeletal	Sodium nitrate	Human	↓ Oxygen consumption during maximal exercise	120

DBP = diastolic blood pressure; I/R = ischemia reperfusion injury; MAP = mean arterial pressure; NO = nitric oxide; SBP = systolic blood pressure.

triggers by which mitochondria modulate signal transduction cascades involved in the induction of cellular defense mechanisms and adaptive responses, particularly in response to hypoxia and other environmental stressors.<sup>128</sup> As mentioned above, myoglobin and complex IV are nitrite reductases, and nitrite may exert nitric oxide-like effects on mitochondria. This suggests that nitrite could play a role in regulating cellular energetic and oxygen utilization, especially in conditions of physiologic hypoxia. This hypothesis was tested in healthy volunteers during exercise where working muscle is subjected to low  $PO_2$  and pH. In a double-blind, placebo-controlled, cross over study, Larsen *et al.* found that the oxygen cost during standardized exercise was reduced after 3 days of dietary supplementation with sodium nitrate compared with placebo.<sup>124</sup> There was no difference in lactate formation, indicating that there was no compensatory increase in glycolytic energy contribution, and thus metabolic efficiency seemed to be improved. Subsequent studies

have confirmed and extended these results with beetroot juice as the nitrate source, as well as sodium nitrate salt.<sup>120,129–131</sup> In these studies, oxygen cost was also reduced during maximal performance, and time-to-exhaustion was significantly extended after beetroot juice. The molecular mechanisms behind these remarkable effects of nitrate have not been determined in detail, but data point toward the mitochondria as the central targets.<sup>132</sup>

## I/R Injury

After the discovery of nitric oxide as a signaling molecule for vasodilation, the production and role of nitric oxide in I/R injury has piqued interest. Among the factors that are suggested to contribute to I/R injury are endothelial and microvascular dysfunction, proinflammatory activation, and oxidative stress.<sup>133</sup> By scavenging nitric oxide, the latter may contribute to reduced nitric oxide bioavailability, which is a central event in I/R

**Table 2.** Therapeutic Effects of Inorganic Nitrite

Organ System	Species	Effect	Ref.
Brain I/R	Rat	↓ Infarct size, ↑ Cerebral blood flow, ↓ Oxidative stress	141
Cardiac allograft	Rat	↑ Survival post transplant	163
Cardiac I/R	Rat	↓ Infarct size, ↑ Ventricular function	139
Cardiac I/R	Mouse	↓ Infarct size	140
Cardiac I/R	Rat	↓ Infarct size, ↓ Necrosis, ↑ Ventricular function	147
Cardiac I/R	Mouse	↓ Infarct size	125
Cardiac I/R*	Mouse	↓ Infarct size	148
Cardiac I/R	Dog	↓ Infarct size, ↓ Apoptosis, Improved microvascular perfusion and contraction	146
Cardiac I/R	Mouse	↓ Infarct Size, ↓ ROS production, ↑ Ventricular function	81
Cardiac arrest	Mouse	↓ Mitochondrial oxidative stress, ↑ Cardiac function, ↑ Survival, ↓ Neurological damage	149
Chronic muscle Ischemia*	Mouse	↑ Restoration of tissue blood flow, ↑ Limb vascular density, ↑ Endothelial proliferation	150
Liver I/R	Mouse	↓ ALT, ↓ AST	140
Liver I/R	Mouse	↓ ALT, ↓ AST, ↑ Mitochondrial respiration recovery	125
Liver I/R	Mouse	↓ ALT, ↓ AST	144
Renal hypertension*	Rat	↓ SBP, ↓ Proteinuria, ↓ Renal damage	123
Renal I/R	Rat	↓ AST, ↓ Creatinine, ↓ Renal dysfunction	142
Renal I/R	Mouse	↓ Renal dysfunction markers, ↓ Inflammation	105
Sepsis	Mouse	↓ Hypothermia, ↓ Mitochondrial damage, ↓ Oxidative stress, ↓ Tissue infarction, ↑ Survival	151
Sickle cell disease	Human	↑ Regional blood flow	110

\* Multiple or long term treatment.

ALT = alanine transaminase; AST = aspartate aminotransferase; I/R = ischemia reperfusion; ROS = reactive oxygen species; SBP = systolic blood pressure.

injury.<sup>134</sup> Early research indicated a therapeutic role for nitric oxide in cardioprotection in myocardial infarction models,<sup>135</sup> and L-arginine treatment before reperfusion was also organ-protective.<sup>133,136,137</sup>

In 1995, Zweier *et al.* showed endogenous NOS-independent nitric oxide production in the ischemic heart. As the duration of ischemia increased, more nitrite was converted into nitric oxide.<sup>138</sup> In 2004, Webb *et al.* reported protective effects of nitrite in isolated perfused heart preparations subjected to I/R injury.<sup>139</sup> They could show conversion of nitrite to nitric oxide, which was dependent on XOR. This was interesting because XOR is generally thought to contribute to I/R injuries *via* production of reactive oxygen species. However, the findings by Webb *et al.* suggest that during

hypoxic conditions, nitrite supplementation may shift the activity of XOR from generation of damaging superoxide ( $O_2^-$ ) to protective nitric oxide.

Duranski *et al.* then demonstrated potent cytoprotective effects of low-dose nitrite *in vivo* in mouse models of myocardial infarction and liver ischemia.<sup>140</sup> The effects were independent of NOS and abolished by coadministration of the nitric oxide scavenger cPTIO, suggesting nitrite-derived nitric oxide as an active mediator. Furthermore, the efficiency profile of nitrite therapy on liver and heart function was U-shaped, with a maximum protective effect reached at a dose of 48 nmol of nitrite. It is noteworthy that a similar systemic load of nitrite can be achieved in humans by ingestion of only 100 g of a nitrate-rich vegetable, such as beetroot

or spinach. A number of subsequent studies in different animal species have confirmed protective effects of low-dose nitrite in various settings of I/R injury, including models of stroke,<sup>141</sup> cerebral vasospasm,<sup>108</sup> kidney ischemia,<sup>105,142</sup> hepatic injury,<sup>125,143,144</sup> lung injury,<sup>109,145</sup> acute myocardial infarction,<sup>146–148</sup> cardiac arrest,<sup>149</sup> and chronic limb ischemia (table 2).<sup>150</sup>

Other areas where the therapeutic action of nitrite administration has been investigated are sepsis and sickle cell disease. In mouse models of septic shock, induced by either tumor necrosis factor or Gram-negative lipopolysaccharide, Cauwels *et al.* showed that administration of nitrite attenuated hypothermia, mitochondrial damage, oxidative stress, tissue infarction, and mortality. Higher doses were needed in endotoxemic mice compared with the mice receiving tumor necrosis factor.<sup>151</sup> These salutary effects were dependent on soluble guanylyl cyclase because they were largely abolished in guanylyl cyclase  $\alpha$ -1 subunit-null mice. The underlying physiologic mechanisms remain to be elucidated, but improved microcirculation or mitochondrial function was suggested.

Sickle cell disease is characterized by hemolysis, regional and pulmonary microvascular occlusion, and inflammation. In addition, cell-free, hemoglobin-mediated consumption of nitric oxide leads to reduced nitric oxide bioavailability. In a Phase I/II study, Mack *et al.* tested the safety and vasodilating effects of nitrite by intraarterial forearm infusions of nitrite to patients with sickle cell disease. Nitrite dose-dependently increased forearm blood flow, although the response was blunted compared with healthy controls. Nitrite infusions were well tolerated and did not induce hypotension or clinically significant methemoglobinemia. The authors conclude that the vasodilating and cytoprotective properties of nitrite make it a plausible candidate for future clinical trials in sickle cell patients.

Although the mechanism of nitrate-nitrite-mediated cytoprotection is not fully elucidated, Shiva *et al.* have identified the mitochondria as targets for protection.<sup>125</sup> They show that nitrite-mediated protection occurs through reversible inhibition of mitochondrial complex I, which dampens electron transfer to the respiratory chain, thereby decreasing the production of oxygen radicals. This mechanism also prevents mitochondrial permeability transition pore opening and cytochrome *c* release, which are mechanisms involved in apoptosis. Complex I inhibition appears to occur through *S*-nitrosylation of cysteine thiol residues, although the exact details still need to be elucidated.<sup>152,153</sup> It has been shown that nitric oxide is a mediator of the ischemic preconditioning cell survival program,<sup>154</sup> and it is worth noting that Shiva *et al.* found that nitrate administered as long as 24 h before injury was also protective.<sup>125</sup> It is noteworthy that nitric oxide has also been suggested to play a role in the preconditioning effects of volatile anesthetics,<sup>24</sup> but whether nitrite is involved in this process has not been investigated.

Together, these findings convincingly suggest a potential role for nitrite as a useful adjunctive therapy in preventing

I/R injuries in several organs and tissues, and human trials are presently under way.

As anesthesiologists or ICU physicians, we are faced with the risk of or overt I/R injury almost on a daily basis. Many of these situations can be anticipated (*e.g.*, after coronary artery bypass surgery, aortic aneurysm surgery, or neurosurgery). Many of these patients have a preexisting morbidity with metabolic syndrome, atherosclerosis, or diabetes in which reduced nitric oxide bioavailability is common because of decreased eNOS activity or increased nitric oxide scavenging by reactive oxygen species. In addition, preoperative fasting does not only reduce glycogen depots but also prevents the possibility to fuel the nitrate-nitrite-nitric oxide pathway. Based on the present findings showing protective effects of nitrate and nitrite in numerous models of I/R injury, it is of great interest to study whether preemptive administration of nitrate or nitrite, or perhaps a combination, could have beneficial effects. A combination of nitrate and nitrite salts for oral administration is theoretically attractive. Nitrite would provide immediate effects after absorption, whereas nitrate would work like a prodrug with a slow and sustained release of nitrite over a prolonged period of time *via* the enterosalivary recirculation described in the section on enterosalivary circulation of nitrate.

## Inhalation of Nitric Oxide and Nitrite

Nitric oxide inhalation is one of the few clinically approved nitric oxide-based therapies that have emerged from basic research.<sup>155–157</sup> It is used in infants with primary pulmonary hypertension of the newborn to reduce pulmonary artery pressure.<sup>158</sup> It is noteworthy that inhalation of nitric oxide does not only vasodilate pulmonary vessels but has also distant effects.<sup>159,160</sup> Humans breathing nitric oxide gas exhibit increases in peripheral forearm blood flow, which is associated with increases in plasma nitrite.<sup>161</sup> This suggests that nitrite could be a stable endocrine carrier of nitric oxide-like bioactivity in the circulation.

Recently, inhaled nitrite has been shown to have beneficial effects in animal models of pulmonary hypertension. Hunter *et al.* used nebulized nitrite to reduce pulmonary hypertension induced by hypoxia or a thromboxane analog.<sup>114</sup> During hypoxia-induced pulmonary hypertension, inhaled nitrite elicited a rapid and sustained reduction in pulmonary artery pressure with concomitant appearance of nitric oxide in exhaled air. This effect was coupled with deoxygenation of hemoglobin. The authors advocate that inhaled nitrite is a simple and inexpensive potential therapy for neonatal hypertension. Very recently, Zuckerbraun *et al.* used more chronic rodent models of pulmonary hypertension to test the effects of inhaled nitrite.<sup>145</sup> Again, pulmonary hypertension was prevented by inhaled nitrite but also right ventricular hypertrophy and failure. In these experiments, nitrite conversion to nitric oxide was dependent on XOR. In addition, hypoxia-induced proliferation of cultured pulmonary artery smooth muscle cells was inhibited by nitrite. On-

going studies will reveal whether inhaled nitrite will be an additional therapeutic tool in the clinic.

## Solid Organ Transplantation

Despite significant improvements in the management of solid organ transplantations, these procedures are still associated with a significant risk of allograft rejection. Both immunologic and nonimmunologic factors, including I/R injury, contribute to these events. In cardiac transplantation, allograft vasculopathy remains a dreaded complication leading to rejection.<sup>162</sup> Because nitric oxide has been shown to play a critical role in the maintenance of vascular integrity, and in light of the previously reported studies with salutary effects of nitrate and nitrite in I/R injury models, Zahn *et al.* investigated the effects of oral nitrite supplementation on cardiac allograft rejection in rats.<sup>163</sup> Animals were followed for 120 days, and treatment started 7 days before transplantation. Supplementation of drinking water with nitrite enhanced graft survival to more than 120 days compared with 50 days in control animals on a normal diet. In contrast, in animals on a low nitrate/nitrite diet, allograft survival was significantly reduced to 31 days. These differences were accompanied by amelioration of histopathologic changes in the allografts as well as in decreased tissue messenger RNA concentrations of interferon- $\gamma$  and tumor necrosis factor- $\alpha$ . Future studies will expand on these findings by also testing the addition of nitrite in organ preservation fluids and administration to donors and recipients combined.

Other ways to provide bioactive nitrogen oxide species therapeutically during transplantation procedures have been investigated. Apart from systemic administration of traditional nitric oxide donors, inhalation of nitric oxide has been studied during orthotopic liver transplantation in humans. It was hypothesized that nitric oxide inhalation would generate relatively stable nitric oxide-containing intermediates with effects in the transplanted liver. In a randomized, prospective, placebo-controlled study, Lang *et al.* inhaled nitric oxide (80 ppm) perioperatively and found improvement in posttransplantation liver function parameters and decreased hospital length of stay.<sup>164</sup> It did not affect inflammatory markers after reperfusion but significantly decreased hepatocyte apoptosis. The authors conclude that their findings support the clinical use of inhaled nitric oxide as an extrapulmonary therapeutic to improve organ function after transplantation. It is noteworthy that circulating nitrite increased significantly during nitric oxide inhalation, and arteriovenous gradients were observed, indicating metabolism of this anion to nitric oxide or other bioactive nitrogen oxides. In another study, the same group used inhaled nitric oxide in a human model of I/R injury (knee surgery) to show attenuation of the inflammatory response measured as reduced expression of CD11b/CD18, P-selectin, and lipid hydroperoxidase.<sup>165</sup> Again, increased plasma concentrations of nitrite accompanied these effects.

## Antimicrobial Effects of Nitrite

Acidification of nitrite results in formation of nitric oxide and other nitrogen oxide species with potent antimicrobial effects against a broad range of potential pathogens.<sup>29,62,166</sup> More recently, these antibacterial effects of nitrite have been investigated from a clinical perspective. Yoon *et al.* used acidified nitrite in an animal model of cystic fibrosis and were successful in clearing the airways of *Pseudomonas aeruginosa*, a common pathogen in patients with this disease.<sup>167</sup>

As mentioned above, nitrate is continuously excreted at relatively high concentrations in the urine. During a urinary tract infection, bacteria will reduce nitrate to nitrite, and in the clinic, nitrite test strips are routinely used to indicate an ongoing infection. Nitrite is reduced to nitric oxide and other nitrogen oxide species with potent antibacterial effects, if the urine is mildly acidic (pH 5–6).<sup>168</sup> Moreover, nitrite reduction to nitric oxide is greatly potentiated in the presence of the water-soluble and reducing agent, vitamin C.<sup>169</sup> It is noteworthy that acidification of urine with different compounds, including vitamin C and cranberry juice, has been used in traditional medicine for prevention and treatment of urinary tract infections.<sup>170</sup> *In vitro*, the antibacterial potency of nitrite and ascorbic acid is comparable with traditional antibiotics.<sup>171</sup> The use of indwelling urinary catheters is a major risk factor for catheter-associated urinary tract infection. In spite of optimal care and preventive measures, catheter-associated urinary tract infection is still one of the most common nosocomial infections.<sup>172</sup> Carlsson *et al.* used nitrite and ascorbic acid to generate antibacterial nitrogen species, including nitric oxide in an *in vitro* model of the urinary bladder.<sup>173</sup> By filling the retention balloon of a silicon urinary catheter with these compounds, they were able to generate sufficient amounts of nitric oxide that easily diffused into the surrounding urine. Two different strains of *Escherichia coli* that were grown in the urine were efficiently killed by this procedure. Later, the same group observed similar *in vitro* results on a variety of common urinary pathogens in a more advanced flow-through model of urinary tract infection (unpublished data, Eddie Weitzberg, M.D., Ph.D., Department of Physiology and Pharmacology, Karolinska Institute, Stockholm, Sweden, November 2009).

## Dietary Aspects

As mentioned before, vegetables are the main source of nitrate in our diet. Epidemiologic studies convincingly show that diets rich in fruits and vegetables, such as the Mediterranean diet, protect against development of cardiovascular disease and type 2 diabetes.<sup>115</sup> Moreover, intervention studies, such as the classic Dietary Approaches to Stop Hypertension trial, have shown blood pressure-lowering effects of such diets.<sup>116</sup> However, the active component(s) responsible for this protection has not been identified, and trials with single nutrients have generally failed. It is striking that the reduction in blood pressure seen by a modest dose of inorganic nitrate is similar or even greater than that seen with the

vegetable- and fruit-rich diet in the Dietary Approaches to Stop Hypertension trial. With the accumulating data on the beneficial effects of nitrate in the cardiovascular system, it is possible that nitrate might be one active ingredient in these healthy diets.<sup>174</sup> This development is remarkable considering that nitrate is just about the only naturally occurring compound in vegetables that is considered unwanted and potentially harmful. Although much more research is needed to establish the role of nitrate in our diet, the possibility of boosting nitric oxide production by dietary intervention is intriguing and may have important implications for public health.

It is noteworthy that enteral and parenteral nutrition contains extremely low amounts of nitrate and nitrite (unpublished data, Eddie Weitzberg, M.D., Ph.D., Department of Physiology and Pharmacology, Karolinska Institute, Stockholm, Sweden, 2004).

During a hospital stay, the primary use of enteral or parenteral feeding creates a situation of nitrate/nitrite starvation. Many of the patients subjected to anesthesia and intensive care have preexisting cardiovascular diseases with compromised endogenous nitric oxide production. Because accumulating evidence suggests that moderate doses of nitrate and nitrite have beneficial effects in the cardiovascular system, it is of great interest to study whether these anions can provide any improvement in anesthesiology and intensive care. In a wider context, future clinical studies will elucidate whether nitrate can offer a nutritional approach to prevention and treatment of cardiovascular disease and whether such positive effects will outweigh any negative health effects traditionally attributed to this anion.

## Summary and Future Perspectives

The recently discovered nitrate-nitrite-nitric oxide pathway provides an alternative route to supply nitric oxide-like bioactivity in addition to the classic L-arginine-NOS pathway. There are two main sources of nitrate fueling this pathway: nitrate from oxidized endogenous nitric oxide or dietary intake. Regardless of the nitrate source, oral commensal bacteria are essential in the bioactivation of nitrate, exemplifying a symbiotic host-microbial relationship. It is noteworthy that the several enzymatic and nonenzymatic routes that further reduce nitrite to nitric oxide are all enhanced during hypoxia and low pH situations when nitric oxide generation by the NOSs may be compromised.

A growing scientific interest in this pathway during the last 10 yr has provided therapeutic suggestions in a wide range of clinically interesting areas. Nitrate and nitrite has been shown to be beneficial in models of I/R injury to the heart, brain, liver, kidney, and lungs. Furthermore, administration of nitrate or nitrite positively affects gastric mucosal integrity, blood pressure, endothelial function, oxygen consumption during exercise, and basal mitochondrial function. In comparison with the traditional organic nitrates used in cardiovascular medicine, nitrate and nitrite do not seem to

induce tolerance, and their conversion to nitric oxide and other bioactive nitrogen oxides is enhanced by low  $\text{PO}_2$  and pH (*i.e.*, in areas of poor perfusion). Together, these findings have promoted ongoing clinical studies that may support a future use of these inorganic anions in clinical practice.

Although the therapeutic effects of exogenously delivered nitrate in animal models are unequivocal, the physiologic relevance of endogenously generated nitrate and nitrite is still unresolved. This is not trivial because in contrast to the NOS-dependent physiology, which has been explored by the use of selective NOS inhibitors, there are no specific nitrite reductase inhibitors available. Furthermore, the dual origin of nitrate and nitrite represents a major problem in experimental design.

The nutritional implications of nitrate and nitrite biology are exciting. The amounts of these anions needed for the effects on the cardiovascular system, described in this review, are readily achieved by our everyday diet. Future studies will elucidate whether the cardiovascular benefits of a diet rich in vegetables, such as the Mediterranean diet, are related to nitrate. If that is the case, we may have to reconsider our current thinking, and what is presently considered a harmful constituent may in the future be regarded as an essential nutrient.

Considering the aforementioned effects of nitrate and nitrite, there are several interesting issues related to anesthesiology and intensive care that are worth investigating. What are the consequences of preoperative fasting? Is the lack of nitrate and nitrite in our parenteral and enteral formulas harmful? What is the relevance of low gastric nitric oxide concentrations in intubated ICU patients? Could preemptive administration of nitrate or nitrite ameliorate perioperative I/R injury? Hopefully, future studies will be able to resolve some of these questions.

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