

Anesthesiology:  
December 2010 - Volume 113 - Issue 6 - pp 1460-1475  
doi: 10.1097/ALN.0b013e3181fcf3cc  
Education: Review Article

## **Nitrate-Nitrite-Nitric Oxide Pathway: Implications for Anesthesiology and Intensive Care**

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Received from the Department of Physiology and Pharmacology, Section of Anesthesiology and Intensive Care, Karolinska Institutet, Stockholm, Sweden. Submitted for publication August 21, 2010. Accepted for publication September 2, 2010. Support was provided by the Swedish Research Council, Torsten and Ragnar Söderbeks Foundation, Swedish Heart and Lung Foundation, Stockholm City Council, Vinnova (Chronic Inflammation Diagnosis and Therapy CIDaT), Karolinska Institutet (all in Stockholm, Sweden) and European Union 7th Framework Program (Flaviola), Brussels, Belgium. Dr. Weitzberg and Dr. Lundberg are named coinventors on patent applications (U.S. patent application nos. 12/528.794 and 12/528.798) relating to therapeutic use of inorganic nitrate and nitrite salts. The figures and tables in this article were prepared by Annemarie B. Johnson, C.M.I., Medical Illustrator, Wake Forest University School of Medicine Creative Communications, Wake Forest University Medical Center, Winston-Salem, North Carolina.

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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 aspects 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 ( $N_2$ ), 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 ( $NH_4^+$ ). Ammonium can then be oxidized to a variety of nitrogen oxides, including nitrite ( $NO_2^-$ ) and nitrate ( $NO_3^-$ ). The cycle is completed by the denitrification process where nitrate is serially reduced to nitrite, nitric oxide, nitrous oxide, and, finally, nitrogen gas ( $N_2$ ), 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 secretory 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>

Fig. 1

[Image Tools](#)

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

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## 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 activation 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 oxyhemoglobin, 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>

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### 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>

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### 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 reaction 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>

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## 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 gastric nitric oxide, the widespread use of H<sub>2</sub> 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.

Fig. 2

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## 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 nonenzymatic 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 *bc*<sub>1</sub><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.

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## 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 mmol nitrate · kg<sup>-1</sup> · day<sup>-1</sup>) 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 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.*

Table 1

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## 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 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  $P_{O_2}$  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>

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## 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 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>

Table 2

### [Image Tools](#)

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.

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## **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. Ongoing studies will reveal whether inhaled nitrite will be an additional therapeutic tool in the clinic.

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## **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.

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### 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).

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### 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.

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### **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  $P_{O_2}$  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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## References

1. Mitchell H, Shonle H, Grindley H: The origin of nitrates in the urine. *J Biol Chem* 1916; 24:461–90  
[Cited Here...](#)
2. Green LC, Tannenbaum SR, Goldman P: Nitrate synthesis in the germfree and conventional rat. *Science* 1981; 212:56–8  
[Cited Here...](#)
3. Furchgott RF, Zawadzki JV: The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* 1980; 288:373–6  
[Cited Here...](#)
4. Ignarro LJ, Buga GM, Wood KS, Byrns RE, Chaudhuri G: Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. *Proc Natl Acad Sci U S A* 1987; 84:9265–9  
[Cited Here...](#)
5. Palmer RM, Ferrige AG, Moncada S: Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature* 1987; 327:524–6  
[Cited Here...](#)
6. Moncada S, Higgs A: The L-arginine-nitric oxide pathway. *N Engl J Med* 1993; 329:2002–12  
[Cited Here...](#)
7. Murad F: Shattuck Lecture. Nitric oxide and cyclic GMP in cell signaling and drug development. *N Engl J Med* 2006; 355:2003–11  
[Cited Here...](#)
8. Lundberg JO, Weitzberg E, Lundberg JM, Alving K: Intra-gastric nitric oxide production in humans:

Measurements in expelled air. *Gut* 1994; 35:1543–6

[Cited Here...](#)

9. Benjamin N, O'Driscoll F, Dougall H, Duncan C, Smith L, Golden M, McKenzie H: Stomach NO synthesis. *Nature* 1994; 368:502

[Cited Here...](#)

10. Spiegelhalder B, Eisenbrand G, Preussmann R: Influence of dietary nitrate on nitrite content of human saliva: Possible relevance to *in vivo* formation of N-nitroso compounds. *Food Cosmet Toxicol* 1976; 14:545–8

[Cited Here...](#)

11. Zweier JL, Li H, Samouilov A, Liu X: Mechanisms of nitrite reduction to nitric oxide in the heart and vessel wall. *Nitric Oxide* 2010; 22:83–90

[Cited Here...](#)

12. Weitzberg E, Lundberg JO: Nonenzymatic nitric oxide production in humans. *Nitric Oxide* 1998; 2:1–7

[Cited Here...](#)

13. Lundberg JO, Weitzberg E, Gladwin MT: The nitrate-nitrite-nitric oxide pathway in physiology and therapeutics. *Nat Rev Drug Discov* 2008; 7:156–67

[Cited Here...](#)

14. van Faassen EE, Bahrami S, Feelisch M, Hogg N, Kelm M, Kim-Shapiro DB, Kozlov AV, Li H, Lundberg JO, Mason R, Nohl H, Rassaf T, Samouilov A, Slama-Schwok A, Shiva S, Vanin AF, Weitzberg E, Zweier J, Gladwin MT: Nitrite as regulator of hypoxic signaling in mammalian physiology. *Med Res Rev* 2009; 29:683–741

[Cited Here...](#)

15. Lundberg JO, Gladwin MT, Ahluwalia A, Benjamin N, Bryan NS, Butler A, Cabrales P, Fago A, Feelisch M, Ford PC, Freeman BA, Frenneaux M, Friedman J, Kelm M, Kevil CG, Kim-Shapiro DB, Kozlov AV, Lancaster JR Jr., Lefler DJ, McColl K, McCurry K, Patel RP, Petersson J, Rassaf T, Reutov VP, Richter-Addo GB, Schechter A, Shiva S, Tsuchiya K, van Faassen EE, Webb AJ, Zuckerbraun BS, Zweier JL, Weitzberg E: Nitrate and nitrite in biology, nutrition and therapeutics. *Nat Chem Biol* 2009; 5:865–9

[Cited Here...](#)

16. Lundberg JO, Feelisch M, Björne H, Jansson EA, Weitzberg E: Cardioprotective effects of vegetables: Is nitrate the answer? *Nitric Oxide* 2006; 15:359–62

[Cited Here...](#)

17. Bryan NS: Cardioprotective actions of nitrite therapy and dietary considerations. *Front Biosci* 2009; 14:4793–808

[Cited Here...](#)

18. Bian K, Doursout MF, Murad F: Vascular system: Role of nitric oxide in cardiovascular diseases. *J Clin Hypertens (Greenwich)* 2008; 10:304–10

[Cited Here...](#)

19. Alonso D, Radomski MW: Nitric oxide, platelet function, myocardial infarction and reperfusion therapies. *Heart Fail Rev* 2003; 8:47–54  
[Cited Here...](#)
20. Ricciardolo FL, Sterk PJ, Gaston B, Folkerts G: Nitric oxide in health and disease of the respiratory system. *Physiol Rev* 2004; 84:731–65  
[Cited Here...](#)
21. Calabrese V, Mancuso C, Calvani M, Rizzarelli E, Butterfield DA, Stella AM: Nitric oxide in the central nervous system: Neuroprotection *versus* neurotoxicity. *Nat Rev Neurosci* 2007; 8:766–75  
[Cited Here...](#)
22. Bogdan C: Nitric oxide and the immune response. *Nat Immunol* 2001; 2:907–16  
[Cited Here...](#)
23. Moncada S, Erusalimsky JD: Does nitric oxide modulate mitochondrial energy generation and apoptosis? *Nat Rev Mol Cell Biol* 2002; 3:214–20  
[Cited Here...](#)
24. Toda N, Toda H, Hatano Y: Nitric oxide: Involvement in the effects of anesthetic agents. *Anesthesiology* 2007; 107:822–42  
[Cited Here...](#)
25. Miclescu A, Gordh T: Nitric oxide and pain: “Something old, something new.” *Acta Anaesthesiol Scand* 2009; 53:1107–20  
[Cited Here...](#)
26. Stamler JS: S-Nitrosothiols and the bioregulatory actions of nitrogen oxides through reactions with thiol groups. *Curr Top Microbiol Immunol* 1995; 196:19–36  
[Cited Here...](#)
27. Stamler JS, Lamas S, Fang FC: Nitrosylation. the prototypic redox-based signaling mechanism. *Cell* 2001; 106:675–83  
[Cited Here...](#)
28. Stamler JS: S-Nitrosothiols in the blood: Roles, amounts, and methods of analysis. *Circ Res* 2004; 94:414–7  
[Cited Here...](#)
29. Lundberg JO, Weitzberg E, Cole JA, Benjamin N: Nitrate, bacteria and human health. *Nat Rev Microbiol* 2004; 2:593–602  
[Cited Here...](#)
30. Heiss C, Lauer T, Dejam A, Kleinbongard P, Hamada S, Rassaf T, Matern S, Feelisch M, Kelm M: Plasma nitroso compounds are decreased in patients with endothelial dysfunction. *J Am Coll Cardiol* 2006; 47:573–9  
[Cited Here...](#)
31. Huang PL: eNOS, metabolic syndrome and cardiovascular disease. *Trends Endocrinol Metab* 2009; 20:295–302

[Cited Here...](#)

32. Vallance P, Moncada S: Role of endogenous nitric oxide in septic shock. *New Horiz* 1993; 1:77–86

[Cited Here...](#)

33. Moshage H, Kok B, Huizenga JR, Jansen PL: Nitrite and nitrate determinations in plasma: A critical evaluation. *Clin Chem* 1995; 41:892–6

[Cited Here...](#)

34. Shiva S, Wang X, Ringwood LA, Xu X, Yuditskaya S, Annavajjhala V, Miyajima H, Hogg N, Harris ZL, Gladwin MT: Ceruloplasmin is a NO oxidase and nitrite synthase that determines endocrine NO homeostasis. *Nat Chem Biol* 2006; 2:486–93

[Cited Here...](#)

35. Moncada S, Palmer RM, Higgs EA: Nitric oxide: Physiology, pathophysiology, and pharmacology. *Pharmacol Rev* 1991; 43:109–42

[Cited Here...](#)

36. Wagner DA, Schultz DS, Deen WM, Young VR, Tannenbaum SR: Metabolic fate of an oral dose of <sup>15</sup>N-labeled nitrate in humans: Effect of diet supplementation with ascorbic acid. *Cancer Res* 1983; 43:1921–5

[Cited Here...](#)

37. Campillo B, Bories PN, Benvenuti C, Dupeyron C: Serum and urinary nitrate concentrations in liver cirrhosis: Endotoxemia, renal function and hyperdynamic circulation. *J Hepatol* 1996; 25:707–14

[Cited Here...](#)

38. Yang F, Comtois AS, Fang L, Hartman NG, Blaise G: Nitric oxide-derived nitrate anion contributes to endotoxic shock and multiple organ injury/dysfunction. *Crit Care Med* 2002; 30:650–7

[Cited Here...](#)

39. Kleinbongard P, Dejam A, Lauer T, Rassaf T, Schindler A, Picker O, Scheeren T, Gödecke A, Schrader J, Schulz R, Heusch G, Schaub GA, Bryan NS, Feelisch M, Kelm M: Plasma nitrite reflects constitutive nitric oxide synthase activity in mammals. *Free Radic Biol Med* 2003; 35:790–6

[Cited Here...](#)

40. Jungersten L, Ambring A, Wall B, Wennmalm A: Both physical fitness and acute exercise regulate nitric oxide formation in healthy humans. *J Appl Physiol* 1997; 82:760–4

[Cited Here...](#)

41. Crawford JH, Chacko BK, Pruitt HM, Pikhova B, Hogg N, Patel RP: Transduction of NO-bioactivity by the red blood cell in sepsis: Novel mechanisms of vasodilation during acute inflammatory disease. *Blood* 2004; 104:1375–82

[Cited Here...](#)

42. Herulf M, Svenungsson B, Lagergren A, Ljung T, Morcos E, Wiklund NP, Lundberg JO, Weitzberg E: Increased nitric oxide in infective gastroenteritis. *J Infect Dis* 1999; 180:542–5

[Cited Here...](#)

43. Fujiwara N, Osanai T, Kamada T, Katoh T, Takahashi K, Okumura K: Study on the relationship

between plasma nitrite and nitrate level and salt sensitivity in human hypertension: Modulation of nitric oxide synthesis by salt intake. *Circulation* 2000; 101:856–61

[Cited Here...](#)

44. Lundberg JO: Cardiovascular prevention by dietary nitrate and nitrite. *Am J Physiol Heart Circ Physiol* 2009; 296:H1221–3

[Cited Here...](#)

45. Lundberg JO, Govoni M: Inorganic nitrate is a possible source for systemic generation of nitric oxide. *Free Radic Biol Med* 2004; 37:395–400

[Cited Here...](#)

46. Ward MH, deKok TM, Levallois P, Brender J, Gulis G, Nolan BT, VanDerslice J, International Society for Environment Epidemiology: Workgroup report: Drinking-water nitrate and health—recent findings and research needs. *Environ Health Perspect* 2005; 113:1607–14

[Cited Here...](#)

47. Tannenbaum SR, Correa P: Nitrate and gastric cancer risks. *Nature* 1985; 317:675–6

[Cited Here...](#)

48. Mirvish SS: Role of N-nitroso compounds (NOC) and N-nitrosation in etiology of gastric, esophageal, nasopharyngeal and bladder cancer and contribution to cancer of known exposures to NOC. *Cancer Lett* 1995; 93:17–48

[Cited Here...](#)

49. Powlson DS, Addiscott TM, Benjamin N, Cassman KG, de Kok TM, van Grinsven H, L'Hirondel JL, Avery AA, van Kessel C: When does nitrate become a risk for humans? *J Environ Qual* 2008; 37:291–5

[Cited Here...](#)

50. Govoni M, Jansson EA, Weitzberg E, Lundberg JO: The increase in plasma nitrite after a dietary nitrate load is markedly attenuated by an antibacterial mouthwash. *Nitric Oxide* 2008; 19:333–7

[Cited Here...](#)

51. Li H, Duncan C, Townend J, Killham K, Smith LM, Johnston P, Dykhuizen R, Kelly D, Golden M, Benjamin N, Leifert C: Nitrate-reducing bacteria on rat tongues. *Appl Environ Microbiol* 1997; 63:924–30

[Cited Here...](#)

52. Duncan C, Dougall H, Johnston P, Green S, Brogan R, Leifert C, Smith L, Golden M, Benjamin N: Chemical generation of nitric oxide in the mouth from the enterosalivary circulation of dietary nitrate. *Nat Med* 1995; 1:546–51

[Cited Here...](#)

53. Björne H, Govoni M, Törnberg DC, Lundberg JO, Weitzberg E: Intragastric nitric oxide is abolished in intubated patients and restored by nitrite. *Crit Care Med* 2005; 33:1722–7

[Cited Here...](#)

54. Gago B, Lundberg JO, Barbosa RM, Laranjinha J: Red wine-dependent reduction of nitrite to nitric oxide in the stomach. *Free Radic Biol Med* 2007; 43:1233–42

[Cited Here...](#)

55. Webb AJ, Patel N, Loukogeorgakis S, Okorie M, Aboud Z, Misra S, Rashid R, Miall P, Deanfield J, Benjamin N, MacAllister R, Hobbs AJ, Ahluwalia A: Acute blood pressure lowering, vasoprotective, and antiplatelet properties of dietary nitrate *via* bioconversion to nitrite. *Hypertension* 2008; 51:784–90  
[Cited Here...](#)
56. Petersson J, Carlström M, Schreiber O, Phillipson M, Christoffersson G, Jägare A, Roos S, Jansson EA, Persson AE, Lundberg JO, Holm L: Gastroprotective and blood pressure lowering effects of dietary nitrate are abolished by an antiseptic mouthwash. *Free Radic Biol Med* 2009; 46:1068–75  
[Cited Here...](#)
57. Sobko T, Reinders C, Norin E, Midtvedt T, Gustafsson LE, Lundberg JO: Gastrointestinal nitric oxide generation in germ-free and conventional rats. *Am J Physiol Gastrointest Liver Physiol* 2004; 287:G993–7  
[Cited Here...](#)
58. Fang FC: Antimicrobial reactive oxygen and nitrogen species: Concepts and controversies. *Nat Rev Microbiol* 2004; 2:820–32  
[Cited Here...](#)
59. Björne H, Weitzberg E, Lundberg JO: Intra-gastric generation of antimicrobial nitrogen oxides from saliva—physiological and therapeutic considerations. *Free Radic Biol Med* 2006; 41:1404–12  
[Cited Here...](#)
60. Duncan C, Li H, Dykhuizen R, Frazer R, Johnston P, MacKnight G, Smith L, Lamza K, McKenzie H, Batt L, Kelly D, Golden M, Benjamin N, Leifert C: Protection against oral and gastrointestinal diseases: Importance of dietary nitrate intake, oral nitrate reduction and enterosalivary nitrate circulation. *Comp Biochem Physiol A Physiol* 1997; 118:939–48  
[Cited Here...](#)
61. Dykhuizen RS, Frazer R, Duncan C, Smith CC, Golden M, Benjamin N, Leifert C: Antimicrobial effect of acidified nitrite on gut pathogens: Importance of dietary nitrate in host defense. *Antimicrob Agents Chemother* 1996; 40:1422–5  
[Cited Here...](#)
62. Dykhuizen RS, Fraser A, McKenzie H, Golden M, Leifert C, Benjamin N: *Helicobacter pylori* is killed by nitrite under acidic conditions. *Gut* 1998; 42:334–7  
[Cited Here...](#)
63. Björne HH, Petersson J, Phillipson M, Weitzberg E, Holm L, Lundberg JO: Nitrite in saliva increases gastric mucosal blood flow and mucus thickness. *J Clin Invest* 2004; 113:106–14  
[Cited Here...](#)
64. Petersson J, Phillipson M, Jansson EA, Patzak A, Lundberg JO, Holm L: Dietary nitrate increases gastric mucosal blood flow and mucosal defense. *Am J Physiol Gastrointest Liver Physiol* 2007; 292:G718–24  
[Cited Here...](#)
65. Miyoshi M, Kasahara E, Park AM, Hiramoto K, Minamiyama Y, Takemura S, Sato EF, Inoue M: Dietary nitrate inhibits stress-induced gastric mucosal injury in the rat. *Free Radic Res* 2003; 37:85–90  
[Cited Here...](#)

66. Jansson EA, Petersson J, Reinders C, Sobko T, Björne H, Phillipson M, Weitzberg E, Holm L, Lundberg JO: Protection from nonsteroidal anti-inflammatory drug (NSAID)-induced gastric ulcers by dietary nitrate. *Free Radic Biol Med* 2007; 42:510–8

[Cited Here...](#)

67. Boivin MA, Fiack CA, Kennedy JC, Iwamoto GK: Etiology of decreased gastric nitric oxide in the critically ill. *J Investig Med* 2006; 54:484–9

[Cited Here...](#)

68. Modin A, Björne H, Herulf M, Alving K, Weitzberg E, Lundberg JO: Nitrite-derived nitric oxide: A possible mediator of ‘acidic-metabolic’ vasodilation. *Acta Physiol Scand* 2001; 171:9–16

[Cited Here...](#)

69. Brooks J: The action of nitrite on haemoglobin in the absence of oxygen. *Proc R Soc Med* 1937; 137:368–82

[Cited Here...](#)

70. Doyle MP, Pickering RA, DeWeert TM, Hoekstra JW, Pater D: Kinetics and mechanism of the oxidation of human deoxyhemoglobin by nitrites. *J Biol Chem* 1981; 256:12393–8

[Cited Here...](#)

71. Cosby K, Partovi KS, Crawford JH, Patel RP, Reiter CD, Martyr S, Yang BK, Waclawiw MA, Zalos G, Xu X, Huang KT, Shields H, Kim-Shapiro DB, Schechter AN, Cannon RO 3rd, Gladwin MT: Nitrite reduction to nitric oxide by deoxyhemoglobin vasodilates the human circulation. *Nat Med* 2003; 9:1498–505

[Cited Here...](#)

72. Nagababu E, Ramasamy S, Abernethy DR, Rifkind JM: Active nitric oxide produced in the red cell under hypoxic conditions by deoxyhemoglobin-mediated nitrite reduction. *J Biol Chem* 2003; 278:46349–56

[Cited Here...](#)

73. Huang KT, Keszler A, Patel N, Patel RP, Gladwin MT, Kim-Shapiro DB, Hogg N: The reaction between nitrite and deoxyhemoglobin. Reassessment of reaction kinetics and stoichiometry. *J Biol Chem* 2005; 280:31126–31

[Cited Here...](#)

74. Huang Z, Shiva S, Kim-Shapiro DB, Patel RP, Ringwood LA, Irby CE, Huang KT, Ho C, Hogg N, Schechter AN, Gladwin MT: Enzymatic function of hemoglobin as a nitrite reductase that produces NO under allosteric control. *J Clin Invest* 2005; 115:2099–107

[Cited Here...](#)

75. Stamler JS, Jia L, Eu JP, McMahon TJ, Demchenko IT, Bonaventura J, Gernert K, Piantadosi CA: Blood flow regulation by *S*-nitrosohemoglobin in the physiological oxygen gradient. *Science* 1997; 276:2034–7

[Cited Here...](#)

76. Angelo M, Singel DJ, Stamler JS: An *S*-nitrosothiol (SNO) synthase function of hemoglobin that utilizes nitrite as a substrate. *Proc Natl Acad Sci U S A* 2006; 103:8366–71

[Cited Here...](#)

77. Schwab DE, Stamler JS, Singel DJ: Nitrite-methemoglobin inadequate for hypoxic vasodilation. *Nat Chem Biol* 2009; 5:366–7  
[Cited Here...](#)
78. Isbell TS, Sun CW, Wu LC, Teng X, Vitturi DA, Branch BG, Kevil CG, Peng N, Wyss JM, Ambalavanan N, Schwiebert L, Ren J, Pawlik KM, Renfrow MB, Patel RP, Townes TM: SNO-hemoglobin is not essential for red blood cell-dependent hypoxic vasodilation. *Nat Med* 2008; 14:773–7  
[Cited Here...](#)
79. Shiva S, Huang Z, Grubina R, Sun J, Ringwood LA, MacArthur PH, Xu X, Murphy E, Darley-USmar VM, Gladwin MT: Deoxymyoglobin is a nitrite reductase that generates nitric oxide and regulates mitochondrial respiration. *Circ Res* 2007; 100:654–61  
[Cited Here...](#)
80. Rassaf T, Flögel U, Drexhage C, Hendgen-Cotta U, Kelm M, Schrader J: Nitrite reductase function of deoxymyoglobin: Oxygen sensor and regulator of cardiac energetics and function. *Circ Res* 2007; 100:1749–54  
[Cited Here...](#)
81. Hendgen-Cotta UB, Merx MW, Shiva S, Schmitz J, Becher S, Klare JP, Steinhoff HJ, Goedecke A, Schrader J, Gladwin MT, Kelm M, Rassaf T: Nitrite reductase activity of myoglobin regulates respiration and cellular viability in myocardial ischemia-reperfusion injury. *Proc Natl Acad Sci U S A* 2008; 105:10256–61  
[Cited Here...](#)
82. Flögel U, Merx MW, Godecke A, Decking UK, Schrader J: Myoglobin: A scavenger of bioactive NO. *Proc Natl Acad Sci U S A* 2001; 98:735–40  
[Cited Here...](#)
83. Petersen MG, Dewilde S, Fago A: Reactions of ferrous neuroglobin and cytoglobin with nitrite under anaerobic conditions. *J Inorg Biochem* 2008; 102:1777–82  
[Cited Here...](#)
84. Zhang Z, Naughton DP, Blake DR, Benjamin N, Stevens CR, Winyard PG, Symons MC, Harrison R: Human xanthine oxidase converts nitrite ions into nitric oxide (NO). *Biochem Soc Trans* 1997; 25: 524S  
[Cited Here...](#)
85. Godber BL, Doel JJ, Sapkota GP, Blake DR, Stevens CR, Eisenthal R, Harrison R: Reduction of nitrite to nitric oxide catalyzed by xanthine oxidoreductase. *J Biol Chem* 2000; 275:7757–63  
[Cited Here...](#)
86. Millar TM, Stevens CR, Benjamin N, Eisenthal R, Harrison R, Blake DR: Xanthine oxidoreductase catalyses the reduction of nitrates and nitrite to nitric oxide under hypoxic conditions. *FEBS Lett* 1998; 427:225–8  
[Cited Here...](#)
87. Harrison R: Physiological roles of xanthine oxidoreductase. *Drug Metab Rev* 2004; 36:363–75  
[Cited Here...](#)

88. Jansson EA, Huang L, Malkey R, Govoni M, Nihlén C, Olsson A, Stensdotter M, Petersson J, Holm L, Weitzberg E, Lundberg JO: A mammalian functional nitrate reductase that regulates nitrite and nitric oxide homeostasis. *Nat Chem Biol* 2008; 4:411–7

[Cited Here...](#)

89. Huang L, Borniquel S, Lundberg JO: Enhanced xanthine oxidoreductase expression and tissue nitrate reduction in germ free mice. *Nitric Oxide* 2010; 22:191–5

[Cited Here...](#)

90. Kozlov AV, Staniek K, Nohl H: Nitrite reductase activity is a novel function of mammalian mitochondria. *FEBS Lett* 1999; 454:127–30

[Cited Here...](#)

91. Castello PR, David PS, McClure T, Crook Z, Poyton RO: Mitochondrial cytochrome oxidase produces nitric oxide under hypoxic conditions: Implications for oxygen sensing and hypoxic signaling in eukaryotes. *Cell Metab* 2006; 3:277–87

[Cited Here...](#)

92. Nohl H, Staniek K, Sobhian B, Bahrami S, Redl H, Kozlov AV: Mitochondria recycle nitrite back to the bioregulator nitric monoxide. *Acta Biochim Pol* 2000; 47:913–21

[Cited Here...](#)

93. Brown GC, Cooper CE: Nanomolar concentrations of nitric oxide reversibly inhibit synaptosomal respiration by competing with oxygen at cytochrome oxidase. *FEBS Lett* 1994; 356:295–8

[Cited Here...](#)

94. Bolaños JP, Peuchen S, Heales SJ, Land JM, Clark JB: Nitric oxide-mediated inhibition of the mitochondrial respiratory chain in cultured astrocytes. *J Neurochem* 1994; 63:910–6

[Cited Here...](#)

95. Cleeter MW, Cooper JM, Darley-Usmar VM, Moncada S, Schapira AH: Reversible inhibition of cytochrome c oxidase, the terminal enzyme of the mitochondrial respiratory chain, by nitric oxide. Implications for neurodegenerative diseases. *FEBS Lett* 1994; 345:50–4

[Cited Here...](#)

96. Taylor CT, Moncada S: Nitric oxide, cytochrome C oxidase, and the cellular response to hypoxia. *Arterioscler Thromb Vasc Biol* 2010; 30:643–7

[Cited Here...](#)

97. Li H, Cui H, Kundu TK, Alzawahra W, Zweier JL: Nitric oxide production from nitrite occurs primarily in tissues not in the blood: Critical role of xanthine oxidase and aldehyde oxidase. *J Biol Chem* 2008; 283:17855–63

[Cited Here...](#)

98. Golwala NH, Hodenette C, Murthy SN, Nossaman BD, Kadowitz PJ: Vascular responses to nitrite are mediated by xanthine oxidoreductase and mitochondrial aldehyde dehydrogenase in the rat. *Can J Physiol Pharmacol* 2009; 87:1095–101

[Cited Here...](#)

99. Chen Z, Zhang J, Stamler JS: Identification of the enzymatic mechanism of nitroglycerin bioactivation.

Proc Natl Acad Sci U S A 2002; 99:8306–11

[Cited Here...](#)

100. Kozlov AV, Dietrich B, Nohl H: Various intracellular compartments cooperate in the release of nitric oxide from glycerol trinitrate in liver. *Br J Pharmacol* 2003; 139:989–97

[Cited Here...](#)

101. Khatsenko O: Interactions between nitric oxide and cytochrome P-450 in the liver. *Biochemistry (Mosc)* 1998; 63:833–9

[Cited Here...](#)

102. Gautier C, van Faassen E, Mikula I, Martasek P, Slama-Schwok A: Endothelial nitric oxide synthase reduces nitrite anions to NO under anoxia. *Biochem Biophys Res Commun* 2006; 341:816–21

[Cited Here...](#)

103. Vanin AF, Bevers LM, Slama-Schwok A, van Faassen EE: Nitric oxide synthase reduces nitrite to NO under anoxia. *Cell Mol Life Sci* 2007; 64:96–103

[Cited Here...](#)

104. Webb AJ, Milsom AB, Rathod KS, Chu WL, Qureshi S, Lovell MJ, Lecomte FM, Perrett D, Raimondo C, Khoshbin E, Ahmed Z, Uppal R, Benjamin N, Hobbs AJ, Ahluwalia A: Mechanisms underlying erythrocyte and endothelial nitrite reduction to nitric oxide in hypoxia: Role for xanthine oxidoreductase and endothelial nitric oxide synthase. *Circ Res* 2008; 103:957–64

[Cited Here...](#)

105. Milsom AB, Patel NS, Mazzon E, Tripatara P, Storey A, Mota-Filipe H, Sepodes B, Webb AJ, Cuzzocrea S, Hobbs AJ, Thiemermann C, Ahluwalia A: Role for endothelial nitric oxide synthase in nitrite-induced protection against renal ischemia-reperfusion injury in mice. *Nitric Oxide* 2010; 22:141–8

[Cited Here...](#)

106. Butler AR, Feelisch M: Therapeutic uses of inorganic nitrite and nitrate: From the past to the future. *Circulation* 2008; 117:2151–9

[Cited Here...](#)

107. Tsuchiya K, Kanematsu Y, Yoshizumi M, Ohnishi H, Kirima K, Izawa Y, Shikishima M, Ishida T, Kondo S, Kagami S, Takiguchi Y, Tamaki T: Nitrite is an alternative source of NO *in vivo*. *Am J Physiol Heart Circ Physiol* 2005; 288:H2163–70

[Cited Here...](#)

108. Pluta RM, Dejam A, Grimes G, Gladwin MT, Oldfield EH: Nitrite infusions to prevent delayed cerebral vasospasm in a primate model of subarachnoid hemorrhage. *JAMA* 2005; 293:1477–84

[Cited Here...](#)

109. Dias-Junior CA, Gladwin MT, Tanus-Santos JE: Low-dose intravenous nitrite improves hemodynamics in a canine model of acute pulmonary thromboembolism. *Free Radic Biol Med* 2006; 41:1764–70

[Cited Here...](#)

110. Mack AK, McGowan Ii VR, Tremonti CK, Ackah D, Barnett C, Machado RF, Gladwin MT, Kato GJ: Sodium nitrite promotes regional blood flow in patients with sickle cell disease: A phase I/II study. *Br*

J Haematol 2008; 142:971–8

[Cited Here...](#)

111. Ingram TE, Pinder AG, Bailey DM, Fraser AG, James PE: Low-dose sodium nitrite vasodilates hypoxic human pulmonary vasculature by a means that is not dependent on a simultaneous elevation in plasma nitrite. *Am J Physiol Heart Circ Physiol* 2010; 298:H331–9

[Cited Here...](#)

112. Maher AR, Milsom AB, Gunaruwan P, Abozguia K, Ahmed I, Weaver RA, Thomas P, Ashrafian H, Born GV, James PE, Frenneaux MP: Hypoxic modulation of exogenous nitrite-induced vasodilation in humans. *Circulation* 2008; 117:670–7

[Cited Here...](#)

113. Dejam A, Hunter CJ, Tremonti C, Pluta RM, Hon YY, Grimes G, Partovi K, Pelletier MM, Oldfield EH, Cannon RO 3rd, Schechter AN, Gladwin MT: Nitrite infusion in humans and nonhuman primates: Endocrine effects, pharmacokinetics, and tolerance formation. *Circulation* 2007; 116:1821–31

[Cited Here...](#)

114. Hunter CJ, Dejam A, Blood AB, Shields H, Kim-Shapiro DB, Machado RF, Tarekegn S, Mulla N, Hopper AO, Schechter AN, Power GG, Gladwin MT: Inhaled nebulized nitrite is a hypoxia-sensitive NO-dependent selective pulmonary vasodilator. *Nat Med* 2004; 10:1122–7

[Cited Here...](#)

115. Willett WC: Diet and health: What should we eat? *Science* 1994; 264:532–7

[Cited Here...](#)

116. Liese AD, Nichols M, Sun X, D'Agostino RB Jr, Haffner SM: Adherence to the DASH Diet is inversely associated with incidence of type 2 diabetes: The insulin resistance atherosclerosis study. *Diabetes Care* 2009; 32:1434–6

[Cited Here...](#)

117. Joshipura KJ, Hu FB, Manson JE, Stampfer MJ, Rimm EB, Speizer FE, Colditz G, Ascherio A, Rosner B, Spiegelman D, Willett WC: The effect of fruit and vegetable intake on risk for coronary heart disease. *Ann Intern Med* 2001; 134:1106–14

[Cited Here...](#)

118. Joshipura KJ, Ascherio A, Manson JE, Stampfer MJ, Rimm EB, Speizer FE, Hennekens CH, Spiegelman D, Willett WC: Fruit and vegetable intake in relation to risk of ischemic stroke. *Jama* 1999; 282:1233–9

[Cited Here...](#)

119. Larsen FJ, Ekblom B, Sahlin K, Lundberg JO, Weitzberg E: Effects of dietary nitrate on blood pressure in healthy volunteers. *N Engl J Med* 2006; 355:2792–3

[Cited Here...](#)

120. Larsen FJ, Weitzberg E, Lundberg JO, Ekblom B: Dietary nitrate reduces maximal oxygen consumption while maintaining work performance in maximal exercise. *Free Radic Biol Med* 2010; 48:342–7

[Cited Here...](#)

121. Richardson G, Hicks SL, O'Byrne S, Frost MT, Moore K, Benjamin N, McKnight GM: The ingestion of inorganic nitrate increases gastric S-nitrosothiol levels and inhibits platelet function in humans. *Nitric Oxide* 2002; 7:24–9

[Cited Here...](#)

122. Kapil V, Milsom AB, Okorie M, Maleki-Toyserkani S, Akram F, Rehman F, Arghandawi S, Pearl V, Benjamin N, Loukogeorgakis S, Macallister R, Hobbs AJ, Webb AJ, Ahluwalia A: Inorganic nitrate supplementation lowers blood pressure in humans: Role for nitrite-derived NO. *Hypertension* 2010; 56:274–81

[Cited Here...](#)

123. Kanematsu Y, Yamaguchi K, Ohnishi H, Motobayashi Y, Ishizawa K, Izawa Y, Kawazoe K, Kondo S, Kagami S, Tomita S, Tsuchiya K, Tamaki T: Dietary doses of nitrite restore circulating nitric oxide level and improve renal injury in L-NAME-induced hypertensive rats. *Am J Physiol Renal Physiol* 2008; 295:F1457–62

[Cited Here...](#)

124. Larsen FJ, Weitzberg E, Lundberg JO, Eklom B: Effects of dietary nitrate on oxygen cost during exercise. *Acta Physiol (Oxf)* 2007; 191:59–66

[Cited Here...](#)

125. Shiva S, Sack MN, Greer JJ, Duranski M, Ringwood LA, Burwell L, Wang X, MacArthur PH, Shoja A, Raghavachari N, Calvert JW, Brookes PS, Lefer DJ, Gladwin MT: Nitrite augments tolerance to ischemia/reperfusion injury *via* the modulation of mitochondrial electron transfer. *J Exp Med* 2007; 204:2089–102

[Cited Here...](#)

126. Erusalimsky JD, Moncada S: Nitric oxide and mitochondrial signaling: From physiology to pathophysiology. *Arterioscler Thromb Vasc Biol* 2007; 27:2524–31

[Cited Here...](#)

127. Shiva S: Mitochondria as metabolizers and targets of nitrite. *Nitric Oxide* 2010; 22:64–74

[Cited Here...](#)

128. Unitt DC, Hollis VS, Palacios-Callender M, Frakich N, Moncada S: Inactivation of nitric oxide by cytochrome c oxidase under steady-state oxygen conditions. *Biochim Biophys Acta* 2010; 1797:371–7

[Cited Here...](#)

129. Bailey SJ, Winyard P, Vanhatalo A, Blackwell JR, DiMenna FJ, Wilkerson DP, Tarr J, Benjamin N, Jones AM: Dietary nitrate supplementation reduces the O<sub>2</sub> cost of low-intensity exercise and enhances tolerance to high-intensity exercise in humans. *J Appl Physiol* 2009; 107:1144–55

[Cited Here...](#)

130. Vanhatalo A, Bailey SJ, Blackwell JR, DiMenna FJ, Pavey TG, Wilkerson DP, Benjamin N, Winyard PG, Jones AM: Acute and chronic effects of dietary nitrate supplementation on blood pressure and the physiological responses to moderate-intensity and incremental exercise. *Am J Physiol Regul Integr Comp Physiol* 2010; 299:R1121–31

[Cited Here...](#)

131. Bailey SJ, Fulford J, Vanhatalo A, Winyard PG, Blackwell JR, DiMenna FJ, Wilkerson DP,

Benjamin N, Jones AM: Dietary nitrate supplementation enhances muscle contractile efficiency during knee-extensor exercise in humans. *J Appl Physiol* 2010; 109:135–48

[Cited Here...](#)

132. Gouspillou G, Bourdel-Marchasson I, Rouland R, Calmettes G, Franconi JM, Deschodt-Arsac V, Diolez P: Alteration of mitochondrial oxidative phosphorylation in aged skeletal muscle involves modification of adenine nucleotide translocator. *Biochim Biophys Acta* 2010; 1797:143–51

[Cited Here...](#)

133. Yellon DM, Hausenloy DJ: Myocardial reperfusion injury. *N Engl J Med* 2007; 357:1121–35

[Cited Here...](#)

134. Schulz R, Kelm M, Heusch G: Nitric oxide in myocardial ischemia/reperfusion injury. *Cardiovasc Res* 2004; 61:402–13

[Cited Here...](#)

135. Lefer DJ: Myocardial protective actions of nitric oxide donors after myocardial ischemia and reperfusion. *New Horiz* 1995; 3:105–12

[Cited Here...](#)

136. Abdel Baky NA, Zaidi ZF, Fatani AJ, Sayed-Ahmed MM, Yaqub H: Nitric oxide pros and cons: The role of L-arginine, a nitric oxide precursor, and idebenone, a coenzyme-Q analogue in ameliorating cerebral hypoxia in rat. *Brain Res Bull* 2010; 83:49–56

[Cited Here...](#)

137. Weyrich AS, Ma XL, Lefer AM: The role of L-arginine in ameliorating reperfusion injury after myocardial ischemia in the cat. *Circulation* 1992; 86:279–88

[Cited Here...](#)

138. Zweier JL, Wang P, Samouilov A, Kuppusamy P: Enzyme-independent formation of nitric oxide in biological tissues. *Nature Med* 1995; 1:804–9

[Cited Here...](#)

139. Webb A, Bond R, McLean P, Uppal R, Benjamin N, Ahluwalia A: Reduction of nitrite to nitric oxide during ischemia protects against myocardial ischemia-reperfusion damage. *Proc Natl Acad Sci U S A* 2004; 101:13683–8

[Cited Here...](#)

140. Duranski MR, Greer JJ, Dejam A, Jaganmohan S, Hogg N, Langston W, Patel RP, Yet SF, Wang X, Kevil CG, Gladwin MT, Lefer DJ: Cytoprotective effects of nitrite during *in vivo* ischemia-reperfusion of the heart and liver. *J Clin Invest* 2005; 115:1232–40

[Cited Here...](#)

141. Jung KH, Chu K, Ko SY, Lee ST, Sinn DI, Park DK, Kim JM, Song EC, Kim M, Roh JK: Early intravenous infusion of sodium nitrite protects brain against *in vivo* ischemia-reperfusion injury. *Stroke* 2006; 37:2744–50

[Cited Here...](#)

142. Tripatara P, Patel NS, Webb A, Rathod K, Lecomte FM, Mazzon E, Cuzzocrea S, Yaqoob MM, Ahluwalia A, Thiemermann C: Nitrite-derived nitric oxide protects the rat kidney against

ischemia/reperfusion injury *in vivo*: Role for xanthine oxidoreductase. *J Am Soc Nephrol* 2007; 18:570–80

[Cited Here...](#)

143. Lu P, Liu F, Yao Z, Wang CY, Chen DD, Tian Y, Zhang JH, Wu YH: Nitrite-derived nitric oxide by xanthine oxidoreductase protects the liver against ischemia-reperfusion injury. *Hepatobiliary Pancreat Dis Int* 2005; 4:350–5

[Cited Here...](#)

144. Raat NJ, Noguchi AC, Liu VB, Raghavachari N, Liu D, Xu X, Shiva S, Munson PJ, Gladwin MT: Dietary nitrate and nitrite modulate blood and organ nitrite and the cellular ischemic stress response. *Free Radic Biol Med* 2009; 47:510–7

[Cited Here...](#)

145. Zuckerbraun BS, Shiva S, Ifedigbo E, Mathier MA, Mollen KP, Rao J, Bauer PM, Choi JJ, Curtis E, Choi AM, Gladwin MT: Nitrite potently inhibits hypoxic and inflammatory pulmonary arterial hypertension and smooth muscle proliferation *via* xanthine oxidoreductase-dependent nitric oxide generation. *Circulation* 2010; 121:98–109

[Cited Here...](#)

146. Gonzalez FM, Shiva S, Vincent PS, Ringwood LA, Hsu LY, Hon YY, Aletras AH, Cannon RO 3rd, Gladwin MT, Arai AE: Nitrite anion provides potent cytoprotective and antiapoptotic effects as adjunctive therapy to reperfusion for acute myocardial infarction. *Circulation* 2008; 117:2986–94

[Cited Here...](#)

147. Baker JE, Su J, Fu X, Hsu A, Gross GJ, Tweddell JS, Hogg N: Nitrite confers protection against myocardial infarction: Role of xanthine oxidoreductase, NADPH oxidase and K(ATP) channels. *J Mol Cell Cardiol* 2007; 43:437–44

[Cited Here...](#)

148. Bryan NS, Calvert JW, Elrod JW, Gundewar S, Ji SY, Lefer DJ: Dietary nitrite supplementation protects against myocardial ischemia-reperfusion injury. *Proc Natl Acad Sci U S A* 2007; 104:19144–9

[Cited Here...](#)

149. Dezfulian C, Shiva S, Alekseyenko A, Pendyal A, Beiser DG, Munasinghe JP, Anderson SA, Chesley CF, Vanden Hoek TL, Gladwin MT: Nitrite therapy after cardiac arrest reduces reactive oxygen species generation, improves cardiac and neurological function, and enhances survival *via* reversible inhibition of mitochondrial complex I. *Circulation* 2009; 120:897–905

[Cited Here...](#)

150. Kumar D, Branch BG, Pattillo CB, Hood J, Thoma S, Simpson S, Illum S, Arora N, Chidlow JH Jr, Langston W, Teng X, Lefer DJ, Patel RP, Kevil CG: Chronic sodium nitrite therapy augments ischemia-induced angiogenesis and arteriogenesis. *Proc Natl Acad Sci U S A* 2008; 105:7540–5

[Cited Here...](#)

151. Cauwels A, Buys ES, Thoonen R, Geary L, Delanghe J, Shiva S, Brouckaert P: Nitrite protects against morbidity and mortality associated with TNF- or LPS-induced shock in a soluble guanylate cyclase-dependent manner. *J Exp Med* 2009; 206:2915–24

[Cited Here...](#)

152. Burwell LS, Brookes PS: Mitochondria as a target for the cardioprotective effects of nitric oxide in

ischemia-reperfusion injury. *Antioxid Redox Signal* 2008; 10:579–99

[Cited Here...](#)

153. Galkin A, Moncada S: S-nitrosation of mitochondrial complex I depends on its structural conformation. *J Biol Chem* 2007; 282:37448–53

[Cited Here...](#)

154. Bolli R, Dawn B, Tang XL, Qiu Y, Ping P, Xuan YT, Jones WK, Takano H, Guo Y, Zhang J: The nitric oxide hypothesis of late preconditioning. *Basic Res Cardiol* 1998; 93:325–38

[Cited Here...](#)

155. Frostell C, Fratacci MD, Wain JC, Jones R, Zapol WM: Inhaled nitric oxide. A selective pulmonary vasodilator reversing hypoxic pulmonary vasoconstriction. *Circulation* 1991; 83:2038–47

[Cited Here...](#)

156. Weitzberg E, Rudehill A, Alving K, Lundberg JM: Nitric oxide inhalation selectively attenuates pulmonary hypertension and arterial hypoxia in porcine endotoxin shock. *Acta Physiol Scand* 1991; 143:451–2

[Cited Here...](#)

157. Pepke-Zaba J, Higenbottam TW, Dinh-Xuan AT, Stone D, Wallwork J: Inhaled nitric oxide as a cause of selective pulmonary vasodilatation in pulmonary hypertension. *Lancet* 1991; 338:1173–4

[Cited Here...](#)

158. Kinsella JP, Neish SR, Shaffer E, Abman SH: Low-dose inhalation nitric oxide in persistent pulmonary hypertension of the newborn. *Lancet* 1992; 340:819–20

[Cited Here...](#)

159. Fox-Robichaud A, Payne D, Hasan SU, Ostrovsky L, Fairhead T, Reinhardt P, Kubes P: Inhaled NO as a viable antiadhesive therapy for ischemia/reperfusion injury of distal microvascular beds. *J Clin Invest* 1998; 101:2497–505

[Cited Here...](#)

160. Hambræus-Jonzon K, Chen L, Fredén F, Wiklund P, Hedenstierna G: Pulmonary vasoconstriction during regional nitric oxide inhalation: Evidence of a blood-borne regulator of nitric oxide synthase activity. *Anesthesiology* 2001; 95:102–12

[Cited Here...](#)

161. Cannon RO 3rd, Schechter AN, Panza JA, Ognibene FP, Pease-Fye ME, Waclawiw MA, Shelhamer JH, Gladwin MT: Effects of inhaled nitric oxide on regional blood flow are consistent with intravascular nitric oxide delivery. *J Clin Invest* 2001; 108:279–87

[Cited Here...](#)

162. Taylor DO, Edwards LB, Aurora P, Christie JD, Dobbels F, Kirk R, Rahmel AO, Kucheryavaya AY, Hertz MI: Registry of the International Society for Heart and Lung Transplantation: Twenty-fifth official adult heart transplant report–2008. *J Heart Lung Transplant* 2008; 27:943–56

[Cited Here...](#)

163. Zhan J, Nakao A, Sugimoto R, Dhupar R, Wang Y, Wang Z, Billiar TR, McCurry KR: Orally administered nitrite attenuates cardiac allograft rejection in rats. *Surgery* 2009; 146:155–65

[Cited Here...](#)

164. Lang JD Jr., Teng X, Chumley P, Crawford JH, Isbell TS, Chacko BK, Liu Y, Jhala N, Crowe DR, Smith AB, Cross RC, Frenette L, Kelley EE, Wilhite DW, Hall CR, Page GP, Fallon MB, Bynon JS, Eckhoff DE, Patel RP: Inhaled NO accelerates restoration of liver function in adults following orthotopic liver transplantation. *J Clin Invest* 2007; 117:2583–91

[Cited Here...](#)

165. Mathru M, Huda R, Solanki DR, Hays S, Lang JD: Inhaled nitric oxide attenuates reperfusion inflammatory responses in humans. *Anesthesiology* 2007; 106:275–82

[Cited Here...](#)

166. Dykhuizen RS, Frazer R, Duncan C, Smith CC, Golden M, Benjamin N, Leifert C: Antimicrobial effect of acidified nitrite on gut pathogens: Importance of dietary nitrate in host defense. *Antimicrob Agents Chemother* 1996; 40:1422–25

[Cited Here...](#)

167. Yoon SS, Coakley R, Lau GW, Lyman SV, Gaston B, Karabulut AC, Hennigan RF, Hwang SH, Buettner G, Schurr MJ, Mortensen JE, Burns JL, Speert D, Boucher RC, Hassett DJ: Anaerobic killing of mucoid *Pseudomonas aeruginosa* by acidified nitrite derivatives under cystic fibrosis airway conditions. *J Clin Invest* 2006; 116:436–46

[Cited Here...](#)

168. Lundberg JO, Carlsson S, Engstrand L, Morcos E, Wiklund NP, Weitzberg E: Urinary nitrite: More than a marker of infection. *Urology* 1997; 50:189–91

[Cited Here...](#)

169. Carlsson S, Wiklund NP, Engstrand L, Weitzberg E, Lundberg JO: Effects of pH, nitrite, and ascorbic acid on nonenzymatic nitric oxide generation and bacterial growth in urine. *Nitric Oxide* 2001; 5:580–6

[Cited Here...](#)

170. Jepson RG, Craig JC: Cranberries for preventing urinary tract infections. *Cochrane Database Syst Rev* 2008; CD001321

[Cited Here...](#)

171. Carlsson S, Govoni M, Wiklund NP, Weitzberg E, Lundberg JO: *In vitro* evaluation of a new treatment for urinary tract infections caused by nitrate-reducing bacteria. *Antimicrob Agents Chemother* 2003; 47:3713–8

[Cited Here...](#)

172. Shuman EK, Chenoweth CE: Recognition and prevention of healthcare-associated urinary tract infections in the intensive care unit. *Crit Care Med* 2010; 38:S373–9

[Cited Here...](#)

173. Carlsson S, Weitzberg E, Wiklund P, Lundberg JO: Intravesical nitric oxide delivery for prevention of catheter-associated urinary tract infections. *Antimicrob Agents Chemother* 2005; 49:2352–5

[Cited Here...](#)

174. Sobko T, Marcus C, Govoni M, Kamiya S: Dietary nitrate in Japanese traditional foods lowers diastolic blood pressure in healthy volunteers. *Nitric Oxide* 2010; 22:136–40

