The nitrate–nitrite–nitric oxide pathway in physiology and therapeutics

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Abstract | The inorganic anions nitrate (NO_3^-) and nitrite (NO_2^-) were previously thought to be inert end products of endogenous nitric oxide (NO) metabolism. However, recent studies show that these supposedly inert anions can be recycled *in vivo* to form NO, representing an important alternative source of NO to the classical L-arginine–NO-synthase pathway, in particular in hypoxic states. This Review discusses the emerging important biological functions of the nitrate–nitrite–NO pathway, and highlights studies that implicate the therapeutic potential of nitrate and nitrite in conditions such as myocardial infarction, stroke, systemic and pulmonary hypertension, and gastric ulceration.

Xanthine oxidoreductase
An enzyme involved in purine metabolism that catalyses the oxidation of hypoxanthine to xanthine and the further oxidation of xanthine to uric acid.

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Nitrite (NO_2^-) and nitrate (NO_3^-) are known predominantly as undesired residues in the food chain with potentially carcinogenic effects[1,2], or as inert oxidative end products of endogenous nitric oxide (NO) metabolism. However, from research performed over the past decade, it is now apparent that nitrate and nitrite are physiologically recycled in blood and tissues to form NO and other bioactive nitrogen oxides[3–6]. Therefore, they should now be viewed as storage pools for NO-like bioactivity, thereby complementing the NO synthase (NOS)-dependent pathway. The recognition of this mammalian nitrogen cycle has led researchers to explore the role of nitrate and nitrite in physiological processes that are known to be regulated by NO.

The bioactivation of nitrate from dietary or endogenous sources requires its initial reduction to nitrite, and because mammals lack specific and effective nitrate reductase enzymes, this conversion is mainly carried out by commensal bacteria in the gastrointestinal tract and on body surfaces[7,8]. Nitrite is unique to the nitrogen oxides in its redox position between oxidative (NO_2 radical) and reductive (NO radical) signalling and its relative stability in blood and tissue[9]. Once nitrite is formed, there are numerous pathways in the body for its further reduction to NO, involving haemoglobin[6,10], myoglobin[11,12], xanthine oxidoreductase[13–15], ascorbate[16], polyphenols[17,18] and protons[3,4] (BOX 1). The generation of NO by these pathways is greatly enhanced during hypoxia and acidosis, thereby ensuring NO production in situations for which the oxygen-dependent NOS enzyme activities are compromised[19,20]. Nitrite reduction to NO and NO-modified proteins during physiological and pathological hypoxia appear to contribute to physiological hypoxic signalling, vasodilation, modulation of cellular respiration and the cellular response to ischaemic stress[6,11,21–26].

Here, we review the metabolism and biological roles of NO within the body, and discuss the potential therapeutic use of nitrate or nitrite to treat various disorders, including those associated with vasoconstriction or ischaemia–reperfusion, as well as gastric ulcers.

NOS-independent NO generation

The NOS enzymes utilize L-arginine and molecular oxygen to produce the free-radical gas •NO, a critical regulator of vascular homeostasis, neurotransmission and host defence[27,28]. NO is an autocrine and paracrine signalling molecule whose lifetime and diffusion gradients are limited by scavenging reactions involving haemoglobin, myoglobin and other radicals. However, as discussed here, NO can be stabilized in the blood and tissue by oxidation to nitrate and nitrite, which can be considered as endocrine molecules that are transported in the blood, accumulate in tissue and have the potential to be converted back to NO under physiological and pathological conditions.

Interestingly, the L-arginine–NOS pathway is oxygen dependent, whereas the nitrate–nitrite–NO pathway is gradually activated as oxygen tensions falls. In this sense, NOS-independent NO formation (FIG. 1) can be viewed as a back-up system to ensure that there is sufficient NO formation when oxygen supply is limited, which is analogous to the complementary role of anaerobic glycolysis in energetics. The exact oxygen tension at which NOS-dependent NO generation fails to signal is unknown, in part owing to uncertainties about the *in vivo* K_m of

Box 1 | **The biological chemistry of some reactions involving nitrite**

Nitrite (NO_2^-) is formed in the body via the oxidation of nitric oxide (NO) or through the reduction of nitrate (NO_3^-).The non-enzymatic reaction of NO with oxygen in tissues is relatively slow, whereas its oxidation by the multicopper oxidase ceruloplasmin in plasma is rapid. Commensal bacteria in the oral cavity and gastrointestinal tract contribute to nitrite formation via a one-electron reduction of nitrate.

Reduction of nitrite to NO occurs in blood and tissues and proceeds through several enzymatic and non-enzymatic pathways, some of which are listed below. The acidic reduction of nitrite results in the generation of NO but also other nitrogen oxides, with nitrosating (N_2O_3) and nitrating (nitrogen dioxide, $^{\bullet}NO_2$) properties. In the presence of ascorbic acid or polyphenols, the acidic reduction of nitrite is greatly enhanced with less generation of N_2O_3 and $^{\bullet}NO_2$. Oxidation of nitrite occurs in the red blood cell and results in the formation of nitrate and methaemoglobin (Met-Hb).

Nitrite formation

Auto-oxidation of NO

$4\ ^{\bullet}NO + O_2 + 2\ H_2O \rightarrow 4NO_2^- + 4\ H^+$

Ceruloplasmin

$^{\bullet}NO + Cu^{2+} \rightarrow NO^+ + Cu^+$

$NO^+ + H_2O \rightarrow HNO_2 + H^+$

$HNO_2 \leftrightarrow H^+ + NO_2^-$

Bacterial nitrate reductase

$NO_3^- + e^- + H^+ \rightarrow NO_2^- + H_2O$

Nitrite reduction

Deoxyhaemoglobin/myoglobin

$NO_2^- + Fe^{2+} + H^+ \rightarrow {}^{\bullet}NO + Fe^{3+} + OH^-$

Xanthine oxidoreductase

$NO_2^- + Mo^{4+} + H^+ \rightarrow {}^{\bullet}NO + Mo^{5+} + OH^-$

Protons

$NO_2^- + H^+ \rightarrow HNO_2$

$2\ HNO_2 \rightarrow 2\ N_2O_3 + H_2O$

$N_2O_3 \rightarrow {}^{\bullet}NO + {}^{\bullet}NO_2$

Ascorbate

$NO_2^- + H^+ \rightarrow HNO_2$

$2\ HNO_2 + Asc \rightarrow 2\ {}^{\bullet}NO + dehydroAsc + 2\ H_2O$

Polyphenols (Ph-OH)

$NO_2^- + H^+ \rightarrow HNO_2$

$Ph\text{-}OH + HNO_2 \rightarrow Ph\text{-}{}^{\bullet}O + {}^{\bullet}NO + H_2O$

Nitrite oxidation

Haemoglobin

$4\ NO_2^- + 4\ HbO_2 + 4H^+ \rightarrow 4NO_3^- + 4\ Met\text{-}Hb + 2\ H_2O + O_2$

the NOS enzymes for oxygen and the fact that the rate of NO oxidative metabolism is reduced at low oxygen. However, it is clear that at very low oxygen tensions NO generation in tissues is independent of NOS activity and dependent on nitrite[5,22,26]. This principle has driven hypotheses that nitrite participates in hypoxic vasodilation and in the regulation of oxygen consumption at the mitochondrial level. It also predicts a role for nitrite in cytoprotective signalling in the setting of pathological ischaemia and reperfusion.

Sources of nitrate and nitrite

There are two major sources of nitrate and nitrite in the body: the endogenous L-arginine–NO synthase pathway and the diet. NO, generated by NOS enzymes, is oxidized in the blood and tissues to form nitrate and nitrite[27]. The reaction of NO with oxyhaemoglobin produces nitrate and methaemoglobin[27], whereas the oxidation of NO forms nitrite, a process that is catalysed in plasma by the multi-copper oxidase and NO oxidase ceruloplasmin[29]. In NOS knockout mice, the circulating nitrite levels are reduced by up to 70%[30], and nitrite levels are also lower in mice and humans lacking ceruloplasmin[29]. Normal plasma levels of nitrate are in the 20–40 μM range, while nitrite levels are substantially lower (50–300 nM)[8,25,31,32]. Regular exercise increases endothelial NOS (eNOS) expression and activity[33], which results in higher circulating levels of nitrate[33–35]. In systemic inflammatory disorders such as sepsis and severe gastroenteritis, nitrate and nitrite levels are greatly increased owing to massive inducible NOS (iNOS) induction[27,36]. By contrast, in diseases with endothelial dysfunction and reduced eNOS activity, plasma levels of nitrate and nitrite are often low[37].

Dietary nitrate intake is considerable and many vegetables are particularly rich in this anion[38]. For example, a plate of green leafy vegetables such as lettuce or spinach contains more nitrate[38] than is formed endogenously over a day by all three NOS isoforms combined[39]. Drinking water can also contain considerable amounts of nitrate, although in many countries the levels are strictly regulated. Nitrite can be found in some food stuffs, most notably as a preservative in cured meat and bacon.

The entero-salivary circulation of nitrate

In 1994, two groups independently described intragastric NO generation from salivary nitrite in humans[3,4]. This process does not require NOS activity, but instead involves the entero-salivary circulation of inorganic nitrate (FIG. 2). Dietary nitrate is rapidly absorbed in the upper gastrointestinal tract. In the blood, it mixes with the nitrate formed from the oxidation of endogenous NO produced from the NOS enzymes. After a meal rich in nitrate, the levels in plasma increase greatly and remain high for a prolonged period of time (plasma half-life of nitrate is 5–6 hours). The nitrite levels in plasma also increase after nitrate ingestion[8]. Although much of the nitrate is eventually excreted in the urine, up to 25% is actively taken up by the salivary glands and is concentrated up to 20-fold in saliva[8,40].

Once in the oral cavity, commensal facultative anaerobic bacteria use nitrate as an alternative electron acceptor to oxygen during respiration, effectively reducing salivary nitrate to nitrite by the action of nitrate reductases[7,38]. Human nitrate reduction requires the presence of these bacteria — suggesting a functional symbiosis relationship — as mammalian cells cannot effectively metabolize this anion. The salivary nitrate levels can approach 10 mM and nitrite levels 1–2 mM after a dietary nitrate load[8]. When saliva enters the acidic stomach (1–1.5 l per day), much of the nitrite is rapidly protonated to form nitrous acid (HNO_2; pKa ~3.3), which decomposes further to form NO and other nitrogen oxides[3,4]. Nitrite reduction to NO is greatly enhanced by reducing compounds such as vitamin C and polyphenols, both of which are abundant in the diet[17,18,41]. The importance of oral bacteria in gastric NO generation is perhaps most clearly illustrated in experiments using germ-free sterile rats, in which gastric NO formation is negligible even after a dietary load of nitrate[42]. In addition to the

Autocrine
A form of hormonal signalling in which a cell secretes a chemical messenger that binds to receptors on the same cell, leading to changes in the cell.

Paracrine
A form of cell signalling in which the target cell is close to (para = alongside of or next to) the signal-releasing cell.

Endocrine
A form of cell signalling in which chemical mediators are released directly into local blood vessels and travel to distant organs to regulate the target organ's function.

Hypoxic vasodilation
A physiological phenomenon in which blood vessels dilate in response to low oxygen levels.

stomach, a reductive pathway from nitrate to nitrite and then NO has also been demonstrated in the oral cavity[7], on the skin surface[43] in the lower gastrointestinal tract[44] and in urine[45].

While the scientific community had focused on the potentially harmful effects of nitrate and nitrite[1], the well-known antibacterial effects of NO[46–49] suggested a role for gastric NO in host defence[3,4] (FIG. 3). Interestingly, enteropathogens can survive for a surprisingly long time in acid alone, but the combination of acid and nitrite results in effective killing[3,50,51]. NO and other reactive nitrogen oxides formed from acidified nitrite act on multiple bacterial targets including DNA, proteins and components of the cell wall[38,47].

Another proposed physiological role for gastric NO is in the regulation of mucosal blood flow and mucus generation. Recent studies using the rat gastric mucosa as an *in vivo* bioassay tested the effects of human saliva on these two important determinants of gastric integrity. When the rat gastric mucosa was exposed to human nitrite-rich saliva, NO gas was immediately generated and both mucosal blood flow and mucus thickness increased in a cyclic GMP-dependent manner[52]. Furthermore, nitrate addition to drinking water for 1 week produces similar effects[53]. During the nitrate treatment, nitrite accumulates in the gastric mucus and when this mucus is removed, the blood flow immediately returns to basal levels, indicating a continuous slow release of 'NO-like' bioactivity from nitrite trapped in the mucus[53]. A role for salivary nitrite in regulating gastric gastrin release has also been suggested[54].

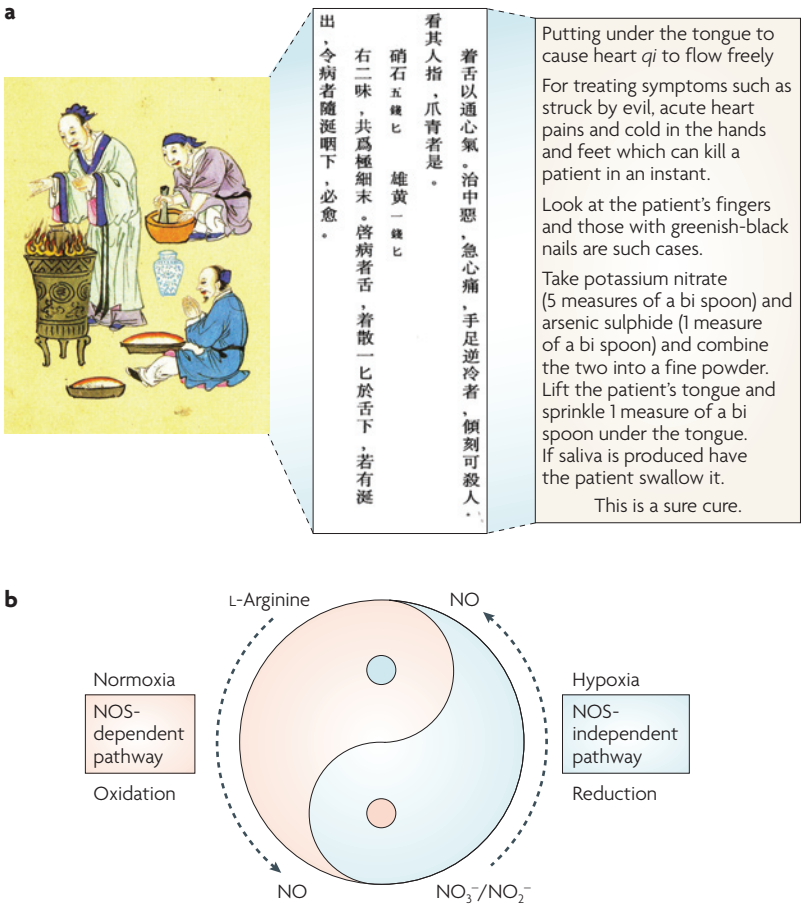

Figure 1 | **The nitrate–nitrite–NO pathway. a** | A medical recipe from Dunhuang. The nitrate–nitrite–nitric oxide (NO) pathway has been harnessed therapeutically since the medieval times as evidenced by a translation of medieval Buddhist manuscripts, which was discovered in a Buddhist grotto near the town of Dunhuang by a Daoist monk (Abbot Wang) at the beginning of the twentieth century after being hidden for 900 years. This was brought to our attention and translated by Anthony Butler, Zhou Wuzong and John Moffett. It illustrates the early appreciation of the effect of nitrate, readily available for meat-curing and gunpowder and reduced to nitrite in saliva, on cardiovascular conditions (angina and digital ischaemia). The text is written vertically beginning on the right and progressing leftwards. The term qi refers to a 'fluid' that, in a healthy person, flows harmoniously around the body. Its flow is disrupted during sickness. A bi spoon was a ceremonial spoon used in medicine. Chinese physicians often added realgar to a recipe as its colour is that of healthy blood. It would have had no effect because of its low solubility. **b** | Two parallel pathways for the generation of bioactive NO in mammals. NO is a key signalling molecule that serves to regulate a wide range of physiological functions. It is classically produced from L-arginine and oxygen by a family of enzymes, the NO synthases (NOSs). More recently, a fundamentally different mechanism for the generation of NO in mammals has been described. In this pathway, the inorganic anions nitrate and nitrite are reduced to form bioactive NO in blood and tissues during physiological hypoxia. Although NO generation by NOS becomes limited as oxygen levels fall, the nitrate–nitrite–NO pathway is enhanced. By the parallel action of both of these pathways, sufficient NO generation is ensured along the physiological and pathological oxygen and proton gradients.

Vasodilatory effects of nitrite

Although the vasodilatory properties of pharmacological doses of exogenous nitrite have been known for more than half a century[55–57], a physiological role of this anion in vasoregulation has been dismissed, even in more recent studies[58]. However, artery-to-vein gradients in nitrite across the human forearm, with increased consumption during exercise stress, suggests that nitrite is metabolized across the peripheral circulation[25]. Furthermore, humans breathing NO gas exhibit increases in peripheral forearm blood flow that is associated with increases in plasma nitrite[59], suggesting that nitrite could be a stable endocrine carrier and transducer of NO-like bioactivity within the circulation[60]. Consistent with a potentially greater efficacy under hypoxic or metabolic stress, the potency of nitrite increases dramatically with decreases in buffer pH in aortic ring experiments[24]. This hypothesis was tested by infusion of sodium nitrite into the forearm brachial artery of healthy volunteers, which was surprisingly potent, increasing blood flow even at blood concentrations below 1 μM and producing substantial vasodilation[6]. More recent dose-response experiments in normal human volunteers reveal significant vasodilation of the forearm circulation already at concentrations as low as 300 nM[61] and a significant decrease in blood pressure after nitrate ingestion, associated with an increase in plasma nitrite levels from 140–220 nM[62].

Pathways for systemic nitrite reduction

Haemoglobin as an allosterically regulated nitrite reductase. The ability of nitrite to vasodilate the circulation in the presence of NO-scavenging red blood cells under physiological conditions is unexpected and suggests new pathways to intravascular bioactivation. During infusion of nitrite into the human forearm circulation, the vasodilatory effects are associated with

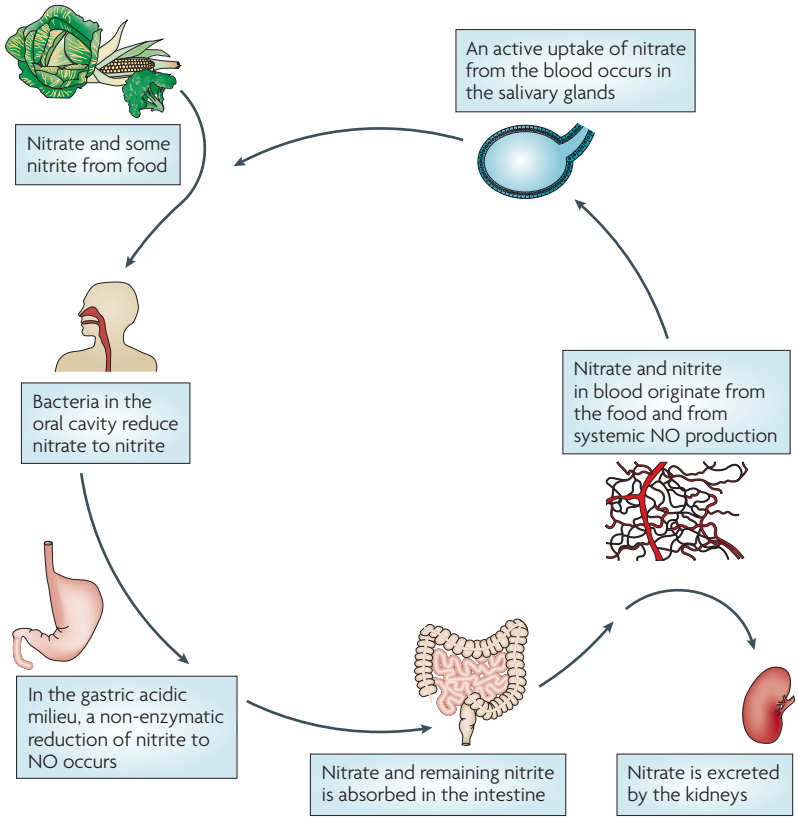

Figure 2 | **The entero-salivary circulation of nitrate in humans.** Ingested inorganic nitrate from dietary sources is rapidly absorbed in the small intestine. Although much of the circulating nitrate is eventually excreted in the urine, up to 25% is actively extracted by the salivary glands and concentrated in saliva. In the mouth, commensal facultative anaerobic bacteria effectively reduce nitrate to nitrite by the action of nitrate reductase enzymes. Nitrate reduction to nitrite requires the presence of these bacteria, as mammalian cells cannot effectively metabolize this anion. In the acidic stomach, nitrite is spontaneously decomposed to form nitric oxide (NO) and other bioactive nitrogen oxides, which regulate important physiological functions. Nitrate and remaining nitrite is absorbed from the intestine into the circulation and can convert to bioactive NO in blood and tissues under physiological hypoxia.

Methaemoglobin
A form of the oxygen-carrying protein haemoglobin in which the iron in the haem group is in the Fe^{3+} state, not the Fe^{2+} of normal haemoglobin. Methaemoglobin is unable to carry oxygen.

Facultative anaerobic bacteria
A bacterium, that makes ATP by aerobic respiration if oxygen is present but is also capable of switching to anaerobic respiration.

Electron acceptor
A chemical entity that accepts electrons transferred to it from another compound. It is an oxidizing agent that, by virtue of it accepting electrons, is itself reduced in the process.

the formation of NO in the blood, as measured by the rate of formation of iron-nitrosylated haemoglobin (NO bound to the haem of haemoglobin) during artery to vein transit[6]. This rate of NO formation increases as haemoglobin oxygen saturation decreases, suggesting a hypoxia-regulated mechanism of nitrite bioactivation.

These physiological findings are consistent with a nitrite reductase activity of deoxyhaemoglobin as described by Brooks in 1947 and Doyle and colleagues in 1981 (REFS 63,64). According to this chemistry, nitrite reacts with ferrous deoxyhemoglobin ($HbFe^{2+}$) and a proton (H^+) to generate NO and methaemoglobin ($HbFe^{3+}$), which is analogous to the coupled proton and electron-transfer reactions of bacterial nitrite reductases. The NO can then bind to a second deoxyhaemoglobin to form iron-nitrosyl-haemoglobin ($HbFe^{2+}$–NO) as outlined in equations 1 and 2.

$$\text{Nitrite } (NO_2^-) + \text{deoxyhaemoglobin } (Fe^{2+}) + H^+ \rightarrow \text{NO} + \text{methaemoglobin } (Fe^{3+}) + OH^- \quad (1)$$

$$\text{NO} + \text{deoxyhaemoglobin } (Fe^{2+}) \rightarrow HbFe^{2+}\text{-NO} \quad (2)$$

This simple reaction has physiological implications in that it uses naturally occurring nitrite as a substrate, requires deoxygenation of haemoglobin so it has hypoxic sensor properties, requires a proton so it has pH sensor properties, and generates NO, the most potent vasodilator known. These chemical properties, and supporting physiological studies, suggest that haemoglobin may function as an allosterically regulated nitrite reductase that may contribute to hypoxic signalling and hypoxic vasodilation[6,10,65,66] (FIG. 3). The chemistry of this reaction, mechanisms of NO export from the red blood cell and physiological contribution to hypoxic blood flow regulation are the subjects of active research (FIG. 4). Interestingly, recent studies suggest that NO formed from nitrite reduction (equation 1) can react with a second nitrite that is bound to methaemoglobin ($HbFe^{3+}$)[67]. Remarkably, when nitrite binds to methaemoglobin, it forms a nitrogen dioxide radical ($^{\bullet}NO_2$) character ($HbFe^{3+}$–NO_2^- forms $HbFe^{2+}$–$^{\bullet}NO_2$), which reacts with NO in a radical–radical reaction to form N_2O_3. N_2O_3 is more stable in a haem-rich environment than NO and has the potential to escape from the red blood cell. The overall stoichiometry of the reaction of equation 1 and the second reaction of NO with nitrite–methaemoglobin is shown in equation 3. Note that in this reaction haemoglobin is catalytic and redox cycles convert two molecules of nitrite into N_2O_3.

$$2\ NO_2^- + \text{deoxyhemoglobin } (Fe^{2+}) + H^+ \rightarrow N_2O_3 + \text{deoxyhaemoglobin } (Fe^{2+}) + OH^- \quad (3)$$

Myoglobin, xanthine oxidoreductase and other pathways. Myoglobin has a high affinity for oxygen and a low haem redox potential that contributes to rapid nitrite reduction to NO when deoxygenated; in fact, deoxymyoglobin will reduce nitrite to NO at a rate 30-times faster than haemoglobin[11,66]. These chemical properties suggest that when myoglobin becomes deoxygenated, such as in the subendocardium of the heart or in exercising skeletal muscle, it will rapidly convert nitrite to NO (via the same nitrite reductase reaction as described above). Indeed, myoglobin has recently been shown to convert nitrite to NO in the cardiomyocyte and in the working heart[11,12]. NO formed by myoglobin can bind to cytochrome *c* oxidase of the mitochondrial electron transport chain, reducing electron flow and oxygen utilization[11]. Consistent with these studies, nitrite reduction to NO and nitrite-dependent modulation of cardiac consumption is abolished in the myoglobin knockout mouse[12]. These studies suggest that nitrite and myoglobin play an important role in regulating cardiac energetics and oxygen utilization under conditions of physiological hypoxia. Supporting this possibility, Larsen and colleagues found that whole-body oxygen consumption in young healthy volunteers was significantly reduced during submaximal exercise after dietary supplementation with nitrate compared with placebo treatment[68]. This surprising effect was associated with the metabolism of plasma nitrite.

Several enzymes, including xanthine oxidoreductase[13–15,69], complexes of the mitochondrial electron transport chain[70–72], cytochrome P450s[73] and even the

NOS enzyme[74], have been shown to use nitrite as an alternative electron acceptor to molecular oxygen thereby forming NO (FIG. 3). For example, xanthine oxidoreductase is known to reduce molecular oxygen to superoxide (O_2^-), but at low oxygen tensions and pH values this enzyme can also reduce nitrite to NO at the molybdenum site of the enzyme. In terms of a potential role in vasoregulation and NO signalling, these four pathways all require low oxygen tensions to effectively generate NO and these enzymes also produce superoxide, which is expected to react rapidly with and scavenge any NO that is synthesized. It is likely that nitrite can competitively reduce vascular reactive oxygen species (ROS) formation by these enzymes, by direct diversion of electrons away from oxygen, thus limiting superoxide formation. Decreases in superoxide will effectively

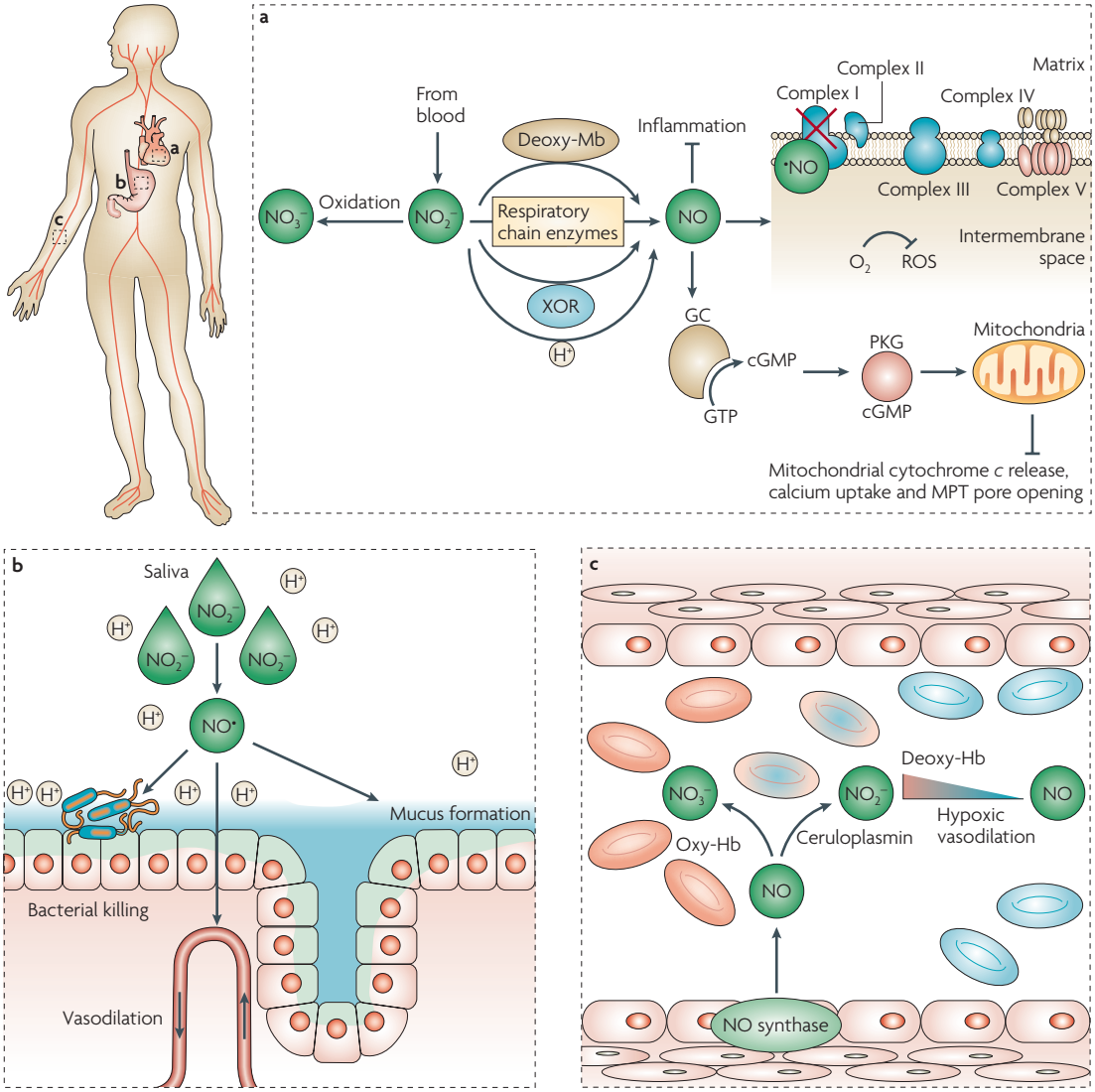

Figure 3 | **Pathways for nitrite reduction to NO and its proposed physiological roles. a** | In the tissues, such as the heart, there are numerous pathways for the generation of NO from nitrite, all greatly potentiated during hypoxia, including xanthine oxidoreductase (XOR), deoxygenated myoglobin (deoxy-Mb), enzymes of the mitochondrial chain and protons. Nitrite-dependent NO formation and *S*-nitrosothiol formation can modulate inflammation, inhibit mitochondrial respiration and mitochondrial derived reactive oxygen species formation, and drive cyclic GMP-dependent signalling under anoxia. NO-dependent cytochrome *c* oxidase (complex IV) inhibition can also drive reactive oxygen species (ROS)-dependent signalling. **b** | The formation of bioactive nitric oxide (NO) from the inorganic anion nitrite is generally enhanced under acidic and reducing conditions. In the acidic gastric lumen, NO is generated non-enzymatically from nitrite in saliva after formation of nitrous acid (HNO_2) and then decomposition into NO and other reactive nitrogen oxides. This NO helps to kill pathogenic bacteria and it also stimulates mucosal blood flow and mucus generation, thereby enhancing gastric protection. Detrimental effects have also been suggested, including nitrite-dependent generation of nitrosamines with potentially carcinogenic effects. **c** | In the blood vessels, nitrite forms vasodilatory NO after a proposed reaction with deoxygenated haemoglobin (deoxy-Hb) and contributes to physiological hypoxic blood flow regulation. GC, guanylate cyclase; MPT, mitochondrial permeability transition; Oxy-Hb, oxygenated haemoglobin; PKG, protein kinase G.

cyclic GMP
A cyclic nucleotide derived from guanosine triphosphate (GTP) that acts as a second messenger, much like cyclic AMP.

Allosteric
Allosteric regulation is the regulation of an enzyme or protein by binding an effector molecule at a site other than the protein's active site.

Mitochondrial electron transport chain
An electron transport chain associates energy-rich electron donors (for example, NADH) and mediates the biochemical reactions that produce ATP, which is the energy currency of life.

increase vascular NO bioavailabilty. In addition to secondary NO generation, a role of nitrite as an intrinsic signalling molecule that can directly modify target haem or thiol groups on proteins has been proposed[23].

Therapeutic opportunities

Vasodilation. Numerous studies have now confirmed the vasodilating effects of low-dose nitrite in mice, rats, sheep, dogs, primates and humans[75–81]. Therapeutic delivery of nitrite to vasodilate ischaemic vascular beds shows great promise in preclinical studies (FIG. 5). Patients suffering from spontaneous haemorrhage of a subarchnoidal artery aneurism are at risk for developing delayed cerebral artery spasm. In primate models, this spasm is associated with acute depletion of cerebral spinal fluid nitrite levels. Two-week infusions of systemic nitrite effectively prevented this complication[80].

Primary pulmonary hypertension of the newborn (PPHN) is a condition that is associated with a high pulmonary vascular resistance and extremely low systemic oxygenation. In sheep models of PPHN, inhaled nitrite was converted to NO gas in the lung and selectively vasodilated the pulmonary circulation[78]. In such diseases, which are characterized by regional ischaemia and vasoconstriction, nitrite may provide an ideal stable and naturally occurring therapeutic NO donor.

The vasodilatory and biological activities of the inorganic anions nitrite and nitrate must be distinguished from the organic nitrates (that is, nitroglycerin) and nitrites (amyl-nitrite). Clearly, the organic nitrates and nitrites are much more potent than nitrite in terms of vasoactivity. Although the anti-anginal and vasodilatory organic nitrates and nitrites are metabolized *in vivo* into vasodilatory NO and nitrite[82], this bioactivation requires metabolism by mitochondrial aldehyde dehydrogenase and other enzymes, which are all subject to induced tolerance[83,84]. Tolerance is characterized by a lack of nitroglycerin biological activity with chronic drug exposure. Studies from as far back as 1930 suggest that inorganic nitrite does not induce tolerance[85], implying that nitrite may represent an active metabolite of nitroglycerin that can bypass enzymatic nitroglycerin metabolism and tolerance.

Tissue protection in ischaemia–reperfusion injury. Systemic NOS-independent NO formation from nitrite was first demonstrated in the ischaemic heart[5]. Studies in animal models of ischaemia and reperfusion have now revealed a central role of nitrite in hypoxic signalling. Physiological and therapeutic levels of nitrite exert potent cytoprotection after prolonged ischaemia and blood-flow reperfusion in liver[22,86], heart[22,79,87], brain[88] and kidney[89]. These findings suggest an opportunity for nitrite therapy for human diseases associated with ischaemia–reperfusion, such as myocardial infarction, stroke, solid-organ transplantation, cardiopulmonary arrest and sickle-cell disease (FIG. 5).

Dose-response studies in mice suggest a broad efficacy to safety range of nitrite of three orders of magnitude, with doses as low as 0.1 μmoles per kg to 100 μmoles per kg providing significant protection. Interestingly, the protective effect of nitrite is evident at very low plasma concentrations (less than 200 nM), but is lost as plasma concentrations rise above 100–1,000 μM[22]. The lowest dose of nitrite given in these studies only increased the plasma levels of nitrite by 20%. Intriguingly, a similar or even greater increase in plasma nitrite is seen after ingesting a portion of spinach or lettuce[8]; this evokes provocative questions about a putative role of nitrate as an active ingredient of the cardioprotective mediterranean diet[9,38,90,91] (BOX 2).

The mechanism of nitrite-mediated cytoprotection appears to be NO-dependent and mitochondria-targeted. Studies using various inhibitors and genetic knockout mice provide some clues to the potentially important pathways. All the published animal studies have demonstrated a loss of cytoprotection when animals were treated with the NO scavenger carboxy-PTIO (2-(4-carboxyphenyl)-4,5-dihydro-4,4,5,5-tetramethyl-1H-imidazolyl-1-oxy-3-oxide)[22,79,86,88], suggesting the importance of NO in the mechanism of cytoprotection. Pretreatment of animals with a NOS inhibitor[22,79] or use of eNOS knockout mice[22] did not inhibit cytoprotection, proving that the nitrite effect is NOS-independent.

The pathway(s) by which nitrite forms NO in hypoxic tissue remains to be determined. Two groups suggest the involvement of xanthine oxidoreductase in the reduction of nitrite to NO on the basis of reduced efficacy after treatments with allopurinol, a xanthine oxidase inhibitor[79,87,89]. The fact that nitrite remains protective in isolated buffer-perfused organ models, such as the Langendorff heart, suggests that the haemoglobin pathway is not necessary for this function. We have considered the possibility that in the heart, myoglobin can serve this function and have recently demonstrated that deoxymyoglobin has nitrite reductase activity, which can modulate mitochondrial respiration[11,21].

ROS generation by mitochondria is a necessary component of mitochondrial signalling in cytoprotection[92–95]. However, the large burst of oxidizing ROS generated after reperfusion following ischaemia can also contribute to cellular injury, necrosis and apoptosis[96,97]. *S*-Nitrosation of complex I of the electron transport chain inhibits the activity of this complex[98] and decreases mitochondrial-derived ROS formation during reperfusion, an effect associated with cellular cytoprotection[99,100]. Nitrite can similarly nitrosate complex I during ischaemia and reperfusion[21]. This modification limits complex I-dependent reperfusion ROS formation, activation of the mitochondrial permeability transition pore, and cytochrome *c* release. Interestingly, the effects of nitrite on mitochondria and tissue cytoprotection occur both acutely (immediately before reperfusion) and remotely (if given 24 hours before reperfusion), suggesting a potential role for nitrite as an effector of ischaemic preconditioning[21].

The inhibitory effect of NO[101–104] and nitrite on mitochondrial respiration that is associated with mitochondrial-dependent cytoprotection presents an interesting paradox. The energetic cost of reversibly inhibiting mitochondrial respiration appears to be offset by reduced ROS generation during reperfusion. A paradigm is emerging that damping electron flow to

Reactive oxygen species (ROS). Include oxygen ions, free radicals and peroxides that are both inorganic and organic. They are generally highly reactive owing to the presence of unpaired valence shell electrons. ROS form as a natural by-product of the normal metabolism of oxygen and have important roles in cell signalling. However, during times of environmental stress ROS levels can increase dramatically, which can result in significant damage to cell structures.

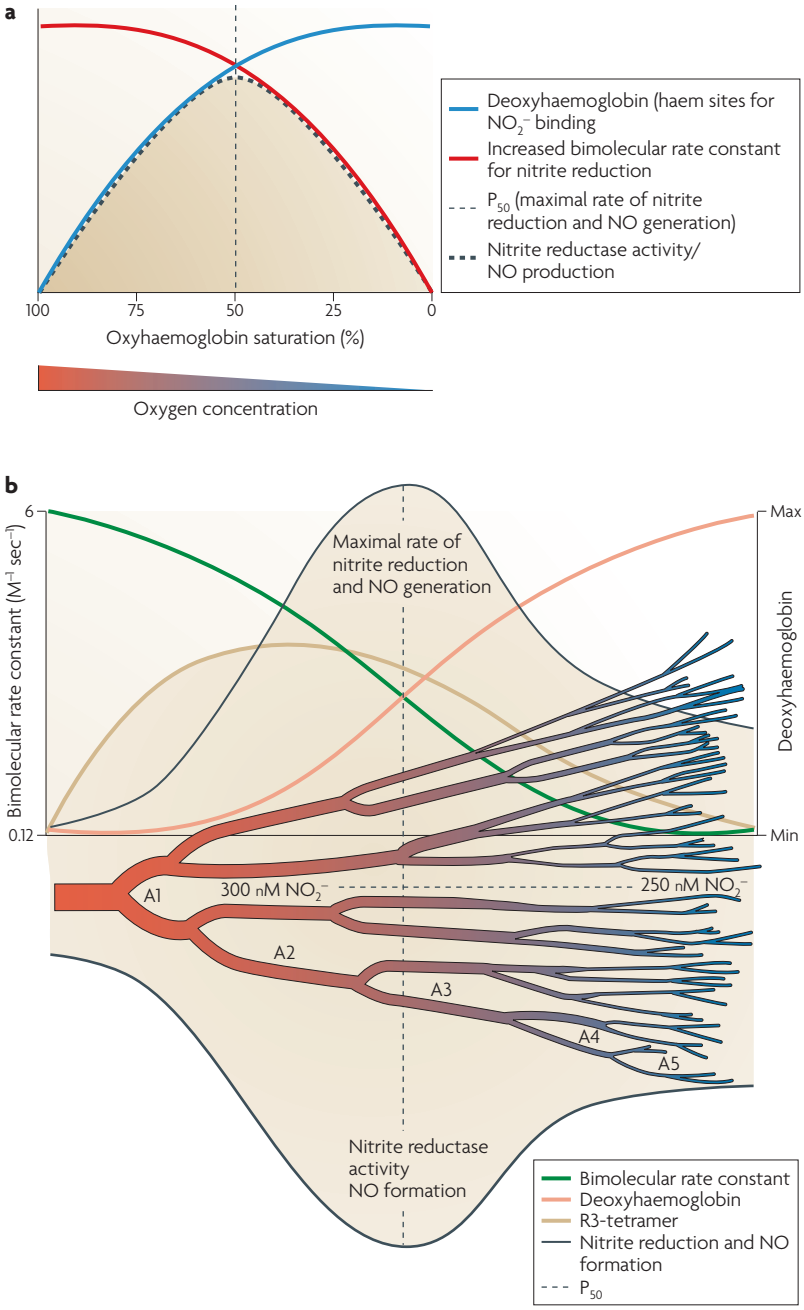

Figure 4 | **R-state or allosteric autocatalysis of nitrite reduction by haemoglobin. a** | Allosteric nitrite reduction by haemoglobin. The rate at which haemoglobin converts nitrite into nitric oxide (NO) is maximal when it is 50% saturated with oxygen, the midpoint of the haemoglobin–oxygen dissociation curve (P_{50}). This effect is mechanistically determined by two opposing chemistries, the availability of deoxyhaems (reaction substrate) to bind nitrite, which is maximal in T-state or deoxygenated haemoglobin, and the amount of R-state or oxygenated haemoglobin tetramer, which increases the intrinsic reactivity of the haem with nitrite. The latter process occurs because R-state or oxygenated haemoglobin has a decreased haem redox potential, which is analogous to the low haem redox potential of myoglobin. This low redox potential of the R-state ferrous haem favours an equilibrium distribution of electrons to nitrite, resulting in the increased reactivity of nitrite with the unliganded ferrous haems of R-state haemoglobin and myoglobin. The lowered haem redox potential is kinetically manifested as an increased bimolecular rate constant for the reaction of nitrite with R-state haemoglobin compared with T-state haemoglobin. Because the observed rate of nitrite reduction to form NO is equal to the product of the bimolecular rate constant times the deoxyhaem (reactant) concentration, this rate is expected to be maximal at approximately 50% oxygen saturation, or the intrinsic haemoglobin P_{50}; this allows for physiological hypoxic 'sensing' and NO generation. **b** | Physiological model. In the mammalian circulation, the allosteric state of haemoglobin tetramers is modulated primarily by oxygen ligation such that the *in vivo* intrinsic reactivity (bimolecular rate constant) of the deoxyhaem is therefore dictated by oxygen binding to other haems on the same tetramer. This model therefore predicts that the most effective tetrameric nitrite reductase would be the R-state haemoglobin that rapidly deoxygenates during arterial to capillary transit. This molecule would transition through R4 (R-state with four oxygens bound) to R3 (R-state with three oxygens bound) to R2 intermediates and then shift to T2 (T-state with two oxygens bound) and ultimately T1. These R3 and R2 tetramers would be the most effective nitrite reductases (bimolecular rate of 6 M^{-1} sec^{-1} for R-haem compared with 0.03 M^{-1} sec^{-1} for T-haem). This chemistry is consistent with experiments showing that nitrite reduction and NO signalling are most effective in systems subjected to rapid deoxygenation in the presence of oxyhaemoglobin and nitrite or when haemoglobin is 50% saturated with oxygen. A1–A5 reflect the arteriolar size, which decreases at branch points.

Organic nitrates
Drugs used principally in the treatment of angina pectoris and acting mainly by dilating the blood vessels by the formation of nitric oxide.

S-nitrosation
The conversion of thiol groups (-SH), including cysteine residues in proteins, to form *S*-nitrosothiols. *S*-Nitrosation has been suggested to be a mechanism for dynamic, post-translational regulation of proteins. In addition, *S*-nitrosothiols can act as

oxygen (thus limiting superoxide formation) during reperfusion, by NO-dependent complex I and IV inhibition[98,101] or by depleting oxygen during reperfusion (post-conditioning), may reduce reperfusion ROS generation and limit downstream apoptotic signalling[105]. Data also suggest that NO-dependent inhibition of cytochrome *c* oxidase before ischaemia, that is, during normoxia, can produce the opposite effect of increasing basal ROS formation, creating a preconditioning environment that is also adaptive[92–95].

Several recent studies of NO gas inhalation in both animals and humans suggest a transformation of NO in the lung into a more long-lived bioactive NO-species that can be transported in blood[59,106]. Moreover, inhaled NO reduces myocardial infarction volume in mice[107] and pigs[108] and the extent of liver injury after orthotopic transplantation in humans[109]. These effects are associated with significant increases in circulating nitrite, with no significant changes in blood *S*-nitrosothiol levels. NO treatment significantly reduced the overall incidence of brain injury in premature newborns with respiratory failure, an effect consistent with endocrine transport of an NO-intermediate in blood to the central nervous system[110]. Thus, increasing evidence suggests that nitrite is mediating extrapulmonary effects of NO gas inhalation.

The promising animal data discussed here indicate that nitrite possesses the characteristics of a useful adjunctive therapy for acute myocardial infarction, including

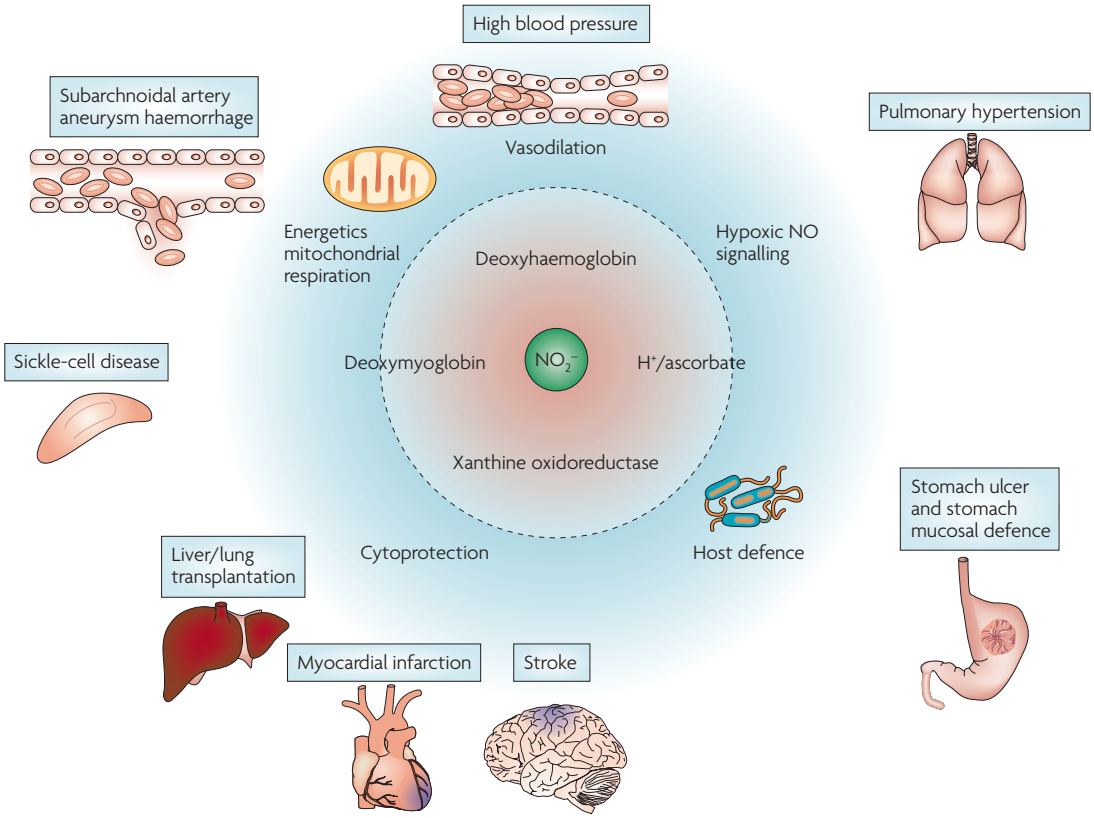

Figure 5 | **Therapeutic opportunities for inorganic nitrite.** The inorganic anion nitrite (NO_2^-) can be metabolized in blood and tissues to form nitric oxide (NO), a pluripotent biological messenger. Nitrite reduction to NO is catalysed by various enzymatic and non-enzymatic pathways and is greatly enhanced during hypoxia and ischaemic stress, which may be of therapeutic value. In animal models, nitrite is strongly cytoprotective and protects against ischaemia–reperfusion injury. These findings suggest an opportunity for nitrite therapy for human diseases such as myocardial infarction, stroke, solid-organ transplantation and sickle-cell disease. Nitrite is also cytoprotective in the stomach, where it can prevent drug-induced gastric ulcers. The vasodilatory and blood-pressure lowering effects of nitrite could be useful in pulmonary and systemic hypertension, as well as in the treatment and prevention of delayed cerebral vasospasm after subarachnoidal artery aneurysm haemorrhage.

significant cardioprotection following prolonged ischaemia, simple administration and minimum associated regional and systemic side effects. Based on these considerations, a human Phase II clinical trial of intravenous nitrite for ST segment elevation myocardial infarction is currently being planned by the US National Heart, Lung, and Blood Institute in cooperation with European centres.

Gastric ulcers. A common and potentially serious side effect of aspirin-like drugs (non-steroidal anti-inflammatory drugs; NSAIDs) is the development of gastric ulcers secondary to the inhibition of prostaglandin synthesis by these agents[111]. Similarly, in animal models, pharmacological inhibition of the NOS enzymes increases the susceptibility to ulcerogenic compounds[112]. Experiments with isoform-selective inhibitors suggest that the constitutive isoforms of cyclooxygenase (COX1) and NOS (eNOS and neuronal NOS; nNOS) are protective, while the opposite may be true for the inducible enzymes (COX2 and iNOS)[113,114]. In a recent study, rats were given sodium nitrate in the drinking water for 1 week followed by acute exposure to an NSAID (diclofenac) by gastric gavage[115]. Dietary nitrate increased gastric NO levels and potently protected against the macroscopic injury caused by NSAID exposure (FIG. 5). Additionally, nitrate pretreatment decreased mucosal myeloperoxidase activity and expression of iNOS, which is indicative of reduced tissue inflammation. The protection afforded by nitrate probably relates to increased gastric mucosal blood flow and mucus generation and reduced epithelial permeability[52,53].

The gastroprotective effect of nitrate was abolished in rats if they were pretreated with topical antibiotics in the mouth before nitrate supplementation, thereby illustrating the importance of the oral microflora in the bioactivation of nitrate[116]. An additional protective effect of nitrate on ulcer development may occur through inhibition of *Helicobacter pylori*[117].

In critically ill patients, endotracheal intubation and sedation interrupt the entero-salivary nitrate cycle, which results in depleted gastric NO, nitrite and *S*-nitrosothiol levels[118]. It has been suggested that the insufficient levels of gastric NO contribute to the gastric lesions and bacterial overgrowth commonly found in these patients[118].

Box 2 | **Dietary aspects of nitrate and nitrite**

The metabolism of dietary nitrate can result in intragastric formation of nitrosamines, which may be carcinogenic[1]. However, despite more than 40 years of extensive research, there is still no clear evidence for a link between nitrate intake and gastric cancer in humans[133]. Moreover, it is well known that a diet rich in vegetables is associated with a lower blood pressure and a reduced long-term risk for the development of cardiovascular disease[134,135]. Recently, Larsen and colleagues[62] performed a double-blind placebo-controlled crossover evaluation of dietary nitrate supplementation in healthy young volunteers and found a significant reduction in resting blood pressure with a nitrate dose corresponding to the amount found in 150–250 g of a green leafy vegetable. Remarkably, the reduction in blood pressure was similar to that described in healthy controls consuming a diet rich in fruits and vegetables in the Dietary Approaches to Stop Hypertension (DASH) trial[136]. Consistent with this, new preclinical studies now indicate that dietary levels of nitrite and nitrate significantly modulate the susceptibility to cardiac and liver ischaemia–reperfusion injury and gastric and intestinal mucosal integrity, respectively[115,127]. In recent studies in rats and mice, oral intake of nitrite either immediately before or even 24 hours before myocardial infarction significantly reduced infarction volume[21]. In this context, nitrate may be considered as a 'prodrug', which produces a sustained delivery of nitrite to the systemic circulation following entero-salivary circulation[8]. The possibility of boosting nitric oxide production by dietary intervention may have important implications for public health, in particular cardiovascular disease. The central role of commensal bacteria in the bioactivation of nitrate is intriguing and suggests that a symbiotic host–microbial relationship is involved in the regulation of cardiovascular function.

Future clinical studies will elucidate whether nitrate can offer a nutritional approach to the prevention and treatment of disease. If such investigations point towards a protective effect of nitrate, the current strictly regulated levels of nitrate in food and drinking water may need to be reconsidered.

Antimicrobial effects. Nitrite is used as a preservative in meat products to inhibit the growth of pathogens, most notably *Clostridium botulinum*, and these antibacterial effects have been attributed to NO formation[119]. The discovery of endogenous nitrite reduction to NO in the acidic stomach triggered researchers to explore therapeutic uses for acidified nitrite as an antimicrobial agent. Indeed, acidified nitrite results in the generation of NO and other nitrogen oxides, which have potent antibacterial activity against a range of pathogens, including *Salmonella*, *Yersinia* and *Shigella* species, *H. pylori*, and *Pseudomonas aerguinosa*[3,16,117,120]. These antibacterial effects of nitrite have recently been investigated in the airways. In an animal model resembling cystic fibrosis, acidified nitrite successfully cleared the airways of mucoid *P. aeruginosa*, a pathogen commonly infecting the airways of patients with cystic fibrosis[121].

Infected urine typically contains considerable amounts of nitrite, owing to bacterial reduction of urinary nitrate. Although nitrite is stable at neutral or alkaline conditions, it is reduced to NO and has potent antibacterial effects if the urine is mildly acidified (to pH 5–6); these effects are potentiated in the presence of the reducing agent vitamin C[16]. In fact, the *in vitro* antibacterial potency of nitrite and ascorbic acid is fully comparable to that of traditional antibiotics such as nitrofurantoin and trimetoprim. Acidification of urine — for example, by vitamin C intake — has been used in traditional medicine for the prevention and treatment of urinary tract infections. This effect may be related to the formation of antibacterial nitrogen oxides from the acidified nitrite[38,122].

Opportunities for drug development

As mentioned above, several different therapeutic indications for nitrite have been successfully tested recently both in animal models and in humans (FIG. 5). Depending on the condition to be treated, the development of several different nitrite-containing formulations and methods of administration are anticipated.

Topical administration of acidified inorganic nitrite. An inorganic nitrite salt such as sodium nitrite ($NaNO_2$) is combined with an acidifying agent (for example, ascorbic acid). This mixture rapidly releases NO and other nitrogen oxides and has been evaluated for its antimicrobial activity. Topical application of acidified nitrite to the skin has proved effective in various skin infections[123–125], and in the airways, acidified nitrite has been shown to kill mucoid *Pseudomonas* in an animal model of cystic fibrosis[121]. Carlsson and colleagues used the inflatable retention balloon of a urinary catheter as a depot for nitrite and ascorbic acid, leading to direct intravesicular delivery of antimicrobial nitrogen intermediates[126]. In their *in vitro* studies, NO was generated in the retention balloon and diffused into the surrounding urine where it effectively killed the urinary pathogen *Escherichia coli*. They suggested that this could be a new approach to prevent catheter-associated urinary-tract infections, the most common hospital-acquired infection.

Enteral administration of inorganic nitrite and nitrate. It is clear that both nitrate and nitrite are readily absorbed and biologically active when given orally, and therapeutic effects have been observed in animal models of ischaemia–reperfusion injury[127] and in protection against gastric ulcerations[115,116,128]. In addition, short-term dietary nitrate supplementation has been shown to lower blood pressure in healthy volunteers[62]. A combination of nitrate and nitrite salts for oral administration is theoretically attractive, as the nitrite would ensure immediate effects soon after absorption, while the nitrate would continuously provide a slow release of nitrite over a prolonged period of time via the entero-salivary recirculation described above. Similar to the recently developed NO–NSAIDs, in which the active drug is combined with an organic nitrate[129], the addition of inorganic nitrate to an ulcerogenic drug such as aspirin or another NSAID is also a possible new composition.

Organic nitro compounds as donors of nitrite. The bioavailability of nitrite after enteral administration of inorganic nitrate or nitrite can be difficult to control because of the variable metabolism of these anions within the gastrointestinal tract. However, the use of organic allylic nitro compounds as nitrite donors may overcome this potential problem[130], as *in vitro* experiments have shown that such compounds can release nitrite and NO in the presence of thiols (L-cysteine) and ascorbic acid. Traditional organic nitrates (nitroglycerine) and nitrites (amyl nitrite) used in cardiovascular medicine are also metabolized to nitrite *in vivo*. Whether the organic allylic nitro compounds or other donors of nitrite can offer any additional advantages over these

compounds and the native inorganic anions, in terms of controlled delivery, bioavailability and tolerance, remains to be studied.

Short or long-term infusions of inorganic nitrite. In the development of nitrite for therapeutic intravenous use, it is anticipated that the dose and duration of treatment will have to be adjusted depending on the condition and the desired effect. In animal studies, large doses of nitrite infused over a long period of time are needed to effectively alleviate the vasospasm associated with subarachnoidal haemorrhage[80]. In models of ischaemia–reperfusion injury, however, the dose of nitrite needed for protective effects is remarkably low[21,22].

Toxicity. The two major health concerns with inorganic nitrite and nitrate are the risk for development of methaemoglobinaemia and their potential carcinogenic effects[2]. Any toxicity of the nitrate ion is thought to occur after its bioconversion to nitrite, which is considerably more reactive. Formation of methaemoglobin occurs when the oxygen-carrying ferrous ion (Fe^{2+}) of the haem group of the haemoglobin molecule is oxidized by nitrite to the ferric state (Fe^{3+}). This converts haemoglobin to methaemoglobin, which cannot bind oxygen. Clinically significant methaemoglobinaemia with cyanosis occurs when the levels increase above a certain level (approximately 5%). In animal studies looking at the tissue protective and vasodilatory effects of intravenous nitrite, the increase in methaemoglobin is generally undetectable or modest even after prolonged delivery[80], suggesting that methaemoglobin is not a major problem in these dose ranges. In fact, the estimated EC_{50} for nitrite in human adults, based on methaemoglobin formation, is 1 g[131], whereas calculations from animal data suggest that less than 40 mg nitrite would be necessary for the treatment of myocardial infarction in a 70 kg adult.

In 2001, the US Department of Health and Human Services National Toxicology Program published extensive toxicology and carcinogenesis studies of sodium nitrite in rats and mice. Sodium nitrite was delivered in drinking water for 14-week and 2-year periods and genetic toxicology studies were conducted in *Salmonella typhimurium*, and in rat and mouse bone marrow and peripheral blood. Consistent with recent epidemiological studies in humans[2], there was no significant evidence of carcinogenic activity of nitrite, despite dose escalations sufficient to produce profound methaemoglobinaemia and weight loss in rodents[131]. A recent epidemiological study evaluating dietary exposure of nitrite (cured meat) has suggested a possible link to the development of emphysema in at-risk subjects[132]; further population studies will be required to validate this observation. For most of the therapeutic indications discussed in this article, the low dose and short duration of treatment suggest that the risk of any carcinogenic effects is negligible. In fact as stated above, a large consumption of nitrate-containing vegetables may provide similar or even greater systemic loads of both nitrate and nitrite. If nitrite is to be used in much higher doses over prolonged periods of time, this issue will naturally have to be addressed.

Conclusions

The nitrate–nitrite–NO pathway may be viewed as complementary to the classical L-arginine–NOS pathway. These pathways work partly in parallel, but when oxygen availability is reduced and NOS activity is decreased, nitrite reduction to NO becomes more pronounced. So, in pathological conditions when regional and systemic ischaemia prevail, it may be beneficial to support the nitrate and nitrite stores pharmacologically or by dietary intervention.

We must now revise our long-standing view that nitrate and nitrite are only harmful substances in our diet or inert metabolites of endogenous NO. Instead, accumulating evidence suggests that the nitrate–nitrite–NO pathway critically subserves physiological hypoxic NO signalling, providing an opportunity for novel NO-based therapeutics.

1. Tannenbaum, S. R. & Correa, P. Nitrate and gastric cancer risks. *Nature* **317**, 675–676 (1985).
2. Mensinga, T. T., Speijers, G. J. & Meulenbelt, J. Health implications of exposure to environmental nitrogenous compounds. *Toxicol. Rev.* **22**, 41–51 (2003).
3. Benjamin, N. *et al.* Stomach NO synthesis. *Nature* **368**, 502 (1994).
The first suggestion of NOS-independent NO generation from inorganic nitrite and *in vitro* demonstration of its role in gastric host defence.
4. Lundberg, J. O., Weitzberg, E., Lundberg, J. M. & Alving, K. Intragastric nitric oxide production in humans: measurements in expelled air. *Gut* **35**, 1543–1546 (1994).
The first demonstration of NOS-independent NO generation from inorganic nitrate and nitrite in humans.
5. Zweier, J. L., Wang, P., Samouilov, A. & Kuppusamy, P. Enzyme-independent formation of nitric oxide in biological tissues. *Nature Med.* **1**, 804–809 (1995).
The first report demonstrating NOS-independent NO generation from nitrite in ischaemic heart tissue.
6. Cosby, K. *et al.* Nitrite reduction to nitric oxide by deoxyhemoglobin vasodilates the human circulation. *Nature Med.* **9**, 1498–1505 (2003).
The first report demonstrating vasodilation in humans by infusions of nitrite at near physiological levels. Experiments show a novel function of deoxyhaemoglobin as a functional nitrite reductase contributing to vasodilation.
7. Duncan, C. *et al.* Chemical generation of nitric oxide in the mouth from the enterosalivary circulation of dietary nitrate. *Nature Med.* **1**, 546–551 (1995).
8. Lundberg, J. O. & Govoni, M. Inorganic nitrate is a possible source for systemic generation of nitric oxide. *Free Radic. Biol. Med.* **37**, 395–400 (2004).
9. Gladwin, M. T. *et al.* The emerging biology of the nitrite anion. *Nature Chem. Biol.* **1**, 308–314 (2005).
10. Nagababu, E., Ramasamy, S., Abernethy, D. R. & Rifkind, J. M. Active nitric oxide produced in the red cell under hypoxic conditions by deoxyhemoglobin-mediated nitrite reduction. *J. Biol. Chem.* **278**, 46349–46356 (2003).
11. Shiva, S. *et al.* Deoxymyoglobin is a nitrite reductase that generates nitric oxide and regulates mitochondrial respiration. *Circ. Res.* **100**, 654–661 (2007).
12. Rassaf, T. *et al.* Nitrite reductase function of deoxymyoglobin: oxygen sensor and regulator of cardiac energetics and function. *Circ. Res.* **100**, 1749–1754 (2007).
13. Zhang, Z. *et al.* Human xanthine oxidase converts nitrite ions into nitric oxide (NO). *Biochem. Soc. Trans.* **25**, 524S (1997).
An early report demonstrating nitrite reduction by xanthine oxidoreductase and suggesting a physiological role for this enzyme in NO generation.
14. Godber, B. L. *et al.* Reduction of nitrite to nitric oxide catalyzed by xanthine oxidoreductase. *J. Biol. Chem.* **275**, 7757–7763 (2000).
15. Millar, T. M. *et al.* Xanthine oxidoreductase catalyses the reduction of nitrates and nitrite to nitric oxide under hypoxic conditions. *FEBS Lett.* **427**, 225–228 (1998).
16. Carlsson, S., Wiklund, N. P., Engstrand, L., Weitzberg, E. & Lundberg, J. O. Effects of pH, nitrite, and ascorbic acid on nonenzymatic nitric oxide generation and bacterial growth in urine. *Nitric Oxide* **5**, 580–586 (2001).
17. Peri, L. *et al.* Apples increase nitric oxide production by human saliva at the acidic pH of the stomach: a new biological function for polyphenols with a catechol group? *Free Radic. Biol. Med.* **39**, 668–681 (2005).
18. Gago, B., Lundberg, J. O., Barbosa, R. M. & Laranjinha, J. Red wine-dependent reduction of nitrite to nitric oxide in the stomach. *Free Radic. Biol. Med.* **43**, 1233–1242 (2007).

19. Giraldez, R. R., Panda, A., Xia, Y., Sanders, S. P. & Zweier, J. L. Decreased nitric-oxide synthase activity causes impaired endothelium-dependent relaxation in the postischemic heart. *J. Biol. Chem.* **272**, 21420–21426 (1997).
20. Oestergaard, L. *et al.* Diminished NO release in chronic hypoxic human endothelial cells. *Am. J. Physiol. Heart Circ. Physiol.* **293**, H2894–H2903 (2007).
21. Shiva, S. *et al.* Nitrite augments tolerance to ischemia/reperfusion injury via the modulation of mitochondrial electron transfer. *J. Exp. Med.* **204**, 2089–2102 (2007).
Shows that the cytoprotective effects of nitrite in ischaemia–reperfusion injury occur via the dynamic regulation of mitochondrial electron transfer, through reversible inhibition of complex I and subsequent limitation of oxidative damage.
22. Duranski, M. R. *et al.* Cytoprotective effects of nitrite during *in vivo* ischemia-reperfusion of the heart and liver. *J. Clin. Invest.* **115**, 1232–1240 (2005).
The first report demonstrating cytoprotective effects of low-dose nitrite *in vivo* after ischaemia–reperfusion injury of the heart and liver. This study provides proof of *in vivo* nitrite signalling at near physiological levels.
23. Bryan, N. S. *et al.* Nitrite is signalling molecule and regulator of gene expression in mammalian tissue. *Nature Chem. Biol.* **1**, 290–297 (2005).
A report suggesting that the nitrite anion is a physiological signalling molecule independent of intermediary NO formation.
24. Modin, A. *et al.* Nitrite-derived nitric oxide: a possible mediator of 'acidic-metabolic' vasodilation. *Acta Physiol. Scand.* **171**, 9–16 (2001).
25. Gladwin, M. T. *et al.* Role of circulating nitrite and *S*-nitrosohemoglobin in the regulation of regional blood flow in humans. *Proc. Natl Acad. Sci. USA* **97**, 11482–11487 (2000).
26. Bryan, N. S. *et al.* Cellular targets and mechanisms of nitros(yl)ation: an insight into their nature and kinetics *in vivo*. *Proc. Natl Acad. Sci. USA* **101**, 4308–4313 (2004).
27. Moncada, S. & Higgs, A. The L-arginine-nitric oxide pathway. *N. Engl. J. Med.* **329**, 2002–2012 (1993).
28. Ignarro, L. J. Nitric oxide as a unique signaling molecule in the vascular system: a historical overview. *J. Physiol. Pharmacol.* **53**, 503–514 (2002).
29. Shiva, S. *et al.* Ceruloplasmin is a NO oxidase and nitrite synthase that determines endocrine NO homeostasis. *Nature Chem. Biol.* **2**, 486–493 (2006).
30. Kleinbongard, P. *et al.* Plasma nitrite reflects constitutive nitric oxide synthase activity in mammals. *Free Radic. Biol. Med.* **35**, 790–796 (2003).
31. Kelm, M., Preik-Steinhoff, H., Preik, M. & Strauer, B. E. Serum nitrite sensitively reflects endothelial NO formation in human forearm vasculature: evidence for biochemical assessment of the endothelial L-arginine-NO pathway. *Cardiovasc. Res.* **41**, 765–772 (1999).
32. Rassaf, T., Feelisch, M. & Kelm, M. Circulating NO pool: assessment of nitrite and nitroso species in blood and tissues. *Free Radic. Biol. Med.* **36**, 413–422 (2004).
33. Green, D. J., Maiorana, A., O'Driscoll, G. & Taylor, R. Effect of exercise training on endothelium-derived nitric oxide function in humans. *J. Physiol.* **561**, 1–25 (2004).
34. Lewis, T. V., Dart, A. M., Chin-Dusting, J. P. & Kingwell, B. A. Exercise training increases basal nitric oxide production from the forearm in hypercholesterolemic patients. *Arterioscler. Thromb. Vasc. Biol.* **19**, 2782–2787 (1999).
35. Jungersten, L., Ambring, A., Wall, B. & Wennmalm, A. Both physical fitness and acute exercise regulate nitric oxide formation in healthy humans. *J. Appl. Physiol.* **82**, 760–764 (1997).
36. Crawford, J. H. *et al.* Transduction of NO-bioactivity by the red blood cell in sepsis: novel mechanisms of vasodilation during acute inflammatory disease. *Blood* **104**, 1375–1382 (2004).
37. Kleinbongard, P. *et al.* Plasma nitrite concentrations reflect the degree of endothelial dysfunction in humans. *Free Radic. Biol. Med.* **40**, 295–302 (2006).
38. Lundberg, J. O., Weitzberg, E., Cole, J. A. & Benjamin, N. Nitrate, bacteria and human health. *Nature Rev. Microbiol.* **2**, 593–602 (2004).
39. Wennmalm, Å. *et al.* Nitric oxide synthesis and metabolism in man. *Ann. NY Acad. Sci.* **714**, 158–164 (1994).
40. Spiegelhalder, B., Eisenbrand, G. & Preussman, R. Influence of dietary nitrate on nitrite content of human saliva: possible relevance to *in vivo* formation of *N*-nitroso compounds. *Food Cosmet. Toxicol.* **14**, 545–548 (1976).
41. Weitzberg, E. & Lundberg, J. O. Nonenzymatic nitric oxide production in humans. *Nitric Oxide* **2**, 1–7 (1998).
42. Sobko, T. *et al.* Gastrointestinal nitric oxide generation in germ-free and conventional rats. *Am. J. Physiol. Gastrointest. Liver Physiol.* **287**, G993–G997 (2004).
43. Weller, R. *et al.* Nitric oxide is generated on the skin surface by reduction of sweat nitrate. *J. Invest. Dermatol.* **107**, 327–331 (1996).
44. Sobko, T. *et al.* Gastrointestinal bacteria generate nitric oxide from nitrate and nitrite. *Nitric Oxide* **13**, 272–278 (2005).
45. Lundberg, J. O. *et al.* Urinary nitrite: more than a marker of infection. *Urology* **50**, 189–191 (1997).
46. Nathan, C. F. & Hibbs, J. B. Role of nitric oxide synthesis in macrophage antimicrobial activity. *Curr. Opin. Immunol.* **3**, 65–70 (1991).
47. Fang, F. C. Perspectives series: host/pathogen interactions. Mechanisms of nitric oxide-related antimicrobial activity. *J. Clin. Invest.* **99**, 2818–2825 (1997).
48. Stuehr, D. & Marletta, M. A. Mammalian nitrate biosynthesis: mouse macrophages produce nitrite and nitrate in response to *Escherichia coli* lipopolysaccharide. *Proc. Natl Acad. Sci. USA* **82**, 7738–7742 (1985).
49. Hibbs, J. B., Jr, Taintor, R. R. & Vavrin, Z. Macrophage cytotoxicity: role for L-arginine deiminase and imino nitrogen oxidation to nitrite. *Science* **235**, 473–476 (1987).
50. Dykhuizen, R. S. *et al.* Antimicrobial effect of acidified nitrite on gut pathogens: importance of dietary nitrate in host defense. *Antimicrob. Agents Chemother.* **40**, 1422–1425 (1996).
51. Bjorne, H., Weitzberg, E. & Lundberg, J. O. Intragastric generation of antimicrobial nitrogen oxides from saliva — physiological and therapeutic considerations. *Free Radic. Biol. Med.* **41**, 1404–1412 (2006).
52. Bjorne, H. H. *et al.* Nitrite in saliva increases gastric mucosal blood flow and mucus thickness. *J. Clin. Invest.* **113**, 106–114 (2004).
53. Petersson, J. *et al.* Dietary nitrate increases gastric mucosal blood flow and mucosal defense. *Am. J. Physiol. Gastrointest. Liver Physiol.* **292**, G718–G724 (2007).
54. Holm, M., Olbe, L. & Fandriks, L. Intragastric CO_2 and nitric oxide participate in the regulation of peptone-induced gastrin release in humans. *Scand. J. Gastroenterol.* **35**, 1260–1265 (2000).
55. Weiss, S., Wilkins, R. W. & Haynes, F. W. The nature of the collapse induced by sodium nitrite. *J. Clin. Invest.* **16**, 73–84 (1937).
56. Furchgott, R. F. & Bhadrakom, S. Reactions of strips of rabbit aorta to epinephrine, isopropylarterenol, sodium nitrite and other drugs. *J. Pharmacol. Exp. Ther.* **108**, 129–143 (1953).
57. Ignarro, L. J. *et al.* Mechanism of vascular smooth muscle relaxation by organic nitrates, nitrites, nitroprusside and nitric oxide: evidence for the involvement of *S*-nitrosothiols as active intermediates. *J. Pharmacol. Exp. Ther.* **218**, 739–749 (1981).
58. Lauer, T. *et al.* Plasma nitrite rather than nitrate reflects regional endothelial nitric oxide synthase activity but lacks intrinsic vasodilator action. *Proc. Natl Acad. Sci. USA* **98**, 12814–12819 (2001).
59. Cannon, R. O. 3rd *et al.* Effects of inhaled nitric oxide on regional blood flow are consistent with intravascular nitric oxide delivery. *J. Clin. Invest.* **108**, 279–287 (2001).
60. Schechter, A. N. & Gladwin, M. T. Hemoglobin and the paracrine and endocrine functions of nitric oxide. *N. Engl. J. Med.* **348**, 1483–1485 (2003).
61. Dejam, A., Hunter, C. J. & Gladwin, M. T. Effects of dietary nitrate on blood pressure. *N. Engl. J. Med.* **356**, 1590 (2007).
62. Larsen, F. J., Ekblom, B., Sahlin, K., Lundberg, J. O. & Weitzberg, E. Effects of dietary nitrate on blood pressure in healthy volunteers. *N. Engl. J. Med.* **355**, 2792–2793 (2006).
A report demonstrating a reduction in blood pressure in humans by ingestion of dietary levels of inorganic nitrate. The authors suggest that nitrate is bioactivated *in vivo* to form nitrite and vasodilatory NO and that this may regulate basal blood pressure.
63. Brooks, J. the action of nitrite on haemoglobin in the absence of oxygen. *Proc. R. Soc. Med.* **137**, 368–382 (1937).
64. Doyle, M. P., Pickering, R. A., DeWeert, T. M., Hoekstra, J. W. & Pater, D. Kinetics and mechanism of the oxidation of human deoxyhemoglobin by nitrites. *J. Biol. Chem.* **256**, 12393–12398 (1981).
65. Huang, K. T. *et al.* The reaction between nitrite and deoxyhemoglobin. Reassessment of reaction kinetics and stoichiometry. *J. Biol. Chem.* **280**, 31126–31131 (2005).
66. Huang, Z. *et al.* Enzymatic function of hemoglobin as a nitrite reductase that produces NO under allosteric control. *J. Clin. Invest.* **115**, 2099–2107 (2005).
67. Basu, S. *et al.* Catalytic generation of N_2O_3 by the concerted nitrite reductase and anhydrase activity of haemoglobin. *Nature Chem. Biol.* **3**, 785–794 (2007).
68. Larsen, F. J., Lundberg, J. O., Weitzberg, E. & Ekblom, B. Effect of dietary nitrate on oxygen cost during exercise. *Acta Physiol. (Oxf)* **191**, 59–66 (2007).
69. Li, H., Samouilov, A., Liu, X. & Zweier, J. L. Characterization of the magnitude and kinetics of xanthine oxidase-catalyzed nitrite reduction. Evaluation of its role in nitric oxide generation in anoxic tissues. *J. Biol. Chem.* **276**, 24482–24489 (2001).
70. Nohl, H. *et al.* Mitochondria recycle nitrite back to the bioregulator nitric monoxide. *Acta Biochim. Pol.* **47**, 913–921 (2000).
71. Castello, P. R., David, P. S., McClure, T., Crook, Z. & Poyton, R. O. Mitochondrial cytochrome oxidase produces nitric oxide under hypoxic conditions: implications for oxygen sensing and hypoxic signaling in eukaryotes. *Cell. Metab.* **3**, 277–287 (2006).
72. Kozlov, A. V., Staniek, K. & Nohl, H. Nitrite reductase activity is a novel function of mammalian mitochondria. *FEBS Lett.* **454**, 127–130 (1999).
73. Kozlov, A. V., Dietrich, B. & Nohl, H. Various intracellular compartments cooperate in the release of nitric oxide from glycerol trinitrate in liver. *Br. J. Pharmacol.* **139**, 989–997 (2003).
74. Vanin, A. F., Bevers, L. M., Slama-Schwok, A. & van Faassen, E. E. Nitric oxide synthase reduces nitrite to NO under anoxia. *Cell. Mol. Life Sci.* **64**, 96–103 (2007).
75. Kozlov, A. V. *et al.* Mechanisms of vasodilatation induced by nitrite instillation in intestinal lumen: possible role of hemoglobin. *Antioxid. Redox Signal.* **7**, 515–521 (2005).
76. Tsuchiya, K. *et al.* Nitrite is an alternative source of NO *in vivo*. *Am. J. Physiol. Heart Circ. Physiol.* **288**, H2163–H2170 (2004).
77. Tsuchiya, K. *et al.* Malfunction of vascular control in lifestyle-related diseases: formation of systemic hemoglobin-nitric oxide complex (HbNO) from dietary nitrite. *J. Pharmacol. Sci.* **96**, 395–400 (2004).
78. Hunter, C. J. *et al.* Inhaled nebulized nitrite is a hypoxia-sensitive NO-dependent selective pulmonary vasodilator. *Nature Med.* **10**, 1122–1127 (2004).
79. Webb, A. *et al.* Reduction of nitrite to nitric oxide during ischemia protects against myocardial ischemia-reperfusion damage. *Proc. Natl Acad. Sci. USA* **101**, 13683–13688 (2004).
The first report demonstrating cardioprotective effects of nitrite in heart preparations via xanthine oxidoreductase-mediated nitrite reduction to NO.
80. Pluta, R. M., Dejam, A., Grimes, G., Gladwin, M. T. & Oldfield, E. H. Nitrite infusions to prevent delayed cerebral vasospasm in a primate model of subarachnoid hemorrhage. *JAMA* **293**, 1477–1484 (2005).
81. Dias-Junior, C. A., Gladwin, M. T. & Tanus-Santos, J. E. Low-dose intravenous nitrite improves hemodynamics in a canine model of acute pulmonary thrombo-embolism. *Free Radic. Biol. Med.* **41**, 1764–1770 (2006).
82. Murad, F. Shattuck Lecture. Nitric oxide and cyclic GMP in cell signaling and drug development. *N. Engl. J. Med.* **355**, 2003–2011 (2006).
83. Chen, Z., Zhang, J. & Stamler, J. S. Identification of the enzymatic mechanism of nitroglycerin bioactivation. *Proc. Natl Acad. Sci. USA* **99**, 8306–8311 (2002).
84. Li, H., Cui, H., Liu, X. & Zweier, J. L. Xanthine oxidase catalyzes anaerobic transformation of organic nitrates to nitric oxide and nitrosothiols: characterization of this mechanism and the link between organic nitrate and guanylyl cyclase activation. *J. Biol. Chem.* **280**, 16594–16600 (2005).

85. Crandall, L. A., Leake, A. S., Loevenhart, A. S. & Muehlberger, C. W. Acquired tolerance to and cross tolerance between the nitrous and nitric acid esters and sodium nitrite in man. *J. Pharmacol. Exp. Therapeut.* **103** (1930).
86. Lu, P. *et al.* Nitrite-derived nitric oxide by xanthine oxidoreductase protects the liver against ischemia-reperfusion injury. *Hepatobiliary Pancreat. Dis. Int.* **4**, 350–355 (2005).
87. Baker, J. E. *et al.* Nitrite confers protection against myocardial infarction: role of xanthine oxidoreductase, NADPH oxidase and K(ATP) channels. *J. Mol. Cell Cardiol.* **43**, 437–444 (2007).
88. Jung, K. H. *et al.* Early intravenous infusion of sodium nitrite protects brain against *in vivo* ischemia-reperfusion injury. *Stroke* **37**, 2744–2750 (2006).
89. Tripatara, P. *et al.* Nitrite-derived nitric oxide protects the rat kidney against ischemia/reperfusion injury *in vivo*: role for xanthine oxidoreductase. *J. Am. Soc. Nephrol.* **18**, 570–580 (2007).
90. Lundberg, J. O., Feelisch, M., Bjorne, H., Jansson, E. A. & Weitzberg, E. Cardioprotective effects of vegetables: is nitrate the answer? *Nitric Oxide* **15**, 359–362 (2006).
91. Classen, H. G., Stein-Hammer, C. & Thoni, H. Hypothesis: the effect of oral nitrite on blood pressure in the spontaneously hypertensive rat. Does dietary nitrate mitigate hypertension after conversion to nitrite? *J. Am. Coll. Nutr.* **9**, 500–502 (1990).
92. Pain, T. *et al.* Opening of mitochondrial K(ATP) channels triggers the preconditioned state by generating free radicals. *Circ. Res.* **87**, 460–466 (2000).
93. Oldenburg, O., Cohen, M. V. & Downey, J. M. Mitochondrial K(ATP) channels in preconditioning. *J. Mol. Cell. Cardiol.* **35**, 569–575 (2003).
94. Oldenburg, O. *et al.* Bradykinin induces mitochondrial ROS generation via NO, cGMP, PKG, and mitoKATP channel opening and leads to cardioprotection. *Am. J. Physiol. Heart Circ. Physiol.* **286**, H468–H476 (2004).
95. Xu, Z., Ji, X. & Boysen, P. G. Exogenous nitric oxide generates ROS and induces cardioprotection: involvement of PKG, mitochondrial KATP channels, and ERK. *Am. J. Physiol. Heart Circ. Physiol.* **286**, H1433–H1440 (2004).
96. Das, D. K. Cellular, biochemical, and molecular aspects of reperfusion injury. Introduction. *Ann. NY Acad. Sci.* **723**, xiii–xvi (1994).
97. Zweier, J. L., Flaherty, J. T. & Weisfeldt, M. L. Direct measurement of free radical generation following reperfusion of ischemic myocardium. *Proc. Natl Acad. Sci. USA* **84**, 1404–1407 (1987).
98. Clementi, E., Brown, G. C., Feelisch, M. & Moncada, S. Persistent inhibition of cell respiration by nitric oxide: crucial role of *S*-nitrosylation of mitochondrial complex I and protective action of glutathione. *Proc. Natl Acad. Sci. USA* **95**, 7631–7636 (1998).
99. Burwell, L. S., Nadtochiy, S. M., Tompkins, A. J., Young, S. & Brookes, P. S. Direct evidence for *S*-nitrosation of mitochondrial complex I. *Biochem. J.* **394**, 627–634 (2006).
100. Dahm, C. C., Moore, K. & Murphy, M. P. Persistent *S*-nitrosation of complex I and other mitochondrial membrane proteins by *S*-nitrosothiols but not nitric oxide or peroxynitrite: implications for the interaction of nitric oxide with mitochondria. *J. Biol. Chem.* **281**, 10056–10065 (2006).
101. Cleeter, M. W., Cooper, J. M., Darley-Usmar, V. M., Moncada, S. & Schapira, A. H. Reversible inhibition of cytochrome c oxidase, the terminal enzyme of the mitochondrial respiratory chain, by nitric oxide. Implications for neurodegenerative diseases. *FEBS Lett.* **345**, 50–54 (1994).
102. Carr, G. J. & Ferguson, S. J. Nitric oxide formed by nitrite reductase of *Paracoccus denitrificans* is sufficiently stable to inhibit cytochrome oxidase activity and is reduced by its reductase under aerobic conditions. *Biochim. Biophys. Acta* **1017**, 57–62 (1990).
103. Brown, G. C. & Cooper, C. E. Nanomolar concentrations of nitric oxide reversibly inhibit synaptosomal respiration by competing with oxygen at cytochrome oxidase. *FEBS Lett.* **356**, 295–298 (1994).
104. Bolanos, J. P., Peuchen, S., Heales, S. J., Land, J. M. & Clark, J. B. Nitric oxide-mediated inhibition of the mitochondrial respiratory chain in cultured astrocytes. *J. Neurochem.* **63**, 910–916 (1994).
105. Nadtochiy, S. M., Burwell, L. S. & Brookes, P. S. Cardioprotection and mitochondrial *S*-nitrosation: effects of *S*-nitroso-2-mercaptopropionyl glycine (SNO-MPG) in cardiac ischemia-reperfusion injury. *J. Mol. Cell. Cardiol.* **42**, 812–825 (2007).
106. Fox-Robichaud, A. *et al.* Inhaled NO as a viable antiadhesive therapy for ischemia/reperfusion injury of distal microvascular beds. *J. Clin. Invest.* **101**, 2497–2505 (1998).
107. Hataishi, R. *et al.* Inhaled nitric oxide decreases infarction size and improves left ventricular function in a murine model of myocardial ischemia-reperfusion injury. *Am. J. Physiol. Heart Circ. Physiol.* **291**, H379–H384 (2006).
108. Liu, X. *et al.* Nitric oxide inhalation improves microvascular flow and decreases infarction size after myocardial ischemia and reperfusion. *J. Am. Coll. Cardiol.* **50**, 808–817 (2007).
109. Lang, J. D., Jr *et al.* Inhaled NO accelerates restoration of liver function in adults following orthotopic liver transplantation. *J. Clin. Invest.* **117**, 2583–2591 (2007).
110. Kinsella, J. P. *et al.* Early inhaled nitric oxide therapy in premature newborns with respiratory failure. *N. Engl. J. Med.* **355**, 354–364 (2006).
111. Hawkey, C. J. Nonsteroidal anti-inflammatory drug gastropathy. *Gastroenterology* **119**, 521–535 (2000).
112. Wallace, J. L. & Miller, M. J. Nitric oxide in mucosal defense: a little goes a long way. *Gastroenterology* **119**, 512–520. (2000).
113. Evans, S. M. & Whittle, B. J. Role of bacteria and inducible nitric oxide synthase activity in the systemic inflammatory microvascular response provoked by indomethacin in the rat. *Eur. J. Pharmacol.* **461**, 63–71 (2003).
114. Hawkey, C. J. & Langman, M. J. Non-steroidal anti-inflammatory drugs: overall risks and management. Complementary roles for COX-2 inhibitors and proton pump inhibitors. *Gut* **52**, 600–608 (2003).
115. Jansson, E. A. *et al.* Protection from nonsteroidal anti-inflammatory drug (NSAID)-induced gastric ulcers by dietary nitrate. *Free Radic. Biol. Med.* **42**, 510–518 (2007).
116. Miyoshi, M. *et al.* Dietary nitrate inhibits stress-induced gastric mucosal injury in the rat. *Free Radic. Res.* **37**, 85–90 (2003).
117. Dykhuizen, R. S. *et al. Helicobacter pylori* is killed by nitrite under acidic conditions. *Gut* **42**, 334–337 (1998).
118. Bjorne, H., Govoni, M., Tornberg, D. C., Lundberg, J. O. & Weitzberg, E. Intragastric nitric oxide is abolished in intubated patients and restored by nitrite. *Crit. Care Med.* **33**, 1722–1727 (2005).
119. Reddy, D., Lancaster, J. R. Jr & Cornforth, D. P. Nitrite inhibition of *Clostridium botulinum*: electron spin resonance detection of iron-nitric oxide complexes. *Science* **221**, 769–770 (1983).
120. Dykhuizen, R. *et al.* Antimicrobial effect of acidified nitrite on gut pathogens: importance of dietary nitrate in host defence. *Antimicrob. Agents Chemother.* **40**, 1422–1425 (1996).
121. Yoon, S. S. *et al.* Anaerobic killing of mucoid *Pseudomonas aeruginosa* by acidified nitrite derivatives under cystic fibrosis airway conditions. *J. Clin. Invest.* **116**, 436–446 (2006).
122. Carlsson, S., Govoni, M., Wiklund, N. P., Weitzberg, E. & Lundberg, J. O. *In vitro* evaluation of a new treatment for urinary tract infections caused by nitrate-reducing bacteria. *Antimicrob. Agents Chemother.* **47**, 3713–3718 (2003).
123. Weller, R., Ormerod, A. D., Hobson, R. P. & Benjamin, N. J. A randomized trial of acidified nitrite cream in the treatment of tinea pedis. *J. Am. Acad. Dermatol.* **38**, 559–563 (1998).
124. Weller, R., Price, R. J., Ormerod, A. D., Benjamin, N. & Leifert, C. Antimicrobial effect of acidified nitrite on dermatophyte fungi, *Candida* and bacterial skin pathogens. *J. Appl. Microbiol.* **90**, 648–652 (2001).
125. Ormerod, A. D., White, M. I., Shah, S. A. & Benjamin, N. Molluscum contagiosum effectively treated with a topical acidified nitrite, nitric oxide liberating cream. *Br. J. Dermatol.* **141**, 1051–1053 (1999).
126. Carlsson, S., Weitzberg, E., Wiklund, P. & Lundberg, J. O. Intravesical nitric oxide delivery for prevention of catheter-associated urinary tract infections. *Antimicrob. Agents Chemother.* **49**, 2352–2355 (2005).
127. Dezfulian, C., Raat, N., Shiva, S. & Gladwin, M. T. Role of the anion nitrite in ischemia-reperfusion cytoprotection and therapeutics. *Cardiovasc. Res.* **75**, 327–338 (2007).
128. Larauche, M. *et al.* Protective effect of dietary nitrate on experimental gastritis in rats. *Br. J. Nutr.* **89**, 777–786 (2003).
129. Wallace, J. L., Ignarro, L. J. & Fiorucci, S. Potential cardioprotective actions of NO-releasing aspirin. *Nature Rev. Drug Discov.* **1**, 375–382 (2002).
130. Chakrapani, H., Gorczynski, M. J. & King, S. B. Allylic nitro compounds as nitrite donors. *J. Am. Chem. Soc.* **128**, 16332–16337 (2006).
131. National Toxicology Programe. Toxicology and carcinogenesis studies of sodium nitrite (CAS NO. 7632-00-0) in F344/N rats and B6C3F1 mice (drinking water studies). *Natl Toxicol. Program Tech. Rep. Ser.* **495**, 7–273 (2001).
132. Jiang, R., Paik, D. C., Hankinson, J. L. & Barr, R. G. Cured meat consumption, lung function, and chronic obstructive pulmonary disease among United States adults. *Am. J. Respir. Crit. Care Med.* **175**, 798–804 (2007).
133. Ward, M. H. *et al.* Workgroup report: drinking-water nitrate and health — recent findings and research needs. *Environ. Health Perspect.* **113**, 1607–1614 (2005).
134. Joshipura, K. J. *et al.* The effect of fruit and vegetable intake on risk for coronary heart disease. *Ann. Intern. Med.* **134**, 1106–1114 (2001).
135. Hu, F. B. & Willett, W. C. Optimal diets for prevention of coronary heart disease. *JAMA* **288**, 2569–2578 (2002).
136. Appel, L. J. *et al.* A clinical trial of the effects of dietary patterns on blood pressure. DASH Collaborative Research Group. *N. Engl. J. Med.* **336**, 1117–1124 (1997).

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Competing interests statement

The authors declare competing financial interests: see web version for details.

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