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Original Contribution

INORGANIC NITRATE IS A POSSIBLE SOURCE FOR SYSTEMIC GENERATION OF NITRIC OXIDE

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Abstract—Nitrate and nitrite have been considered stable inactive end products of nitric oxide (NO). While several recent studies now imply that nitrite can be reduced to bioactive NO again, the more stable anion nitrate is still considered to be biologically inert. Nitrate is concentrated in saliva, where a part of it is reduced to nitrite by bacterial nitrate reductases. We tested if ingestion of inorganic nitrate would affect the salivary and systemic levels of nitrite and *S*-nitrosothiols, both considered to be circulating storage pools for NO. Levels of nitrate, nitrite, and *S*-nitrosothiols were measured in plasma, saliva, and urine before and after ingestion of sodium nitrate (10 mg/kg). Nitrate levels increased greatly in saliva, plasma, and urine after the nitrate load. Salivary *S*-nitrosothiols also increased, but plasma levels remained unchanged. A 4-fold increase in plasma nitrite was observed after nitrate ingestion. If, however, the test persons avoided swallowing after the nitrate load, the increase in plasma nitrite was prevented, thereby illustrating its salivary origin. We show that nitrate is a substrate for systemic generation of nitrite. There are several pathways to further reduce this nitrite to NO. These results challenge the dogma that nitrate is biologically inert and instead suggest that a complete reverse pathway for generation of NO from nitrate exists.

Keywords—Nitric oxide, Vasodilation, Hypertension, Nitric oxide synthase, Vegetarian, *S*-Nitrosothiols, Nitrite, Nitric oxide synthase-independent, Free radicals

一氧化氮(NO)是由L-精氨酸合成的重要血管稳态调节因子。 这种气体除了是一种有效的血管扩张剂外，还能抑制血小板的功能。 血液中NO的半衰期被认为很短，主要是由于与血红蛋白反应后迅速失活。 因此，NO被认为是一种旁分泌化学介质，仅在其生产现场附近产生影响。 最近有人提出，NO可以与其他化合物结合，它们可以作为NO的稳定载体，从而保持其生物活性。

NO或化学相关物种与硫醇(SH-)基团的反应，例如在蛋白质中，可以导致S-亚硝基硫醇(SNO)的形成)。 SNO被认为是血液中NO的重要载体/供体，从而增加了其半衰期，并允许更多的远端效应。 NO失活的一个主要途径是氧化为亚硝酸盐和硝酸盐。 这两种阴离子长期以来被认为是NO的稳定惰性终产物。 对亚硝酸盐不再适用，因为最近的几项研究表明，存在不同的途径将这种阴离子回收到血液和组织中的生物活性NO。 结果表明，当试验系统中的pH降低到缺血期间组织中测量的水平时，生理量的亚硝酸盐起到血管扩张剂的作用

INTRODUCTION

Nitric oxide (NO) produced from L-arginine by NO synthases is a key regulator of vascular homeostasis. Besides being a potent vasodilator this gas also inhibits platelet function. The half-life of NO in blood is thought to be very short mainly due to rapid inactivation after reaction with hemoglobin. Therefore, NO has been regarded as a paracrine chemical mediator having effects only in the close vicinity of its production site. More recently it has been suggested that NO can combine with other compounds, which can function as stable carriers of NO, thereby conserving its bioactivity. Reaction of NO or chemically related species with thiol (SH–) groups, e.g., in proteins, can result in formation of *S*-nitrosothiols (SNOs). SNOs have been suggested to be important carriers/donors of NO in blood, thereby increasing its half-life and allowing for more distal effects. A major pathway for inactivation of NO is through oxidation to nitrite and nitrate. Both these anions have long been considered as stable inert end products of NO. This is no longer true for nitrite, as several recent studies have shown that different pathways exist to recycle this anion back into bioactive NO in blood and tissues [1–7]. Modin et al. showed that physiological amounts of nitrite acted as a vasodilator when pH in the test system was reduced to levels measured in tissues during ischemia [3]. This was paralelled by formation of NO from the acidified nitrite. In a recent study, Cosby et al. reported that intraarterial infusion of nitrite at near-physiological levels caused local vasodilation in humans through

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血浆中的亚硝酸盐水平在0.1-1uM范围内，组织水平要高得多。 血液中SNO的水平处于低纳米摩尔范围。 因此，似乎亚硝酸盐阴离子构成了比SNO更大的血液中NO的储存池。 理论上，比亚硝酸盐更大的NO池可能是硝酸盐离子，硝酸盐离子在血浆中的浓度比亚硝酸盐高几百倍。 然而，这首先需要将硝酸盐还原为亚硝酸盐，这是哺乳动物酶无法进行的反应。 与真核细胞相反，许多细菌配备了高效的硝酸盐还原酶。 他们使用硝酸盐进行呼吸或作为底物将氮掺入生物质中。 口腔内含有大量的硝酸盐还原细菌。这种共生菌群从唾液中产生亚硝酸盐，其中硝酸盐含量非常丰富。 事实上，大约25%的循环硝酸盐被唾液腺主动吸收并分泌在唾液中，从而为口腔亚硝酸盐产生提供持续的底物。 因此，在细菌的帮助下，存在一种从硝酸盐的大循环池中产生亚硝酸盐的途径。 如上所述，存在几种途径来进一步降低血液和组织中亚硝酸盐到生物活性NO。 然后问题仍然是唾液亚硝酸盐是否能达到全身循环时吞咽。 我们想研究摄入无机硝酸盐是否会导致血浆亚硝酸盐和其他相关氮氧化物水平的增加。

deoxyhemoglobin-dependent reduction to nitric oxide [7]. Nitrite levels in plasma are in the range 0.1–1 μM and tissue levels are considerably higher [3,7]. The levels of SNOs in blood are in the low nanomolar range [8,9]. Thus, it seems as if nitrite anions constitute a much larger storage pool for NO in blood than SNOs. Theoretically, an even greater pool of NO than nitrite could be nitrate ions, which are present in plasma at concentrations several hundred-fold higher than nitrite. This, however, would first require reduction of nitrate to nitrite, a reaction that cannot be carried out by mammalian enzymes. In contrast to eukaryotic cells, many bacteria are equipped with highly efficient nitrate reductases. They use nitrate for respiration or as a substrate for incorporation of nitrogen into biomass. The oral cavity contains large numbers of nitrate-reducing bacteria [10]. This commensal flora generates nitrite from saliva, which is remarkably rich in nitrate. In fact, about 25% of all circulating nitrate is actively taken up by the salivary glands and secreted in saliva, thereby providing a continuous delivery of substrate for oral nitrite generation [11]. Thus, with the aid of bacteria a pathway exists for generation of nitrite from the large circulating pool of nitrate. As indicated above, several pathways exist to further reduce nitrite to bioactive NO in blood and tissues. The question then remains if salivary nitrite can reach the systemic circulation when swallowed. We wanted to study if ingestion of inorganic nitrate would result in increased plasma levels of nitrite and other related nitrogen oxides.

MATERIAL AND METHODS

Study subjects and experimental protocol

The study was approved by the ethics committee at the Karolinska Institute and all subjects gave their informed consent. Nine healthy nonsmoking volunteers (aged 26–46 years, 5 males and 4 females) took part in the study. All subjects had fasted overnight and food or drinks were not allowed during the experiment.

A catheter was inserted into the antecubital vein of the left arm for repeated blood sampling. Samples of blood (5 ml) were drawn 30 min before and again immediately before ingestion of sodium nitrate (10 mg/kg in 100 ml water). After nitrate ingestion, blood was again sampled at 15, 30, 60, and 90 min. Within 30 s of sampling, the blood was centrifuged at 1300*g* for 10 min at 4°C. Saliva (2–4 ml) was collected at the same time points and centrifuged at 1300*g* for 5 min. Urine was collected immediately before nitrate ingestion and then 1, 2, and 3 h after the nitrate load for analysis of nitrate + nitrite. Samples of plasma, urine, and saliva were kept at −80°C for a maximum of 3 days before analysis.

In four of the subjects an additional experiment was performed on a separate day. To test if saliva could be a possible source of plasma nitrate/nitrite, subjects were asked to completely avoid swallowing saliva during the first hour after intake of nitrate. Blood samples were taken at the same intervals as described above for measurements of nitrate/nitrite. After 1 h swallowing was again allowed.

Chemiluminescence assay for nitrogen oxides

Nitrite, nitrate, and SNOs were determined by chemiluminescence after reductive cleavage and subsequent determination of the NO released into the gas phase [12]. The detector (Aerocrine AB, Sweden) is based on the reaction of NO with ozone (O_3) to give nitrogen dioxide (NO_2). A proportion of the latter arises in an electronically excited state (NO_2^*), which, on decay to its ground state, emits light in the near-infrared region and can be quantified by a photomultiplier.

The samples were directly introduced via a gastight syringe into the reduction solution of a microreaction purge vessel coupled with a condenser and heating jacket unit (Sievers, Boulder, CO, USA). The condenser jacket temperature was controlled by a continuous flow of cold water while the temperature of the heating jacket was controlled by a continuous flow of warm water regulated by a constant-temperature circulating bath (MGW Lauda M3). Nitrogen, at the rate of 192 ml/min, was used as the carrier gas of NO. The flow could be adjusted with a needle valve integrated with the purge vessel, and the outlet of the gas stream was passed through a scrubbing bottle containing sodium hydroxide (1 mol/l, 0°C) to trap traces of acid before transfer into the NO analyzer.

A rapid-response chemiluminescence NO system (Aerocrine AB, Stockholm, Sweden) was used to detect the NO signals and collect the data which were further manipulated with Origin for Windows, Version 7.0 (Microcal, Northampton, MA, USA), and reported as area under the curve.

样品通过气密注射器直接引入微反应净化容器的还原溶液中，再加上冷凝器和加热外套单元(Sievers，Boulder，CO，USA)。冷凝器夹套温度由冷水连续流动控制，加热夹套温度由恒温循环浴(MGWLaudaM3)调节的温水连续流动控制)。氮气以192ml/min的速率作为NO的载气。流量可以用与清洗容器集成的针阀调节，气流的出口通过含有氢氧化钠(1mol/l，0jC)的洗涤瓶，在转移到NO分析仪之前捕获酸的痕迹。

Nitrite and S-nitrosothiols 亚硝酸盐和S-亚硝基硫醇

The reducing mixture, consisting of 45 mmol/l potassium iodide (KI) and 10 mmol/l iodine (I_2) in glacial acetic acid, was kept at a constant temperature of 56°C continuously bubbled with nitrogen. Blood and saliva samples were collected in tubes containing EDTA (final concentration 2 mM) and *N*-ethylmaleimide (final concentration 5 mM) and immediately centrifuged.

Nitrite measurements were performed by direct sample injection (100 μl) into the reducing solution,

还原混合物由45mmol/l碘化钾(KI)和10mmol/l碘(I2)在冰醋酸中组成，在56度的恒温下与氮气连续鼓泡。在含有EDTA(最终浓度2mM)和N-乙基马来酰亚胺(最终浓度5mM)的试管中采集血液和唾液样本，并立即离心。

亚硝酸盐测量是通过直接进样(100ul)到还原溶液中进行的，用磺胺预处理样品的峰面积与未处理的样品的峰面积进行简单减法，测定亚硝酸盐的含量[将5%磺胺溶液在1NHCl中的10%(v/v)加入到生物样品中(最终浓度为29mmol/l)，并在室温下孵育15min]。 在这些条件下，亚硝酸盐与磺胺反应生成稳定的重氮离子，而还原混合物不会将其转化为NO。 用新制备的亚硝酸盐标准溶液在超纯水中获得校准曲线。

and the amount of nitrite was quantified by simple subtraction of the peak area of sample aliquots pretreated with sulfanilamide from that of untreated aliquots [10% (v/v) of a 5% solution of sulfanilamide in 1 N HCl is added to the biological sample (final concentration 29 mmol/l) and incubated for 15 min at room temperature]. Under these conditions, nitrite reacts with sulfanilamide to form a stable diazonium ion that is not converted to any appreciable extent to NO by the reducing mixture. The calibration curve was obtained with freshly prepared nitrite standard solutions in ultrapure water.

直接进样(300ul)对还原溶液进行S-硝硫硫醇测定，用磺胺氯化汞预处理样品的峰面积简单减量化，室温下为5%磺胺溶液的30min[10%(v/v 在1NHCl中，从室温下仅用磺胺处理的样品中提取0.2% HgCl2[10%(v/v)的溶液5%磺胺在1NHCl中]。在这些条件下，HgCl2选择性地切割S-NO键[Saville反应，见12]，而不影响其他可检测的NO物种的峰形或回收率。

S-Nitrosothiol measurements were performed by direct sample injection (300 µl) into the reducing solution and quantified by simple subtraction of the peak area of sample aliquots pretreated with sulfanilamide + mercuric chloride at room temperature for 30 min [10% (v/v) of a solution 5% sulfanilamide + 0.2% $HgCl_2$ in 1 N HCl] from that of sample aliquots treated just with sulfanilamide at room temperature for 15 min [10% (v/v) of a solution 5% sulfanilamide in 1 N HCl]. Under these conditions, $HgCl_2$ selectively cleaves the *S*–NO bond [Saville reaction, see 12] without affecting peak shape or recovery of other detectable NO species. The calibration curve was obtained with freshly prepared *S*-nitrosoglutathione.

为了避免富含蛋白质的样品注射（例如血浆）后还原溶液的起泡，在分析前将100ul的抗泡剂(Sievers)添加到还原溶液中。

To avoid foaming of the reducing solution after protein-rich sample injections (e.g., plasma),100 µl of Anti-Foaming Agent (Sievers) was added to the reducing solution prior to analysis.

Nitrate 硝酸盐检测

在95度的1N盐酸（饱和溶液）中，用钒(III)氯化物溶液将硝酸盐还原为NO。由于钒(III)/HCl也将亚硝酸盐转化为NO，因此通过减去亚硝酸盐浓度来量化硝酸盐的数量。为了避免富含蛋白质的样品注射后还原溶液的发泡（例如血浆），在用冷乙醇(血浆/乙醇比=1/3)分析之前，对样品进行脱蛋白）。用新制备的硝酸盐标准溶液在超纯水中得到校准曲线。

Nitrate was reduced to NO with a solution of vanadium(III) chloride in 1 N hydrochloric acid (saturated solution) at 95°C. As vanadium(III)/HCl will also convert nitrite to NO, the amount of nitrate was quantified by subtraction of the nitrite concentration. To avoid foaming of the reducing solution after protein-rich sample injections (e.g., plasma), samples were deproteinized prior to analysis with cold ethanol (plasma/ethanol ratio = 1/3). The calibration curve was obtained with freshly prepared nitrate standard solutions in ultrapure water.

RESULTS

Saliva 唾液

The temporal changes in salivary levels of nitrate, nitrite, and SNOs after nitrate ingestion are shown in Fig. 1. At 30 min, salivary nitrate had increased from 0.19 ± 0.03 to 8.2 ± 1 mM. Nitrite increased from 104 ± 21 to 713 ± 150 µM, and SNOs increased from 25 ± 9.8 to 297 nM.

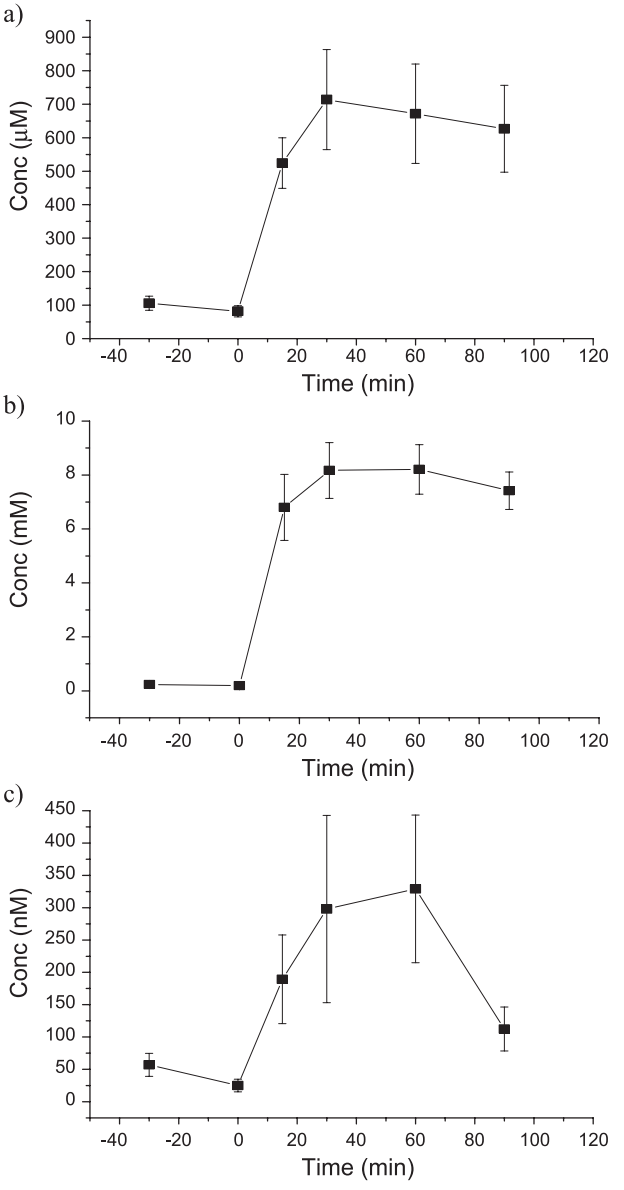

Fig. 1. Temporal changes in salivary levels of nitrite (a), nitrate (b), and *S*-nitrosothiols (c) before and after ingestion of sodium nitrate (10 mg/kg).

Plasma 血浆；原生质，细胞质

The temporal changes in plasma levels of nitrate, nitrite, and SNOs after nitrate ingestion are shown in Fig. 2. At 30 min, plasma nitrate had increased from 30 ± 4 to 432 ± 44 µM (Fig. 2a). Plasma nitrite already started to increase 15 min after nitrate ingestion and continued to increase during the entire observation period (Fig. 2b). At 30 min, plasma nitrite had increased from 123 ± 19 to 229 ± 46 nM. At 90 min after nitrate ingestion, nitrite levels were 392 ± 68 nM. Plasma SNO levels were 6.3 ± 1.4 nM immediately

本研究的主要意外发现是，血浆亚硝酸盐水平在摄入无机硝酸盐后持续增加，而血浆S-亚硝基硫醇保持不变。 这些发现至少有两个潜在的重要影响。 首先，这些结果表明，膳食硝酸盐可以对血浆硝酸盐和亚硝酸盐水平产生重大影响。 其次，本文的结果表明，硝酸盐可以作为进一步产生生物活性NO的底物。

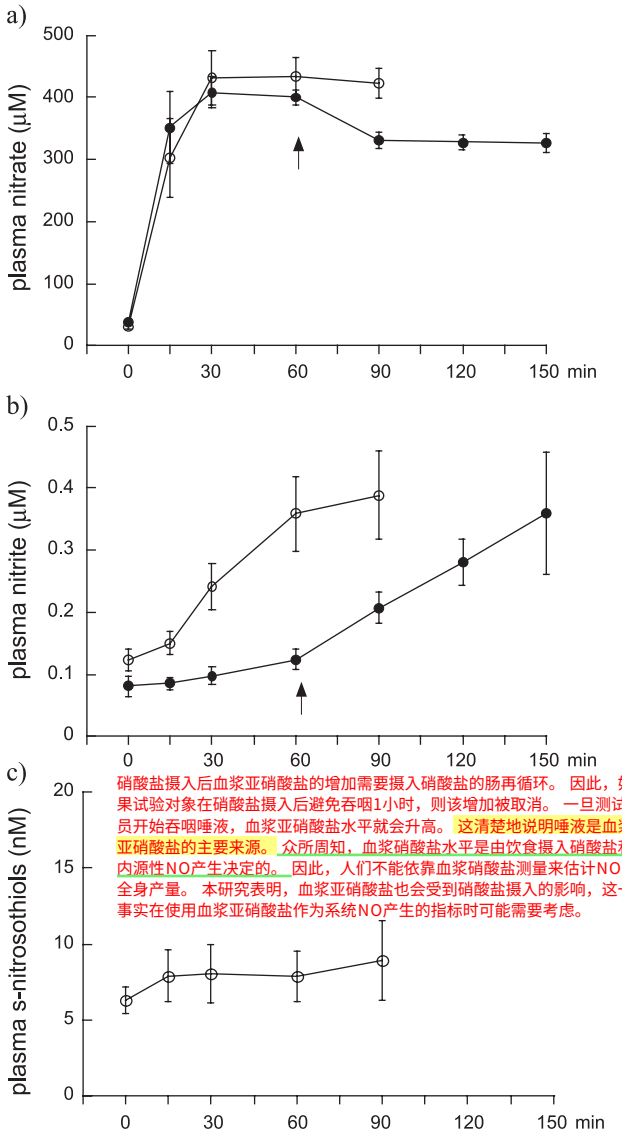

Fig. 2. Plasma levels of nitrate (a), nitrite (b), and *S*-nitrosothiols (c) after ingestion of sodium nitrate (10 mg/kg) on two separate occasions. Unfilled circles represent occasions when the subjects were allowed to swallow during the entire experiment, and filled circles represent experiments in which the subjects were asked not to swallow during the initial 60 min, after which they started to swallow again (indicated by arrows).

硝酸盐摄入后血浆亚硝酸盐的增加需要摄入硝酸盐的肠再循环。 因此，如果试验对象在硝酸盐摄入后避免吞咽1小时，则该增加被取消。 一旦测试人员开始吞咽唾液，血浆亚硝酸盐水平就会升高。 这清楚地说明唾液是血浆亚硝酸盐的主要来源。 众所周知，血浆硝酸盐水平是由饮食摄入硝酸盐和内源性NO产生决定的。 因此，人们不能依靠血浆硝酸盐测量来估计NO的全身产量。 本研究表明，血浆亚硝酸盐也会受到硝酸盐摄入的影响，这一事实在使用血浆亚硝酸盐作为系统NO产生的指标时可能需要考虑。

before the nitrate load and did not change significantly during the course of the experiment (Fig. 2c).

Urine 尿液

Urinary levels of nitrate+nitrite increased after ingestion of sodium nitrate. The levels of nitrate in urine increased from 0.8 ± 0.3 mM at baseline to 6.9 ± 3 mM at 1 h, 9.5 ± 3 mM at 2 h, and 8.1 ± 1.9 mM at 3 h. Nitrite levels were 3.4 ± 1 μM at baseline and did not increase significantly during the experiment (2.1 ± 1 μM at 1 h, 1.7 ± 1 μM at 2 h, and 3.1 ± 1.6 μM at 3 h).

DISCUSSION

The major unexpected finding in this study is that plasma levels of nitrite increased for a sustained period after ingestion of inorganic nitrate while plasma *S*-nitrosothiols remained unchanged. There are at least two potentially important implications of these findings. First, these results show that dietary nitrate can have a major impact on plasma levels of both nitrate and nitrite. Second, the results presented here indicate that nitrate can function as a substrate for further generation of bioactive NO (Fig. 3).

The increase in plasma nitrite after nitrate ingestion requires enterosalivary recirculation of the ingested nitrate. Thus, the increase was abolished if the test subject avoided swallowing for 1 h after the nitrate intake. As soon as the test persons started to swallow saliva, plasma nitrite levels increased. This clearly illustrates that saliva is a major source of plasma nitrite. It is well known that plasma levels of nitrate are determined both by dietary intake of nitrate and by endogenous NO production. Therefore one cannot rely on plasma nitrate measurements to estimate total body production of NO. The present study shows that plasma nitrite also can be influenced by nitrate intake, a fact that may have to be considered when using plasma nitrite as an index of systemic NO production. In a study by Pannala et al. plasma nitrite did not increase significantly after ingestion of nitrate [13]. The reason for this discrepancy is not clear. In the cited study nitrate was administered over a longer period at a lower dose. Also, it is not clear exactly how the blood samples were handled after collection. Because of the short half-life of nitrite in whole blood (<2 min due to reaction with hemoglobin), it may be essential to centrifuge the blood immediately after sampling.

目前还不清楚采集后如何处理血样。 由于全血亚硝酸盐半衰期短(由于与血红蛋白反应<2min)，采样后立即离心血液可能是必不可少的

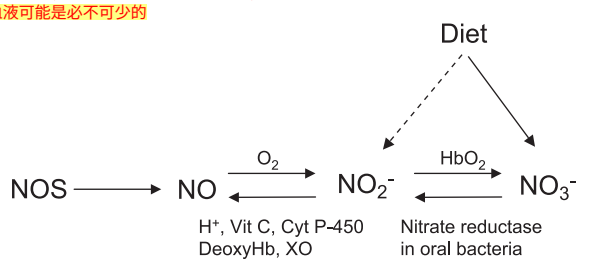

Fig. 3. Tentative reverse pathway for systemic generation of NO from nitrate in humans. Plasma nitrate originates from oxidation of endogenous NO and from dietary sources. About 25% of all plasma nitrate is taken up by the salivary glands and excreted in saliva, where commensal bacteria reduce it to nitrite. Salivary nitrite then enters the circulation after swallowing. Nitrite reduction to bioactive NO occurs in cells and tissues and is enhanced by acidic (H^+) and reducing (e.g., vitamin C) conditions. Several mammalian proteins and enzymes can catalyze nitrite reduction to NO, including xanthine oxidase (XO), cytochrome P450 (Cyt P-450), and deoxyhemoglobin (deoxyHb).

血浆硝酸盐来源于内源性NO的氧化和饮食来源。 大约25%的血浆硝酸盐被唾液腺吸收并在唾液中排泄，在唾液中，共生细菌将其还原为亚硝酸盐。 唾液亚硝酸盐吞咽后进入循环。 亚硝酸盐还原为生物活性NO发生在细胞和组织中，并通过酸性(H)和还原(例如维生素C)条件增强。 几种哺乳动物蛋白质和酶可以催化亚硝酸盐还原为NO，包括黄嘌呤氧化酶(XO)、细胞色素P450 (CytP-450)和脱氧血红蛋白(脱氧HB)。

细菌硝酸盐还原酶在口腔中进行硝酸盐生物转化为亚硝酸盐的中心步骤。

The central step in bioconversion of nitrate to nitrite is carried out in the oral cavity by bacterial nitrate reductases. As shown here and earlier, ingested or endogenous nitrate is greatly concentrated in saliva, and part of this nitrate is reduced to nitrite locally in the oral cavity [10,11,14]. By this mechanism salivary nitrite levels increase greatly after a nitrate load. When swallowed, this nitrite apparently enters the systemic circulation by yet uncharacterized mechanisms. It has been suggested recently that mammalian enzymes could also possess nitrate reductase activity. One candidate is xanthine oxidase, which is structurally related to nitrate reductase. In vitro studies suggest both nitrate-reducing and nitrite-reducing activities of this enzyme [5]. However, the present findings do not support any acute major systemic nitrate reductase activity from mammalian enzymes in healthy subjects, as the increase in plasma nitrite was completely prevented if the test person avoided swallowing after nitrate intake.

一旦硝酸盐还原为亚硝酸盐，就有几条途径从亚硝酸盐中产生NO。

Once the reduction of nitrate to nitrite has occurred there are several pathways to generate NO from nitrite. Reduction of nitrite to NO is enhanced by a low pH and a reducing milieu [1,2,4] but also by several mammalian enzymes and proteins including xanthine oxidase [5], cytochrome P450 [6], and deoxyhemoglobin [7]. In addition, reducing agents, e.g., vitamin C and thiocyanate, can further enhance NO generation from nitrite [10]. Because of the high nitrite content in saliva and the low pH in the stomach, very large quantities of NO are produced there [1,2]. A recent study by Björne et al. shows that salivary nitrite increases gastric mucosal blood flow and mucus thickness through intragastric generation of NO [15]. Other studies have suggested that nitrite can be reduced to NO also in the systemic circulation and in tissues [3–5,7].

亚硝酸盐还原为NO是通过低pH和还原环境来增强的[1,2,4]但也是通过几种哺乳动物酶和蛋白质来增强的，包括黄嘌呤氧化酶[5]、细胞色素P450[6]和脱氧血红蛋白。

胃保护作用：最近的一项研究。结果表明，唾液亚硝酸盐通过胃内产生NO而增加胃粘膜血流量和粘液厚度

Besides endogenous NO production by NO synthases, the main source of nitrate is our diet. The amount of nitrate ingested in this study corresponds to what is found in about 300 g of spinach or lettuce [16]. It is a well-known fact that vegetarians are at reduced risk of developing hypertension and other cardiovascular diseases [17,18]. It is therefore tempting to speculate that the high nitrate content of many vegetables may contribute to these protective effects through prolonged systemic low-dose generation of nitric oxide. Naturally, any long-term cardiovascular protective effects of nitrate need to be confirmed in controlled studies. Interestingly, Richardsson et al. have recently shown that ingestion of inorganic nitrate acutely inhibits platelet function [19], an effect that implies involvement of bioactive NO. In the cited study and in the present study SNOs remained completely unchanged after nitrate ingestion, whereas in this study, nitrite increased markedly. Moreover, basal levels of nitrite were at least 20 times higher than the levels of SNOs. This implies that nitrite rather than SNOs is a substrate for nitric oxide synthase-independent NO generation from nitrate. There has been much recent discussion in the literature about the biological significance of *S*-nitrosothiols as carriers of NO in the circulation. Earlier work suggested high basal levels of SNOs in the circulation and important roles for these compounds in the regulation of regional blood flow [20,21]. More recently, however, these suggestions have been questioned by several authors [8,22–24].

除NO合成酶产生内源性NO外，硝酸盐的主要来源是我们的饮食。

众所周知，素食者患高血压和其他心血管疾病的风险降低。因此，人们很容易推测，许多蔬菜的高硝酸盐含量可能通过长时间的系统性低剂量生成一氧化氮来促进这些保护作用。

In conclusion, the bioactivity of NO is acutely terminated by oxidation to nitrite and nitrate. Recent studies suggest that nitrite can be recycled to bioactive NO in cells and tissues. With the identification of systemic nitrite generation from inorganic nitrate presented here, a reverse pathway for regeneration of NO from nitrate is completed.

总之，NO的生物活性被氧化成亚硝酸盐和硝酸盐而终止。

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