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Original Contribution

Gastroprotective and blood pressure lowering effects of dietary nitrate are abolished by an antiseptic mouthwash

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ABSTRACT

Recently, it has been suggested that the supposedly inert nitrite anion is reduced in vivo to form bioactive nitric oxide with physiological and therapeutic implications in the gastrointestinal and cardiovascular systems. Intake of nitrate-rich food such as vegetables results in increased levels of circulating nitrite in a process suggested to involve nitrate-reducing bacteria in the oral cavity. Here we investigated the importance of the oral microflora and dietary nitrate in regulation of gastric mucosal defense and blood pressure. Rats were treated twice daily with a commercial antiseptic mouthwash while they were given nitrate-supplemented drinking water. The mouthwash greatly reduced the number of nitrate-reducing oral bacteria and as a consequence, nitrate-induced increases in gastric NO and circulating nitrite levels were markedly reduced. With the mouthwash the observed nitrate-induced increase in gastric mucus thickness was attenuated and the gastroprotective effect against an ulcerogenic compound was lost. Furthermore, the decrease in systemic blood pressure seen during nitrate supplementation was now absent. These results suggest that oral symbiotic bacteria modulate gastrointestinal and cardiovascular function via bioactivation of salivary nitrate. Excessive use of antiseptic mouthwashes may attenuate the bioactivity of dietary nitrate.

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Introduction

Nitrate (NO3−) and nitrite (NO2−) have long been considered as inert oxidative end products of nitric oxide (NO) metabolism or unwanted residues in the food chain. However, research conducted during the last decade reveals that these anions can be recycled in vivo to form NO [1,2]. The nitrate–nitrite–NO pathway can be viewed as a complementary source of NO in addition to the classical L-arginine–NO– synthase pathway [2]. Nitrate is of particular interest since it is found in high concentrations in many vegetables in our everyday diet [3]. Ingested nitrate is rapidly absorbed in the proximal intestine and for yet unknown reasons as much as 25% is actively taken up by the salivary glands and secreted in saliva [4]. In the mouth facultative anaerobic bacteria reduce nitrate to nitrite, resulting in high levels of nitrite both in saliva [4–6] and in plasma [7]. In addition to bacterial nitrate reduction, a recent study also suggests the presence of a functional mammalian nitrate reductase that contributes to circulating nitrite levels [8]. Numerous recent studies have shown that nitrite can affect physiological processes in the gastrointestinal tract as well as in the cardiovascular system [2]. In the stomach, the biological effects of nitrite have been attributed to NO and other reactive nitrogen oxides that are formed nonenzymatically when salivary nitrite reacts with protons in the acidic gastric milieu [9,10]. Studies now suggest beneficial roles of intragastric nitrogen oxides in host defense against enteropathogens [10,11] and in regulation of gastric mucosal blood flow [12,13] and mucus formation [12,14]. In further support of a gastroprotective role of salivary nitrite, several studies have shown that dietary nitrate can enhance the mucosal protection against ulcerogenic compounds most notably nonsteroidal anti-inflammatory drugs (NSAIDs) [14–16]. Nitrite also has systemic effects, in particular in the cardiovascular system. In humans low-dose infusion of nitrite has NO-like effects and elicits vasodilatation [17]. In animal models it protects against ischemia–reperfusion injury [18,19]. In addition, dietary nitrate was recently shown to decrease blood pressure in healthy volunteers [20,21], an effect associated with increases in circulating nitrite levels.

Abbreviations: MAP, mean arterial blood pressure; NO, nitric oxide; NO2−, nitrite; NO3−, nitrate; NSAIDs, nonsteroidal anti-inflammatory drugs.

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In this study we have explored the role of oral commensal bacteria in bioactivation of dietary nitrate to nitrite and NO. To address this issue, we studied if NO-dependent biological effects of dietary nitrate in the gastrointestinal tract and cardiovascular system would be affected by the selective removal of oral commensal bacteria using an antiseptic mouthwash.

**Material and methods**

**Experimental protocols**

In a first set of experiments we studied the effect of chlorhexidine mouth spray on oral bacteria. Intragastric NO formation and plasma nitrite were measured in control and chlorhexidine mouth-sprayed rats with and without nitrate supplementation.

In a second set we investigated the effect of nitrate supplementation on gastric mucus thickness. Mucus thickness was measured in vivo in control and chlorhexidine mouth-sprayed rats as well as in conventional and germ-free mice with and without nitrate and nitrite supplementation. We also induced gastric injury with diclofenac in control and chlorhexidine mouth-sprayed rats with and without nitrate supplementation and measured levels of the adhesion molecule P-selectin in gastric wall.

In a third set we implanted telemetric devices and measured blood pressure and heart rate in control and chlorhexidine mouth-sprayed rats with and without nitrate supplementation. We also investigated the effect of nitrite infusion in anesthetized control rats as well as with and without nitrate supplementation on blood pressure in control and mouth-sprayed anesthetized rats.

**Animals**

All experiments were approved by the regional ethics committee for animal experiments in Uppsala, Sweden. Male Sprague-Dawley rats (190–360 g, B and K, Sollentuna, Sverige) were kept under standardized conditions.

**Chlorhexidine antiseptic mouthwash**

In an attempt to selectively suppress the oral microflora we sprayed the oral cavity of rats twice daily for 1 week with a commercial antibacterial mouthwash solution. We then examined the amount of viable bacteria in samples taken from the tongues. Rats were treated topically in the mouth with a commercial chlorhexidine mouth spray (2 mg/ml, Corsodyl, GlaxoSmithKline, Brentford, England). The solution (0.3 ml) was sprayed at the dorsal aspect of the tongue twice daily for 1 week.

**Nitrate and nitrite supplementation**

All animals were given conventional chow (the measured nitrate content was 35 mg/kg) and nitrate-free water, resulting in a calculated total daily nitrate intake of approx 1.5 mg/kg body weight. In selected groups of animals, the drinking water was supplemented with nitrate or nitrite (10 mM NaNO$_3$, 1.0 mM NaNO$_2$, Sigma-Aldrich, Steinheim, Germany) resulting in a total daily intake of approx 140 mg/kg nitrate or 14 mg/kg nitrite. The overall in vivo conversion of nitrate to nitrite has been estimated to be 5–10% [5] and we therefore used 1/10 of the nitrate dose when specifically studying effects of nitrite.

**Quantification of the levels of bacteria on the tongue**

The number of viable bacteria present on the dorsal aspect of the tongue was quantified in control animals and in those treated with the mouth spray. After the intragastric NO measurements (see below), the tongue was removed and frozen in liquid nitrogen. The tongues were kept at-70°C until analyzed. The dorsal part of the tongue was disaggregated in 1 ml sterile PBS containing 0.05% Tween 20, using a mortar. A medium described by Doel et al. [22] was used and the plates were incubated either aerobically or anaerobically (BD GasPak EZ Anaerobe-Container-System, Sparks, MD) for 48 h, before counting the colonies. The detection limit of the determination was 10$^5$ colony-forming units (cfu) per tongue sample.

**Intragastric nitric oxide measurements**

We also examined if the reduction in oral bacteria would impact the levels of NO in the gastric lumen. Luminal NO gas measurements were performed in anesthetized rats (120 mg/kg bw of 5-ethyl-5-(1-methylpropyl)-2-thiobutabarbital sodium (Inactin),) with or without nitrate supplementation for 1 week and in mouth-sprayed rats with or without nitrate supplementation. Using a syringe with a fine needle, 3 ml of NO-free air (NO<3 ppb) was directly introduced into the stomach. After incubating the air for 15 s the gas was aspirated and immediately injected into a chemiluminescence analyzer (Aerocrine AB, Stockholm, Sweden) as described earlier [23].

**Plasma nitrite measurements**

We also studied if the depression of oral bacteria would influence the levels of circulating nitrite. Plasma nitrite was determined in half of the rats used in the NO measurement experiments, and in mouth-sprayed rats given nitrate supplementation. Blood samples (1.0 ml) were taken from the heart, put into Eppendorf tubes with NEM/EDTA and immediately centrifuged. The plasma was stored in-70 °C until analyzed. The nitrite concentrations were measured using a chemiluminescence method described in detail earlier [24].

**In vivo mucus measurements**

The gastric mucus thickness was measured in conventional and mouth-sprayed rats with and without nitrate and nitrite supplementation for 1 week.

The gastric mucosa of anesthetized rats (Inactin, 120 mg/kg bw) was exteriorized for intravital microscopy as described earlier [25]. The mucus thickness was measured with the use of micropipettes pushed into the mucus gel at an angle of 25–35° to the epithelial cell surface and the distance traveled by the micropipette from the luminal surface of the mucus gel to the epithelial cell surface was measured with a digimatic indicator (IDC Series 543, Mitutoyo Corp., Tokyo, Japan). This model is well established for in vivo mucus measurements in the gastrointestinal tract [12,14,26].

**Diclofenac-induced gastric injury**

To induce an acute injury to the gastric mucosa we administered the nonsteroidal anti-inflammatory drug diclofenac by oral gavage 4 h prior to terminating the experiment. The animals were deprived of food for 18 h before the induction of damage with the NSAID diclofenac (30 mg kg$^{-1}$, Voltaren, Novartis, Täby, Sweden), given by gavage (0.5 ml) 4 h before the experiment. The animals were sacrificed and the stomach was removed and opened along the greater curvature, gently washed with saline, and spread for subsequent photography (Fig. 3B). The photographs were analyzed in ImageJ (Version 1.38, NIH) and the ulcer index is expressed as the total damaged area (mm$^2$).

To investigate the delayed proinflammatory effect of diclofenac [14], we examined a separate group of rats 18 h after administration of diclofenac. In this second series of experiments the NSAID (diclofenac, 30 mg kg$^{-1}$) was given 18 h before the experiment. To assess the inflammatory reaction the levels of the adhesion molecule P-selectin...
were measured in the tissue. Four different control groups, not given diclofenac, were used for measurements of baseline values of P-selectin expression in the tissue (controls, nitrate treated, mouth-sprayed, and nitrate treated with mouth spray). Rats were anesthetized (Inactin, 120 mg/kg bw) and the right carotid artery and left jugular vein were cannulated for injection of antibodies and for blood sampling. To measure P-selectin expression, a mixture of 10 μg of 125I-labeled P-selectin MAb (RMP-1) and 10 μg of 131I-labeled nonbinding MAb (P-23) was injected. We investigated the gastric wall. The superficial mucosa was scraped off with a scalpel and analyzed separately. This duo-labeled monoclonal antibody technique has been described in detail earlier [27]. We also measured mucus thickness in control and nitrate-treated animals 18 h after administration of diclofenac.

**Blood pressure measurements**

We also studied if the recently described cardiovascular effects of dietary nitrate [20,21] would be affected by oral treatment with an antibacterial mouthwash solution.

**Measurements in anesthetized animals**

First, we wanted to confirm the vasoactivity of nitrite in this model by intravenous administration. Rats were anesthetized (Inactin, 120 mg/kg bw) and the right femoral artery and vein were cannulated for blood pressure registration and infusion. The blood pressure was monitored continuously for 100 min and after 50 min, a bolus dose of nitrite (NaNO2 0.1 mmol/kg) was infused into the femoral vein. Next we measured blood pressure in animals that had been fed nitrate in the drinking water for 1 week. Mouth-sprayed rats with and without

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**Fig. 1.** Effects of mouth spray on oral bacteria, intragastric NO formation, and plasma nitrite. Levels of viable bacteria found on the tongue in conventional rats and in rats treated topically for 1 week with a chlorhexidine mouth spray (A). The effect of dietary nitrate supplementation on intragastric NO levels (B) and plasma levels of nitrite (C) in conventional and mouth-sprayed rats. *P* < 0.05.

**Fig. 2.** Effects of nitrate and nitrite on gastric mucus thickness. Thickness of the firmly adherent gastric mucus layer in conventional rats (A) and chlorhexidine mouth-sprayed rats (B) given a standard diet (Control) or dietary supplementation with nitrate (nitrate pretreated) or nitrite (nitrite pretreated). *P* < 0.05.
nitrate or nitrite supplementation for 1 week were anesthetized (Inactin, 120 mg/kg bw) and the right femoral artery and vein were cannulated for blood pressure registration and infusion. After recovery from the surgery, blood pressure was registered for 60 min.

Telemetric measurements

To study longer-term effects of dietary nitrate supplementation on blood pressure, and to exclude any interfering effects of anesthesia on the measurements, we conducted a series of experiments using

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**Fig. 3.** Effects of nitrate and nitrite on NSAID-induced gastric injury. Rats received nitrate or nitrite in the drinking water for 1 week prior to oral challenge with diclofenac. In addition, all rats were treated twice daily with an antibacterial mouthwash solution. The acute gastric injury caused by diclofenac is displayed as overall damage score (A) and as representative photos (B). (C) Effects of diclofenac challenge on the thickness of the firmly adherent gastric mucus in control rats and nitrate-pretreated rats. (D) P-Selectin expression in gastric tissue after a gastric challenge with diclofenac in control rats and nitrate-pretreated rats (left), in mouth-sprayed control rats and nitrate-pretreated rats (middle), and finally in control rats and nitrate-pretreated rats not exposed to diclofenac (right). *P < 0.05.
implanted telemetric devices. A telemetric device (PA-C40, DSI, St Paul, MN) was implanted in the abdominal aorta for continuous 24-h blood pressure and heart rate measurements, as described earlier [28]. After surgery, the animals were allowed to recover for 10 days before the telemetric measurements were started. The first group of animals was given regular drinking water for 5 days followed by nitrate-supplemented water for 5 days. The second group was given regular drinking water for 5 days followed by a 10-day period of mouth spray treatment where they were given regular nitrate free water the first 5 days and nitrate-supplemented water the last 5 days. Data were collected for 5 s every second minute throughout the period and the results are presented as a mean value for the entire period.

Statistical analysis

Data are expressed as means ± SEM. Differences between groups of animals were evaluated by one-way ANOVA and within groups by ANOVA for repeated measurements, followed by Fisher’s protected least significant difference test using Statistica for Windows (StatSoft Inc., Tulsa, OK). P values < 0.05 were considered significant.

Results

An antiseptic mouthwash reduces oral bacteria, intragastric NO formation, and plasma nitrite

Amounts of bacteria on the tongue

The mouth-sprayed rats showed a drastic reduction in overall amounts of viable bacteria detected per tongue, as compared with the non-mouth-sprayed animals (Fig. 1A). The antibacterial effect of the chlorhexidine solution was equally effective against anaerobic and aerobic bacteria.

Intragastric NO formation

Nitrate supplementation resulted in a 40-fold increase in the levels of NO gas in the stomach, and this effect of nitrate supplementation was markedly reduced in the mouth-sprayed rats (Fig. 1B).

Plasma nitrite

Nitrate addition to the drinking water resulted in an almost 10-fold increase in plasma nitrite (Fig. 1C) as compared to the control situation. When the oral flora was suppressed with mouth spray, the increase in plasma nitrite after nitrate supplementation was markedly attenuated. In mouth-sprayed rats receiving nitrite in the drinking water (at a 10-fold lower dose compared to nitrate), plasma nitrite was not significantly increased compared to control animals (175 ± 60 nM, n = 4, not displayed in figure).

Nitrate-induced increase in gastric mucus thickness is attenuated by an antiseptic mouthwash: In vivo mucus measurements

Nitrate supplementation resulted in a 20% increase in the thickness of the firmly adherent mucus layer (Fig. 2A). This increase was absent in the rats treated with the antiseptic mouth spray (Fig. 2B). In the experiments where rats were given nitrite in the drinking water, the thickness of the mucus layer increased in both control and mouth-sprayed animals (Figs. 2A and B). Thus, by giving nitrite directly we

Fig. 4. Blood pressure measurements. (A) Effects of intravenous nitrite infusion (0.1 mg/kg) on mean arterial pressure (MAP) in anesthetized rats. (B) MAP measured in anesthetized rats given a dietary supplementation with nitrate or nitrite for 1 week. (C) Mean values of MAP and diastolic pressure measured telemetrically during a control period (5 days) followed by a period with dietary nitrate supplementation (5 days). (D) Blood pressure measured during a control period followed by a second period where the animals were mouth-sprayed and finally a third period with both mouth spray and dietary nitrate supplementation.
could bypass the nitrate-reduction step carried out by the oral microflora, thereby demonstrating that nitrite is an obligate intermediate in bioactivation of nitrate.

**Gastroprotective effect of nitrate is abolished in animals treated with an antiseptic mouthwash**

**Acute gastric damage**
Administration of diclofenac 4 h prior to the experiments resulted in multiple gastric bleedings. We have earlier shown that dietary nitrate in the same dose used here strongly protects against such damage [14], but in mouth-sprayed animals, nitrate was now unable to prevent this injury. Importantly, however, the damage was markedly reduced in mouth-sprayed animals receiving nitrite in the drinking water (Figs. 3A and B).

**Gastric inflammation**
Administration of diclofenac 18 h prior to the experiments resulted in a thinner gastric mucus layer and clear signs of gastric inflammation in control animals. The inflammatory response was detected by upregulated levels of P-selectin in the gastric tissue. In animals given nitrate supplementation, the diclofenac-induced reduction in mucus thickness and the P-selectin upregulation were completely prevented (Figs. 3C and D). Again, in mouth-sprayed animals nitrate was unable to prevent the upregulation of P-selectin in the stomach (Fig. 3D). Neither nitrate supplementation alone (Fig. 3D) nor mouth spray affected the basal levels of P-selectin in the stomach (mouth spray alone, 0.10 ± 0.07; nitrate-treated and mouth-sprayed, 0.15 ± 0.08 ng P-selectin Ab/g tissue, not displayed in figure).

**Blood pressure reduction seen after nitrate supplementation is absent in animals treated with an antiseptic mouthwash**

**Measurements in anesthetized animals**
As expected, when nitrite was given as a large bolus (0.1 mg/kg) to anesthetized rats it powerfully reduced the mean arterial blood pressure (MAP) (Fig. 4A). Next we measured blood pressure in animals that had been fed nitrate in the drinking water for 1 week. In these animals MAP was significantly lower compared to control animals receiving regular water (Figs. 4A and B). This effect of nitrate was absent in mouth-sprayed animals. However, in mouth-sprayed animals receiving nitrite supplementation instead of nitrate, MAP was again reduced (Fig. 4B).

**Telemetric blood pressure measurements**
Nitrate supplementation decreased MAP and diastolic blood pressure in all rats (Fig. 4C), whereas a nonsignificant decrease was observed in systolic blood pressure (-1.1 ± 0.7 mm Hg P = 0.15). Again, in animals treated with mouth spray this reduction in blood pressure was absent. Mouth spray per se did not affect the blood pressure (Fig. 4D). Finally, no differences in heart rates were observed between the non-mouth-sprayed and mouth-sprayed animals at any time (data not shown).

**Discussion**
Inorganic nitrate from dietary and endogenous sources is reduced stepwise in vivo, to form nitrite and then NO [1,2]. Formation of bioactive NO takes place locally in the gastrointestinal tract after entero-salivary circulation of nitrate [9,10], as well as systemically in the vasculature [2,29]. The measurable biological effects of nitrate-derived NO include enhanced gastroprotection [13–15] and a reduction in blood pressure [20,21]. We show here that if the oral microflora is suppressed by an antiseptic mouthwash, both the gastroprotection and the blood pressure lowering effect of dietary nitrate are abolished. Nitrate supplementation in the diet resulted in increased circulating nitrite and high concentrations of intragastric NO gas which could not be observed in animals with a suppressed oral flora. This suggests that by bioactivation of salivary nitrate, the nitrate reducing commensal bacteria in the mouth play an active role in modulating gastric mucosal defense as well as in regulating blood pressure.

The nitrate dose used in this animal study was chosen to resemble what is needed in humans to obtain similar cardiovascular effects. In humans a similar or even greater blood pressure reduction is seen already with a 10-fold lower dose of nitrate, which is readily achievable through a diet rich in vegetables [20]. This greater response is likely because humans effectively concentrate (>10-fold) nitrate in saliva, thereby maximizing substrate availability for the oral bacteria [4]. In rats such concentration is not seen [30]. So, when selecting the nitrate dose for this study we compensated for the differences in nitrate metabolism between rodents and humans.

The adherent mucus gel that covers the gastric mucosa constitutes the important first preepithelial line of defense. It serves as a physical barrier against luminal contents and also acts as a protective acid neutralizer, since bicarbonate is secreted from the epithelium into the mucus [31–33]. Nitrate supplementation increased the thickness of this mucus layer as shown before [14], but in rats treated with the antiseptic mouthwash this effect was absent. The amount of bioavailable NO present in the stomach under these conditions was probably insufficient to stimulate mucus secretion. When the animals were given nitrite instead of nitrate, the mucus thickness increased irrespective of whether they were mouth-sprayed or not. Under these circumstances, the oral flora is not needed, since the initial nitrate reduction is bypassed. All together, these data implicate a role for salivary nitrite in physiological regulation of mucus secretion.

The acute gastric injury caused by a nonsteroidal anti-inflammatory drug is markedly reduced by dietary nitrate [14] but as shown here this protection is completely lost by the mouthwash treatment. Clearly, it is the lack of bacterial nitrate reduction that underlies this effect, since nitrate pretreatment was strongly protective. We also measured the expression of the cell adhesion molecule P-selectin, which is commonly used as a marker of the early event in the gastric inflammatory cascade [34,35]. We show that diclofenac challenge increases P-selectin expression in the stomach, and that nitrate supplementation totally prevents this. In addition, nitrate prevented the reduction in mucus thickness induced by diclofenac. Interestingly, these effects of nitrate were abolished when the animals were mouth-sprayed. In aggregate, these findings support previous studies by us [9,12–14] and Miyoshi and colleagues [15] suggesting a role for salivary nitrite in gastroprotection. As shown here and earlier [9,10,36,37], salivary nitrite is reduced to NO in the acidic stomach, and this NO results in a cGMP-dependent [12,13] increase in gastric mucosal blood flow and mucus formation. In addition to increasing gastric mucosal blood flow and mucus generation, nitrite might also protect the tissue by modification of mitochondrial function. Indeed, its tissue protective effects in ischemia reperfusion injury have been shown to involve inhibition of mitochondrial complex I with resulting reduction in reperfusion reactive oxygen species generation and cytochrome c release [38].

The reduction in blood pressure following nitrate intake confirms recent studies in humans [20,21], and we now show that the effect is critically dependent on oral nitrate-reducing bacteria. In addition we show that the blood pressure lowering effects of dietary nitrate is sustained over a 5-day observation period without any signs of tolerance. The cardiovascular effects of dietary nitrate have been attributed to increases in nitrite and then further conversion to vasodilatory NO or closely related species [20,21,39]. Our results confirms the early findings that a large bolus dose of nitrite injected intravenously potently reduces blood pressure [40]. In addition, when nitrite was given orally to animals with a reduced oral flora, a clear
reduction in blood pressure was observed. The mechanisms behind nitrate- and nitrite-derived blood pressure reduction are not yet fully understood and it is not clear exactly where the NO is being formed. It has been suggested that nitrite is reduced to vasodilatory NO in the circulation [17] as well as in the vessel wall [21,41]. Intermediate formation of NO-releasing nitrosothiols [42,43] is also possible. The modest increase in plasma nitrite noted in the mouth-sprayed animals given nitrate supplementation can likely be explained by conversion of nitrate to nitrite by a mammalian nitrate reductase activity in the tissues which was described very recently [8]. The small increase in intragastric NO can be attributed to gastric or intestinal bacteria that might reduce nitrate to nitrite and NO [44,45] or some residual nitrite reduction in the mouth. Although these smaller increases in nitrite did not affect the parameters studied here, it is likely that other nitrite-dependent bioactivity is present already at lower nitrite levels. Indeed, in a recent study nitrite was given orally in a considerably lower dose than the one used here. While this dose did not affect blood pressure, it completely reversed signs of renal damage caused by prolonged drug-induced hypertension [46].

Extensive studies have shown that a diet rich in fruits and vegetables is associated with a lower blood pressure [47], a reduced risk of cardiovascular disease [48], and prevention of gastrointestinal cancer [49]. It has been speculated that parts of these effects can be explained by the high nitrate content in many vegetables [50,51]. This is supported by the potent blood pressure lowering effects of dietary nitrate in humans [20,21] and its cardioprotective effects in animal studies [52]. It remains to be studied if the widespread everyday use of mouthwash has any negative effects in humans and more specifically, if it attenuates the positive health effects of a vegetable-rich diet. Several studies have reported a positive correlation between bacterial infections in the oral cavity and cardiovascular disease, as reviewed by Niedzelska and colleagues [53]. It is clear that a balanced oral hygiene, combining tooth brushing and flossing is of utmost importance to reduce the risk of caries and periodontitis, the latter being a risk factor for development of cardiovascular disease. However, these simple interventions have little or no effect on the overall oral bacterial flora. In contrast, an antibacterial mouthwash can effectively and unselectively kill all different strains of bacteria, good and bad. While this might be desirable in advanced circumstances, based on the findings in the current study we speculate that a prolonged overuse of potent antibacterial mouthwash products may adversely affect the gastrointestinal tract and the cardiovascular system by reducing the number of oral nitrate reducing bacteria. Naturally, this provocative suggestion needs to be confirmed in controlled future studies.

In summary, when a commercial antibacterial mouthwash is administered to rats daily for 1 week, the oral microflora is suppressed to such an extent that the conversion of nitrate to nitrite in the oral cavity is strongly reduced. As a consequence the nitrite-dependent blood pressure lowering and gastrointestinal protective effects of nitrate are abolished. This study suggests that symbiotic oral bacteria play an active role in the regulation of physiological functions in the gastrointestinal tract and the cardiovascular system.

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References


