Blood Pressure–Lowering Effect of Orally Ingested Nitrite Is Abolished by a Proton Pump Inhibitor

Marcelo F. Montenegro, Michaela L. Sundqvist, Filip J. Larsen, Zhengbing Zhuge, Mattias Carlström, Eddie Weitzberg, Jon O. Lundberg

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Abstract—Inorganic nitrate and nitrite from dietary and endogenous sources are metabolized to NO and other bioactive nitrogen oxides that affect blood pressure. The mechanisms for nitrite bioactivation are unclear, but recent studies in rodents suggest that gastric acidity may influence the systemic effects of this anion. In a randomized, double-blind, placebo-controlled crossover study, we tested the effects of a proton pump inhibitor on the acute cardiovascular effects of nitrite. Fifteen healthy nonsmoking, normotensive subjects, aged 19 to 39 years, were pretreated with placebo or esomeprazole (3×40 mg) before ingesting sodium nitrite (0.3 mg kg⁻¹), followed by blood pressure monitoring. Nitrite reduced systolic blood pressure by a maximum of 6±1.3 mm Hg when taken after placebo, whereas pretreatment with esomeprazole blunted this effect. Peak plasma nitrite, nitrate, and nitroso species levels after nitrite ingestion were similar in both interventions. In 8 healthy volunteers, we then infused increasing doses of sodium nitrite (1, 10, and 30 nmol kg⁻¹ min⁻¹) intravenously. Interestingly, although plasma nitrite peaked at similar levels as with orally ingested nitrite (=1.8 µmol/L), no changes in blood pressure were observed. In rodents, esomeprazole did not affect the blood pressure response to the NO donor, DEA NONOate, or vascular relaxation to nitroprusside and acetylcholine, demonstrating an intact downstream NO-signaling pathway. We conclude that the acute blood pressure–lowering effect of nitrite requires an acidic gastric environment. Future studies will reveal if the cardiovascular complications associated with the use of proton pump inhibitors are linked to interference with the nitrate–nitrite–NO pathway. (Hypertension. 2017;69:23-31. DOI: 10.1161/HYPERTENSIONAHA.116.08081.)

Key Words: blood pressure ■ esomeprazole ■ nitrate ■ nitric oxide ■ nitrite ■ proton pump inhibitors

An association between chronic use of proton pump inhibitors (PPIs) and increased risk of cardiovascular disease has been implicated.¹⁻³ For example, it seems that PPIs reduce the efficacy of antiplatelet drugs such as clopidogrel by competing with the hepatic isoenzyme CYP2C19, thereby interfering with its capacity to inhibit platelet aggregation.⁶ Other studies have associated chronic use of PPI with increased cardiovascular risk, independently of treatment with clopidogrel⁴ or CYP2C19 metabolism.⁷,⁸ In addition, a recent study in diabetic patients indicates that PPIs increase blood pressure.⁹ The mechanisms behind these effects are still unclear, but a reduced vascular bioavailability of NO has been suggested.¹ Moreover, it has been speculated that bioactivation of some antiplatelet drugs requires gastric nitrosation under acidic conditions.¹⁰,¹¹

We and others have been studying an alternative NO synthase–independent pathway for NO generation in which inorganic nitrate and nitrite from dietary and endogenous sources are metabolized to NO and other bioactive nitrogen oxides in blood and tissues to affect cardiovascular function, including blood pressure.¹² Dietary nitrate is absorbed rapidly and is extracted from blood by the salivary glands and greatly concentrated in saliva. In the mouth, commensal bacteria reduce nitrate to the more reactive nitrite anion. Nitrite is then swallowed and enters the acidic stomach where it is nonenzymatically metabolized further to form several potentially bioactive nitrogen oxides, including NO. Orally ingested nitrate clearly has robust NO-like effects systemically, including a decrease in blood pressure,¹³⁻¹⁵ improvement in measures of endothelial function,¹⁶ inhibition of platelet activation,¹⁴,¹⁷ and distinct metabolic effects.¹⁸⁻²⁰ Studies have shown that the NO-like effects of nitrate are blocked if bacterial nitrate reduction in the mouth is abrogated with an antiseptic mouthwash, indicating that nitrite is an obligate intermediate in nitrate bioactivation.²¹,²² Direct administration of nitrite to humans or animals has demonstrated many similar effects as nitrate, including protection against ischemia–reperfusion injury and vasodilatory,²³⁻²⁵ antihypertensive,²⁶⁻³² and metabolic effects.³³,³⁴ Several pathways have been described for the reduction of nitrite to more bioactive nitrogen

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From the Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden (M.F.M., M.S., Z.Z., M.C., E.W., J.O.L.); and Åstrand Laboratory, The Swedish School of Sport and Health Sciences, Stockholm, Sweden (F.J.L.).
Correspondence to Jon O. Lundberg, Department of Physiology and Pharmacology, Karolinska Institutet, Nanna Svartz Väg 2, 17177, Stockholm, Sweden. E-mail jon.lundberg@ki.se
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oxide species including NO and S-nitrosothiols. Deoxygenated hemoglobin in red blood cells represents one extensively studied nitrite-reducing pathway, whereas xanthine oxidase in tissues is another one. However, despite much research during the past decade, the mechanisms for nitrate and nitrite bioactivation are still not entirely clear. A puzzling observation is the fact that orally ingested nitrate has robust cardiovascular effects despite modest increases in plasma nitrite, whereas in some clinical trials systemic nitrate administration, resulting in much higher plasma nitrite, has little or no effect on hemodynamic parameters. One possibility is that the metabolism and subsequent bioactivation of nitrate or nitrite are influenced by the route of administration. Indeed, recent studies in rodents suggest that gastric acidity may influence the degree of systemic nitrite bioactivity. Hence, the proposed association between chronic use of PPIs and cardiovascular disease could be linked to a negative influence on nitrate bioactivation via the effect on gastric acidity. In the current study, we evaluated the acute effects of orally ingested nitric oxide on blood pressure in healthy volunteers with or without esomeprazole pretreatment to increase gastric pH.

Methods

Human Studies

The study was conducted in conformity with the Declaration of Helsinki and was approved by the Karolinska Institute regional ethics committee in Stockholm, Sweden. All subjects gave their written, informed consent to participation before the tests. In total, 23 healthy male subjects were recruited in this study. Regular smokers, vegetarians, and subjects who were taking chronic medications were not recruited. Two separate protocols were performed, and the mean age was 20±7 and 36±16 years in protocols 1 and 2, respectively. Study participants were told not to consume vegetables or other nitrite- or nitrate-rich food on the same day and the day before the experiments. The volunteers and investigators were blinded to each pretreatment, and the volunteers were randomized 1:1 to start the crossover study with placebo or esomeprazole pretreatment.

Animal Studies

The experimental protocols were approved by the Institutional Animal Care and Use Committee of the Karolinska Institute in Stockholm, Sweden, and performed according to the National Institutes of Health guidelines for the conduct of experiments in animals.

Protocol 1: Effects of Nitric Oxide on Blood Pressure After Pretreatment With Esomeprazole

In this protocol, we tested the influence of esomeprazole on the acute cardiovascular effects of sodium nitrite. In a randomized, double-blinded, placebo-controlled, crossover study, 15 healthy nonsmoking, normotensive subjects were pretreated with placebo or esomeprazole (40 mg) 16, 8, and 1 hours before intake of sodium nitrite (0.3 mg·kg⁻¹·h⁻¹). Blood pressure and heart rate were automatically recorded (WatchBP-03) repeatedly during 1 hour before (baseline) and 2 hours after nitrite ingestion. The readings were blinded to both participants and the investigators. Venous blood sampling was chosen at time points not to disturb the blood pressure measurements. A minimum of 6 days of washout was allowed between the interventions. Sodium nitrite solutions were freshly prepared and given to the volunteer (0.3 mg·kg⁻¹) in a total volume of 10 mL distilled water.

Protocol 2: Effects of Sodium Nitrite Infusion on Blood Pressure

In this protocol, 8 healthy volunteers received an intravenous infusion of sodium nitrite, and blood pressure was monitored. Nitrite (NaNO₂) approved for use in humans was obtained from Hope Pharmaceuticals (300 mg in 10 mL H₂O) and diluted in sterile 0.9% saline solution. Nitrite was infused cumulatively at 3 different rates (1, 10, and 30 nmol·kg⁻¹·min⁻¹) for 10 minutes at each dose. Blood pressure was measured immediately after each infusion period by using a standard blood pressure device and a stethoscope. A blood sample was collected after the final nitrite infusion period, immediately after completing the last blood pressure recording.

Measurement of Plasma Nitrate and Nitrite

Plasma levels of nitrate and nitrite were assessed using a dedicated high-performance liquid chromatography system (ENO-20; Eicom) as previously described. The method is based on the separation of nitrate by reverse-phase or ion-exchange chromatography, followed by online reduction of nitrate to nitrite with cadmium and reduced copper. Derivatization of reduced nitrite was performed with Griess reagent, and the level of diazo compounds was measured at 540 nm.

Measurement of Plasma Nitroso Species

Plasma samples were analyzed in duplicate for their nitroso species (RSGNO) levels using an ozone-based reductive chemiluminescence assay with tri-iodide solution, as previously described. Briefly, plasma samples (300 µL) were pretreated with acidified sulfanilamide (5%; 9:1, v/v) for 5 minutes to eliminate any nitrite signal and then injected into the tri-iodide solution. The resulting signal corresponds to the sum of the nitroso species (ie, RSNO and other nitroso species, such as N-nitrosamines and Fe-nitrosyl species) remaining in the sample. A standard curve was constructed injecting S-nitrosoglutathione (3–60 nmol/L) in triplicate. We used a CLD 77 NO chemiluminescence analyzer (Eco Physics, Duernten, Switzerland). The sensitivity was set in D1, with the aspirating sample tube running at 130 µL per minute. The data signal was collected using the Chromatography Data System software Azur 5.0.10 (Datalys; Le Touvet, France), and at the end of experiments, the data were exported to a second program, Origin Pro 2016 (OriginLab Corporation, Northampton, MA). Using Origin Pro, we could remove background noise from the data, allowing the peaks to be measured with greater precision and increased accuracy.

Effects of Esomeprazole on Intragastric NO Generation

A previously described noninvasive protocol to measure NO formation in the gastric lumen was used. Because intragastric NO formation is strongly pH dependent, these measurements also serve as a surrogate control, confirming that esomeprazole effectively raises gastric pH as intended. We evaluated NO levels expelled from the stomach in 3 placebo or esomeprazole pretreated healthy male volunteers (crossover design) after ingestion of sodium nitrite (0.3 mg·kg⁻¹) as described above for protocol 1. Voluntary regurgitation of gas was performed 3 to 5 minutes after drinking 300 mL of carbonated water (Ramlösa, Sweden). Expelled air was led into a plastic bag, and the NO content was assessed immediately after chemiluminescence (CLD 77; Eco Physics, Duemten, Switzerland), and peak concentrations of NO were registered on a chart recorder.

Surgical Procedures and Assessment of Cardiovascular Responses in Mice

Male Sprague Dawley rats (250–400 g) were anesthetized using isoflurane (Baxter, Kista, Sweden), mixed with air during spontaneous breathing using a vaporizer (Isotec 3, Datec-Ohmeda, Madison, WI). Anesthesia was induced with 5% isoflurane and maintained at 2% (v/v) throughout experiments. Rats were placed on a heating table, and temperature was kept at 37 °C. A polyethylene catheter (PE10 heat bonded in a PE50) was inserted into the right femoral artery, and the systemic blood pressure was recorded using a data acquisition system connected to a computer (Powerlab 4/30; AD Instruments, Australia). Another similar catheter was introduced into the right femoral vein for intravenous injections of drugs. The absence of somatic motor reflexes in response to tail tapping or blinking in response to a low-pressure corneal stimulation indicated deep anesthesia, and at least 15 minutes of stabilization was allowed before drug infusions. Rats were
pretreated with either vehicle control or esomeprazole (5 mg·kg⁻¹) 2 hours before bolus intravenous injections of DEA NONOate (5.6 µg·kg⁻¹) or sodium nitrite 0.1 and 0.5 mg·kg⁻¹.

**Effects of Nitrite on Isolated Mesenteric Arteries**

Third-order branch of mesenteric resistance vessels from C57BL6 mice (average weight of 30 g) were isolated and dissected in ice-cold Krebs solution (composition in mmol/L: NaCl, 119; KCl, 4.7; CaCl₂, 1.6; KH₂PO₄, 1.2; MgSO₄·7H₂O, 1.2; NaHCO₃, 25.1; glucose, 5.5; and EDTA, 0.026). Arterial rings (2 mm) were mounted on 25-µm tungsten wires in a small-vessel myograph (Model 620M; Danish Myo Technology, Denmark) for recording isometric force by transducers (PowerLab 4/30; AD Instruments). The chambers were filled with Krebs solution (37 °C, pH 7.4) aerated with carbogen (95% O₂; 5% CO₂). Isometric tension was recorded with Powerlab system (Powerlab 4/30). After mounting, vessels were equilibrated for 20 minutes in PSS bubbled with carbogen (95% O₂; 5% CO₂) at 37 °C, pH 7.4. Resting tension of the arteries was set as described previously. After stabilization and washout protocols, 1 µmol/L norepinephrine was used to preconstrict mesenteric resistance vessels to obtain a basal tone of ≈50% of its maximal diameter. When the constriction reached a steady-state plateau, sodium nitrite (10⁻¹⁰–10⁻⁵ mol/L), sodium nitroprusside (10⁻¹¹–10⁻⁴ mol/L), or acetylcholine (10⁻¹¹–10⁻⁴ mol/L) were cumulatively added to the chambers. Cumulative concentration responses were studied with or without previous pretreatment with esomeprazole (10 µmol/L), and the vascular responses were expressed as relative change (%) compared with the norepinephrine response.

**Drugs and Solutions**

All drugs and reagents used were purchased from Sigma Chemical, Co (St Louis, MO). All drugs were dissolved in saline solution immediately before use.

**Statistical Analysis**

The results are expressed as mean±SEM. The comparisons between groups were assessed by t tests, 2-way ANOVA using Bonferroni correction, or 1-way ANOVA followed by Dunnett multiple comparison tests. A *P* value <0.05 was considered significant.

**Results**

**Blood Pressure–Lowering Effect of Orally Ingested Nitrite Is Abolished by Esomeprazole**

Pretreatment with esomeprazole did not significantly alter blood pressure measured before intake of sodium nitrite (placebo: 116.5±2.42; esomeprazole: 119.2±2.12; *P*>0.05; unpaired *t* test). Nitrite ingestion resulted in an acute lowering effect of systolic blood pressure (SBP) when taken after pretreatment with placebo (*P*<0.05 for placebo versus baseline; lowest mean±SEM=−6±1.26 mm Hg; Figure 1A). The drop was maximal at 15 minutes after nitrite ingestion and gradually returned to basal values during the 2-hour observation period.

**Figure 1.** The blood pressure–lowering effect of orally ingested nitrite is abolished by the proton pump inhibitor esomeprazole. Esomeprazole (40 mg) or placebo was taken at 3 separate occasions 16, 8, and 1 hour before ingestion of sodium nitrite (0.3 mg·kg⁻¹). Sodium nitrate induced a decrease in systolic blood pressure (SBP) after placebo, whereas SBP was unaffected after esomeprazole pretreatment (A). At the dose used, sodium nitrite induced no significant changes in diastolic blood pressure (DBP; B). *P*<0.05 by 2-way repeated-measures ANOVA for treatment (placebo vs esomeprazole) and time factors; #*P*<0.05 between groups for matched time point by using Bonferroni post hoc test. *P*<0.05 vs baseline (0 min) within the same group by using repeated-measures ANOVA and Dunnett multiple comparison test. Data are shown as mean±SEM (n=15).

**Figure 2.** Changes in plasma nitrite and nitrate levels after nitrite ingestion. Sodium nitrite increased the plasma levels of both nitrite (A) and nitrate (B). Plasma nitrite levels were higher at 60 and 120 min after esomeprazole compared with placebo pretreatment. Conversely, plasma nitrate levels were somewhat higher in the placebo group compared with the esomeprazole group. Nonsignificant changes (*P*>0.05) by 2-way repeated-measures ANOVA for the factor treatment (placebo vs esomeprazole) and #*P*<0.05 for matched time point by using Bonferroni post hoc test. *P*<0.001 vs baseline (0 min) within the same group by using repeated-measures ANOVA and Dunnett multiple comparison test. Data are shown as mean±SEM (n=14).
In contrast, when the volunteers instead were pretreated with esomeprazole, sodium nitrite induced no significant change in SBP (Figure 1A). At the dose used, sodium nitrite induced no significant changes in diastolic blood pressure with either of the 2 pretreatments (Figure 1B). The baseline heart rate was 66±2 and 66±3 for placebo and esomeprazole arms, respectively, and remained unaffected after nitrite intake (P=0.85 for placebo versus esomeprazole; 2-way repeated-measures ANOVA).

**Nitrite and Nitrate Levels**

Sodium nitrite ingestion increased plasma levels of nitrite and nitrate at both occasions (Figure 2A and 2B, respectively). The peak in plasma nitrite at 30 minutes was near identical between the placebo and esomeprazole arms, respectively, and remained unaffected after nitrite intake (*P<0.05 versus baseline in all time points after nitrite, for both groups; Figure 3A), suggesting that RXNO species were not affected by esomeprazole treatment.

**Increase in Gastric pH Induced by Esomeprazole Blunts Intragastric NO Generation**

Because intragastric NO formation from nitrite is known to be highly pH dependent, we measured NO levels directly after nitrite intake with a similar profile at both occasions (Figure 2B).

**Plasma RXNO Is Increased After Orally Administered Nitrite**

The plasma RXNO levels, which represent a circulating pool of potentially bioactive NO-related species, were assessed using reductive chemiluminescence. After nitrite ingestion, we found a significant increase in RXNO levels with placebo and esomeprazole pretreatment (*P<0.01 versus baseline in all time points after nitrite, for both groups; Figure 3A), suggesting that RXNO species were not affected by esomeprazole treatment.
in expelled stomach gas by chemiluminescence. Indeed, pretreatment with esomeprazole strongly reduced intragastric NO formation after nitrite ingestion (Figure 4A), indirectly confirming that treatment with esomeprazole effectively raises gastric pH, as intended.

Figure 5. Intravenous sodium nitrite does not induce significant changes in systolic blood pressure (SBP). Eight healthy volunteers received intravenous cumulative doses of sodium nitrite (Nitrite). Each dose was infused for a period of 10 min. The nitrite plasma levels reached ≈1.8 µmol/L after sodium nitrite infusions (not shown in figure) and induced no significant changes on blood pressure. Nonsignificant changes (P>0.05) by 2-way repeated-measures ANOVA for the factors treatment (placebo vs nitrite), time, or interaction. Data are shown as mean±SEM (n=8).

Intravenous Infusion of Sodium Nitrite Does Not Acutely Affect SBP

To our surprise, the increase in plasma nitrite after nitrite ingestion was similar with placebo and omeprazole pretreatment while causing hemodynamic changes only in the placebo group. We, therefore, decided to evaluate whether nitrite administered via another route would also affect blood pressure. Eight subjects received intravenous cumulative doses of sodium nitrite infused for 10-minute periods. Plasma nitrite reached 1.8 µmol/L after the highest dose but induced no significant changes in blood pressure (Figure 5). Importantly, these levels of nitrite were similar to the ones observed after nitrite ingestion when SBP was affected.

Esomeprazole Does Not Affect Vascular Responses to an NO Donor or Nitrite

The observed absence of a blood pressure response to nitrite in humans after pretreatment with esomeprazole could theoretically be explained by the interference of this drug with NO formation from nitrite or with downstream NO-signaling pathways. We, therefore, next evaluated whether esomeprazole alone in any way could affect the vascular response to nitrite or NO. First, rats were pretreated with either vehicle control or esomeprazole 2 hours before a bolus intravenous injection of the short-acting NO donor DEA NONOate or with a high dose of sodium nitrite known to cause vasodilation in vitro. Mean arterial blood pressure decreased considerably with both interventions, but the response was similar in placebo- and esomeprazole-treated animals (Figure 6A). The effectiveness of the esomeprazole treatment was confirmed by substantial increases in gastric pH measured at the end of the experiment (data not shown). Additionally, we assessed the effects of esomeprazole in mesenteric resistance vessels precontracted with norepinephrine (1 µmol/L). The vessels were incubated in the presence of vehicle or esomeprazole (10 µmol/L), and concentration–response curves to sodium nitrite, nitroprusside, and acetylcholine were evaluated. Again, no differences were found in vascular relaxation responses when comparing control vehicle versus esomeprazole (P>0.05; Figure 6B through 6D).

Discussion

Here, we show that ingestion of sodium nitrite causes a robust transient decrease in SBP in healthy volunteers. This effect is abolished if the test persons are pretreated with esomeprazole, suggesting that bioactivation of nitrite occurs in the gastric lumen and requires an acidic environment. This is further supported by the fact that intravenous nitrite had no effect on blood pressure despite reaching similar plasma levels as obtained after oral intake.

Many bioactive nitrogen oxide are formed from nitrite in the acidic stomach, and it is possible that these are exported to exert systemic NO-like bioactivity. However, in this study, peak nitrite and RXNO levels in plasma were similar regardless of pretreatment, and therefore, the exact nature of any exported nitrogen oxide species is yet to be determined. RXNO represents the sum of nitrosated and nitrosylated species. S-nitrosothiols were recently suggested to mediate the effect of nitrite on blood pressure in rats, and the involvement of these species also in humans is indeed possible. However, the overall RXNO signal (in which S-nitrosothiols are included) was already weak, leaving little room for detection of selective changes in S-nitrosothiol levels. The fact that intravenously infused nitrite had no effect on blood pressure further supports the notion that nitrite itself is not effectively and rapidly bioactivated in blood and tissues to affect blood pressure. This brings some important mechanistic insight to this highly active field of research because it implies that the most studied nitrite bioactivation pathways, that is, deoxygenated hemoglobin in red blood cells and xanthine oxidase in red blood cells and tissues are of less importance at least for the acute blood pressure effects of dietary nitrate and nitrite in humans. Although plasma nitrite peaked at the same levels with or without esomeprazole, the decline in nitrite was more rapid when the subjects were given placebo. The reason for this is currently unknown. One might argue that esomeprazole in some way affects the red blood cells or other nitrite-reducing cell so that they consume or reduce less nitrite, and this would then explain the slower nitrite decline and the lack of blood pressure–lowering effect of nitrite after the PPI. However, this still seems somewhat unlikely given the fact that the blood pressure response to a high intravenous dose of nitrite in rats was entirely unaffected by esomeprazole. Moreover, the lack of effect of intravenous nitrite on blood pressure in the healthy volunteers also speaks against this notion.

In the current study, no changes in blood pressure were observed when nitrite was intravenously infused, in contrast to when this anion was administered orally. Although surprising, these results are in fact in line with a previous study by Pluta et al, who reported no changes in blood pressure during sodium...
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Nitrite infusions at even higher doses and reaching plasma levels similar or higher than the ones reported here. Similar results showing absence of changes in peripheral blood pressure mediated by nitrite infusions were recently reported also by Omar et al. In this regard, although previous studies have clearly showed that nitrite infusions increase peripheral blood flow at plasma concentrations close to the physiological range, it seems that nitrite-induced changes in blood pressure require higher intravenous nitrite doses or, as suggested by the current study, a different administration route. For example, Webb et al reported changes in blood pressure 2 to 3 hours after oral ingestion of inorganic nitrate, when nitrate was cumulatively converted to nitrite by the entero-salivary pathway. The changes in blood pressure in that study were associated with plasma nitrite levels not exceeding 1 µmol/L. Even lower increases in plasma nitrite were reported by Larsen et al and Kapil et al using orally ingested sodium nitrate, which still decreased blood pressure. Importantly, in all studies looking at effect of dietary nitrate, the nitrite generated locally in the oral cavity from the ingested nitrate enters the blood stream via gastric passage. Altogether, it seems that the route of nitrite administration determines its acute effects on blood pressure.

At this stage, we cannot exclude that nitrite signals locally via gastric nitrosation or nitration reactions. This could affect afferent nerve signaling or the release, modification, and export of another vasoactive hormone or signaling molecule. Indeed, nitrite-mediated nitration of gastric pepsin altering its activity was recently reported. Alternatively, esomeprazole could inhibit systemic nitrite bioactivation, for example, by interfering with nitrite transport systems or nitrite reduction pathways. However, this again seems unlikely because in the rat experiments the acute blood pressure response to an NO donor or a larger dose of intravenous nitrite was unaffected by esomeprazole pretreatment. Moreover, in the in vitro myograph experiments, pretreatment with esomeprazole did not alter the dilatory action of higher doses of sodium nitrite, acetylcholine, or sodium nitroprusside, demonstrating that downstream NO-signaling pathways are not affected by this PPI.

Figure 6. Vascular effects of sodium nitrite, nitroprusside, and acetylcholine in the presence of esomeprazole. Changes in mean arterial blood pressure (MAP) in anaesthetized rats after intravenous administration of an NO donor or nitrite. The rats were pretreated with either vehicle control or esomeprazole (5 mg·kg⁻¹) 2 h before bolus intravenous injections of DEA NONOate (5.6 µg·kg⁻¹) or sodium nitrite 0.1 and 0.5 mg·kg⁻¹. B, The vascular relaxation induced by increasing concentrations of sodium nitrite in mesenteric resistance vessel precontracted with norepinephrine (1 µmol/L), in the presence of vehicle or esomeprazole (10 µmol/L). Similarly, concentration–response curves were performed to both endothelium-independent and endothelium-dependent vasorelaxation compounds, that is, the NO donor sodium nitroprusside (C) and acetylcholine (D), respectively. No differences were found comparing control vehicle vs esomeprazole in vivo and in vitro. Data are shown as mean±SEM (n=5–6).
In the current study, high levels of NO were found in expelled stomach gas after nitrite ingestion, and these were greatly reduced after pretreatment with esomeprazole, consistent with the idea of an effective increase in gastric pH after pretreatment with esomeprazole. This is in line with previous studies demonstrating pH-dependent nonenzymatic NO formation from nitrite immediately on contact with the acidic gastric juice.\textsuperscript{26,35–37} We cannot fully exclude that NO itself can escape directly into the systemic circulation to affect blood pressure. However, considering the high reactivity of the NO radical, in particular with oxyhemoglobin, another more stable NO reaction product is a more likely candidate for exerting such systemic effect.

Studies in rats show that the blood pressure–lowering effects of orally ingested nitrate are also blunted by PPIs.\textsuperscript{38} In an extension, this would imply that the beneficial cardio-vascular effects of nitrate-rich vegetables,\textsuperscript{8} such as lettuce, beetroots, and spinach,\textsuperscript{12} may be partly lost in patients taking PPIs. Interestingly, PPIs have been described to interfere with the efficacy of antiplatelet drugs, such as clopidogrel,\textsuperscript{5} aspirin,\textsuperscript{8} and ticagrelor,\textsuperscript{7} which in turn has been associated with adverse clinical outcomes, especially in high cardiovascular risk populations.\textsuperscript{4} In line with this, inorganic nitrite and nitrate have been shown to inhibit platelet aggregation.\textsuperscript{14,17,48,49} On the basis of our current data, showing that esomeprazole interferes with nitrite- or nitrate-mediated effects on blood pressure, it would be interesting to evaluate whether PPIs will also influence the ability of these anions to modulate platelet function. Importantly, in this study, we only looked at one of the parameters known to be affected by nitrate and nitrite, and it remains to be determined whether other effects, including metabolic effects, are also dependent on gastric acidity.\textsuperscript{12} Moreover, it is clear that nitrite when given systemically does have many effects, including protection against ischemia–reperfusion injury, vasodilation, and platelet inhibition,\textsuperscript{12} although in many cases these seem to require higher concentrations. Thus, it is possible that the mechanism for nitrite bioactivation is different at low near-physiological levels versus high pharmacological doses.

In a recent study in healthy volunteers, we showed that elimination of oral nitrate-reducing bacteria with the use of an antiseptic mouthwash lowered plasma nitrite with a concomitant increase in blood pressure, suggesting that circulating endogenously derived nitrite (from NO synthases) modulates blood pressure.\textsuperscript{50} In light of the present results, we would now modify this statement and suggest that although nitrite is an obligate intermediate in bioactivation of endogenous nitrate, the nitrite found in plasma is likely not the primary source of this NO bioactivity. Rather, nitrite bioactivation mainly takes place in the gastric environment. Importantly, previous knowledge in combination with our current findings clearly suggest that PPIs will affect physiological vascular NO bioactivity also in the absence of any dietary intake of nitrate or nitrite.

In conclusion, the present study suggests that the acute blood pressure–lowering effect of oral nitrite is dependent on an acidic gastric environment. Future studies will reveal whether the cardiovascular complications recently associated with the use of PPIs are linked to reduced nitrite-derived NO bioactivity.

**Perspectives**

This study defines a central role for an acidic gastric environment in bioactivation of the anion nitrite to generate systemic NO-like bioactivity. It helps to shine new light on the mechanisms behind the vasodilatory and blood pressure–lowering effects of dietary nitrate and nitrite and may offer a plausible mechanism behind some of the negative cardiovascular effects associated with the chronic use of PPIs.

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**Disclosures**

J.O. Lundberg and E. Weitzberg are coinventors on patent applications related to the therapeutic use of inorganic nitrate. The other authors report no conflicts.

**References**


**Novelty and Significance**

**What Is New?**

- We demonstrate here that bioactivation of orally ingested nitrite to generate systemic NO-like bioactivity is entirely dependent on an acidic gastric environment.
- The blood pressure–lowering effect of orally ingested nitrite was abolished when test persons were pretreated with a proton pump inhibitor (PPI) to raise gastric pH. Conversely, when nitrite was intravenously administered, no changes on blood pressure were observed despite reaching the same plasma levels as when ingested orally.
- These results suggest that nitrite itself is not effectively bioactivated systemically in blood to generate NO bioactivity and acutely affect cardiovascular function.

**What Is Relevant?**

- The nitrate–nitrite–NO pathway has emerged as an important alternative source of vascular NO formation in addition to the classic pathway where NO is generated from l-arginine by NO synthases. Although several nitrite-reducing pathways have been proposed to be involved in nitrite bioactivation, including deoxygenated hemoglobin in red blood cells and xanthine oxidase in tissues, it still remains unclear exactly how nitrite is bioactivated. Our findings suggest that nitrite bioactivation is dependent on an acidic gastric environment.

- Recent studies suggest cardiovascular complications associated with prolonged use of PPIs. The mechanism for this is unknown but has been suggested to involve a reduced vascular bioavailability of NO. We here show that the blood pressure–lowering effect of ingested inorganic nitrite is blocked by pretreatment with a PPI, which may offer a plausible mechanism behind some negative cardiovascular effects of PPIs.
- These results also place the acidic gastric milieu center stage in the nitrate–nitrite–NO pathway, demonstrating that bioactivation of the nitrite anion to elicit a reduction in blood pressure requires an acidic gastric lumen.

**Summary**

We report clinically relevant evidence that the acute blood pressure–lowering effect of nitrite requires an acidic gastric environment. These findings may offer a plausible mechanism behind some of the negative cardiovascular effects associated with the use of PPIs.
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