Gut feelings: visceral hypersensitivity and functional gastrointestinal disorders
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Citation for published version (APA):

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CHAPTER 6
ROLE OF NITRIC OXIDE IN GASTRIC MOTOR AND SENSORY FUNCTIONS IN HEALTHY SUBJECTS

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GUT 2002;51:212-218
ABSTRACT

BACKGROUND & AIMS: Impaired accommodation and hypersensitivity to distension of the proximal stomach are considered to be important factors in the pathogenesis of dyspeptic complaints. As fundus relaxing agents may be effective in the treatment of these symptoms, insight in the mediators involved in fundic accommodation and associated perceptual responses is important. Therefore, we studied the effect of NO synthase inhibition by NG-monomethyl-L-arginine (L-NMMA), on fundic tone, post-prandial sensations and gastric perception in healthy volunteers.

SUBJECTS & METHODS: 18 healthy volunteers participated in a double blind, placebo controlled, randomised study. They underwent a gastric barostat study to evaluate the effect of L-NMMA on meal- and distension-induced sensations and on fundic relaxation in response to oral meal intake, intra-duodenal lipid and glucagon.

RESULTS: Compared to placebo, L-NMMA decreased fundic volume after oral meal intake (438±55 vs. 304±67 ml; n=8, P<0.05) and during intra-duodenal lipid infusion (384±37 vs. 257±43 ml; n=10, P<0.05) but not after glucagon injection (570±62 vs. 540±52 ml; n=4, P=0.4). In addition, basal fundic volume was significantly reduced by L-NMMA. Scores for nausea and satiation were decreased by L-NMMA after oral meal intake, but not during intra-duodenal lipid infusion. Perception scores to gastric distension were not altered by L-NMMA.

CONCLUSIONS: NO is involved in maintaining basal fundic tone and in meal-induced fundic relaxation in humans, but not in visceral perception.

ABBREVIATIONS: NO: Nitric oxide; L-NMMA: NG-monomethyl-L-arginine; MDP: minimal distending pressure; VAS: visual analogue scale
INTRODUCTION

Normal digestion and perception of sensations associated with meal intake are dependent on a reflex relaxation of the proximal stomach initiated by food ingestion. This so-called gastric accommodation reflex allows storage of the meal without concomitant increase in intra-gastric pressure. Impaired relaxation of the proximal stomach may contribute to the development of meal-induced symptoms in conditions such as functional dyspepsia, diabetes mellitus, postfundoplication syndrome, rumination, postsurgical- and diabetic gastroparesis.\(^{1-5}\) Pharmacological interventions aimed at relaxing the proximal stomach may be effective in conditions characterised not only by impaired gastric accommodation but also increased sensitivity to gastric distension, as seen for example in patients with functional dyspepsia.\(^6\) This has been suggested earlier in studies using the 5-HT\(_3\) agonist sumatriptan, which was shown to increase meal-induced gastric relaxation, to increase the threshold for discomfort during gastric distension in healthy volunteers and to increase caloric intake in dyspeptic patients.\(^1,7\) Therefore, to develop fundus relaxing drugs, insight in the mediators involved in the regulation of proximal gastric tone and associated perceptual responses is important.

Nitric oxide (NO) is recognised as an important neurotransmitter in the human gut, mediating a variety of motility patterns.\(^8,10\) Animal studies have provided abundant evidence that both basal fundic tone and the relaxation of the proximal stomach induced by vagal stimulation, meal ingestion or intra-duodenal lipid are mediated by NO.\(^{11-17}\) In addition, evidence is available that NO is involved in the modulation of visceral perception, for example from rat experiments, showing that intra-peritoneal injection of acetic acid results in an increase of nitrogentic neurones in specific regions of the brain.\(^{18}\) Also, NO synthase immune-reactivity has been demonstrated in lumbo-sacral afferents and pre-ganglionic neurones innervating the pelvic viscera.\(^{19}\)

Data illustrating a role of NO in human proximal gastric motility and perception are rather limited to in vitro studies,\(^{10}\) or in vivo studies investigating the effect of NO donors such as nitrates.\(^{20-22}\) However, to gain more insight in the role of endogenous NO, studies evaluating the effect of an NO synthase inhibitor are required. We previously demonstrated that the selective NO synthase inhibitor L-NMMA (\(\text{N}^\text{G}\)-monomethyl-L-arginine) dose-dependently affects small intestinal- and oesophageal motility, illustrating that this chemical is a useful tool to investigate the role of endogenous NO in man.\(^{8,23}\) Therefore, in the present study, we studied the effect of L-NMMA in healthy volunteers on basal fundic volume and fundic relaxation. In addition, perception of fundic distension and meal-induced sensations were assessed.
SUBJECTS AND METHODS

SUBJECTS
18 healthy male volunteers (mean age 24, range 19-30 yr.) were studied. All subjects were free of gastrointestinal symptoms, without previous gastrointestinal surgery and not taking any medication. Subjects were studied after an overnight fast and were not allowed to smoke or drink alcohol at least 24 hours before the study. All volunteers gave their written and informed consent to participate in the protocol, which had been approved by the Medical Ethics Committee of the Academic Medical Centre, Amsterdam.

METHODS

Gastric barostat
The barostat allows continuous recording of proximal gastric volume using an intra-gastric bag set at a fixed pressure level. In addition, gastric sensitivity can be assessed by distending the intra-gastric bag. Following anaesthesia of the throat (10% xylocaine spray) subjects swallowed a 1200 ml polyethylene bag, tightly wrapped on the distal end of a double lumen polyvinyl tube (Salem Sump tube, Sherwood Medical St Louis, USA; outer diameter 4 mm). The bag was unfolded by inflating it with 500 ml of air and positioned in the proximal stomach by gently pulling the catheter back. The catheter was connected to an electronic barostat that automatically corrected for the compressibility of air (Medtronic Functional Diagnostics, Stockholm, Sweden). Recorded data were stored on a personal computer, using commercially available software (Polygram for Windows, Medtronic Functional Diagnostics, Stockholm, Sweden).

Intra-duodenal infusion
(Study II) A polyurethane naso-duodenal feeding tube was placed for lipid infusion (Flocare®, Châtel Medical Devices S.A., Switzerland: Length 125 cm, outer diameter 3.3 mm). The catheter, containing a guide wire in the central lumen, was positioned 30 cm beyond the pyloric region, under fluoroscopic control. The guide wire was then removed and the catheter was connected to a perfusion pump. Based on previous studies, a 10% lipid solution (Intralipid®, Pharmacia & Upjohn, the Netherlands, energy load 1.1 Kcal/ml) was infused at a rate of 1 ml/min.

Sensation scores
Sensations of bloating, nausea, pain and satiation were assessed individually, using a 10 cm, continuous visual analogue scale (VAS: 0 cm = no sensation, 10 cm = worst ever). Perception of sensations induced by feeding were scored just before and at 5-min intervals after meal intake or following the start of intra-duodenal infusion of lipid (Study I en II respectively). Sensations perceived during gastric distension (Study II) were scored halfway each distension step, both during fasting and during intra-duodenal infusion of lipid.

Drugs

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L-NMMA (N\textsuperscript{G}-monomethyl-L-arginine monoacetate) was supplied by Alexis Corporation, Switzerland, and donated by a generous grant from the Janssen Research Foundation, Belgium. L-NMMA was dissolved in a sterile, 0.9% NaCl solution to a concentration of 15 mg/ml. Equal volumes of the vehicle were used in the control studies. Glucagon (Glucagen®: glucagon hydrochloride) was supplied by Novo Nordisk Pharma, the Netherlands.

**EXPERIMENTAL PROTOCOLS**

Three study protocols were used (see Fig 1). Of the 18 volunteers, 3 participated in both Study I and II and 3 participated in Study II and III. Therefore, the total of subjects studied was 10 for Study I, 10 for Study II and 4 for study III. All studies were designed in a double blind, placebo controlled fashion, performed on two separate days, at least three days apart. Study I was designed to study the effect of L-NMMA on fundic relaxation upon oral intake of a liquid, caloric meal. In addition, sensations evoked by the test meal were studied. In Study II, subjects received lipids intra-duodenally via a naso-duodenal feeding tube. We chose this approach 1) to by-pass possible effects of NO synthase inhibition on gastric emptying and 2) to create a situation of increased sensitivity to gastric distension, as described previously.\textsuperscript{24} The effect of L-NMMA on sensations induced by duodenal lipid and by gastric distension was studied before and during intra-duodenal administration of lipids. In addition, we studied fasting fundic volume and fundic relaxation induced by intra-duodenal lipid. Study III was designed to evaluate a possible post-junctional effect of L-NMMA. Therefore, in Study III, we evaluated the effect of L-NMMA on basal volume and fundic relaxation upon glucagon, known to relax the proximal stomach probably via a direct and NO-independent mechanism.\textsuperscript{25}

**Study I: Fundic accommodation and sensations after oral meal intake**

In this study protocol, both proximal stomach and antropyloroduodenal motility were recorded by combining a gastric barostat test with antropyloroduodenal manometry. However, for clarity, the manometry data will be presented in a separate paper.\textsuperscript{26} Subjects reported at the lab at 7:45 a.m. The barostat bag was positioned in the proximal stomach as described above. An intravenous line was placed in the left arm for intravenous infusion of either placebo or L-NMMA. Heart rate, systolic and diastolic blood pressures were measured every 10 min during the protocol, using an automatic sphygmomanometer (Boso, Jungingen, Germany). After an equilibration period of 30 min, minimal distending pressure (MDP) was determined as the minimum pressure at which balloon volume was >30 ml. Baseline operating pressure was set at MDP+2 mmHg. Intra-gastric bag volume was recorded under basal conditions during 15 min. L-NMMA was infused intravenously at a rate of 12 mg/kg within 5 min (bolus), followed by a maintenance infusion of 6.7 mg/kg/h (Fig. 1). In the control studies, an equal volume of saline was infused at equal rates. Twenty min after the start of the infusion, 200 ml of a liquid test meal (Nutridrink®, Nutricia, Zoetermeer, the Netherlands, energy load 300 Kcal) was consumed with
the aid of a straw. Fundic volume was recorded during the following 60 min. Post-prandial sensations were scored every 5 min.

**Study II: Fundic tone and sensations before and after intra-duodenal lipid**

Subjects reported at the lab at 7:45 a.m. After placement and positioning of the naso-duodenal catheter and the barostat bag, an intravenous line was placed and an equilibration period of 30 min was allowed. Thereafter, MDP was determined as described above. Operating pressure was set at MDP+2 mmHg and baseline intragastric bag volume was recorded for 15 min. Intravenous administration of either L-NMMA (bolus of 12 mg/kg within 5 min, followed by maintenance infusion of 6.7 mg/kg/h) or placebo was then started (Fig. 1). Basal volume during the first 15 min of infusion was recorded, followed by a series of 4 isobaric distensions at 3, 6, 9, and 12 mmHg above MDP, in random order. Each pressure step lasted 60 s, with 120-s intervals at the level of MDP. Perception scores were assessed at each pressure step. Again, operating pressure was set at MDP+2 mmHg and continuous intra-duodenal lipid infusion was started 30 min after the first distension series. Perceived meal-related sensations were scored every 5 min during the lipid infusion. A second series of 4 random, isobaric distensions at MDP+ 3, 6, 9, and 12 mmHg was performed 30 min later, while continuously infusing lipids, and perception scores were assessed at each distension level. Heart rate and blood pressure were measured every 10 min.

**Study III: Glucagon-induced fundic relaxation**

Subjects reported at the lab at 8:00 a.m. Placement of the barostat bag, equilibration, determining of the MDP and baseline volume recording (15 min) was performed as described above. The same dose of L-NMMA was used as in the previous two studies. Similar to Study II, fasting fundic volume was recorded for 55 min (Fig 1). At T=55 min, subjects received 1 mg of glucagon i.v., flushed with 5 ml of saline in the right forearm. Heart rate and blood pressure were measured every 10 min.
DATA ANALYSIS

Fundic volume:
Study I: Basal volume 15 min before and 20 min after the start of the infusion (L-NMMA or placebo) was determined as the mean volume over the given period. Meal-induced relaxation was expressed as the mean volume over 60 min post meal. Volume change, or delta V, was determined as the difference between the mean basal volume (before infusion) and the mean post-prandial volume. Based on previous studies, a volume change of >64 ml was considered normal.\(^1\)

Study II: Basal volume was measured 15 min before and 15 min after the start of the infusion of L-NMMA or placebo. In addition, basal volume was measured between 40 and 55 min after the start of the infusion (i.e. 15 min after the first distension series). Lipid-induced relaxation was expressed as the mean volume over 30 min after the start of the intra-duodenal infusion of lipid. Delta V was determined as the difference between the mean basal volume (before infusion) and the mean post-prandial volume.
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Study III: Similar to Study II, basal volume was measured 15 min before and 15 min after the start of the infusion of L-NMMA or placebo and between 40 and 55 min after the start of the infusion. Fundic relaxation in response to glucagon administration was determined as the mean volume over 5 min following injection. Delta V was determined as the difference between the mean basal volume (before infusion) and the mean volume after glucagon.

**Fundic compliance:**
(Study II) Gastric distension was performed at fixed pressure levels (3, 6, 9 and 12 mmHg) above MDP. The mean of the corresponding volumes over the last 30 s of each distension step was measured and plotted against the corresponding distending pressure. Fundic compliance was calculated as the slope of the pressure-volume curve, as described previously.1

**Meal-induced sensations:**
Following meal ingestion and following the start of intra-duodenal lipid administration (Study I and II respectively), perceived sensations were scored every five min. Scores for bloating, nausea, pain and satiation were analysed individually as repeated measures in time, using a customised mixed effects model.

**Sensation induced by gastric distension:**
(Study II) We performed two series of four distensions, at fixed pressure levels applied in random order, one during fasting and one during intra-duodenal lipid administration (distension series 1 and distension series 2 respectively). For each distension series, the scores for bloating, nausea, pain and satiation were plotted against the corresponding distending pressure.

**Statistical analysis**
For analysis of meal-induced sensation scores, a mixed-effects model was fitted using a standard software package (S-PLUS 2000). As each sensation score is dependent on the previous score, it is not possible to use standard techniques for statistical analysis, such as t-tests. Mixed-effects models are similar to linear regression, but account for the structure of the repeated measures using random effects. Random effects allow the intercept and the value of some other coefficients to vary from person to person. In our analysis of the data on meal-induced sensations, we used a model that accounted for the time post-prandial and the square of the time. The random effects were a constant and the gradient associated with time.

The model used to describe the meal-induced sensations perceived at time $t$ by person $i$ under treatment $j$, $y_{ijt}$ was:

$$y_{ijt} = \alpha_i + \beta_j + \gamma t + \delta \tau + \varepsilon_{ijt} \text{ where } \varepsilon_{ijt} \sim N(0, \sigma^2)$$

Distension-induced sensations were compared using a repeated measures ANOVA with the Greenhouse-Geisser correction, using a standard software package (SPSS 128).
All other data were compared using a Student's t-test (SPSS 9.0) and are presented as mean ± SEM. *P* values < 0.05 were considered statistical significant.

**RESULTS**

**EFFECT OF L-NMMA ON BLOOD PRESSURE AND HEART RATE**

All subjects tolerated the studies well. In study I, mean diastolic blood pressure was significantly increased from 73 ± 2 mmHg after placebo to 84 ± 2 mmHg after L-NMMA infusion (*P*<0.01), whereas the mean heart rate was significantly decreased from 69 ± 5 bpm (placebo) to 58 ± 3 bpm (L-NMMA *P*<0.01). In study II, diastolic blood pressure increased from 75 ± 1 mmHg (placebo) to 82 ± 2 mmHg (L-NMMA, *P*<0.01) and heart rate decreased from 60 ± 2 bpm (placebo) to 54 ± 2 bpm (L-NMMA, *P*<0.01). In Study III, L-NMMA increased diastolic blood pressure from 69 ± 3 mmHg (placebo) to 79 ± 4 mmHg (*P*<0.05) and heart rate decreased from 58 ± 4 bpm (placebo) to 52 ± 3 bpm (*P*<0.05). Systolic blood pressure was not significantly altered by L-NMMA. Cardiovascular effects were sustained during the course of the studies. No side effects were reported.

**EFFECT OF L-NMMA ON BASAL FUNDIC VOLUME AND FUNDIC RELAXATION**

*Basal fundic volume and fundic relaxation induced by oral meal intake (Study I):*

Mean fundic volume was similar before the start of placebo and L-NMMA (Fig 2A). L-NMMA did not significantly alter basal volume 20 min after the start of the infusion (Fig 2A). In 8 out of 10 subjects, ingestion of the meal resulted in a fundic relaxation that was considered normal (delta V > 64 ml) during placebo. The mean observed relaxation (or delta V) in these individuals (n=8) decreased from 274 ± 35 ml (placebo) to 143 ± 55 ml (L-NMMA), but this did not reach statistical significance (*P* = 0.08). In contrast, as shown in Figure 2A, mean post-prandial volume was significantly decreased by L-NMMA compared to placebo.

*Basal fundic volume and fundic relaxation induced by intra-duodenal lipid (Study II):*

Baseline fasting fundic volume was similar before the start of placebo and L-NMMA (Fig 2B). L-NMMA did not significantly alter fasting volume during the first 15 min. However, L-NMMA gradually decreased fasting fundic volume during the course of the study, which was statistically significant over the last 15 min before the start of intra-duodenal lipid administration (40 to 55 min following the start of drug infusion (Fig 2B). All (n=10) subjects showed a marked fundic relaxation following the start of intra-duodenal lipid, which was significantly decreased by L-NMMA compared to placebo (delta V: from 204 ± 32 ml to 129 ± 32 ml, *P*<0.05). In addition, mean post-prandial volume was significantly decreased by L-NMMA, compared to placebo (Fig 2B).
Basal fundic volume and fundic relaxation induced by glucagon (Study III):
Baseline fasting fundic volume before the start of placebo or L-NMMA was comparable for both groups (Fig 2C). Similar to Study II, L-NMMA did not significantly alter fasting volume during the first 15 min, but significantly decreased mean fasting fundic volume over the last 15 min of the recording time, 40 to 55 min following the start of drug infusion (Fig 2C). Glucagon administration induced an instant and marked relaxation of the fundus in all (n=4) subjects, which was not altered by L-NMMA (delta V: 397 ± 71 ml and 356 ± 66 ml for placebo and L-NMMA respectively, P= 0.2). In addition, the mean volume after glucagon was not significantly altered by L-NMMA compared to placebo (Fig 2C).

**Figure 2.** Effect of L-NMMA and placebo on basal and post-prandial fundic volume (A) after oral ingestion of a liquid meal, (B) after intra-duodenal infusion of lipid and (C) after injection of glucagon. Data are expressed as mean ± SEM. *P<0.05, paired t-test, versus placebo; #P<0.05 paired t-test, versus basal (before infusion).
EFFECT OF L-NMMA ON VISCERAL PERCEPTION

**Sensations after oral meal intake (Study I):**

At baseline, subjects reported comparable sensation scores (See Fig. 3, T=0). Following the meal, subjects reported increased sensations of bloating, satiation, pain and nausea. The scores of bloating and pain were not altered by L-NMMA, whereas subjects reported significantly decreased scores for nausea and satiation during L-NMMA infusion compared to placebo (Fig. 3).

**Sensations after duodenal lipid infusion (Study II):**

Sensation scores were comparable before the start of intra-duodenal lipid administration. No significant scores for nausea and pain were reported during intra-duodenal administration of lipid, both during placebo and during L-NMMA (maximum score for nausea: 0.6 ± 0.4 and 0.4 ± 0.3 cm respectively; maximum score for pain: 0.3 ± 0.2 and 0.3 ± 0.1 cm respectively) Although sensation scores for bloating and satiation tended to increase slightly during intra-duodenal lipid, subjects perceived only very low scores, both during placebo and during L-NMMA.

**Figure 3.** Effect of L-NMMA and placebo (see legend) on sensation scores for bloating, satiation, pain and nausea after oral ingestion of a nutrient liquid meal (Study I). Scores for satiation and nausea after oral meal intake were significantly decreased by L-NMMA, compared to placebo (*P<0.05, customised mixed model). Data are expressed as the observed rough mean ± SEM.
(maximum score for bloating: 1.4 ± 0.5 and 1.6 ± 0.7 cm respectively; maximum score for satiation: 1.4 ± 0.6 and 1.4 ± 0.6 cm respectively). L-NMMA did not significantly alter any of the sensation scores, compared to placebo.

Sensation induced by gastric distension (Study II):
Subjects reported only mild sensations of pain and nausea during fundic distension while fasting. As shown in Figure 4, intra-duodenal lipid tended to increase the perception of pain and nausea, although this did not reach statistical difference. Scores for bloating and satiation were more pronounced in both distension series and significantly increased during intra-duodenal lipid, compared to placebo (Fig. 4). No significant effect on sensation scores during distension was seen between L-NMMA and control studies both during fasting and during intra-duodenal lipid infusion (Fig. 4).

![Figure 4](image-url)

**Figure 4.** Effect of L-NMMA (filled symbols) and placebo (open symbols) on sensation scores for bloating, satiation, pain and nausea in response to fundic distension during fasting and during intra-duodenal lipid infusion (see legend). Data are expressed as mean ± SEM.
EFFECT OF L-NMMA ON FUNDIC COMPLIANCE
Fundic compliance during fasting was not significantly altered (placebo 50 ± 4 vs. L-NMMA 46 ± 6 ml/mmHg). Likewise, during infusion of lipid, mean fundic compliance was not significantly affected by L-NMMA (placebo 46 ± 3 vs. L-NMMA 50 ± 5 ml/mmHg). The pressure-volume curves are shown in figure 5.

![Pressure-volumé curve](image)

**FIGURE 5.** Effect of L-NMMA (filled symbols) and placebo (open symbols) on the pressure-volume curves in response to isobaric fundic distension during fasting and during intra-duodenal lipid infusion (see legend).

DISCUSSION

In this study, we evaluated the effect of NO-synthase inhibition by L-NMMA on proximal gastric volume and perception in healthy human subjects. We showed that L-NMMA decreased basal fundic volume and reduced fundic relaxation both after ingestion of a liquid meal and during intra-duodenal lipid infusion, indicating that NO is involved in modulating fundic tone. Finally, no effect of L-NMMA on perception was seen, indicating that, at least in healthy volunteers, NO has no major role in visceral perception.

Animal studies have provided abundant evidence that both basal tone and the relaxation of the proximal stomach, induced by vagal stimulation, meal ingestion or intra-duodenal lipid, are mediated by NO.\(^{11-17}\) Similarly, we showed that NO synthesis inhibition by L-NMMA contracted the gastric fundus, resulting in a reduction of basal fundic volume. The effect was only observed after prolonged infusion of L-NMMA, most likely due to the time-dependent inhibitory effect of L-
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NMMA on NO synthase activity. In addition, L-NMMA reduced fundic volume following ingestion of a nutrient liquid meal and during intra-duodenal lipid. It should be emphasised though that the reduction of post-prandial volume observed during L-NMMA may simply reflect functional antagonism, resulting from the contractile effect of L-NMMA on basal tone. However, the fundic relaxation induced by glucagon, known to act by a direct and NO independent mechanism, was not affected by L-NMMA. Therefore, we concluded that the reduction of the post-prandial volume by L-NMMA resulted from an effect on NO release, illustrating that NO is involved in the meal-induced relaxation of the human proximal stomach. These findings are in line with the recent in vitro study by Tonini et al. demonstrating that relaxation of muscle strips of the human proximal stomach induced by nerve stimulation is inhibited by NO synthase inhibition.

With respect to the site of action, L-NMMA may act at all possible levels, since NO is found to act throughout the central nervous system and the enteric nervous system. The fact that fundic relaxation induced by glucagon was not inhibited by L-NMMA excludes a possible post-junctional effect on the fundic smooth muscle. In addition, we did not observe an effect of NO synthase inhibition on plasma pancreatic polypeptide concentrations, indicating that vagal efferent output was not altered by L-NMMA. Finally, no significant effect on reaction time was noted (data not shown), suggesting no sedative effect of L-NMMA. Thus, although a central action of L-NMMA can not be excluded under the current experimental conditions, these findings suggest that L-NMMA increases fundic tone by reducing nitrergic, neuronal input at a peripheral level.

Impaired accommodation to a meal, as encountered in number of clinical conditions, is considered an important patho-physiological mechanism responsible for dyspeptic complaints. For example, in functional dyspepsia, impaired accommodation is associated with early satiety and weight loss. Therefore, we anticipated that reduction of gastric relaxation by L-NMMA should increase post-prandial sensation scores. In contrast, scores for nausea and satiation were significantly decreased. As NO is also involved in the modulation of visceral sensation, this effect could result from interaction of L-NMMA with perception. However, no changes in perception of gastric distension were reported during fasting. Furthermore, when the stomach was distended during intra-duodenal infusion of lipid, which is known to cause increased sensitivity in healthy subjects, no effect of L-NMMA was observed, excluding an interference with visceral perception. The observation that during L-NMMA infusion, subjects only reported decreased sensation scores after oral meal intake may therefore result from an effect of L-NMMA on gastric emptying. Alternatively, since gastric wall tension seems to be the major determinant of gastric perception, reduced perception of meal-induced sensations may also be explained by the reduction in volume and the associated reduction in wall tension. The fact that the healthy volunteers also experienced mild degrees of nausea and pain during the experiment probably reflects the artefact that is introduced by measuring meal-induced sensations while having a barostat balloon inflated in the stomach. Our finding that reduction of meal-induced relaxation is not accompanied by an increase in perceived sensations
questions the direct relationship between impaired accommodation and symptoms, as found in functional dyspepsia. Similar conclusions were drawn from a recent study showing that in patients with functional dyspepsia, abolishing gastric relaxation by the cholecystokinin-A antagonist dexloxiglumide was associated with reduced rather than increased dyspeptic symptoms.32

In contrast to several animal studies, we did not observe an effect of L-NMMA on visceral perception. This may be explained by the knowledge that NO has been shown to be mainly involved in the perception of visceral pain.18,19,30,33 The healthy volunteers in our study predominantly reported bloating and satiation upon gastric distension, rather than pain. One might however anticipate that NO synthase inhibition may have an effect on perception in dyspeptic patients with visceral hypersensitivity.6 Animal models of visceral hyperalgesia indeed show up-regulation of NO synthase producing neurones in the spinal cord. More importantly, NO synthase blockade normalised the hyperalgesic response, but had no effect on perception in control animals.33,34 Therefore, L-NMMA may only affect visceral perception in the presence of hypersensitivity. Further studies investigating the effect of NO synthase inhibition in patients with functional dyspepsia are therefore warranted.

The use of NO donors in conditions characterised by impaired accommodation and / or hypersensitivity to gastric distension is controversial. Nitrates are known to induce gastric relaxation and earlier studies showed some relief of symptoms in functional dyspepsia, but not in diabetes.20,21 Moreover, significant side effects, in particular headaches, are associated with the use of nitrates. Therefore, development of fundus relaxing drugs may need to be aimed at selectively activating NO producing neurones at the level of the myenteric plexus. For example, the 5-HT\textsubscript{1} agonist sumatriptan has been shown to relax the proximal stomach by activating NO producing neurones.11,35 Reduction of gastric tone by sumatriptan allowed higher gastric distension volumes in healthy volunteers before the threshold of discomfort was reached, and increased the amount of calories inducing maximum satiety in functional dyspeptics.1,7 Although these studies are promising, confirmation in larger clinical studies is awaited.

In summary, we showed that NO is involved in basal fundic tone and fundic relaxation in healthy volunteers, but not in gastric perception. Reduction of meal-induced relaxation did not result in increased perception of post-prandial sensations. As NO inhibition did not affect gastric perception to distension and had no effect on sensations scored during intra-duodenal lipid, the reduced scores for nausea and satiation by L-NMMA after oral meal intake is most likely caused by delayed gastric emptying.
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