Effects of long-term oral l-arginine on esophageal motility and gallbladder dynamics in healthy humans

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Effects of long-term oral l-arginine on esophageal motility and gallbladder dynamics in healthy humans. Am. J. Physiol. 274 (Gastrointest. Liver Physiol. 37): G984–G991, 1998.—Inhibitory nitrergic neurons are known to play a role in the regulation of motility patterns of the distal esophagus, the lower esophageal sphincter (LES), and the gallbladder. Our study aim was to investigate the effects of “long-term” (i.e., prolonged) oral intake of l-arginine (L-Arg), the endogenous source for nitric oxide (NO) synthesis, on postprandial LES pressure (LESP), esophageal motility, gastroesophageal reflux, and gallbladder motility. L-Arg (30 g/day) or glycine (placebo; 13 g/day; isosmolar) was given orally to 10 healthy male volunteers for 8 days, according to a randomized, crossover design. Twenty-four-hour urinary nitrite/nitrate excretion was measured to indicate NO synthesis. Basal early postprandial L-Arg ingestion (2.2 kPa) than after glycine ingestion (2.7 kPa) (P < 0.05). L-Arg abolished the physiological late postprandial rise in LESP. Transient LES relaxations were longer lasting after L-Arg ingestion (P < 0.02). Esophageal motility and reflux were not affected (not significant). Fasting and residual gallbladder volumes were greater after L-Arg ingestion (P < 0.05). Urinary nitrite/nitrate excretion was higher after L-Arg intake (P < 0.05). In conclusion, long-term oral l-Arg suppresses late postprandial LESP increase, prolongs transient LES relaxations, and increases fasting and residual gallbladder volumes. These effects may be mediated by increased NO synthesis.

NITRIC OXIDE (NO), produced from l-arginine by enzymatic oxidation of a terminal guanidino nitrogen atom of l-arginine by the enzyme NO synthase (NOS), is involved in a number of biological actions. NO was originally shown to act as a relaxing agent in vascular tissue, with NO formed by an enzyme in vascular endothelial cells (28). NO is now known to be formed in several other cells and tissues, e.g., macrophages and neurons, involving different isoforms of NOS (24). NO plays an important role in the regulation of gastrointestinal motility, serving as a neurotransmitter in nonadrenergic noncholinergic (NANC) pathways of the gastrointestional tract (32).

Excitatory cholinergic and inhibitory NANC nerves are known to play a role in the peristalsis of the esophageal body (1) and in the relaxation of the lower esophageal sphincter (LES) (4). There appears to be a gradient of decreasing cholinergic and increasing NANC influence along the esophagus in the aboral direction (1). NO is a mediator of LES relaxation induced by swallowing, esophageal distension, and vagal efferent nerve stimulation (29, 40). In the esophageal body, NO is involved in the latency period and latency gradient as well as in the contraction amplitude of esophageal peristalsis, especially in the distal part of the esophagus (1, 14, 41). The L-arginine-NO pathway may also play a role in transient LES relaxations (TLESRs), i.e., abrupt decreases in LES pressure (LESP) to the level of intragastric pressure that are not triggered by swallowing (23) and that are the main mechanism underlying gastroesophageal reflux (7, 33).

The L-arginine-NO pathway is involved in the regulation of gallbladder motility (9, 25); gallbladder muscle relaxation is also mediated by NANC inhibitory nerve activity (21).

In vivo studies on the esophagus and gallbladder have so far only concerned the effects of intravenous L-arginine or NO donors or the effects of NOS inhibitors with subsequent reversion of the inhibitory effect by intravenous L-arginine. The effects of prolonged oral administration of L-arginine on both the esophagus and the gallbladder have not been studied.

Therefore, our aims were 1) to investigate whether “long-term” (prolonged administration, as opposed to single bolus or infusion) oral L-arginine intake increases NO production in healthy humans, 2) to investigate whether these increased NO levels affect esophageal motility, LESP, TLESRs, and the occurrence of gastroesophageal reflux, and 3) to study the effects of increased NO levels on gallbladder motility.

METHODS

First we performed a pilot study with a randomized, double-blind, placebo-controlled, crossover design aimed at verifying that glycine has no effects on the esophagus and gallbladder and could therefore be used as an amino acid placebo in a subsequent L-arginine study. The effects of the amino acid glycine on esophageal motility, LESP, basal gallbladder volume, and gallbladder emptying were evaluated.

Six healthy male volunteers [age 27.0 ± 3.2 years (mean ± SD); body mass index 23.0 ± 2.7 kg/m²] participated. Glycine and a placebo solution, identical to the glycine solution as to caloric content (using glucose as a caloric substrate for the placebo solution), chloride amount, osmolarity, pH, taste, and volume, were given as a drink (300 ml/day) four times a day for 7 days, with a washout period of at least 7 days. Esophageal motility and LESP were measured after 6 days of glycine or placebo intake, after a standardized meal (2,810 kJ; 30 g fat, 30 g protein, and 70 g carbohydrates), by using stationary esophageal manometry. Fasting gallbladder volume was measured using ultrasonography both before and after glycine or placebo intake. Residual gallbladder volume was measured only after 6 days of glycine or placebo intake.

No significant differences were observed in esophageal motility, LESP, and
Informed consent was obtained from each subject.

During each period the subjects kept a daily record of health complaints. The study was ended by 24-h ambulatory esophageal pH and pressure recording. Stationary esophageal manometry was immediately followed by 15 cm proximal to the LES border of the LES; the pH transducers were at 3, 6, 9, 12, and 15 cm above the LES. Both catheters were fixed to the esophagus at 5, 10, and 15 cm above the LES, through the nose, the pressure transducers were positioned at 5 and 15 cm proximal to the upper fundus of the stomach. The catheter was connected to a low-compliance pneumohydraulic perfusion system and perfused with deionized water at a constant rate of 0.3 ml/min. Pressures were measured by external transducers (DPT-200; Medische, Hillegom, The Netherlands) and stored in a digital portable data logger (MMS, Enschede, The Netherlands) using a sample frequency of 8 Hz. A separate solid-state catheter (OD 2 mm; Braun Medical, Oss, The Netherlands) was used to record pharyngeal pressure peaks caused by swallowing. Both catheters were fixed to the nose.

Stationary esophageal manometry. Stationary manometric recordings were obtained with a six-channel water-perfused catheter (OD 4.8 mm) with a Dent sleeve. After introduction through the nose, the catheter was positioned with three side holes in the esophagus at 5, 10, and 15 cm above the LES, with the sleeve in the LES, and with one side hole in the fundus of the stomach. The catheter was connected to a low-compliance pneumohydraulic perfusion system and perfused with deionized water at a constant rate of 0.3 ml/min. Pressures were measured by external transducers (DPT-200; Medische, Hillegom, The Netherlands) and stored in a digital portable data logger (MMS, Enschede, The Netherlands) using a sample frequency of 8 Hz. A separate solid-state catheter (OD 2 mm; Braun Medical, Oss, The Netherlands) was used to record pharyngeal pressure peaks caused by swallowing. Both catheters were fixed to the nose.

Amino acid drinks. Solutions of the amino acids L-arginine and glycine were prepared by the pharmacy at the University Hospital Utrecht. The composition of the daily dose was as follows: 36.28 g L-arginine-HCl (30 g L-arginine) or an isosmolar amount of glycine-HCl (19.31 g; 13 g glycine), 150 ml sucrose syrup, lemon extract (for flavor improvement), preservative, and water to a volume of 300 ml. The pH of the L-arginine and glycine solutions differed slightly, ~3.0 for the L-arginine drink and ~1.5 for the glycine drink. The subjects were instructed to drink 75 ml of amino acid solution four times a day, accompanied by a meal when possible. Use of a daily L-arginine dose of 30 g for 8 days is based on previous studies in healthy humans and in patients and is described as a safe dose (2, 6, 13).

Ten other healthy male volunteers [age 24.2 ± 4.1 years (mean ± SD); body mass index 22.1 ± 3.3 kg/m²], who were nonsmokers and not on medication, received the amino acids L-arginine and glycine, each over an 8-day period, according to a randomized double-blind crossover design. A washout period of 6 days was allowed between the 8 days of amino acid ingestion. Figure 1 shows a scheme of the protocol. On the first day of both periods, after an overnight fast, a blood sample was taken for measurements of plasma levels of electrolytes and amino acids, and blood pressure and gallbladder volume were measured, after which amino acid intake was started. To standardize food and nitrate intake within subjects, a standardized meal (1 slice of white bread, 5 g margarine, 50 g cheese, 1 boiled egg, 200 ml yogurt, and 50 g lean red meat) was consumed, followed immediately by stationary esophageal manometry. The lack of a glycine effect on the esophagus and LES is consistent with the findings of McCallum et al. (20). Therefore, glycine was chosen as a suitable placebo for the study of the effects of L-arginine.

Protocol. Ten other healthy male volunteers [age 24.2 ± 4.1 years (mean ± SD); body mass index 22.1 ± 3.3 kg/m²], who were nonsmokers and not on medication, received the amino acids L-arginine and glycine, each over an 8-day period, according to a randomized double-blind crossover design. A washout period of 6 days was allowed between the 8 days of amino acid ingestion. Figure 1 shows a scheme of the protocol. On the first day of both periods, after an overnight fast, a blood sample was taken for measurements of plasma levels of electrolytes and amino acids, and blood pressure and gallbladder volume were measured, after which amino acid intake was started. To standardize food and nitrate intake within subjects, a standardized meal (1 slice of white bread, 5 g margarine, 50 g cheese, 1 boiled egg, 200 ml yogurt, and 50 g lean red meat) was consumed, followed immediately by stationary esophageal manometry. The lack of a glycine effect on the esophagus and LES is consistent with the findings of McCallum et al. (20). Therefore, glycine was chosen as a suitable placebo for the study of the effects of L-arginine.
tion and to note times spent in the supine position on a diary form. Intake of acidic food and beverages (pH < 5) and extreme physical activity were to be avoided.

Gallbladder ultrasonography. Gallbladder volumes were measured by real-time ultrasonography (Scanner 250, 3.5/5 MHz convex transducer; Pie Medical, Maastricht, The Netherlands). Subcostal sonographic images were obtained in duplicate with the subjects supine. Longitudinal and transverse images of the gallbladder at its largest dimensions were obtained. Gallbladder volume was calculated on-line according to the sum-of-cylinders method (8). The fasting volumes on days 1 and 7 and the residual volume on day 7 were calculated. The ejection volume was calculated as the difference between fasting and residual gallbladder volume.

Blood analysis. Plasma levels of sodium, potassium, chloride, bicarbonate, and urea were measured. Plasma levels of free amino acids (arginine, glycine, citrulline, and ornithine) were measured by ion-exchange chromatography (Biotronic LC 5001). For free amino acid measurement, a volume of norleucine-containing sulfosalicylic acid, equal to 3.3% of the plasma volume, was added to the blood plasma and a thymol crystal was added. The blood samples were frozen (−20°C) to await further analysis.

Urine analysis. Urine was collected for 24 h for measurement of creatinine and nitrite/nitrate excretion. Excretion of nitrite and nitrate, the oxidation products of NO, is indicated as NOx. NOx concentration was determined by automated flow analysis (12). Urinary NOx excretion (in mmol NOx/mol urinary creatinine) was checked for inaccuracy of urine collection by the subjects.

Analysis of pressure recordings. Recordings obtained during stationary esophageal manometry were analyzed for basal LESP and relaxations of the LES, using computer algorithms. The algorithms first calculated the LESP with respect to fundic pressure (as kPa above fundic pressure). This was done by subtracting the fundic pressure signal from the LESP signal derived from the sleeve device. Second, relaxations of the LES were detected automatically, using the following criteria: relaxations should occur at a minimum rate of 0.4 kPa in 3 s, at a pressure of <0.1 kPa above gastric fundic pressure for at least 1 s. The LES relaxations were then classified as “swallow-induced” or “spontaneous/transient”. LES relaxations were considered as transient when one of the following two criteria, according to Mittal et al. (23), was met: 1) absence of a pharyngeal swallow signal for 4 s before to 2 s after the onset of LES relaxation with a decrease rate for LESP of >0.13 kPa/s to an LESP of ≤0.1 kPa within 10 s; or 2) relaxation to ≤0.1 kPa for >10 s, irrespective of the timing of LES relaxation in response to swallowing; LES relaxations associated with multiple rapid swallows were excluded. Mean basal LESP was calculated for 15-min periods for each subject. This was done after exclusion of LESP relaxations and after contractions, using intervals beginning at the start of LES relaxation and ending 8 s after the end of LES relaxation. The percentages of peristaltic, simultaneous, and nontransmitted contractions were evaluated for the esophageal pressure signals from both stationary and ambulatory esophageal manometry. The peristaltic contractions were analyzed for mean amplitude, duration, and propagation velocity. Stationary esophageal manometry recordings were analyzed for dry and wet swallows separately. Ambulatory manometry recordings were analyzed separately for times in the upright and the supine positions. Meal and beverage periods were excluded from the analysis.

Analysis of esophageal pH recordings. Recordings of esophageal pH were analyzed automatically by means of locally developed computer software (34). Reflux parameters from the five channels (percentage of time with pH < 4, number of episodes with pH < 4, number of episodes with pH < 4 lasting 5 min or more, and mean duration of episodes) were calculated separately for periods of upright and supine positions and for the total recording time. The values for numbers of reflux episodes were normalized to periods of 16 h (upright position), 8 h (supine position), and 24 h (total recording time).

Statistical analysis. Differences in LESP for the entire recording period were analyzed using ANOVA for repeated measures. When differences were significant, further analysis with contrast methods was used to analyze in detail the time at which a change in LESP occurred. Missing values in LESP were replaced by the series mean of that 15-min period to make paired analysis possible. TLESR frequency and duration were compared by Student’s t-test for paired data. Differences in esophageal pressure and pH parameters were analyzed using Student’s t-test for paired data (for normally distributed parameters) or Wilcoxon’s test (for nonparametric parameters). Differences between L-arginine and glycine in pH parameters at all esophageal levels were analyzed using the ANOVA for repeated measures. Gallbladder volumes were compared by means of Student’s t-test for paired data. Values for blood and urine parameters were analyzed for differences using Student’s t-test for paired data. Statistical significance was defined as a two-tailed P < 0.05.

RESULTS

Data obtained from one of the subjects had to be excluded from analysis, because his plasma L-arginine levels showed no increase after 6 days of L-arginine intake. His intake of L-arginine was considered to be unreliable. Stationary esophageal manometry data could be analyzed for only seven subjects, as data for two subjects were lost through computer failure. Ambulatory manometry data were analyzed for eight subjects, due to early ending of esophageal manometry in one subject. Reflux data were not complete for all levels in all subjects, because of technical failures. Plasma levels were missing for one subject on day 7 of L-arginine. Urinary data during glycine intake were missing for one subject.

LES. During the first 60 min postprandial, the mean LESP (n = 7) for L-arginine and glycine intake differed (Fig; 2; P < 0.02), with a mean LESP of 2.16 ± 0.06 vs. 2.65 ± 1.20 kPa for L-arginine vs. glycine, respectively. During the glycine period, a significant increase in LESP was observed starting at ~75 min postprandial (P < 0.01), reaching a plateau of 3.80 ± 0.41 kPa at 135 min postprandial. There was no such late postprandial increase of LESP during L-arginine intake, and LESP remained at the early postprandial levels (LESP of 2.14 ± 0.10 kPa at 135 min postprandial). The frequency of TLESRs did not differ significantly for L-arginine and for glycine, being 12.0 ± 4.6 and 10.7 ± 4.9 TLESRs, respectively, for 3 h postprandial. One subject had an extremely high frequency of 33 and 36 TLESRs (in 3 h) after L-arginine and glycine, respectively, and two subjects showed no TLESRs. The duration of TLESRs was significantly prolonged, by ~16%, after L-arginine intake; durations were 18.0 ± 1.3 and 15.1 ± 1.0 s for L-arginine and glycine, respectively (P < 0.02; n = 5).
Eosophageal body. Amplitude, duration, and propagation velocity of esophageal contractions did not differ significantly between L-arginine and glycine intake. This was found for both wet and dry swallows, during stationary esophageal manometry and ambulatory esophageal manometry, even after separate analysis for the upright or supine position and for preprandial periods. In addition, no differences were found in the type of contractions, i.e., peristaltic, simultaneous, or nontransmitted contractions.

Gastroesophageal reflux. Table 1 shows reflux parameters (% reflux time and number of reflux episodes) for different levels in the esophagus, after both L-arginine and glycine. These reflux parameters showed a gradual decrease from the distal to the proximal recording site, following a linear pattern, both for L-arginine and glycine. The mean duration of reflux episodes was the same for all esophageal levels (not shown in Table 1). The values for the 24-h reflux parameters were not significantly different between L-arginine and glycine intake, whether for the upright or supine position and for preprandial periods. In addition, no differences were found in the residual volumes, even after separate analysis for the upright or supine position and for preprandial periods. In addition, no differences were found in the fasting gallbladder volume after L-arginine intake, the absolute residual volume after L-arginine intake, and the residual volumes after the meal on day 7 were also different, being 7.7 ± 1.6 vs. 3.4 ± 0.9 ml for L-arginine vs. glycine, respectively (P < 0.05). The fasting gallbladder volume was significantly increased, by 21.9 ± 1.7%, compared with the fasting gallbladder volume after the start of L-arginine intake (P < 0.05). The residual volumes after the meal on day 7 were also different, being 7.7 ± 1.6 vs. 3.4 ± 0.9 ml for L-arginine vs. glycine, respectively (P < 0.05). The gallbladder emptying percentage after L-arginine was smaller than after glycine (67.4 ± 6.2% and 81.7 ± 4.3%, respectively; P < 0.05). However, due to the simultaneous increase of both fasting and residual volume after L-arginine intake, the absolute ejection volume was not significantly different, being 15.2 ± 1.6 vs. 15.0 ± 1.5 ml for L-arginine vs. glycine, respectively.

Blood chemistry. Table 2 shows the plasma levels of free amino acids (arginine, glycine, citrulline, ornithine), urea and electrolytes (sodium, potassium, chloride, and bicarbonate) before L-arginine and glycine intake (day 1), respectively, and after 6 days of intake (day 7).

Table 1. Reflux parameters for different levels in the esophagus

<table>
<thead>
<tr>
<th></th>
<th>Supine</th>
<th></th>
<th>Upright</th>
<th></th>
<th>Total Time</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>%Reflux time</td>
<td>No. of episodes</td>
<td>%Reflux time</td>
<td>No. of episodes</td>
<td>%Reflux time</td>
<td>No. of episodes</td>
</tr>
<tr>
<td>L-Arginine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 cm</td>
<td>1.04 ± 0.49</td>
<td>2.1 ± 0.7</td>
<td>8.07 ± 1.5</td>
<td>55.4 ± 11.8</td>
<td>5.29 ± 1.14</td>
<td>53.1 ± 10.6</td>
</tr>
<tr>
<td>6 cm</td>
<td>1.13 ± 0.55</td>
<td>2.0 ± 1.2</td>
<td>6.43 ± 1.46</td>
<td>43.0 ± 8.2</td>
<td>4.33 ± 0.88</td>
<td>41.7 ± 7.8</td>
</tr>
<tr>
<td>9 cm</td>
<td>0.64 ± 0.35</td>
<td>1.1 ± 0.5</td>
<td>4.80 ± 1.22</td>
<td>30.1 ± 5.4</td>
<td>3.14 ± 0.74</td>
<td>28.7 ± 4.6</td>
</tr>
<tr>
<td>12 cm</td>
<td>0.44 ± 0.22</td>
<td>0.8 ± 0.4</td>
<td>3.86 ± 0.98</td>
<td>22.9 ± 4.0</td>
<td>2.51 ± 0.58</td>
<td>22.9 ± 3.5</td>
</tr>
<tr>
<td>15 cm</td>
<td>0.42 ± 0.24</td>
<td>1.3 ± 0.6</td>
<td>4.12 ± 1.49</td>
<td>24.3 ± 5.8</td>
<td>2.60 ± 0.92</td>
<td>23.1 ± 5.8</td>
</tr>
<tr>
<td>Glycine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 cm</td>
<td>0.33 ± 0.21</td>
<td>2.0 ± 1.1</td>
<td>7.67 ± 1.41</td>
<td>62.9 ± 9.2</td>
<td>4.73 ± 0.82</td>
<td>58.4 ± 7.8</td>
</tr>
<tr>
<td>6 cm</td>
<td>0.26 ± 0.16</td>
<td>0.9 ± 0.6</td>
<td>6.09 ± 1.41</td>
<td>46.5 ± 8.6</td>
<td>3.70 ± 0.83</td>
<td>40.9 ± 7.0</td>
</tr>
<tr>
<td>9 cm</td>
<td>0.09 ± 0.07</td>
<td>0.5 ± 0.3</td>
<td>4.26 ± 1.14</td>
<td>32.4 ± 5.5</td>
<td>2.57 ± 0.72</td>
<td>29.4 ± 4.8</td>
</tr>
<tr>
<td>12 cm</td>
<td>0.05 ± 0.05</td>
<td>0.3 ± 0.3</td>
<td>3.76 ± 0.96</td>
<td>23.5 ± 4.1</td>
<td>2.34 ± 0.63</td>
<td>21.4 ± 3.5</td>
</tr>
<tr>
<td>15 cm</td>
<td>0.04 ± 0.04</td>
<td>0.3 ± 0.3</td>
<td>3.42 ± 0.81</td>
<td>23.6 ± 4.9</td>
<td>2.04 ± 0.54</td>
<td>21.8 ± 5.1</td>
</tr>
</tbody>
</table>

Values are means ± SE and are given at levels of 3 cm (n = 7), 6 cm (n = 7), 9 cm (n = 7), 12 cm (n = 8), and 15 cm (n = 5) above the lower esophageal sphincter (LES). No. of episodes has been normalized to periods of 16 h (upright position), 8 h (supine position), and 24 h (total recording time). There were no significant differences between L-arginine and glycine treatment, although there was a tendency (P = 0.1) toward higher % reflux time at all esophageal levels during the supine period for L-arginine.
Intake of L-arginine significantly changed the plasma levels of arginine, ornithine, urea, chloride, and bicarbonate ($P < 0.005$). Ornithine and urea are metabolic products of L-arginine, which explains the observed increases of plasma ornithine and urea after L-arginine intake. Glycine intake changed the plasma levels of glycine, chloride and bicarbonate significantly ($P < 0.005$). As expected, glycine intake did not significantly change the plasma levels of arginine. No differences were observed between the values for blood parameters before L-arginine and glycine ($P > 0.05$).

Urine chemistry. NOx excretion after 7 days of L-arginine intake was significantly higher [63.7 ± 6.8 mmol/mol creatinine (mean ± SE)] than after 7 days of glycine intake (54.5 ± 6.8 mmol/mol creatinine) ($P < 0.05$) (Fig. 4). No differences in 24-h urinary creatinine excretion were observed between L-arginine and glycine (17.8 ± 1.6 and 17.4 ± 0.8 mmol/24 h, respectively; $P > 0.05$).

Seven of the subjects had no abdominal complaints during the protocol. Two subjects reported a feeling of nausea after intake of glycine, but interruption of amino acid intake was not necessary. Blood pressure did not change during the intake of either L-arginine or glycine.

**DISCUSSION**

This study provides evidence that after several days of an increased daily intake of L-arginine NO production increases, as was shown by enhanced urinary NOx excretion. Esophageal motility was not affected, but LES tone and relaxation were altered. The postprandial LESP was lowered, the normal late postprandial LESP increase was suppressed, and the duration of TLESRs was prolonged, while the occurrence of reflux was not affected significantly. Long-term oral L-arginine also increased fasting and residual gallbladder volume. These effects of L-arginine on the LES and the gallbladder may be mediated by NO. It is not clear why oral L-arginine selectively affected only the LES and the gallbladder, without an effect on esophagus and blood pressure. It is possible that differences in sensitivity to NO changes between the organs exist or that organ-specific long-term adaptation occurs.

**Table 2. Plasma levels of arginine, glycine, citrulline, ornithine, urea, sodium, potassium, chloride, and bicarbonate**

<table>
<thead>
<tr>
<th></th>
<th>L-Arginine</th>
<th>Glycine</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Day 1</td>
<td>Day 7</td>
</tr>
<tr>
<td>Arginine, μmol/l</td>
<td>63.5 ± 6.3</td>
<td>178.5 ± 13.0*</td>
</tr>
<tr>
<td>Glycine, μmol/l</td>
<td>198.2 ± 13.9</td>
<td>180.3 ± 14.8</td>
</tr>
<tr>
<td>Citrulline, μmol/l</td>
<td>27.0 ± 4.8</td>
<td>21.1 ± 4.7</td>
</tr>
<tr>
<td>Ornithine, μmol/l</td>
<td>69.3 ± 6.1</td>
<td>121.5 ± 8.9*</td>
</tr>
<tr>
<td>Urea, mmol/l</td>
<td>2.8 ± 0.2</td>
<td>3.5 ± 0.3*</td>
</tr>
<tr>
<td>Sodium, mmol/l</td>
<td>141.0 ± 0.6</td>
<td>140.1 ± 0.5</td>
</tr>
<tr>
<td>Potassium, mmol/l</td>
<td>4.1 ± 0.1</td>
<td>3.9 ± 0.1</td>
</tr>
<tr>
<td>Chloride, mmol/l</td>
<td>101.3 ± 0.6</td>
<td>106.0 ± 0.8*</td>
</tr>
<tr>
<td>Bicarbonate, mmol/l</td>
<td>28.6 ± 0.8</td>
<td>22.9 ± 0.8*</td>
</tr>
</tbody>
</table>

Values are means ± SE and are given for L-arginine (n = 8) and glycine (n = 9) before intake (day 1) and after 6 days of intake (day 7). *P < 0.005 (day 1 vs. day 7).

Fig. 3. Gallbladder volumes before (day 1) and after 6 days (day 7) of L-arginine or glycine intake, respectively. Individual curves (lines) and group means (bars) are shown for fasting gallbladder volume (FV) and residual volume (RV) at 120 min postprandial for L-arginine and glycine, separately. $^*P < 0.05$.

Fig. 4. Twenty-four hour urinary nitrate excretion (NOx; mmol/mol creatinine) after glycine vs. L-arginine intake ($^*P < 0.05$). Individual values with corresponding means ± SE are shown.
Urinary NOx excretion has been shown to be a good parameter for endogenous NO synthesis from L-arginine (5, 18), although the measurement of endogenously generated NOx excretion may be confounded by several factors. The most important of these factors is dietary NOx intake. We therefore regulated the food intake for each subject by using a food diary and standardized dinners, starting one day before urine collection. A second source for NOx, NO inhaled via tobacco smoke, could be eliminated since all subjects were nonsmokers.

The route of administration of L-arginine also affects NOx biosynthesis. Oral L-arginine administration results in a more extensive transfer of labeled nitrogen atoms of L-arginine to urinary NOx than does intravenous administration (5). Also, continuous infusion of low-dose [15N]arginine intragastrically results in a greater recovery of [15N]NOx than does a large oral bolus of labeled L-arginine (5, 18). We therefore assumed that long-term repeated oral administration of L-arginine, as used in our study, would yield maximal NO synthesis from L-arginine and be a better model of the physiological situation than would acute high-dose L-arginine intravenously. The difference in NOx excretion with long-term oral L-arginine and that with glycine was small but significant, indicating a small increase in endogenous NOx excretion from L-arginine-derived NO production. However, the results we obtained do not allow us to conclude whether NOx is derived from the oxidation of NO produced by constitutive NOS (especially neuronal NOS associated with NANC innervation) or of NO produced by inducible NOS, which is expressed in many cell types after immune stimulation. Because all subjects were in good health during the experiment, we assumed that the increased urinary NOx excretion seen with L-arginine reflects activation of the constitutive L-arginine-NO pathway.

We found an effect of long-term oral L-arginine on LES and the gallbladder. The early postprandial LESP with L-arginine was lowered, and the physiological late postprandial LESP increase (33) was suppressed. The effect on early postprandial LESP corresponds with the decreased basal LESP seen in humans after administration of the NO donor molsidomine (39). Our findings are also consistent with the increase in LES resting pressure seen after administration of the NOS inhibitor Nω-nitro-L-arginine methyl ester in dogs (3). In our study, L-arginine had no effect on TLESR frequency, but the mean duration of TLESRs was significantly prolonged after L-arginine in our human study. The mechanism determining meal-induced TLESRs is suggested to involve CCK, with NO as the neurotransmitter released at the postganglionic site in the vagal pathway (3, 23). Frequency of TLESRs triggered by gastric distension is shown to be lowered by the NOS inhibitor Nω-nitro-L-arginine methyl ester in dogs (3). In our study, L-arginine had no effect on TLESR frequency. A possible explanation may be that NO increase (by L-arginine) and NO decrease (by NOS inhibitors) do not necessarily have an equal opposite effect. Second, the increase in NO in our study was only small. The mechanism controlling TLESR duration is not known, although the CCK-A receptor antagonist devazepide reduced the duration of TLESRs in dogs, while NOS inhibition was not effective (3). On the basis of our findings, we suggest that the L-arginine-NO pathway may also be involved in the duration of TLESRs. TLESRs are now recognized as the most important mechanism of gastroesophageal reflux. TLESRs associated with reflux have been shown to be significantly longer than those not associated with reflux (33). In our study we did not observe an effect of L-arginine on the frequency of reflux episodes. However, the duration of reflux episodes during the supine period tended to be increased after L-arginine. As the decrease in reflux
occurrence from distal to proximal in the esophagus was equal for L-arginine and glycine, this might indicate that the acid clearance was not affected by L-arginine. Moreover, esophageal motility as an important mechanism for acid clearance was not changed after L-arginine in our study. Longer TLESRs and longer duration of reflux episodes at all esophageal levels might indicate that the volume of refluxate is increased. However, since TLESRs and reflux were not measured simultaneously in our study, we need to be very careful in associating the two, since that requires further investigation.

We found no effects of L-arginine on tubular esophageal motility, i.e., amplitude, duration, and propagation velocity of contractions, although effects in response to NO donors and NOS inhibitors have been reported for the amplitude and propagation velocity of esophageal contractions, especially in the distal esophagus (1, 14, 16, 39, 41). L-Arginine alone has no effect on esophageal peristalsis either in vitro or in vivo in experimental animals (26, 41). The absence of any effect of L-arginine on these parameters in our study may be explained by the fact that the increases in NO synthesis were probably slight compared with the changes in NO levels in studies using NO donors or NOS inhibitors.

In addition to an effect of L-arginine on the LES, we found an increase in fasting and residual gallbladder volume and a reduction in gallbladder emptying after L-arginine. Similar results have been described for intravenous L-arginine (9) and for the NO donor glyceryl trinitrate (11). This suggests that the effect of L-arginine in our study may, as hypothesized, have been NO mediated and that NO in vivo might have an inhibitory influence on gallbladder motility. It is not known whether the effect of L-arginine on gallbladder volume occurs through stimulation of NO synthesis in NANC nerves or in the gallbladder smooth muscle cells themselves. NOS-positive neurons have been identified in both the mucosa and neurons innervating the muscularis in humans (31). The effect of L-arginine on gallbladder emptying may also be explained by an inhibition of CCK-induced gallbladder contractions, since the NOS inhibitor L-NMMA reduces fasting gallbladder volume and augments CCK- or meal-induced gallbladder emptying in vivo in humans (15). In accordance with this, NO completely abolishes CCK-induced gallbladder contraction in vitro (31).

In conclusion, long-term oral L-arginine affects the LES by lowering the basal postprandial LESP and by suppressing the physiological late postprandial LESP increase. Furthermore, fasting and residual gallbladder volumes are increased after L-arginine ingestion. These effects of L-arginine might both be mediated through the L-arginine-NO pathway. Further studies are needed to assess the role of CCK and other gastrointestinal hormones in the L-arginine-induced changes in LES and gallbladder motility.

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