Pharmacokinetics of L-arginine during chronic administration to patients with hypercholesterolaemia

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ABSTRACT

Acute administration of L-arginine, the precursor of endothelial nitric oxide, has been shown to improve endothelial function in hypercholesterolaemic rabbits and humans. Animal studies suggest that this beneficial effect, which is thought to be related to the increased availability of nitric oxide, may not be sustained during chronic oral administration. Pharmacokinetic alterations may contribute to this observation. The present study was designed to examine the disposition of L-arginine in hypercholesterolaemic subjects during long-term administration. Plasma L-arginine concentrations were determined by HPLC in 10 patients (eight women and two men; mean age 46 ± 16 years) after an intravenous dose of 10 or 30 g and an oral dose of 5 or 7 g. Pharmacokinetic studies were performed at regular intervals (4 weeks) during a 12-week period of oral L-arginine administration (14–21 g/day). The average plasma L-arginine concentrations before (baseline) and during administration were 16.1 ± 1.2 and 22.5 ± 1.3 μg/ml respectively (P < 0.05). Plasma concentrations of L-arginine remained above baseline throughout weeks 2–12. The L-arginine exposure, expressed as a normalized area-under-the-curve for 8 h (AUC0–8) after oral or intravenous doses during the first visit, was 894.4 ± 118.7 and 1837.8 ± 157.0 units respectively. There were no significant changes in peak plasma L-arginine concentrations or in the AUC0–8 after oral and intravenous doses during subsequent visits (P > 0.05). The mean non-renal clearance of L-arginine during the four visits remained constant.

Knowledge of the pharmacokinetics of L-arginine may be useful in the design of clinical trials involving this agent, as well as in the interpretation of the pharmacodynamics of this important precursor of nitric oxide.

INTRODUCTION

The amino acid L-arginine is the only known substrate for nitric oxide synthase, the enzyme which catalyses the production of nitric oxide (NO) in vascular endothelial cells [1,2]. NO is not only a potent vasodilator but decreases platelet aggregation and adhesion, monocyte–vessel wall interaction and smooth muscle cell proliferation; all of these are important events in early atherosclerosis [3]. NO production may represent an

Key words: L-arginine, hypercholesterolaemia, pharmacokinetics.
Abbreviations: AUC, area under the curve; GH, growth hormone; NO, nitric oxide.
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important component of the endogenous defence against atherosclerosis.

There is evidence that hypercholesterolaemia is associated with an impaired availability of NO in the endothelium [4] and platelets [5]. Animal [6–9] and human [5,10–12] observations raise the possibility that chronic administration of L-arginine may preserve endothelium-dependent vasodilatation, improve the availability of NO in platelets and even limit development of atherosclerosis in hypercholesterolaemia. Other potential indications for L-arginine include congestive heart failure [13], renal disease [14], aging [15] and diabetes [16].

Interestingly, while dietary supplementation with L-arginine for 4 weeks has been shown to improve endothelial function in young hypercholesterolaemic adults [12], studies carried out in rabbits have shown an attenuation of the beneficial effects of L-arginine on endothelial function after 10 to 14 weeks of chronic oral administration [17,18], suggesting the possibility of pharmacokinetic and/or pharmacodynamic tolerance. Although one experimental observation suggests that this phenomenon could be related to the development of refractoriness or a form of tolerance to NO [19], increased clearance, decreased absorption or decreased release of endogenous L-arginine during chronic administration could also be the cause. Indeed, in rabbits given oral L-arginine, plasma arginine concentrations declined gradually with time over 14 weeks [18]. Whether this observation is relevant to humans has not been explored previously. Therefore, the present study was designed primarily to investigate the pharmacokinetics of L-arginine before and during chronic oral administration. Hypercholesterolaemic subjects, who are now regarded as a potential target population for this treatment, were enrolled in this study.

**METHODS**

**Subjects**

Ten hypercholesterolaemic subjects [eight women and two men; mean age ± S.D. 43 ± 16 (range 20–60) years] were enrolled in this protocol. All subjects had a low-density lipoprotein concentration of at least 160 mg/dl and a normal physical examination; screening blood tests (SMA-20 panel) and urinalysis were normal before the study. All individuals were non-smokers and had no previous significant medical history (in particular of cardiac, renal, hepatic, gastrointestinal, neuropsychiatric, allergic or endocrine disease). Written informed consent was obtained after full explanation of the study procedure. This study, which was carried out in accordance with the Declaration of Helsinki (1989) of the World Medical Association, was approved by the Administrative Panel on Human Subjects in Medical Research, Stanford University.

**Protocol**

**Pharmacokinetics**

No medications were permitted for at least 1 week before the beginning of the protocol and throughout the 12 weeks of L-arginine administration. Consumption of alcohol and caffeine was restricted for 24 h before and during the pharmacokinetic studies. The subjects were admitted to the Clinical Research Center on four occasions; at the time of the first dose and for additional experimental sessions during the 12-week period of oral L-arginine administration. During each admission, oral L-arginine (5 or 7 g) was given in the morning after an overnight fast. Intravenous L-arginine was given 8 h after the oral dose. The dose of intravenous L-arginine (10 or 30 g) was administered over 30 min. Meals were permitted 2 h after oral and intravenous administration. Plasma and urine samples were collected at multiple time points up to 24 h and stored at −70 °C. After the first pharmacokinetic study, the subjects were asked to take 5 or 7 g of L-arginine orally three times a day (total of 15 or 21 g in three divided doses). With an oral dose of 21 g/day, two of the initial five subjects experienced some minor gastrointestinal discomfort. As a result, the oral dose was decreased to 15 g/day in these two subjects. One patient developed flank pain caused by a calcium oxalate kidney stone during the fifth week of L-arginine treatment and decided to withdraw from the study. In a separate series of preliminary experiments in which the plasma L-arginine concentrations of healthy subjects (n = 7) ingesting a normal diet (containing 5–6 g of L-arginine) were monitored at regular intervals over 24 h, we observed that L-arginine concentrations were influenced by meals and by the time of day. Therefore, the same evaluation of endogenous L-arginine concentrations over 24 h was performed in 5 of the 10 hypercholesterolaemic subjects in the absence of administration of supplemental L-arginine.

**Hormones**

Plasma samples from the five subjects who received 30 g of L-arginine infused intravenously on all four occasions were analysed for their growth hormone (GH) concentrations. The intravenous infusion of L-arginine stimulated plasma GH release in all subjects. The data for GH stimulation from each study were expressed as pre-dose or baseline plasma GH concentrations, peak GH concentrations after the intravenous L-arginine infusion, maximal changes from pre-dose concentrations (peak concentrations after the infusion minus the pre-dose concentrations) and the area under the curve (AUC) for plots of time versus the plasma GH concentration above baseline. The parameters were calculated for each individual in the study and averaged between the five subjects. The average parameters for the four visits were subsequently com-
pared. Similarly, blood samples were obtained before each intravenous study in five subjects to measure the pre-dose plasma concentration of insulin.

Compliance
Compliance with the thrice daily dosing regimen was monitored electronically using MEMS® (Aprex Corporation, Menlo Park, CA, U.S.A.) throughout the 12-week period of the study. These devices recorded the times of the opening and closing of the L-arginine container. The stored data were downloaded and analysed at the end of the 12 weeks of L-arginine administration.

Data analysis
Exposure to L-arginine was expressed as the area-under-the-curve (AUC$_{EX}$) for plasma L-arginine concentration versus time. Plasma L-arginine concentrations were used to calculate the AUC of L-arginine produced by the oral (AUC$_{EX}$Oral) or intravenous (AUC$_{EX}$IV) administration of L-arginine for a period of 8 h after each route of administration. Plasma L-arginine concentrations over 24 h obtained from the five hypercholesterolaemic patients consuming a normal diet and without supplemental L-arginine were used to adjust the AUC$_{EX}$Oral and AUC$_{EX}$IV for basal changes in L-arginine concentrations. For the five hypercholesterolaemic subjects who were not included in this 24 h study with a normal diet, the average endogenous L-arginine plasma concentrations from all 12 control subjects studied (5 hypercholesterolaemic and 7 normal subjects) were used to adjust the AUCs. Non-renal clearance of L-arginine was calculated as Dose IV/AUC$_{EX}$IV after intravenous dosing, where Dose IV was adjusted for renal excretion in those subjects receiving an intravenous infusion of 30 g. Absolute bioavailability was estimated according to the following formula: [(AUC$_{EX}$Oral/Dose Oral)/(AUC$_{EX}$IV/Dose IV)]. Compliance data obtained from the MEMS® TrackCap® were interpreted using MEMSVIEW™ software, which provided detailed and summary measures of compliance for each subject enrolled in the 12-week study.

Statistics
Changes in pre-dose plasma L-arginine concentrations, AUC, bioavailability and clearance were examined using analysis of variance (two-tailed ANOVA) followed by Bonferroni multiple comparison correction. Statistical significance was set at $P < 0.05$. The data are expressed as means ± S.E.M. except where stated otherwise. Pre-dose plasma insulin concentrations, peak plasma GH concentrations and average compliance between each visit were also tested by ANOVA.

RESULTS
The characteristics of the subjects are shown in Table 1. No adverse effects were reported during intravenous infusion of L-arginine.

Pharmacokinetics
As previously observed in a separate 24 h study without supplemental L-arginine in healthy volunteers, a significant variation in plasma concentrations of L-arginine was detected over 24 h in hypercholesterolaemic subjects (Figure 1). Plasma concentrations of L-arginine increased after the consumption of a meal (approximately 2 h after the beginning of the study) and rose gradually during the day. Plasma L-arginine concentrations then gradually decreased, beginning 1–2 h after the evening meal (around 18.00 hours), and had returned to baseline by the next day.

Table 1 Characteristic and biochemical profiles of the 10 subjects with hypercholesterolaemia
Values are expressed as means ± S.D. LDL, low-density lipoprotein.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (M/F)</td>
<td>2/8</td>
</tr>
<tr>
<td>Age (years)</td>
<td>43 ± 16</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>74 ± 9</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>165 ± 8</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>27 ± 3</td>
</tr>
<tr>
<td>Total serum cholesterol (mg/dl)</td>
<td>248 ± 43</td>
</tr>
<tr>
<td>Plasma LDL (mg/dl)</td>
<td>179 ± 23</td>
</tr>
<tr>
<td>Serum albumin (g/dl)</td>
<td>4.1 ± 0.3</td>
</tr>
<tr>
<td>Fasting plasma glucose (mg%)</td>
<td>86 ± 8</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>121 ± 18</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>73 ± 11</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>66 ± 9</td>
</tr>
</tbody>
</table>

Figure 1 Average plasma L-arginine concentrations over 24 h in 12 subjects who did not receive supplemental L-arginine
Values are means ± S.E.M. The subjects consumed a normal hospital diet at the times indicated by the arrows.

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morning. This pattern was consistently observed in both healthy subjects and hypercholesterolaemic patients. Figures 2 and 3 illustrate the plasma concentrations of L-arginine after oral and intravenous administration of L-arginine on four occasions (before and during chronic oral administration) in a representative subject. The morning pre-dose plasma L-arginine concentrations during chronic administration were approximately 40% higher compared with the value before treatment (22.3 ± 1.3 versus 16.1 ± 1.2 µg/ml; Table 2 and Figure 4). The AUC\textsubscript{EX, oral} and AUC\textsubscript{EX, IV} (Figure 5), the average pre-dose plasma concentrations of L-arginine on visits 2, 3 and 4, the average peak plasma L-arginine concentrations after oral and intravenous administration, and the absolute bioavailability remained constant during the 12 weeks of L-arginine supplementation (Table 2). Non-renal clearance also remained unchanged (Figure 5 and Table 2). After both oral and intravenous administration, the intra-subject variability for L-arginine pharmacokinetics was low as suggested by individual S.D. observed over the four visits (Table 3).

### Hormones
The average pre-dose GH plasma concentrations, peak plasma concentrations of GH, the maximum changes from pre-dose concentrations and the AUCs above pre-

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**Table 2** Pharmacokinetic data for L-arginine from the initial and three subsequent visits  
Values are expressed as means ± S.E.M. *P < 0.05 by ANOVA. AUCs are expressed as µg × min\(^{-1}\) × ml\(^{-1}\) × g\(^{-1}\) of administered dose.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Initial visit</th>
<th>Visit 2</th>
<th>Visit 3</th>
<th>Visit 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma L-arginine concn. (µg/ml)</td>
<td>16.1 ± 1.2</td>
<td>21.0 ± 1.7*</td>
<td>23.6 ± 2.2*</td>
<td>22.7 ± 2.3*</td>
</tr>
<tr>
<td>Pre-dose</td>
<td>42.9 ± 3.4</td>
<td>42.8 ± 4.4</td>
<td>46.2 ± 4.4</td>
<td>46.5 ± 5.1</td>
</tr>
<tr>
<td>Peak after oral dose</td>
<td>466.4 ± 32.1</td>
<td>493.9 ± 50.7</td>
<td>444.2 ± 45.4</td>
<td>475.1 ± 51.4</td>
</tr>
<tr>
<td>Peak after intravenous dose</td>
<td>894 ± 119</td>
<td>830 ± 146</td>
<td>651 ± 109</td>
<td>760 ± 104</td>
</tr>
<tr>
<td>AUC after oral dose</td>
<td>1838 ± 157</td>
<td>1762 ± 126</td>
<td>1813 ± 156</td>
<td>1926 ± 189</td>
</tr>
<tr>
<td>AUC after intravenous dose</td>
<td>586 ± 50.6</td>
<td>594 ± 38.2</td>
<td>599 ± 57.5</td>
<td>564.4 ± 60.4</td>
</tr>
<tr>
<td>Bioavailability</td>
<td>0.52 ± 0.09</td>
<td>0.46 ± 0.09</td>
<td>0.37 ± 0.08</td>
<td>0.43 ± 0.07</td>
</tr>
<tr>
<td>Non-renal clearance (ml/min)</td>
<td>586 ± 50.6</td>
<td>594 ± 38.2</td>
<td>599 ± 57.5</td>
<td>564.4 ± 60.4</td>
</tr>
</tbody>
</table>
203L-Arginine pharmacokinetics in hypercholesterolaemia

Figure 5 Exposure to exogenous l-arginine expressed as the area-under-the-curve for plasma l-arginine concentration versus time (AUCEX)

The average AUCEXOral (clear bar) and AUCEXIV (patterned bar) are compared on each occasion. The AUCs have been adjusted for the administered dose. Non-renal clearance (solid bar) is also shown with values on the right-hand vertical axis.

Table 3 Pharmacokinetic data for l-arginine: individual mean values over the four visits (n = 10)

<table>
<thead>
<tr>
<th>Subject</th>
<th>Pre-dose l-arginine (µg/ml)</th>
<th>AUCoral</th>
<th>AUCEXoral</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>21.3 ± 4.4</td>
<td>886.2 ± 376.1</td>
<td>1062.4 ± 289.6</td>
</tr>
<tr>
<td>S2</td>
<td>12.1 ± 3.3</td>
<td>244.7 ± 202.7</td>
<td>1372.9 ± 252.7</td>
</tr>
<tr>
<td>S3</td>
<td>20.5 ± 3.0</td>
<td>1040.4 ± 176.6</td>
<td>1491.5 ± 185.0</td>
</tr>
<tr>
<td>S4</td>
<td>22.9 ± 6.2</td>
<td>1213.5 ± 250.4</td>
<td>1574.5 ± 423.7</td>
</tr>
<tr>
<td>S5</td>
<td>15.9 ± 1.5</td>
<td>253.6 ± 198.0</td>
<td>1452.8 ± 142.5</td>
</tr>
<tr>
<td>S6</td>
<td>25.5 ± 4.6</td>
<td>856.9 ± 108.4</td>
<td>2335.5 ± 226.3</td>
</tr>
<tr>
<td>S7</td>
<td>23.6 ± 9.3</td>
<td>695.5 ± 272.2</td>
<td>1594.7 ± 120.5</td>
</tr>
<tr>
<td>S8</td>
<td>26.3 ± 6.3</td>
<td>599.0 ± 173.7</td>
<td>2230.3 ± 244.8</td>
</tr>
<tr>
<td>S9</td>
<td>16.2 ± 2.8</td>
<td>778.7 ± 251.3</td>
<td>1966.6 ± 360.2</td>
</tr>
<tr>
<td>S10</td>
<td>22.5 ± 3.2</td>
<td>1163 ± 263.5</td>
<td>2331.3 ± 597.8</td>
</tr>
</tbody>
</table>

The capacity of intravenously administered l-arginine to stimulate GH secretion did not change during 12 weeks of oral l-arginine. Similarly, no change in average pre-dose plasma insulin concentrations was detected (Table 4). Fasting plasma glucose, plasma low-density lipoprotein, serum albumin and body weight were not affected by chronic administration.

Compliance

Compliance was in the range of 2.5 ± 0.12 doses per day, corresponding to approximately 83% of the assigned dosing. The average number of doses per day throughout the 12-week period of l-arginine administration is shown in Figure 6. To determine whether compliance changed over the 12 weeks of the study, we calculated the average number of doses per day between each visit (2.6 ± 0.6, 2.7 ± 0.1 and 2.5 ± 0.1 doses per day for the intervals between visits 1–2, 2–3 and 3–4 respectively); no difference in compliance was detected between these three intervals (P > 0.05).

Table 4 Plasma growth hormone (GH) and insulin concentrations on the initial and subsequent visits

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Initial visit</th>
<th>Visit 2</th>
<th>Visit 3</th>
<th>Visit 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma GH (ng/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-dose</td>
<td>5.3 ± 1.7</td>
<td>3.4 ± 1.0</td>
<td>3.0 ± 1.0</td>
<td>5.1 ± 1.4</td>
</tr>
<tr>
<td>Peak after intravenous dose</td>
<td>17.5 ± 4.2</td>
<td>16.1 ± 4.4</td>
<td>12.6 ± 4.3</td>
<td>14.1 ± 4.2</td>
</tr>
<tr>
<td>Peak above baseline</td>
<td>12.2 ± 4.1</td>
<td>12.7 ± 4.1</td>
<td>8.8 ± 3.6</td>
<td>9.1 ± 3.3</td>
</tr>
<tr>
<td>AUC above baseline</td>
<td>590.0 ± 326</td>
<td>570.2 ± 291</td>
<td>506.0 ± 238</td>
<td>523.0 ± 316</td>
</tr>
<tr>
<td>Pre-dose plasma insulin (µ-units/ml)</td>
<td>12.6 ± 2.2</td>
<td>9.8 ± 1.5</td>
<td>12.0 ± 1.4</td>
<td>11.6 ± 1.6</td>
</tr>
</tbody>
</table>
DISCUSSION

In the present study, 10 hypercholesterolaemic subjects were given L-arginine for 12 weeks at doses that are believed to produce beneficial effects. We found that L-arginine disposition remained unchanged throughout this period of observation. Pre-dose morning plasma L-arginine concentrations were increased throughout the 12 weeks of administration. Systemic exposure to supplemental L-arginine, expressed as AUC\textsubscript{EX,Oral} or AUC\textsubscript{EX,IV} after L-arginine administration, was unchanged during the chronic oral administration of L-arginine. Additionally, no significant changes in stimulated secretion of GH were detected after intravenous administration of L-arginine during the course of the study.

We found significant variations in plasma concentrations of L-arginine over 24 h in both healthy volunteers and hypercholesterolaemic subjects given a normal diet (5–6 g/day of L-arginine) but no exogenous supplementation of L-arginine. This spontaneous variation, which was integrated in our study to calculate the availability of L-arginine, prevented us from determining the half-life and consequently the volume of distribution of the amino acid. This baseline variation in L-arginine concentrations over 24 h is of interest and will need to be considered when interpreting pharmacokinetic data for L-arginine.

Studies in animals and humans suggest that chronic administration of oral L-arginine may offer a new therapeutic approach to lower risk in patients with hypercholesterolaemia. Most of the beneficial effects of L-arginine on the vascular system in hypercholesterolaemia seem to be related to its endothelial conversion into NO [20,21]. Whereas oral administration of L-arginine (21 g/day) during 4 weeks has been shown to improve endothelial function in hypercholesterolaemic patients [12], studies in hypercholesterolaemic rabbits suggest that tolerance to this property may occur after a 10–14 week treatment [17,18]. Interspecies variability and/or difference in treatment duration may account for these conflicting results. General mechanisms of tolerance can be classified as those involving alterations in pharmacokinetics or those related to changes in pharmacodynamic responses to drugs with time. Tolerance resulting from pharmacokinetic changes can be caused by decreased bioavailability or increased clearance of the drug with time, leading to decreased concentrations of the drug at its site of action. In the case of compounds produced endogenously, such as L-arginine, decreased production could also be a mechanism. Plasma L-arginine concentrations in rabbits have been shown to decline gradually during chronic treatment and eventually reach a level similar to that of control animals after 14 weeks [18]. In a previous study in hypercholesterolaemic patients, plasma concentrations of L-arginine rose significantly from 115 to 231 μmol/l after 4 weeks of administration and this rise was associated with an improvement in endothelial function [12]. However, in this clinical study, plasma L-arginine measurements were not obtained during the first treatment days to exclude a possible decline with time. Our results clearly demonstrate that
the increase in the plasma concentrations of L-arginine observed after intravenous or oral administration is sustained during chronic oral administration for 12 weeks with an average bioavailability of 40–50 %. These findings contrast with the gradual decrease in plasma L-arginine concentrations observed during a 14-week oral treatment in rabbits [18]. To be effective as a therapeutic agent on a long-term basis for a chronic disease such as hypercholesterolaemia, orally administered L-arginine must remain bioavailable throughout the duration of treatment. Previous findings in rabbits [18] suggest that a homoeostatic mechanism restores plasma arginine levels to control values, despite continued oral intake of excess L-arginine. Plasma L-arginine changes reflect the balance between complex interorgan processes leading to movement of the amino acid into and out of the circulation. Endogenous synthesis of arginine occurs primarily in kidney, and to a lesser extent in liver, via conversion of citrulline into arginine [14]. However, the liver does not contribute significantly to the maintenance of the plasma concentrations of L-arginine, since the amino acid synthesized in this organ is routed towards its local utilization [14].

The mean dietary intake of L-arginine in industrialized countries is 3–6 g/day [22]; 60 % of this exogenous source appears in the general circulation [14]. Isotopic studies have shown that the net rate of de novo arginine synthesis in healthy humans is not affected by an arginine-free diet lasting 6 to 7 days [23,24]. Consequently, whole-body arginine homoeostasis in healthy adults is believed to depend principally on modulation in the level of dietary arginine intake and/or on regulation of the rate of its catabolism by arginase to ornithine and glutamate [23]. As described for other basic amino acids, L-arginine is absorbed in the intestine, transported via the basolateral membrane of enterocytes to the portal blood and then rapidly taken up by the splanchnic circulation [25]. Absorption from the diet occurs through amino acid transporters in the brush border of the intestinal epithelium [26]. After a meal, when the concentrations of individual amino acids in the gut lumen increase, the carriers become saturated and simple passive diffusion may exceed carrier-mediated uptake. L-Arginine is transported across cell membranes by the common saturable transport system for basic amino acids called system Y+ [26–28]. Studies in humans have also demonstrated that total body arginine homoeostasis is related to the rate of degradation by hepatic arginase [29]. The activity of this enzyme is directly related to the concentration of arginine substrate [30]. Excess arginine intake in animals is also associated with increased urinary excretion of the amino acid [31] as the renal tubular reabsorption of arginine exhibits a transport maximum [32]. Whereas adaptive responses to increased oral intake of L-arginine (alteration in its intestinal transporter, induction of hepatic arginase or increased renal clearance) seem to play a critical role in hypercholesterolaemic rabbits, our results in humans suggest that a new set point for L-arginine homoeostasis is achieved during the exogenous intake of this amino acid.

Although animal and human studies have demonstrated that acute L-arginine infusion improves endothelial dysfunction in hypercholesterolaemia, there has been considerable debate about the persistence of this effect during chronic treatment [17,18]. The precise mechanism responsible for the vascular effects of L-arginine is believed to involve an increased bioavailability of NO not only as a substrate for NO synthase but also independently through an antioxidant effect [20,21]. Theoretically, however, the supply of L-arginine should not be rate-limiting for NO generation. Indeed, the $K_m$ of NO synthase for L-arginine is 1–2 μM whereas the plasma and the intracellular concentrations are in the order of 40–90 μM and 1 mM respectively [33]. The term ‘arginine paradox’ has been used to describe situations in which exogenous L-arginine administration seems to increase NO synthase activity even when concentrations of L-arginine are well in excess of the $K_m$ [34]. Interestingly, it has been proposed that this paradox, which is primarily observed when the effects of the amino acid are studied in vivo [5–12], is accounted for by a release of insulin. Indeed, Giugliano et al. [35] have clearly shown that the increase in plasma insulin observed after intravenous infusion of L-arginine is the factor responsible for the peripheral vasodilatation and the decrease in platelet aggregation in healthy subjects. However, these findings were obtained with high plasma concentrations of L-arginine and are probably not applicable to studies in which oral supplementation of L-arginine is used in hypercholesterolaemic patients, a transient effect on endothelial function similar to that observed in rabbits [34,36]. During chronic oral exogenous administration of L-arginine, such as in our study, plasma L-arginine concentrations only increase by two-fold, whereas in the study by Giugliano et al. [35], concentrations rose dramatically. Other theoretical scenarios whereby L-arginine administration could directly drive activity of the endothelial NO synthase include a change of the $K_m$ of NO synthase for arginine in hypercholesterolaemia or the presence of endogenous inhibitors of NO synthase which has been reported in hypercholesterolaemic rabbits [34,36]. In the present study, in which endothelial function was not evaluated, it is not possible to draw any conclusion about pharmacodynamic tolerance for NO-mediated vasodilatation. Despite sustained plasma concentrations of L-arginine during chronic oral treatment over 12 weeks in hypercholesterolaemic patients, a transient effect on endothelial function similar to that observed in rabbits remains possible. Prolonged pharmacodynamic studies exceeding 10 weeks would be worthwhile to assess whether an attenuation of the NO-mediated vasodilatation could occur.

At the concentrations observed after intravenous administration, L-arginine stimulates the release of GH
This effect is not stereospecific and has also been reported for \(\text{L-arginine}\), which is not a substrate for NO synthase \([33]\). Thus, it seems unlikely that this hormonal secretion results from an excess substrate for NO synthase. We found that the stimulation of GH secretion by intravenous \(\text{L-arginine}\) was not attenuated during the 12 weeks of treatment with oral \(\text{L-arginine}\), suggesting that no pharmacodynamic tolerance developed for this specific response. This finding supports a previous observation showing that baseline plasma concentrations of \(\text{L-arginine}\) during oral supplementation with \(\text{L-arginine}\) over 4 weeks \([12]\). As mentioned above, plasma concentrations of \(\text{L-arginine}\) during oral treatment do not affect plasma insulin concentrations. Accordingly, we found no change in average pre-dose plasma insulin over the 12 weeks of treatment with \(\text{L-arginine}\). Since insulin plasma concentrations were not measured during intravenous administration of \(\text{L-arginine}\) in our study, a pharmacodynamic tolerance to intravenous \(\text{L-arginine}\)-induced insulin release cannot be excluded. In view of the contribution of insulin release to the relaxant effect of intravenous \(\text{L-arginine}\) \([35]\), this possibility could be investigated in further pharmacodynamic studies.

In conclusion, our results demonstrate that the increase in the plasma concentrations of \(\text{L-arginine}\) observed after its intravenous or oral administration in hypercholesterolaemic patients is not altered by chronic oral administration for 12 weeks. These pharmacokinetic data may be useful in the design of clinical trials involving \(\text{L-arginine}\), as well as in the interpretation of the pharmacodynamics of this important precursor of NO.

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