

Original Article

Hypercholesterolaemia and treatment with statins do not alter L-arginine-induced changes of renal haemodynamics

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Abstract

Background. Hypercholesterolaemia has been found to impair endothelial function in the systemic and coronary circulations and lipid-lowering therapy with statins has been shown to improve this abnormality.

Methods. We examined the impact of hypercholesterolaemia on L-arginine-induced renal vascular relaxation by a cross-sectional study, and the effects of lipid-lowering therapy by a double-blind, randomized, placebo-controlled study. Using constant infusion input clearance technique (PAH and inulin respectively), changes of renal plasma flow (RPF) and glomerular filtration rate (GFR) in response to intravenous infusions of L-arginine (100 mg/kg/30 min and 500 mg/kg/30 min) were studied in 21 hypercholesterolaemic humans (age 57 ± 9 years, LDL-cholesterol 211 ± 35 mg/dl) and in 20 young healthy (age 26 ± 2 years, LDL-cholesterol 90 ± 27 mg/dl) and 20 older healthy age-matched control individuals (age 50 ± 8 years, LDL-cholesterol 106 ± 20 mg/dl). In addition, changes of blood pressure, heart rate, urinary excretion of sodium, and cyclic guanosine monophosphate were measured. Patients were analysed before and after 3 months treatment with either fluvastatin (40 mg twice daily, $n=11$) or placebo ($n=10$).

Results. In hypercholesterolaemic patients, L-arginine increased RPF and GFR ($P < 0.01$) and urinary excretion of sodium ($P < 0.05$) in a dose-dependent manner. Interestingly, changes were similar between the hypercholesterolaemic patients and the young and the age-matched control individuals (Δ RPF 100 mg/kg/30 min, 40 ± 51 ml/min vs 40 ± 52 ml/min, $P = \text{NS}$; Δ RPF 500 mg/kg/30 min, 114 ± 85 ml/min vs 130 ± 78 ml/min, $P = \text{NS}$). L-arginine significantly lowered systemic arterial pressure and increased heart rate in all groups. Despite significant reductions

in LDL-cholesterol levels (291 ± 35 mg/dl vs 213 ± 30 mg/dl, $P < 0.001$), treatment with fluvastatin did not alter the renal haemodynamic response pattern to L-arginine infusion when compared to baseline values and to those with placebo.

Conclusion. In contrast to studies performed in the vasculature of the human forearm or the coronary circulation, our results suggest that hypercholesterolaemia is not associated with an impaired L-arginine-induced renal vascular relaxation.

Keywords: hypercholesterolaemia; L-arginine; nitric oxide; renal haemodynamics

Introduction

Endothelial dysfunction is a common finding in cardiovascular disease and is known to precede the appearance of structural changes of the vasculature. The crucial role of the endothelial cell layer in the regulation of vascular tone has been attributed to the release of endothelium-derived vasoactive autacoids. Most information has been raised about nitric oxide (NO) that accounts for the biological effects of endothelium-derived relaxing factors. NO is synthesized by endothelial cells from the amino acid L-arginine through the activity of the enzyme NO-synthase, and causes vascular relaxation via stimulation of cyclic guanosine monophosphate (cGMP) production in smooth-muscle cells via soluble guanylate cyclase. In addition, NO inhibits platelet adhesion and smooth-muscle cell proliferation and thus counteracts mechanisms commonly involved in the development of atherosclerosis.

Cardiovascular risk factors such as diabetes, arterial hypertension, and hypercholesterolaemia are associated with an impaired endothelial function [1], a fact that has in part been related to interference with the NO system. In hypercholesterolaemia, several abnormalities within the NO pathway have been

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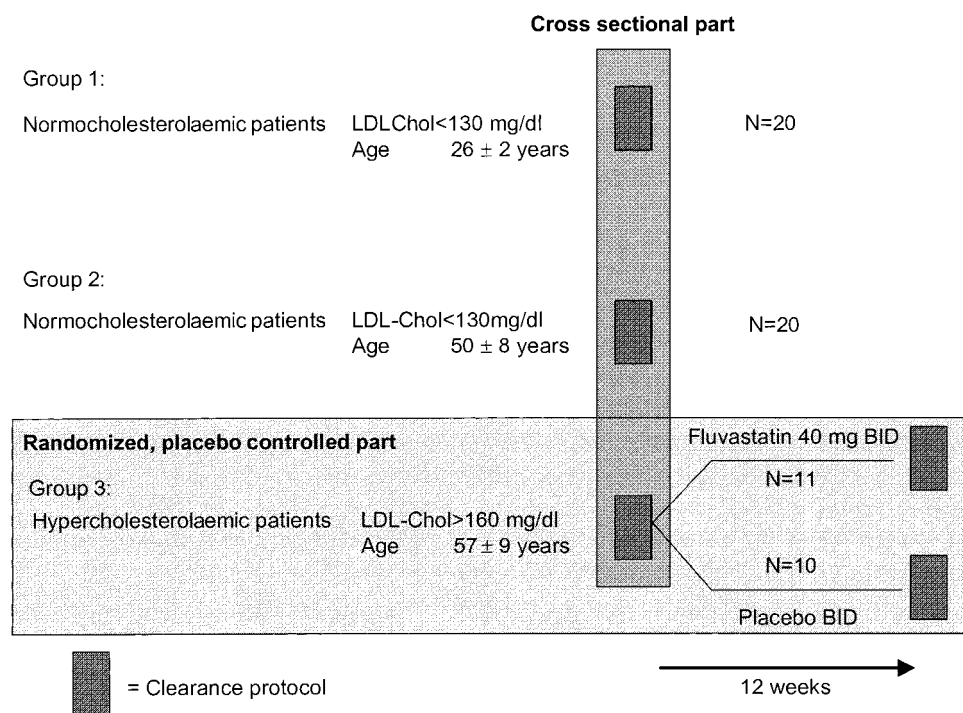


Fig. 1. Study design.

proposed to aggravate endothelial function, yet the precise mechanism remains unknown. Possible explanations include alterations in the formation, the release, and the metabolism of NO. The latter seems to play an important role, since oxidized lipoproteins (oxidative stress) have been shown to promote the break-down and thus the inactivation of NO. Furthermore, oxidized low-density lipoprotein down-regulates endothelial NO-synthase expression, an effect that can be reversed by statin treatment [2].

Many different studies have demonstrated an impaired endothelial function in hypercholesterolaemic humans by investigating the response to local infusions of endothelium-dependent vasodilators such as acetylcholine in the human forearm or the coronary circulation [3]. Further studies in the same vascular beds disclosed that cholesterol-lowering therapy as well as substrate provision with the NO precursor L-arginine could restore endothelial function [4,5].

To date, there is very little information as to whether hypercholesterolaemia impairs renal endothelial function. Lack of data is the result of the difficulties with non-invasive testing of renal endothelial function. The role of nitric oxide within the renal vasculature can be assessed either by studying changes of renal haemodynamics (renal plasma flow (RPF), glomerular filtration rate (GFR)) in response to systemic infusions of competitive inhibitors of NO-synthase such as N^G-monomethyl-L-arginine (L-NMMA), or via application of the precursor amino-acid L-arginine. Systemic infusions of NO-synthase inhibitors have been tested mainly in young healthy individuals [6,7], since intravenous administration can induce severe rises in systemic blood pressure. The use of systemic L-arginine

infusions is less dangerous and has been applied by several investigators in different clinical settings [8,9]. To our knowledge, neither the effects of systemic application of NO-synthase inhibitors nor those of systemic infusions of L-arginine on the renal vasculature have thus far been tested in hypercholesterolaemic subjects.

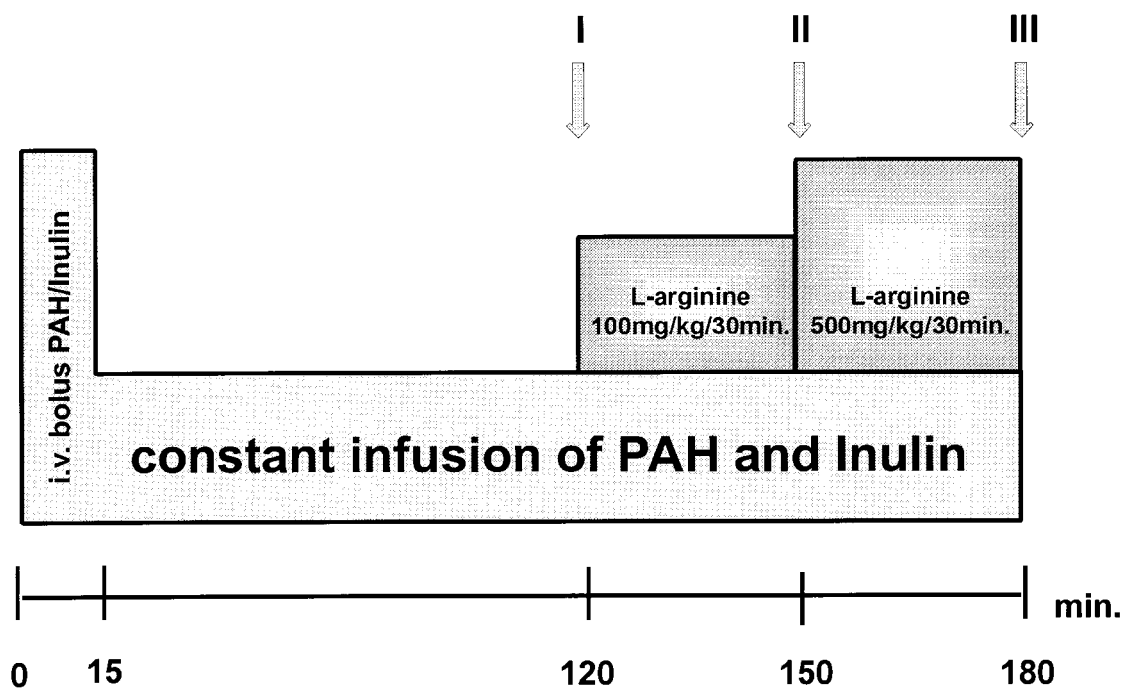
Therefore, the aim of the present study was to assess the impact of hypercholesterolaemia and cholesterol-lowering therapy on renal endothelial function by studying renal vascular relaxation in response to systemic infusions of L-arginine in hypercholesterolaemic subjects.

Subjects and methods

Study cohort

Our study population consisted of 21 hypercholesterolaemic patients (aged 57 ± 9 years, 13 men, 8 women) and 20 healthy control individuals (age 26 ± 2 years, 14 men, 6 women) and 20 matched healthy control subjects (age 50 ± 8 , 12 men, 8 women). The study design is depicted in Figure 1. All patients were initially screened by general practitioners for enrolment in this double-blind, placebo-controlled, randomized clinical trial. Young and age-matched control individuals were selected at the campus of the University of Erlangen-Nürnberg and by advertisement in a local newspaper, respectively. Both control groups served as independent control groups and normal reference values of L-arginine-induced changes of renal haemodynamic parameters.

Hypercholesterolaemic subjects were consecutively referred to our clinical research laboratory if they met the following criteria. Inclusion criteria were: age between 30 and



I-III = blood samples (determination of PAH, inulin)

I+III = urine collection (determination of fractional urinary sodium and cyclic GMP excretion), serum L-arginine levels

Fig. 2. Clearance infusion protocol.

75 years, LDL-cholesterol levels ≥ 160 mg/dl, triglycerides ≤ 350 mg/dl, plasma glucose < 110 mg/dl, and serum creatinine levels < 1.2 mg/dl. Exclusion criteria were: patients with diabetes mellitus, smokers, and patients suffering from any chronic cardiovascular, hepatic, or renal disease. Control subjects were included according to age (18–30 years and 30–75 years respectively), LDL-cholesterol levels (≤ 130 mg/dl), triglycerides (≤ 200 mg/dl), plasma glucose (< 110 mg/dl), serum creatinine levels (< 1.2 mg/dl), and absence of any cardiovascular risk factors. In all subjects casual blood pressure was measured with a standard sphygmomanometer after 5 min rest in seated position, and with the cuff-size adjusted according to the individual's arm circumference, as outlined elsewhere [10]. Subjects were considered hypertensive if averaged casual blood pressure (mean value of four consecutive measurements on two different occasions) exceeded the value of 140/90 mmHg. Control individuals had to be normotensive to participate in the study.

To ensure normal blood pressure values within this group, 24-h ambulatory blood pressure readings were additionally performed (SpaceLabs 90207, Redmont, USA).

In all participants a thorough physical examination and an extensive laboratory work-up was performed. Daily sodium intake was estimated by 24-h urine collection in both study groups. To confirm the diagnosis of hypercholesterolaemia, lipid profiles were measured before discontinuation of lipid-lowering therapy, 4 weeks after cessation of treatment, and on the day of the first clearance study (at least 4 weeks later) in which L-arginine was infused to test renal endothelial function. Fasting LDL-cholesterol levels had to be > 160 mg/dl after the wash-out period and at the time-point of the first clearance study.

The study was conducted in a double-blind, placebo-controlled, randomized fashion. Patients were randomly allocated to receive either fluvastatin 40 mg twice daily, $n=11$ (Novartis Pharma GmbH, Nürnberg, Germany) or placebo ($n=10$) indistinguishable from fluvastatin. Treatment lasted for 12 weeks after which the clearance protocol mentioned below was repeated.

L-arginine-induced haemodynamic changes in the renal circulation measured in patients with hypercholesterolaemia were compared to those obtained in the two normolipidaemic control groups, in whom a similar protocol was used. Prior to study enrolment, written informed consent was obtained from each participant and the study protocol was approved by our local Clinical, Ethical and Investigation Committee.

Clearance protocol

L-arginine-induced renal vascular relaxation was assessed as part of a clearance infusion protocol (Figure 2). To determine RPF and GFR, we applied the constant infusion input clearance technique as suggested by Cole and associates [11]. The advantage of this technique lies in the avoidance of bladder catheterization or reliance on spontaneous voiding.

Briefly, one intravenous line was inserted for infusion another one for withdrawal of blood samples and infusion of L-arginine in the contralateral arm. After a priming dose of *p*-aminohippurate sodium (PAH, Nephrotest[®], Merck Sharp & Dohme, Hertfordshire, UK) and inulin (Inutest[®], Fresenius, Graz, Austria) that was adjusted according to body weight, a constant infusion of both tracer substances was given to achieve steady-state conditions (15–20 mg/dl for inulin and 1.5–3 mg/dl for PAH) after 2 h. The resting phase

Table 1. General characteristics of the study population

	Group 1 Young control group (<i>n</i> = 20)	Group 2 Age-matched control group (<i>n</i> = 20)	Group 3 Hypercholesterolaemic subjects (<i>n</i> = 21)	<i>P</i> -value (group 1 vs 2 + 3)
Age (years)	26 ± 2	50 ± 8	57 ± 9	<0.001
Body mass index (kg/m ²)	23.0 ± 1.9	25.9 ± 3.4	26.1 ± 4.6	<0.05
Total cholesterol (mg/dl)*	162 ± 32	193 ± 31	289 ± 39	<0.001
LDL-cholesterol (mg/dl)*	90 ± 27	97 ± 23	211 ± 35	<0.001
HDL-cholesterol (mg/dl)*	53 ± 13	57 ± 21	56 ± 15	NS
Triglycerides (mg/dl)*	75 ± 24	105 ± 62	132 ± 53	<0.001
Casual systolic blood pressure (mmHg)	124 ± 7	124 ± 12	146 ± 20	<0.001
Casual diastolic blood pressure (mmHg)	76 ± 8	83 ± 5	87 ± 10	<0.05
Serum creatinine (mg/dl)	0.72 ± 0.11	0.76 ± 0.14	0.77 ± 0.18	NS
Urinary sodium excretion (mmol/day)	180 ± 78	204 ± 80	208 ± 70	NS

*Values obtained 4 weeks after discontinuation of lipid-lowering therapy.

was followed by infusion of L-arginine (L-arginine hydrochloride, Braun, Melsungen, Germany) in two different dosages: 100 mg/kg/30 min and 500 mg/kg/30 min respectively. In age-matched control individuals only L-arginine 100 mg/kg/30 min was given. We used hydrochloride solutions of L-arginine instead of free base preparations since alkaline buffers have been reported to stimulate the release of NO from cultured bovine aortic endothelial cells [12]. All L-arginine infusions were prepared immediately before intravenous administration. In order to maintain urinary flow, 0.9% physiological saline solution was co-infused with L-arginine at a constant rate of 125 ml/h. The total fluid volume infused throughout the entire clearance protocol was 1500 ml. At the end of each phase, blood samples were drawn to measure serum PAH and inulin concentrations, and all male subjects were asked to void their bladders in a recumbent position to measure the urinary excretion of sodium and cGMP.

Throughout the clearance study, blood pressure and heart rate were monitored at fixed intervals (every 5 min) by means of an oscillometric device (Dinamap, Critikon, Norderstedt, Germany). Five minutes prior to the collection of blood samples, the time interval was changed to one measurement per minute, and mean values were computed out of five consecutive measurements. All clearance protocols were performed in the morning (8 a.m.), with subjects having fasted overnight.

PAH was measured according to the method of Bratton and Marshall, as modified by Smith *et al.* [13]. Inulin was measured indirectly by converting inulin to fructose and subsequently measuring fructose by an enzymatic method (716260 Boehringer Mannheim, Mannheim, Germany). Each blood sample was measured in duplicate with a coefficient variation of <5%. Urinary cGMP was measured by radioimmunoassay.

Statistics

All statistical analysis was carried out using SPSS software package. One-way analysis of variance was used for comparison of different groups in the cross-sectional part, and the paired *t*-test for comparison of changes within each group due to treatment in the prospective randomized part. Pearson correlation coefficients were calculated when indicated. Unless otherwise specified, values are expressed as

means ± SD. A two-tailed *P* value <0.05 was considered significant.

Results

Study population (Table 1)

Baseline characteristics of the study population are given in Table 1. Mean LDL-cholesterol levels were significantly higher in hypercholesterolaemic subjects compared to control individuals (211 ± 35 mg/dl vs 90 mg/dl vs 106 ± 20 mg/dl respectively, *P* < 0.001). A history of hypercholesterolaemia was known for 88 months on average and 10 patients received previously lipid-lowering therapy for a mean duration of 50 months prior to study entry. By definition, nine hypercholesterolaemic patients were considered hypertensive whereas none in the control groups had an elevated arterial blood pressure. Five patients were currently treated with standard antihypertensive medication (ACE inhibitor, calcium-channel blocker or beta blocker) that was maintained at constant daily dose throughout the study.

Effects of L-arginine on renal haemodynamic parameters (Table 2)

Infusion of L-arginine dose-dependently increased RPF (*P* < 0.01) and GFR (*P* < 0.01) in patients with hypercholesterolaemia. In parallel, renal blood flow increased (*P* < 0.01) whereas the filtration fraction slightly decreased (*P* < 0.05). Changes of RPF and GFR in response to the lower dose of L-arginine inversely correlated with patients age (*r* = -0.49 and *r* = -0.39 respectively, *P* < 0.05), but this effect was not seen with the higher dose of L-arginine. Interestingly, apart from different baseline values, changes of renal haemodynamic parameters were similar between patients with hypercholesterolaemia and normolipidaemic control groups, irrespective whether control individuals were young or old.

Table 2. Impact of L-arginine infusions on renal haemodynamics

	Group 1 Young control group (n = 20)	Group 2 Age-matched control group (n = 20)	Group 3 Hypercholesterolaemic subjects (n = 21)	P-value (group 1 vs 2 + 3)
Baseline RPF (ml/min)	661 ± 86	453 ± 103	481 ± 100	<0.001
Δ RPF 100 mg/kg/30 min	40 ± 52**	55 ± 52**	40 ± 51**	NS
Δ RPF 500 mg/kg/30 min	130 ± 78***	not done	114 ± 85***	NS
Baseline GFR (ml/min)	129 ± 28	107 ± 19	124 ± 22	NS
Δ GFR 100 mg/kg/30 min	5.6 ± 10.3*	3.5 ± 9.2*	5.3 ± 6.7*	NS
Δ GFR 500 mg/kg/30 min	14.1 ± 12.8***	not done	13.4 ± 8.5***	NS
Baseline FF (ml/min)	19.7 ± 4.1	23.6 ± 4.2	26.4 ± 4.8	NS
Δ FF 100 mg/kg/30 min	-0.2 ± 1.7	-0.7 ± 1.9	-0.8 ± 2.1	NS
Δ FF 500 mg/kg/30 min	-1.4 ± 2.6*	not done	-2.5 ± 3.6**	NS
Baseline RBF (ml/min)	1170 ± 201	783 ± 192	831 ± 183	NS
Δ RBF 100 mg/kg/30 min	71 ± 92**	83 ± 89**	69 ± 94**	NS
Δ RBF 500 mg/kg/30 min	229 ± 137***	not done	196 ± 147***	NS

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ from baseline; RPF, renal plasma flow; GFR, glomerular filtration rate; FF, filtration fraction; RBF, renal blood flow.

To corroborate the significance of our 'negative finding' a power calculation was performed under the following terms: (i) a standard deviation of 52 ml/min for the primary objective, namely changes in RPF due to L-arginine infusion; (ii) a difference of 50 ml/min in RPF of the groups considered to be of clinical relevance, and (iii) a two-sided type -1 error of $\alpha = 0.05$, predefined in the study protocol. With a sample size of $n = 20$ in each group, the power of our study was greater than $P = 0.80$. This corresponds to the almost identical increase of RPF to L-arginine in the three groups.

Thus, in comparison to previously analysed control subjects, L-arginine-induced changes in the renal circulation were not impaired due to hypercholesterolaemia.

Effects of L-arginine on blood pressure and heart rate (Table 3)

The impact of L-arginine on blood pressure and heart rate is given in Table 3. The higher concentration of L-arginine lowered both systolic ($P < 0.01$) and diastolic ($P < 0.001$) blood pressure while heart rate increased ($P < 0.05$). Interestingly, the fall of systolic and diastolic blood pressures was greater in patients compared to young healthy control individuals ($P < 0.01$). In contrast, changes of heart rate in response to L-arginine 500 mg/kg/30 min were more marked in control individuals ($P < 0.05$).

For the low dose of L-arginine, a correlation between the rise in RPF and fall in mean arterial pressure was found in the control group ($r = -0.39$, $P < 0.05$), while this effect was yet not statistically significant in the patient group.

Effects of L-arginine on urinary excretion of sodium, cGMP, and serum L-arginine concentrations (Figure 3)

In 11 subjects of the control young group and seven subjects of the patient group urinary samples could be

obtained to determine fractional excretion of sodium and cGMP. In both groups L-arginine 500 mg/kg/30 min increased urinary sodium excretion compared to baseline values ($P < 0.05$, Figure 2). Between the two groups, changes were not statistically different (NS). Urinary cGMP only significantly increased in the control group ($P < 0.05$), whereas because of a high SD, changes in hypercholesterolaemic subjects did not reach statistical significance. Again, changes between the two groups were not statistically different (NS).

Baseline serum L-arginine concentrations were similar between control individuals and hypercholesterolaemic subjects and within the physiological range of 50–150 $\mu\text{mol/l}$ (92.5 ± 25.6 $\mu\text{mol/l}$ vs 105.3 ± 39.7 $\mu\text{mol/l}$ vs 101.3 ± 31 $\mu\text{mol/l}$, $P = \text{NS}$). Infusion of L-arginine 500 mg/kg/30 min roughly induced a 70-fold increase in serum L-arginine levels in all groups (6972 ± 1809 $\mu\text{mol/l}$ vs 7022 ± 1364 $\mu\text{mol/l}$ vs 6892 ± 1574 $\mu\text{mol/l}$, $P = \text{NS}$).

Effects of fluvastatin on the response pattern to L-arginine (Table 4)

Three months' treatment with fluvastatin significantly reduced total serum cholesterol and LDL-cholesterol concentrations compared to placebo (treated group, total cholesterol, 291 ± 35 mg/dl vs 213 ± 30 mg/dl, $P < 0.001$; LDL-cholesterol, 206 ± 33 mg/dl vs 128 ± 25 mg/dl, $P < 0.001$; placebo group, total cholesterol, 281 ± 34 vs 277 ± 33 mg/dl, $P = \text{NS}$; LDL-cholesterol, 210 ± 33 mg/dl vs 200 ± 34 mg/dl, $P = \text{NS}$).

Cholesterol-lowering therapy had no effect on baseline renal haemodynamic parameters. Moreover, despite significant improvement of cholesterol profile (-26.8% reduction in total cholesterol on average), changes in renal perfusion, GFR, and filtration fraction induced by L-arginine infusions were not statistically different between fluvastatin and placebo (Table 4). In addition, the renal haemodynamic response pattern was not different from the one found prior to pharmacological intervention (Table 4).

Table 3. Impact of L-arginine infusions on blood pressure and heart rate

	Group 1 Young control group (n=20)	Group 2 Age-matched control group (n=20)	Group 3 Hypercholesterolaemic subjects (n=21)	P-value (group 1 vs 2+3)
Baseline SBP (mmHg)	118 ± 9	119 ± 11	141 ± 22	<0.001
Δ SBP 100 mg/kg/30 min	-1.2 ± 7.1	-2.1 ± 6.3	-3.0 ± 7.1	NS
Δ SBP 500 mg/kg/30 min	-5.6 ± 6.9**	not done	-13.3 ± 8.6	<0.01
Baseline DBP (mmHg)	69 ± 5	72 ± 7	84 ± 13	<0.001
Δ DBP 100 mg/kg/30 min	-1.1 ± 3.5	1.5 ± 3.8	-2.0 ± 4.0*	NS
Δ DBP 500 mg/kg/30 min	-4.1 ± 3.4	not done	-8.8 ± 5.3	<0.01
Baseline HR (beats/min)	59 ± 7	63 ± 8	65 ± 10	NS
Δ HR 100 mg/kg/30 min	1.7 ± 4.6	0.8 ± 4.2	0.2 ± 4.1	NS
Δ HR 500 mg/kg/30 min	6.1 ± 5.9***	not done	2.5 ± 4.4*	<0.05

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ from baseline; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate.

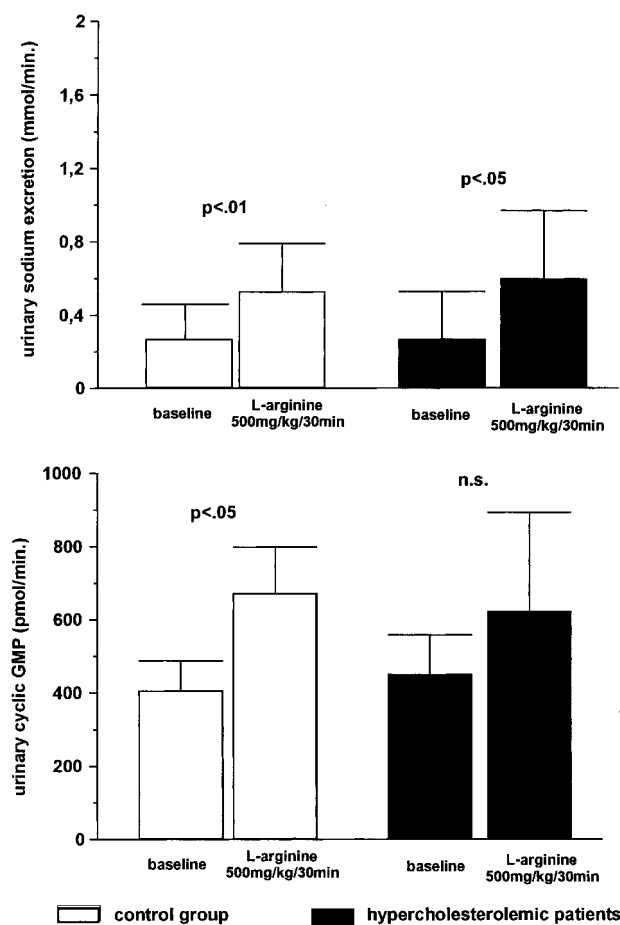


Fig. 3. Impact of L-arginine infusion (500 mg/kg/30 min) on urinary sodium excretion (means ± SD) and urinary excretion of cGMP (means ± SEM).

Discussion

In the current study, L-arginine-mediated renal vasodilatation in hypercholesterolaemic humans was not distinct from that found in a young and old control group with LDL cholesterol levels below 160 mg/dl.

L-arginine infusions dose-dependently increased RPF and GFR, lowered arterial blood pressure, and increased heart rate and fractional excretion of urinary sodium and cGMP, findings that have also previously been made by other authors [14,15]. However, the response pattern to L-arginine infusion was similar between patients with hypercholesterolaemia and control individuals and therefore, not surprisingly, remained unaffected after LDL-cholesterol-lowering therapy with fluvastatin.

Given this negative finding, special emphasis has to be put on the power of our study. No previous data are available to determine what change of renal blood flow could be considered to be of clinical relevance. With the assumption that a change in renal blood flow greater than the observed SD (52 ml/min) would be of clinical significance, and with a sample size of $n=20$ in each group, the power calculation revealed a $P > 0.80$.

Our finding of an unchanged renal haemodynamic response to L-arginine infusions is somewhat surprising, since there is an accumulating body of evidence that endothelial function is impaired in hypercholesterolaemia but is restored by cholesterol-lowering therapy. Thus, our findings are in contrast to most other studies in which L-arginine has been locally applied to assess endothelium-dependent vasodilatation in the coronary [4] or the human forearm vasculature. All these studies could demonstrate a beneficial effect of cholesterol-lowering therapy on endothelial-dependent vasodilatation, since firstly, the response to endothelium-dependent vasodilators such as acetylcholine improved, and secondly, concomitant infusions of L-arginine substantially ameliorated endothelium-dependent vasodilatation. In light of these data our results seem discordant. However, there are also reports that could not find any vasodilatory effect of L-arginine administration on the human forearm vasculature [16].

Our results are consistent with experimental data derived from an animal model. Carroll *et al.* [17] could demonstrate that cholesterol feeding did not alter renal haemodynamic responsiveness to acetylcholine and angiotensin II in rabbits, although hypercholesterolaemia has previously been found to attenuate

Table 4. Impact of therapy on L-arginine induced renal vascular relaxation

	Placebo (n = 10)		Fluvastatin (n = 11)		Placebo vs fluvastatin (P-value)	
	at baseline	after therapy	at baseline	after therapy	at baseline	after therapy
Baseline RPF (ml/min)	488 ± 87	490 ± 112	476 ± 115	464 ± 112	NS	NS
Δ RPF 100 mg/kg/30 min	32 ± 27**	33 ± 38*	46 ± 57*	33 ± 40	NS	NS
Δ RPF 500 mg/kg/30 min	116 ± 88***	111 ± 76***	112 ± 63***	90 ± 66**	NS	NS
Baseline GFR (ml/min)	124 ± 24	122 ± 19	124 ± 18	121 ± 18	NS	NS
Δ GFR 100 mg/kg/30 min	6.1 ± 5.2**	3.5 ± 4.4*	3.7 ± 7.7	1.1 ± 6.8	NS	NS
Δ GFR 500 mg/kg/30 min	14.6 ± 7.1***	8.1 ± 7.6**	9.9 ± 7.2**	7.1 ± 6.9*	NS	NS
Baseline MAP (mmHg)	103 ± 17	103 ± 17	111 ± 24	109 ± 19	NS	NS
Δ FF 100 mg/kg/30 min	-2.8 ± 7.3	-1.9 ± 7.2	-2.2 ± 6.1	-2.0 ± 6.4	NS	NS
Δ FF 500 mg/kg/30 min	-13.3 ± 6.5***	-13.5 ± 8.6**	-8.0 ± 7.5**	-13.2 ± 8.2***	NS	NS
Baseline HR (beats/min)	65 ± 8	64 ± 8	65 ± 10	65 ± 6	NS	NS
Δ RBF 100 mg/kg/30 min	1.1 ± 4.6	0.7 ± 5.3	0.6 ± 3.6	1.4 ± 2.6	NS	NS
Δ RBF 500 mg/kg/30 min	2.9 ± 4.0*	5.6 ± 7.6*	2.1 ± 4.9	2.0 ± 3.1	NS	NS

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ from baseline; RPF, renal plasma flow; GFR, glomerular filtration rate; MAP, mean arterial blood pressure; HR, heart rate.

endothelium-dependent vasodilatation in isolated thoracic aortas and cerebral vessels of this species [18]. Thus these experimental data suggest that there exist regional differences within the vasculature. Our study supports this hypothesis further. In addition, six patients of the current study had previously participated in a study in which the effects of hypercholesterolaemia and cholesterol-lowering therapy were investigated in the human forearm vasculature by use of strain-gauge plethysmography [14]. All six subjects showed a clear and blunted response to an endothelium-dependent vasodilator (acetylcholine) compared to healthy age-matched control individuals. Consistently, in a study group of over one hundred normocholesterolaemic subjects (LDL-cholesterol < 160 mg/dl) we were able to show that the response to systemic infusions of angiotensin II in the renal circulation is not determined by LDL-cholesterol levels whereas in the systemic vasculature LDL-cholesterol levels were the main determinant of vasoconstriction.

Previous studies found the use of systemic L-arginine infusions to be a reliable, suitable, and safe tool for non-invasive testing of renal endothelial function [15]. However, although having been applied by several investigators, the stereospecific, NO-related effects of systemic L-arginine infusions used to assess renal endothelium-dependent vasodilatation as part of a clearance protocol have not so far been established. Current findings are largely based on the assumption that L-arginine infusions present an adequate tool to test renal endothelial function, and that measurement of either plasma or urinary cGMP above all reflects an increased NO synthesis. We addressed this issue by performing a double-blind, cross-over pilot study prior to this project in which the renal haemodynamic properties of L-arginine and its enantiomer D-arginine (100 mg/kg/30 min and 500 mg/kg/30 min) were compared in 20 young and healthy volunteers [14]. L-arginine-induced changes of RPF and GFR were significantly greater at both dosages used than those

found with D-arginine. In addition, only L-arginine infusions lowered systemic blood pressure significantly. The hypotensive effect of L-arginine, which was also found in this study, is in agreement with findings made by other investigators [15].

We cannot rule out with certainty a bias due to systemic effects of L-arginine infusion. However, a decrease in systemic blood pressure is usually accompanied by a decrease in renal blood flow. In our study, we observed that a decrease of blood pressure was linked to an increase of RPF. This negative correlation argues against any major influence of systemic haemodynamic factors.

The observed natriuretic effect of L-arginine is also in accordance with results from other investigators and was found to be significantly greater with L-arginine than with D-arginine in our comparison of both amino acids [14]. Interestingly, in the aforementioned study, when given the higher dose of D-arginine, a significant rise in RPF and GFR was observed. This finding indicates that part of the response to L-arginine can be related to unspecific (NO-unrelated) properties due to the oncotic effect of the amino acid *per se*, a criticism that has also been shared by other authors [19]. Indeed, administration of the higher dose of each amino acid compound significantly increased serum osmolarity suggesting the presence of a concomitant volume effect [14]. This finding may also explain that a negative correlation of L-arginine-induced changes in RPF and GFR with age was only found for the lower dose of L-arginine in the present study, since for the higher dose such a correlation might have been masked due to unspecific effects of the amino acid. The presence of unspecific, NO-unrelated effects is also supported by our measurement of L-arginine serum concentrations. The higher dose of L-arginine (500 mg/kg/30 min) induced an approximately 70-fold increase in L-arginine serum levels with absolute concentrations being as high as 7 mmol/l. This is of importance, since concentrations in the range of 4–5 mmol/l have been

shown to produce non-stereospecific reductions in the tone of blood vessels *in vivo* [38]. Nevertheless, the different response pattern to L-arginine vs D-arginine suggests that a substantial portion of the observed changes of renal haemodynamic parameters, particularly if only 100 mg/kg is used, is substrate specific, and is most probably related to an increase in NO bioavailability. Due to the unspecific effects, we refrained from using the higher dose of L-arginine (500 mg/kg/30 min) in age-matched control individuals who were studied at a later time.

The measurement of urinary cGMP levels as an indicator of an increased NO formation has to be evaluated critically as well since the bioassay is not specific in terms of relation to the NO-system. Cyclic GMP may well derive from other metabolic sources such as atrial natriuretic peptide (ANP). Only recently, Sala *et al.* [20] reported a significant rise in urinary cGMP in response to acute volume loading with physiological saline solution, and this effect was accompanied by a marked increase in plasma ANP concentrations. Excess substrate provision with L-arginine at a dose used in this study also leads to extracellular volume expansion, and this may well stimulate ANP release. Accordingly, urinary cGMP reflects only a poor parameter of NO synthesis and release, although it was measured in this study.

In conclusion, patients with hypercholesterolaemia did not show a blunted renal haemodynamic response pattern to systemic infusions of L-arginine. Consistently, lipid-lowering therapy did not change the effect of L-arginine on renal perfusion.

Thus our results are in contrast to findings made in the vasculature of the human forearm or the coronary circulation. One hypothesis for this discrepancy might be that the effects of hypercholesterolaemia as well as cholesterol-lowering therapy on renal endothelial function are distinct from those found in other vascular beds.

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References

- Casino PR, Kilcoyne CM, Quyyumi AA *et al.* The role of nitric oxide in endothelium-dependent vasodilation of hypercholesterolemic patients. *Circulation* 1993; 88: 2541–2547
- Laufs U, La Fata V, Plutzky J, Liao JK. Upregulation of endothelial nitric oxide synthase by HMG CoA reductase inhibitors. *Circulation* 1998; 97: 1129–1135
- Creager MA, Cooke JP, Mendelsohn ME *et al.* Impaired vasodilation of forearm resistance vessels in hypercholesterolemic humans. *J Clin Invest* 1990; 86: 228–234
- Drexler H, Zeiher AM, Meinzer K, Just H. Correction of endothelial dysfunction in coronary microcirculation of hypercholesterolemic patients by L-arginine. *Lancet* 1991; 338: 1546–1550
- John S, Schlaich MP, Langenfeld MRW *et al.* Increased bioavailability of nitric oxide after lipid-lowering therapy in hypercholesterolemic patients: a randomized, placebo-controlled, double-blind study. *Circulation* 1998; 98: 211–216
- Bech JN, Nielsen CB, Pedersen EB. Effects of systemic NO synthesis inhibition on RPF, GFR, U_{Na} , and vasoactive hormones in healthy humans. *Am J Physiol* 1996; 39: F845–F851
- Wolzt M, Schmetterer L, Ferber W *et al.* Effect of nitric oxide synthase inhibition on renal hemodynamics in humans: reversal by L-arginine. *Am J Physiol* 1997; 272: F178–F182
- Higashi Y, Oshima T, Ozono R *et al.* Effects of L-arginine infusion on renal hemodynamics in patients with mild essential hypertension. *Hypertension* 1995; 25: 898–902
- Mimran A, Ribstein J, du Cailar G. Contrasting effect of antihypertensive treatment on the renal response to L-arginine. *Hypertension* 1995; 26: 937–941
- Schmieder RE, Langenfeld MRW, Friedrich A *et al.* Angiotensin II related to sodium excretion modulates left ventricular structure in human essential hypertension. *Circulation* 1996; 94: 1304–1309
- Cole RB, Giangiacomo J, Ingelfinger JR, Robsa AU. Measurement of renal function without urine collection. A critical evaluation of the constant-infusion-technique for determination of inulin and para-aminohippurate clearance. *N Engl J Med* 1972; 287: 1109–1114
- Mitchell JA, de Nucci G, Warner TD, Vane JR. Alkaline buffers release EDRF from bovine cultured endothelial cells. *Br J Pharmacol* 1991; 103: 1295–1302
- Smith HW, Finkelstern N, Aliminosa L *et al.* The renal clearance of substituted hippuric acid derivatives and other aromatic acids in dogs and man. *J Clin Invest* 1945; 24: 388–398
- Schlaich MP, Jocobi J, Delles C *et al.* Is L-arginine an adequate tool to assess endothelial function of the human renal vasculature. *Clin Sci* 2000; 99: 293–302
- Guigliano D, Marfella R, Verrazzo G *et al.* L-arginine for testing endothelium dependent vascular function in health and disease. *Am J Physiol* 1997; 273: 606–612
- Wennmalm A, Edlund A, Granstrom EF, Wiklund O. Acute supplementation with the nitric oxide precursor L-arginine does not improve cardiovascular performance in patients with hypercholesterolemia. *Atherosclerosis* 1995; 118: 223–231
- Carroll JF, Mizelle HL, Cockrell K *et al.* Cholesterol feeding does not alter renal hemodynamic response to acetylcholine and angiotensin II in rabbits. *Am J Physiol* 1997; 272: R940–947
- Cooke JP, Singer AH, Tsao P *et al.* Antiatherogenic effects of L-arginine in the hypercholesterolemic rabbit. *J Clin Invest* 1992; 90: 1168–1172
- MacAllister J, Calver AL, Collier J *et al.* Vascular and hormonal responses to arginine: provision of substrate for nitric oxide or non-specific effect. *Clin Sci* 1995; 89: 183–190
- Sala C, Valentini P, Tortora G *et al.* Dissociation between plasma and urinary increments of cyclic GMP during saline infusion in essential hypertensive patients. *J Hypertens* 1998; 16: S144

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