

# CORRECTION OF ENDOTHELIAL DYSFUNCTION IN CORONARY MICROCIRCULATION OF HYPERCHOLESTEROLAEMIC PATIENTS BY L-ARGININE

Hypercholesterolaemia impairs endothelial function, possibly by interference with the intracellular formation of endothelium-derived relaxing factor from its precursor L-arginine. Whether L-arginine reverses hypercholesterolaemia-induced endothelial dysfunction in the coronary circulation was thus investigated.

Epicardial artery cross-sectional area and coronary blood flow velocity were measured in 8 hypercholesterolaemic patients (mean serum cholesterol 7.8 [SE 0.3] mmol/l) and 7 age-matched controls before and after graded intracoronary infusions of the endothelium-dependent agent acetylcholine (0.036, 0.36, 3.6  $\mu$ g/min). The effect of intracoronary infusion of L-arginine (160  $\mu$ mol/min via the guiding catheter) on these measurements was then examined. In controls, acetylcholine induced a moderate dose-dependent constriction of the epicardial artery segment of the left anterior descending artery and increased coronary blood flow (by 239% [SE 57] at the highest dose). In patients with hypercholesterolaemia, the vasoconstrictive effect of acetylcholine on epicardial segments was similar to that in controls, but the increase in coronary blood flow with acetylcholine was significantly attenuated (highest dose: 61% [19],  $p < 0.02$  vs controls). L-arginine restored the acetylcholine-induced increase in blood flow in patients with hypercholesterolaemia (198% [61] vs baseline) but did not affect coronary blood flow in controls.

The findings suggest that hypercholesterolaemia impairs endothelium-dependent dilatation of the coronary microcirculation and that this impairment can be restored by short-term administration of L-arginine. The possibility that L-arginine might form the basis of treatment for coronary endothelial abnormalities induced by hypercholesterolaemia could be worth investigating.

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## [Introduction](#)

Hypercholesterolaemia impairs endothelium-dependent relaxation, an effect that occurs long before the formation of atherosclerotic lesions.( [n1-n4](#)) This impairment of endothelial function itself may have far-reaching adverse effects, by enabling abnormal reactions between the vascular wall and platelets, neutrophils, and macrophages, and these in turn may contribute to the atherogenic process.( [n5](#)) Early restoration of endothelial function may prevent the later consequences of dysfunctional endothelial cells.( [n6](#))

Suggestions as to how hypercholesterolaemia reduces endothelium-dependent relaxation include a decrease in the synthesis( [n1](#), [n7](#)) and release( [n8](#)) of endothelium-derived relaxing factor (EDRF) or an increase in inactivation of EDRF after its release from endothelial cells.( [n9-n11](#)) Nitric oxide, which accounts for the biological activity of EDRF,( [n12](#)) is derived from the metabolism of L-arginine to citrulline; thus, L-arginine represents a precursor for the synthesis of nitric oxide and EDRF.( [n13](#)) Although sufficient endogenous L-arginine might be present in normal vessels, data from experiments suggest that the availability of L-arginine is a rate-limiting factor in the production of EDRF in hypercholesterolaemic animals.( [n6](#)) The inhibitory effect of oxidised low-density lipoprotein (LDL) cholesterol (presumed to have accumulated in the vascular wall of patients with hypercholesterolaemia( [n14](#))) may be due to the ability of LDL-cholesterol to interfere with receptor-operated release of L-arginine from intracellular stores or the synthesis of the amino acid.( [n15](#)) If so, L-arginine supplements might reverse this intracellular deficit and increase the synthesis of EDRF, and hence improve endothelium-dependent relaxation--as has been shown by infusion of L-arginine to hypercholesterolaemic rabbits.( [n16-n18](#)) In man, hypercholesterolaemia produces endothelial dysfunction both in the epicardial coronary arteries and in the coronary microcirculation; this abnormality precedes angiographically visible atherosclerotic lesions in large coronary arteries.( [n3](#)) To examine the effect of L-arginine on endothelial dysfunction in hypercholesterolaemic patients, we have used acetylcholine, which causes endothelium-dependent relaxation of normal human coronary arteries but, by a direct effect on vascular smooth muscle, it exerts a paradoxical vasoconstrictor effect in the face of impaired endothelial function. We have examined the effect of acetylcholine on coronary artery dimensions and blood flow before and after administration of L-arginine.

## [Patients and methods](#)

### Patients

The subjects were patients undergoing routine diagnostic cardiac catheterisation for evaluation of chest pain. Patients with unstable angina, recent myocardial infarction, valvular heart disease, hypertension, or clinical evidence of heart failure were excluded. The study protocol was approved by the ethics committee of the University of Freiburg. The research protocol and the experimental nature of the study were explained to patients by one of the primary investigators (H. D., A. M. Z.), who also discussed the rationale and risks of the intervention. The consent form described the experimental nature of the protocol. It was made clear in the consent process that patients could withdraw from the protocol at any time. Of the 18 patients approached 15 agreed to participate in the study. Patients were classified into two groups according to whether their serum LDL-cholesterol was greater or less than 4.7 mmol/l (180 mg/dl). Blood was taken for serum lipid measurements in the fasting state 24 to 48 h before infusion of acetylcholine or L-arginine.

The control group (LDL-cholesterol 4.7 mmol/l or less) consisted of 7 patients (mean age 54.8 years [SE 2.0], range 49 to 68) without substantial coronary artery disease on coronary angiography. In 6 of these patients, angiograms of the left anterior descending coronary artery (the vessel under study) showed slight luminal irregularities. None of the controls had old myocardial infarction, diabetes mellitus, and left ventricular hypertrophy. The mean serum cholesterol was 5.6 (SE 0.2) mmol/l (range 4.8-6.1), mean LDL-cholesterol was 3.4 (0.1) mmol/l

(range 3.1-3.6), and mean high-density-lipoprotein (HDL) cholesterol was 1.4 (0.2) mmol/l (range 1.1-2.3).

The hypercholesterolaemic group (serum cholesterol > 4.7 mmol/l) consisted of 8 patients (mean age 51.5 [SE 2.0], range 45 to 58) with untreated hypercholesterolaemia and angiographically only slight luminal irregularities of the left anterior descending coronary artery (vessel under study). 6 of these patients had high-grade stenosis of the right coronary artery and had successfully undergone coronary angioplasty the day before the study. In the 8 patients mean serum cholesterol was 7.8 (SE 0.3) mmol/l (range 6.9 to 9.6), mean serum LDL-cholesterol was 5.5 (0.3) mmol/l (range 4.8 to 7.5), and mean serum HDL-cholesterol levels was 1.3 (0.1) mmol/l (range 0.8 to 1.8). None of them had a history of diabetes mellitus or raised blood glucose. 5 of these patients had a history of hypertension without evidence of left-ventricular hypertrophy and had been started on antihypertensive therapy by the primary physician. It is noteworthy that endothelial function of the coronary microcirculation is not compromised in hypertensive patients unless there is left-ventricular hypertrophy or hypercholesterolaemia. ([n20](#)) There is experimental evidence that left-ventricular hypertrophy impairs endothelial function in the coronary microcirculation in the absence of hypertension. ([n21](#))

### Study design

All medication, including all vasoactive agents, was discontinued at least 24 h before the study. After diagnostic coronary angiography or 24 h after angioplasty, an additional 10 000 units heparin were given and an 8 F guiding catheter was introduced into the left main coronary artery. A 5 F bipolar pacing catheter was placed in the right ventricle and programmed to prevent the heart rate from dropping below 40 beats/min. A 2.5 F 'Monorail' doppler catheter (Schneider, Zurich, Switzerland) with a 20 MHz pulsed doppler crystal was calibrated and then advanced into the left anterior descending artery by a 0.014 inch guide wire. The doppler catheter was positioned to obtain a stable flow velocity signal.

Saline, acetylcholine (100 mg/10 ml, Dispersa, Germering, Germany), and papaverine (25 mg/ml, Karlspharma, Karlsruhe, Germany) were infused through the lumen of the doppler catheter. L-arginine (Sigma, St Louis, Missouri, USA) and glyceryl trinitrate were infused via the guiding catheter. The agents were given in the following sequence:

- (i) baseline 1 (0.9% saline for 2 min);
- (ii) increasing of acetylcholine (0.036, 0.36, 3.6  $\mu$ g/min each for 2 min);
- (iii) baseline 2 (0.9% saline for 2 min);
- (iv) L-arginine infusion (160  $\mu$ mol/min for 20 min);
- (v) repeat graded infusions of acetylcholine (0.036, 0.36, 3.6  $\mu$ g/min each for 2 min) 8 min after start of L-arginine infusion;
- (vi) baseline 3 (0.9% saline for 2 min);

(vii) injection of 7 mg papaverine to exert maximum endothelium-independent increase in coronary blood flow;( [n18](#)) and

(viii) injection of 0.25 mg glyceryl trinitrate into the left main stem to assess the vasodilatory ability of the epicardial coronary arteries.

Fresh solutions of L-arginine were prepared by dissolving the aminoacid in 0.9% saline, which was then passed through a sterilising filter (pore size 0.22  $\mu$  m).

Throughout the study, phasic and mean intracoronary blood-flow velocity, heart rate, and aortic pressure (via the guiding catheter) were continuously measured. We have previously shown that no significant changes in either heart rate or arterial pressure occur during intracoronary infusions of acetylcholine at the doses used for the study.( [n3](#)) All drugs were infused with an infusion pump (Braun, Melsungen, Germany) set at a flow rate of 2 ml/min At the end of each infusion (and also 5 min after the start of the L-arginine infusion) biplane coronary angiograms were taken.

#### Quantitative coronary angiography

Coronary angiography was done with a simultaneous biplane multidirectional isocentric radiographic system (Siemens Bicolor, Erlangen, Germany). End-diastolic cineframes of biplane cineangiograms were videodigitised and stored in a Mipron I image analysis system (Kontron Electronics, Eching, Germany) in a 512 x 512 matrix with an 8-bit gray scale as described previously.( [n22](#))

Quantitative coronary angiography by automatic contour detection was done by a validated method that incorporates a geometric edge differentiation technique.( [n22](#)) Calculation of the exact radiological magnification factor of the measured segment was used to scale the data from pixels to millimetres as previously described.( [n23](#)) The accuracy and precision of this technique, as well as the reproducibility of serial measurements under routine clinical conditions, have been established.( [n3](#), [n22](#)) Whenever possible, measurements were obtained from both views of the biplane images with the radio-opaque tip of the doppler catheter for identification of corresponding vessel segments, and the vessels' cross-sectional area was calculated from both views, on the assumption that the cross-section was elliptical. Only single-plane analysis was done for those coronary segments showing overlap with other parts of the coronary tree in open view; in the 5 (33%) cases such vessel cross-sectional area was calculated on the assumption that the cross-section was circular. For assessment of directional changes in coronary blood flow, the mean doppler-derived blood-flow velocity (immediately before injection of contrast medium) was multiplied with the computed cross-sectional area of the vessel segment and expressed as  $\text{kHz}\cdot\text{mm}^2$ . To exclude limitations of coronary artery flow due to epicardial coronary artery constriction in response to acetylcholine, the coronary flow was calculated only when the reduction in cross-sectional area of the vessel did not exceed 50% in the most constricted part of the epicardial segment.( [n3](#))

#### Statistical analysis

All data are expressed as mean (SE). For comparisons of haemodynamics and coronary artery dimensions and blood flow, one-way analysis of variance for repeated measures was done, followed by the Student-Newman-Keuls test. Both absolute values and percentage changes from baseline were used for statistical analysis of coronary artery dimensions and blood flow and yielded similar p values. Baseline values of both groups were compared by unpaired t-test. A p value of less than 0.05 was taken as statistical significance.

## Results

### Systemic haemodynamics

Mean arterial pressure was 97 (4) mm Hg in the control group and 99 (4) mm Hg in the group with hypercholesterolaemia. Infusions of L-arginine and of acetylcholine, whether before or after L-arginine, did not affect arterial pressure in either group. Heart rate did not change significantly in either group throughout the study.

### Controls

A dose-dependent constriction of the coronary cross-sectional area occurred in response to acetylcholine in 6 of the 7 controls, the luminal area being reduced by a mean of 15% (table); the mean luminal area was 4.60 (0.49) mm<sup>2</sup> before acetylcholine and 3.79 (0.33) mm<sup>2</sup> after the second dose. 2 patients did not receive the highest dose because epicardial artery cross-sectional area decreased substantially during the second infusion. Coronary blood flow showed a dose-dependent increase with acetylcholine, from a baseline value of 48.2 (11.3) to 157 (36) kHz.mm<sup>2</sup> during infusion of the highest dose (p < 0.05 vs baseline for the second and third doses). During L-arginine infusion, coronary dimensions and blood flow did not change significantly (baseline 2/L-arginine, luminal area 4.26 [0.37]/4.29 [0.46] mm<sup>2</sup>; flow 44.1 [10.8]/43.5 [10.4] kHz mm<sup>2</sup>). L-arginine did not influence the effect of acetylcholine on cross-sectional area (table) or on coronary blood flow in controls (figure). Glyceryl trinitrate, an endothelium-independent vasodilator, substantially increased cross-sectional luminal area from 4.47 (0.44) mm<sup>2</sup> to 5.73 (0.44) mm<sup>2</sup> (mean increase 38 [9])% vs baseline). Papaverine increased blood flow by 367 (42)%.

### Hypercholesterolemic group

In all 8 patients, a dose-dependent constriction occurred in coronary artery segments distal to the site of acetylcholine infusion (table); the mean coronary cross-sectional area decreased from a baseline value of 4.38 (0.35) mm<sup>2</sup> to 3.86 (0.44) mm<sup>2</sup> during the second dose of acetylcholine (0.36 μg/min). 2 patients did not receive the highest dose of acetylcholine because their epicardial artery cross-sectional area decreased substantially during the second infusion.

Coronary blood flow was moderately increased in a dose-dependent way during the intracoronary administration of acetylcholine (p < 0.05 vs baseline for the third dose) (figure) but not to the same extent as in controls. Neither coronary blood flow nor coronary cross-sectional area measured at baseline 2 (after the first series of acetylcholine infusions) and after the administration of L-arginine differed from findings at baseline 1 (baseline 1/baseline 2/L-

arginine-luminal area 4.38 [0.35]/4.28 [0.34]/4.27 [0.43] mm<sup>2</sup>; blood flow 45.3 [7.5]/45.7 [8.0]/45.9 [8.7] kHz.mm<sup>2</sup>). L-arginine did not affect the vasoconstricting effect of acetylcholine on cross-sectional area of the left anterior descending artery (table). By contrast, L-arginine significantly increased the flow response to the second and third doses of acetylcholine (baseline to acetylcholine 3.6 mu g/ml, 45.3 [7.5] to 72.5 [17.0]; after L-arginine, baseline 2 to acetylcholine 3.6 mu g/ml 45.7 [8.0] to 125.9 [34.4] kHz.mm<sup>2</sup>)--ie, after L-arginine the maximum increase in coronary blood flow elicited by acetylcholine was restored to that seen in controls (figure). This beneficial effect of L-arginine was observed in every hypercholesterolaemic patient investigated.

The endothelium-independent dilatation of the left anterior descending coronary artery was preserved in patients with hypercholesterolaemia; glyceryl trinitrate dilated the cross-sectional area from 4.10 (0.35) mm<sup>2</sup> to 5.20 (0.45) mm<sup>2</sup>; the mean increase of 29% was not statistically different from the dilator effect in the control group. The maximum increase in coronary blood flow exerted by the endothelium-independent arteriolar vasodilator papaverine averaged 360 (70)%, which is similar to the effect of papaverine in the control group.

## Discussion

In keeping with experimental findings( [n2](#), [n24](#)) our study showed that endothelium-dependent (acetylcholine-induced) dilatation of the coronary microcirculation (as shown by blood flow measurements) is poorer in hypercholesterolaemic than in normocholesterolaemic subjects. The major finding was that L-arginine augments endothelium-dependent dilatation in hypercholesterolaemic patients but not in normocholesterolaemic controls with normal acetylcholine-induced coronary arteriolar relaxation. The moderate vasoconstrictor effect of acetylcholine on epicardial left anterior descending coronary artery was not affected by L-arginine, either in controls or in patients with hypercholesterolaemia.

The maximum acetylcholine-induced increase in coronary blood flow in the control group (239%) is similar to our previous observations in normal subjects (220%).( [n4](#)) The moderate acetylcholine-induced vasoconstriction of epicardial coronary arteries in both groups also accorded with our previous observations.( [n3](#)) Acetylcholine has been reported to induce vasoconstriction of angiographically normal parts of coronary arteries in patients with disease elsewhere in the coronary system,( [n25](#)) a finding that suggests the presence of a diffuse abnormality of endothelial function, even when the epicardial coronary arteries are only slightly affected (as in this study) or seem to be normal as judged by coronary angiography. The vasodilator effect of intracoronary glyceryl trinitrate on epicardial coronary arteries in hypercholesterolaemic patients was similar to that in control subjects, which indicates preserved endothelium-independent relaxation in response to exogenous nitric oxide.

The improvement of endothelium-dependent dilatation within the human coronary microcirculation after the administration of L-arginine accords with the hypothesis that provision of the substrate for synthesis of EDRF enhances formation of this relaxing factor and restores endothelium-dependent dilatation. This effect of exogenous L-arginine was confined to coronary resistance vessels, which do not undergo structural changes and which, as judged by their normal

endothelium-independent arteriolar dilatation response to papaverine, were not adversely affected by hypercholesterolaemia.

L-arginine did not affect the acetylcholine-induced vascular response of the epicardial left anterior descending coronary artery in patients with and without hypercholesterolaemia. Unlike the coronary microcirculation, epicardial coronary arteries do undergo atherosclerotic remodelling of the vessel wall. We found luminal irregularities on angiography in both groups, which indicated atherosclerotic alterations of the epicardial coronary artery despite the absence of luminal narrowing. Although hypercholesterolaemia selectively causes endothelial dysfunction, (n1, n3, n8) atherosclerosis further impairs endothelial function--eg, by depressing the response to bradykinin or to increased flow. (n3, n8) Thus, when atherosclerosis has occurred, the changes in endothelial function cannot be explained solely by the presence of oxidised LDL-cholesterol in the vascular wall. (n15) It may not be too surprising, therefore, that short-term administration of L-arginine did not affect the vasoconstriction of the epicardial left anterior descending artery in response to acetylcholine.

Our finding that infusion of L-arginine alone did not change coronary dimensions (data not shown) or blood flow is consistent with experimental studies showing that moderate doses of L-arginine exert neither endothelium-dependent nor stereospecific effects on rabbit aortic rings. (n26) Moreover, intra-arterial infusion of L-arginine resulting in blood concentrations of L-arginine similar to those in this study (less than 1.3 mmol/l) does not affect forearm blood flow in man. (n27) Thus, there would seem to be no shortage of L-arginine under baseline conditions.

An important point is that pretreatment with L-arginine did not potentiate acetylcholine-induced increase of coronary blood flow in controls. Similarly, agonist-induced endothelium-dependent relaxation is not enhanced by L-arginine in normal isolated aortic rings. (n28) However, in hypercholesterolaemia, the availability of L-arginine may become a limiting factor during stimulation of the release of EDRF. Indeed, the beneficial effect of L-arginine in patients with hypercholesterolaemia was most prominent during maximum stimulation with acetylcholine. Although the stereospecificity of the L-arginine infusion was not tested, since we cannot infuse D-arginine in man, our results are consistent with previous experimental findings. Girerd et al (n17) reported that exogenous L-arginine but not D-arginine restored endothelium-dependent vasodilatation of hind-limb resistance vessels in cholesterol-fed rabbits. Similarly, Cooke et al (n16, n18) reported that L-arginine stereospecifically improved the endothelium-dependent relaxation in thoracic aorta and cerebral vessels from hypercholesterolaemic rabbits. Taken together, these observations suggest that hypercholesterolaemia reversibly reduces intracellular arginine availability.

Endothelial dysfunction of the coronary microcirculation may modify the regulation of myocardial perfusion by neurohumoral agents as well as facilitate small vessel platelet aggregation, since it depresses the inhibitory effect of EDRF on platelet adhesion and aggregation. (n29) Golino et al (n30) have shown that intracoronary infusion of serotonin (to produce a concentration equivalent to that reached during platelet activation) decreased coronary blood flow in patients with coronary artery disease and endothelial dysfunction, whereas coronary blood flow increased in normal subjects. (n30) Thus, endothelial dysfunction of the coronary microcirculation is likely to predispose to intermittent myocardial ischaemia by

restricting coronary perfusion--eg, during platelet activation. In further support of the functional importance of normal endothelial function in the coronary microcirculation, we have shown that, in patients with early atherosclerosis, endothelial dysfunction of the coronary microvasculature is associated with impaired coronary blood flow regulation during cold pressor test.( [n31](#))

Since our study provides evidence that L-arginine, the precursor of EDRF, restores endothelial function of the coronary microcirculation in hypercholesterolaemic patients, L-arginine supplements could be a way of reversing the endothelial dysfunction. Conceivably, such treatment might contribute to prevention of myocardial ischaemia in these patients. Although L-arginine did not restore endothelial dysfunction of epicardial coronary arteries in our patients with angiographically visible atherosclerosis in coronary arteries, the potential of L-arginine for reversal of epicardial endothelial dysfunction at an earlier stage of the disease should not be dismissed--eg, when selective endothelial dysfunction is present in the face of angiographically smooth coronary arteries.

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#### EFFECT OF ACETYLCHOLINE AND L-ARGININE CROSS-SECTIONAL AREA OF LEFT ANTERIOR DESCENDING ARTERY

Acetylcholine dose	Hypercholesterolaemic group		Controls	
	Before L-arginine	After L-arginine	Before L-arginine	After L-arginine
0.036 (Mu)g/min	6 (4)	-6 (6)	2 (5)	-4 (6)
0.36 (Mu)g/min	-13 (6)(*)	-22 (8)(*)	-15 (6)(*)	-11 (8)
3.6 (Mu)g/min	-27 (4)	-19 (10)	-16 (17)	-19 (11)

Results (mean [SEM]) are expressed as a percentage of the baseline value before administration of acetylcholine; n=6 for the highest dose in both groups.

(\*) p <0.05 vs baseline value before administration of acetylcholine.

GRAPHS: cetylcholine-induced changes in coronary blood flow before and after L-arginine in controls (upper) and hypercholesterolaemic subjects (lower).

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