Selective Loss of Microvascular Endothelial Function in Human Hypercholesterolemia

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Background  Endothelial dysfunction is increasingly recognized as an early and important feature of vascular disease. Endothelium-dependent vasodilation is impaired in humans with hypercholesterolemia, although it is unknown whether this defect is selective for some pathways of nitric oxide production or indicates a more generalized abnormality of endothelial function. The aim of this study was to further elucidate the nature of endothelial dysfunction in human hypercholesterolemia by comparing vascular responses of agonists that use different signal transduction pathways to activate production of nitric oxide.

Methods and Results  Forearm flow was measured in 12 hypercholesterolemic patients (total cholesterol, 285±35 mg/dL [mean±SD]) aged 50±11 years and in 12 healthy subjects (total cholesterol, 173±27 mg/dL) aged 48±7 years using strain-gauge plethysmography and brachial artery drug infusions. The endothelium-dependent vasodilators used were acetylcholine (7.5, 15, and 30 µg/min), which uses a pertussis toxin-sensitive signal transduction pathway, and bradykinin (100, 200, and 400 ng/min), which uses a pertussis toxin-insensitive signal transduction pathway to activate nitric oxide production. Sodium nitroprusside (0.8, 1.6, and 3.2 µg/min) was used to test endothelium-independent vasodilation. The maximum flow in response to acetylcholine was markedly impaired in patients compared with healthy subjects (8.0±5.1 versus 17.5±7.7 mL·min⁻¹·100 mL⁻¹, P=.002). However, the maximum forearm flow in response to bradykinin was similar in the two groups (13.0±4.5 versus 16.2±5.5 mL·min⁻¹·100 mL⁻¹, P=.14), as was the maximum flow in response to sodium nitroprusside (7.0±2.8 versus 8.4±2.2 mL·min⁻¹·100 mL⁻¹, P=.13). N⁰-Monomethyl-L-arginine, an inhibitor of nitric oxide synthesis, reduced the maximum forearm vasodilation induced by bradykinin to the same extent in patients and in healthy subjects (−29±8% versus −32±6% reduction in peak flow, P=.80), with similar maximum flows in response to bradykinin (9.2±4.0 versus 10.4±2.6 mL·min⁻¹·100 mL⁻¹, P=.35).

Conclusions  Hypercholesterolemic patients are capable of normal nitric oxide bioavailability in response to bradykinin. Impairment of microvascular endothelial vasodilator function in human hypercholesterolemia is selective, and the defect occurs at the level of the acetylcholine receptor or its signal transduction pathway. (Circulation. 1994;90:35-41.)

Key Words  •  cholesterol  •  bradykinin  •  acetylcholine  •  nitric oxide  •  G proteins

Knowledge of the role of the endothelium in controlling vascular tone has expanded greatly in the past decade.¹⁻³ In response to a large number of agonists and shear stress, the endothelium releases vasodilating substances, the most important of which is nitric oxide, produced from l-arginine by the enzyme nitric oxide synthase.⁴⁻⁵ A large body of evidence now shows that endothelial dysfunction is not only associated with established atherosclerosis in humans but is also detectable when only risk factors for atherosclerosis, such as hypertension and hypercholesterolemia, are present.⁶⁻¹¹ This endothelial dysfunction is probably an early and important feature in the development of and ultimately in the clinical manifestations of vascular disease.¹² A detailed understanding of the pathophysiology of endothelial dysfunction may therefore lead to better understanding of and therapy for atherosclerosis.

In vitro studies indicate that hypercholesterolemia inhibits the production or biological activity of nitric oxide from endothelial cells of large and small arteries of animals.¹³⁻¹⁹ Recent studies in human hypercholesterolemia have shown that the endothelium-dependent vasodilator responses to acetylcholine or methacholine and to substance P are impaired in hypercholesterolemic patients⁷⁻¹⁰,¹²⁻²³ and that this impairment is at least in part mediated by a reduced activity of nitric oxide.²² However, the precise mechanism underlying this defective endothelial function remains unclear. Studies of large arteries ex vivo have shown that the impaired responses to endothelium-dependent vasodilators may be selective. For example, relaxation of arterial rings from hypercholesterolemic animals or atherosclerotic humans were found to be impaired in response to acetylcholine, 5-hydroxytryptamine, ATP, thrombin, histamine, and substance P.²⁴⁻²⁹ In contrast, the responses to other endothelium-dependent vasodilators such as bradykinin, ADP, norepinephrine, and A23187 were unaffected, as were responses to endothelium-independent vasodilators.

However, all of these studies were performed on arterial rings ex vivo, primarily from animal models of atherosclerosis, and did not assess the impact of endothelium-dependent agonists on blood flow, which primarily reflects microvascular vasomotion. Accordingly, the present study was designed to determine if impaired

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endothelium-dependent vasodilation in human hypercholesterolemia is due to a selective defect in intracellular pathways linking receptor activation to nitric oxide production or indicates a generalized abnormality in endothelial vasodilator function at the level of the microcirculation in vivo.

**Methods**

**Study Population**

**Hypercholesterolemic Patients**

Patients aged from 18 to 75 years with fasting total cholesterol levels of ≥250 mg/dL at the time of screening were eligible for study. Twelve asymptomatic hypercholesterolemic patients (five men and seven women aged 50±11 years) without known atherosclerotic cardiovascular disease were enrolled. Their lipid profile showed a total cholesterol of 286±35 mg/dL, high-density lipoprotein (HDL) cholesterol of 47±7 mg/dL, triglycerides of 148±62 mg/dL, and low-density lipoprotein (LDL) cholesterol of 209±38 mg/dL. All patients were free from hypertension, diabetes, or any other systemic disease and were not receiving any medication or hormone replacement therapy. Patients had not taken any cholesterol-lowering agents within the previous 2 months or any antioxidant vitamin supplements in the preceding 6 months. All patients had a normal physical examination, resting ECG, chest radiography, and a normal symptom-limited exercise test using the standard Bruce protocol.

**Healthy Subjects**

Twelve healthy volunteer subjects (six men and six women aged 48±7 years) were also studied. Their lipid profile showed a total plasma cholesterol of 173±27 mg/dL, HDL cholesterol of 51±12 mg/dL, triglycerides of 78±24 mg/dL, and LDL cholesterol of 108±28 mg/dL. These subjects were screened by clinical history, ECG, and blood chemistry to ensure the absence of cardiovascular or other systemic disease, and they were not taking any kind of medication, vitamin, or hormone replacement therapy.

**Protocol**

The study was approved by the National Heart, Lung, and Blood Institute Review Board, and all subjects gave written informed consent. Because bradykinin stimulates the production of vasodilating prostanooids, all study participants were administered 2 324-mg aspirin tablets daily for 7 days before and on the morning of the study to inhibit vascular prostaglandin synthesis. Alcoholic, caffeine, and smoking were prohibited for 24 hours before the study. A cannula (1/8-in, 20-gauge, Arrow) was inserted into the brachial artery of the nondominant arm. A blood sample for lipid profile was obtained. Forearm blood flow studies were performed using strain-gauge plethysmography as previously described for our laboratory. Briefly, a mercury-filled Silastic strain gauge connected to a plethysmograph (model EC4, DE Hokanson) that was connected to a chart recorder (Pharmacia LKB, Biotechnology) was calibrated to measure forearm volume changes. A rapid cuff inflator (model E10, DE Hokanson) was used to occlude venous outflow from the extremity, and a wrist cuff was inflated to 50 mm Hg above systolic pressure 1 minute before each measurement to exclude the hand circulation. Forearm blood flow was expressed as milliliters per minute per 100 mL of forearm volume. Brachial artery pressure was measured directly from the intra-arterial catheter (model 90308, Spacelabs). Forearm vascular resistance was calculated as the mean arterial pressure divided by the forearm blood flow and is expressed as units.

**An intra-arterial infusion of 5% dextrose solution was begun at 1 mL/min and continued throughout drug infusions. Basal measurements were obtained 3 minutes after starting the infusion. Forearm blood flow was then measured during intra-arterial infusions of acetylcholine chloride (Sigma Chemical) at 7.5, 15, and 30 µg/min; bradykinin (Sigma Chemical) at 100, 200, and 400 ng/min; and sodium nitroprusside at 0.8, 1.6, and 3.2 µg/min. Infusion rates (0.25, 0.5, and 1 mL/min) were identical for each drug. Each dose of drug was infused for 5 minutes, and forearm blood flow was measured in the last 2 minutes of each dose. The order of administration of these drugs was randomized, and a 30-minute rest period ensued between infusions. After an additional 30-minute rest period, an infusion of N\(^\text{6}\)-monomethyl-L-arginine (L-NMMA, Calbiochem), an inhibitor of nitric oxide synthesis, replaced the 5% dextrose infusion at a rate of 4 µmol/min (1 mL).**

**Statistical Analysis**

Differences between two mean values were compared by paired or unpaired Student's t test, as appropriate. The forearm flow response to acetylcholine, bradykinin, bradykinin and L-NMMA in the two groups were compared by ANOVA for repeated measures using a multiple linear regression model that included dummy variables to correct for between-subject variability. Because the baseline vascular resistances differed between hypercholesterolemic patients and healthy subjects, the relative forearm vascular resistance responses to these same agonists were analyzed in an identical manner. Data in the text are expressed as mean±1 SD. Error bars on the figures represent ±1 SEM. A two-tailed P value of <.05 was accepted as indicating statistical significance.

**Results**

**Vasodilator Responses to Acetylcholine, Bradykinin, and Sodium Nitroprusside**

The forearm vasodilator response to acetylcholine was significantly lower in hypercholesterolemic patients compared with healthy subjects (Fig 1), with a maximum forearm flow of 8.0±3.1 mL·min\(^{-1}\)·100 mL\(^{-1}\) in patients compared with 17.5±7.7 mL·min\(^{-1}\)·100 mL\(^{-1}\) in healthy subjects (P=.002) (Fig 2). The relative fall in forearm vascular resistance with acetylcholine from a baseline of 44.0±26.8 U for patients and 38.4±13.0 U for healthy subjects was markedly depressed in patients compared with normal subjects (Fig 1). However, the forearm vasodilator response to bradykinin was similar in both groups (Fig 3), with the maximum forearm flow of 13.0±4.5 mL·min\(^{-1}\)·100 mL\(^{-1}\) in patients versus 16.2±5.5 mL·min\(^{-1}\)·100 mL\(^{-1}\) in healthy subjects (Fig 2). The relative fall in forearm vascular resistance with bradykinin from a baseline of 52.0±30.8 U for patients and 36.3±12.9 U for healthy subjects likewise was similar for the two groups (Fig 3). The maximum flows in response to acetylcholine and bradykinin were similar for healthy subjects (17.5±7.7 versus 16.2±5.5 mL·min\(^{-1}\)·100 mL\(^{-1}\); P=.32). In contrast, the maximum flow in response to acetylcholine was significantly lower than the maximum flow in response to bradykinin in hypercholesterolemic patients (8.0±3.1 versus 13.0±4.5 mL·min\(^{-1}\)·100 mL\(^{-1}\); P=.01).

The forearm flow response to sodium nitroprusside was significantly greater for healthy subjects than for patients (Fig 4), although the maximum forearm flow was similar (7.0±2.2 mL·min\(^{-1}\)·100 mL\(^{-1}\) in patients versus 8.4±2.2 mL·min\(^{-1}\)·100 mL\(^{-1}\) in healthy subjects, P=.13). The relative fall in forearm vascular resistance with sodium nitroprusside from a baseline of
45.5±15.7 U for patients and 33.4±12.5 U for healthy subjects was similar for the two groups (Fig 4).

**Effect of Nitric Oxide Inhibition on Bradykinin Response**

L-NMMA resulted in decreases in basal forearm flow in healthy subjects (2.4±0.8 to 2.1±0.6 mL·min⁻¹·100 mL⁻¹) and in hypercholesterolemic patients (2.4±1.1 to 1.8±0.7 mL·min⁻¹·100 mL⁻¹) and in increases in vascular resistance (52.4±46.6 to 56.0±22.4 U for hypercholesterolemic patients and 38.5±9.3 to 45.9±13.3 U for healthy subjects). There was no difference between the change in basal forearm flow (P=.53) or in forearm resistance (P=.70) in response to L-NMMA between hypercholesterolemic patients and healthy subjects.

L-NMMA resulted in a similar reduction in forearm flow response to bradykinin in hypercholesterolemic patients and in healthy subjects (reduction in maximum flow, −29±8% versus −32±6%, P=.80), with the maximum forearm flow of 9.2±4.0 mL·min⁻¹·100 mL⁻¹ in patients versus 10.5±2.6 mL·min⁻¹·100 mL⁻¹ in healthy subjects (Fig 5). There were no differences in the reduction in forearm flow or the increase in vascular resistance during simultaneous bradykinin and L-NMMA infusions compared with infusion of bradykinin alone for the two groups (Fig 6).

**Discussion**

The present study confirms that forearm vascular response to the endothelium-dependent vasodilator acetylcholine is depressed in human hypercholesterolemia.\textsuperscript{19-22} We have previously shown that nitric oxide bioavailability is depressed in response to acetylcholine in hypercholesterolemic patients.\textsuperscript{22} In the present study, the forearm vascular response to bradykinin, an endothelium-dependent vasodilator that uses a different signal transduction pathway than that of acetylcholine, was similar in hypercholesterolemic patients and healthy subjects. Although the maximum forearm flows in response to acetylcholine and bradykinin were similar in healthy subjects, in hypercholesterolemic patients the maximum flow in response to bradykinin was significantly greater than the maximum flow in response to acetylcholine. The impact of L-NMMA, an antagonist of nitric oxide production, on basal forearm flow and on forearm vascular responses to bradykinin was similar in hypercholesterolemic patients and in healthy subjects.
The maximum forearm flow in response to the endothelium-independent vasodilator sodium nitroprusside was similar for healthy subjects and hypercholesterolemic patients, as was the relative decrease in forearm vascular resistance, indicating unimpaired vascular smooth muscle responsiveness in hypercholesterolemic patients. These findings indicate a selective impairment in the acetylcholine receptor–activated signal transduction pathway in hypercholesterolemic patients, with preservation of normal biological availability of nitric oxide via alternative pathways as evidenced by similar decreases in forearm flow and increases in resistance in response to bradykinin after inhibition of nitric oxide production for patients and healthy subjects.

Several groups have previously reported that the endothelium in large arteries from hypercholesterolemic animals may respond to certain endothelium-dependent vasodilator agonists. Shimokawa et al showed that the pertussis toxin–sensitive G protein–dependent signal transduction pathway linked to adenyl cyclase is impaired in hypercholesterolemia with early atherosclerosis at a time when other signal transduction mechanisms are intact. Bradykinin is an endogenous, locally acting hormone that has been demonstrated to have an endothelium-dependent vasodilator action, with the bradykinin receptor linked by a pertussis toxin–insensitive G protein–dependent signal transduction pathway to phospholipase C. In our study, we found that endothelium-dependent vasodilation to bradykinin is preserved in the microcirculation of hypercholesterolemic patients at a time when the acetylcholine response is markedly depressed. Thus, the data indicate that the depression of endothelial responsiveness in human hypercholesterolemia without clinically apparent atherosclerotic disease is selective for certain agonists and signal transduction pathways and is not generalized.

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**Figure 3.** Plots of forearm blood flow (top) and relative fall in forearm vascular resistance from baseline (bottom) in response to serial doses of bradykinin in the healthy subjects and hypercholesterolemic patients. Values represent mean and SEM.

**Figure 4.** Plots of forearm blood flow (top) and relative fall in forearm vascular resistance from baseline (bottom) in response to serial doses of sodium nitroprusside in the healthy subjects and hypercholesterolemic patients. Values represent mean and SEM.
Agonist stimulation of the endothelium results in production of endothelium-derived relaxing factors, including nitric oxide, prostacyclin, and endothelium-derived hyperpolarizing factor. One possible mechanism by which the endothelium-dependent vasodilation to bradykinin was preserved in our hypercholesterolemic patients was that the release of other endothelium-derived relaxing factors was increased to compensate for an impairment of nitric oxide production, despite administration of aspirin to inhibit prostacyclin production. However, L-NMMA increased basal resistance and inhibited the vasodilator response to bradykinin to a similar degree in patients and in healthy subjects, indicating that hypercholesterolemic patients had normal nitric oxide bioavailability in the basal state and in response to bradykinin. We have previously shown that the contribution of nitric oxide to the acetylcholine-mediated vasodilator response is depressed in hypercholesterolemic patients compared with healthy subjects. Thus, depression in nitric oxide bioavailability appears to be selective to acetylcholine and not bradykinin signal transduction pathways in hypercholesterolemic patients.

Minor et al reported increased nitrogen oxide production in atherosclerotic rabbit aorta with evidence for degradation of nitric oxide to biologically inactive compounds. Because we did not measure production of biologically active and inactive nitrogen oxide compounds in our study, we cannot exclude the possibility of increased production of nitric oxide in the hypercholesterolemic patients, balanced with its partial degradation by the action of superoxide anions. Thus, it is possible that the normal basal and bradykinin-stimulated nitric oxide bioavailability in hypercholesterolemic patients indicated by our data represents a balance between increased production and degradation of nitric oxide. Certainly, our data are incompatible with decreased basal and bradykinin-stimulated production of nitric oxide in hypercholesterolemic patients. Our study differed from that of Minor et al in that we studied a vascular bed probably free of atherosclerosis, albeit vulnerable to the effects of hypercholesterolemia, in a group of patients free of clinically apparent atherosclerosis. Accordingly, studies of vascular responses to bradykinin and L-NMMA in atherosclerotic arteries of humans (eg, the coronary circulation) and studies of the effect of inhibitors of superoxide anion production on endothelium-dependent relaxation would be of interest.

The data from this study are compatible with a selective defect of endothelium-dependent vasodilation and nitric oxide bioavailability at the level of the pertussis toxin-sensitive G protein–dependent signal transduction pathway in the microcirculation of hypercholesterolemic patients. Potential mechanisms responsible for endothelial dysfunction in hypercholesterolemia have been reviewed recently, including inhibition of nitric oxide production by oxidatively modified LDL, an effect linked to lysolecithin in the oxidized particle.
and conversion of nitric oxides to biologically inactive nitrogen oxide compounds by the action of superoxide anions. These findings give incentive to further study of how lipoproteins interfere with endothelial cell membrane signaling and provide an impetus to develop specific interventions to prevent or correct these adverse effects on endothelial vasodilator function.

References


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