Responsiveness to Bradykinin in Veins of Hypercholesterolemic Humans

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Background. Hypercholesterolemia impairs endothelium-dependent dilation in arteries. We tested the hypothesis that hypercholesterolemia impairs endothelium-dependent vasodilation by an interaction between elevated plasma lipoproteins and a presumably normal endothelium using human veins in vivo; veins do not generally develop atherosclerosis and are appropriate for testing functional alterations.

Methods and Results. Full dose-response curves were constructed in 13 hypercholesterolemic and 12 normocholesterolemic subjects by infusing bradykinin (0.25 to 508 ng/min) into hand veins preconstricted with the α-adrenergic agonist phenylephrine. The maximal relaxation induced by bradykinin was 80±38% in the controls and 103±40% in subjects with hypercholesterolemia (P=.08). Responsiveness to bradykinin was also determined after infusion of indomethacin (5.4 μg/min), a cyclooxygenase inhibitor, to block the contribution of prostaglandins; maximal responsiveness was greater in hypercholesterolemic subjects (112±41%) than in controls (81±31%) (P=.03). Hypercholesterolemic subjects were more sensitive to bradykinin, with an ED_{50} of 4.2 ng/min versus 10.9 ng/min in controls (P=.05); a similarly increased sensitivity was found in the presence of indomethacin. The response to a maximally effective dose of nitroglycerin was greater in hypercholesterolemic subjects (142±31%) versus 106±28% in controls (P=.007). In five hypercholesterolemic subjects, treated with lovastatin to normalize serum cholesterol concentrations, maximal responsiveness to bradykinin decreased from 103±52% to 80±28%.

Conclusions. These results demonstrate that hypercholesterolemia in humans does not impair endothelium-derived relaxing factor-mediated vasodilation. (Circulation. 1993;88:2754-2761.)

Key Words • hypercholesterolemia • bradykinin • vasodilation

The vascular endothelium is an important modulator of smooth muscle responsiveness, playing a critical role by releasing both vasoconstricting and vasodilating substances. Endothelium-derived relaxing factor (EDRF), a potent vasodilator, is released when the endothelium is stimulated by agonists such as acetylcholine, bradykinin, and substance P. EDRF is believed to be nitric oxide (NO). It has been repeatedly demonstrated that both animal and human atherosclerotic vessels have an impaired response to endothelium-dependent vasodilation. In atherosclerotic vessels, the increased sensitivity to vasoconstricting substances is left unopposed by the impaired action of EDRF. It has been proposed that this imbalance may predispose atherosclerotic coronary arteries to vasoconstriction.

Several studies have shown that EDRF-mediated arterial dilation is impaired in the presence of hypercholesterolemia prior to the development of any obvious pathologic modifications in the vessel wall. This has been demonstrated in hypercholesterolemic animal models as well as in arteries exposed to lipoproteins in vitro. In hypercholesterolemic humans, blunted vasodilation in response to the cholinergic agonist methacholine has been observed in vivo in forearm arterial resistance vessels, where atherosclerosis is not a common finding. These results suggest that hypercholesterolemia per se may impair EDRF action before frank atheroma formation has occurred and could therefore be involved in the development and exacerbation of disease processes associated with increased peripheral resistance. Low-density lipoproteins (LDLs) in particular have been shown to cause a decrease in EDRF-mediated vasodilation, and oxidized LDLs may be responsible for inactivation of EDRF. We tested the hypothesis that hypercholesterolemia impairs EDRF-mediated vasodilation in vivo in humans by the functional interaction between the high concentrations of lipoproteins in the plasma and the intact endothelium. We chose to investigate the responsiveness of human veins in vivo in hypercholesterolemic subjects; in this experimental system, endothelial damage and other pathologic alterations are very unlikely. Human veins do not develop atherosclerosis unless they are exposed to the same hemodynamic conditions as arteries, such as occurs in vein grafts. We examined bradykinin-mediated vasodilation in markedly hypercholesterolemic subjects using the human hand-vein compliance technique, which has the advantage of allowing construction of complete dose-response curves. In addition, we investigated the effect of lowering...
plasma cholesterol concentrations with lovastatin on venous responsiveness to bradykinin.

Methods

Subjects

Studies were performed on 25 subjects (age range, 22 to 80), 13 hypercholesterolemic and 12 normcholesterolemic, who were age and sex matched. Subjects were identified through the Stanford Blood Center volunteers who are regularly screened for plasma cholesterol concentrations. Hypercholesterolemia was defined as a total serum cholesterol level greater than 240 mg/dL and was confirmed by an overnight fasting lipid panel, performed in the Clinical Laboratory of the Veterans Affairs Medical Center, Palo Alto, Calif. Exclusion criteria included a past history of any significant disease state, use of illicit drugs, alcoholism, or chronic use of any medication, including over-the-counter drugs. The current status of health was verified by performing a complete physical examination, ECG, and routine laboratory tests (SMA-20, CBC, and urinalysis). Subjects signed a written informed consent form, approved by the Stanford Administrative Panel on Human Subjects in Medical Research (Stanford, Calif). The subjects were admitted on the morning of the study to the Geriatric Research, Education and Clinical Center at the Palo Alto VA Medical Center. All were nonsmokers except for one subject and were asked to refrain from caffeine for at least 12 hours prior to the study.

Dorsal Hand-Vein Technique

The dorsal hand-vein technique was used to quantitate responsiveness of the dorsal hand vein to bradykinin and nitroglycerin (NTG). This technique, previously modified by Aellig, has subsequently been used in our laboratory. This approach has the advantage of allowing infusion of very small amounts of vasoactive drugs, thus avoiding potentially confounding systemic hemodynamic effects. Also, basal endogenous tone is not a confounding parameter when studies are conducted in veins as opposed to arteries; at a room temperature of 74°F at which we conduct our studies, infusion of α-adrenergic antagonists does not dilate veins above baseline values. Further, complete dose-response curves can be generated by administering sequentially increasing concentrations of drugs. From the curves obtained, it is possible to derive parameters that represent the maximum effect of the drug (Emax) and the sensitivity to the drug (ED50, the dose producing half-maximal effect).

Each subject was studied in the supine position with one arm placed on a padded support with an upward angle of 30° from the horizontal to allow for complete emptying of the veins. A suitable vein was chosen on the dorsum of the hand, and a 23-gauge needle was inserted. Normal saline solution was infused for at least 30 minutes to allow for equilibration of the vein after the initial vasoconstriction due to the needle insertion. The flow rate was kept constant throughout the study at 0.27 mL/min by use of a Harvard infusion pump (Harvard Apparatus Inc, South Natick, Mass). A tripod, holding a linear variable differential transformer (LVDT, Shae-vitz Engineering, Pennsauken, NJ), was mounted on the back of the hand with the central aperture of the LVDT over the top of the vein under investigation at a distance of 10 mm downstream from the tip of the needle. Each reading was taken by measuring the size of the vein under a congestive pressure of 40 mm Hg (ie, by measuring the difference between the position of the core before and after inflation of the blood pressure cuff positioned on the same arm). The central aperture of the LVDT contains a freely movable metallic core; the LVDT, which contains three coiled wires creating an electromagnetic field, detects the movements of the metallic core when the vein enlarges after inflation of the cuff. The signal output of the LVDT is linearly proportional to the vertical movement of the core and is amplified and recorded on a strip-chart recorder. Doses of drugs are expressed as rates of infusion. Each dose was infused for a minimum of 5 minutes, which has been shown to be sufficient to reach the maximum effect at each infusion rate, after which a reading was taken. Results are presented as normalized dose-response curves, in which venodilation is plotted against the logarithm of the dose. The dilation of the vein at baseline after 30 minutes of saline infusion was defined as 100% relaxation. Phenytoinsine, a selective α-agonist, was used to preconstrict the hand vein. Phenytoinsine was infused in the dose range of 12 to 7917 ng/min; the dose of phenytoinsine that produced 50% constriction was determined. This degree of prestriction was defined as 0% dilatation. This dose of phenytoinsine was then infused at a constant rate during the subsequent administration of the vasodilating drugs, bradykinin and NTG. The vasodilation produced by these drugs was calculated as a percentage of the range between 100% and 0% vasodilation. Blood pressure and heart rate were monitored in the opposite arm with a Dinamap Blood Pressure Monitor model 845 (Critikon, Tampa, Fla).

Study Design

After an 80% preconstriction of the vein was obtained with phenytoinsine, a complete dose-response curve to bradykinin was constructed (doses ranging from 0.25 to 508 ng/min). A washout period of 45 minutes followed, during which indomethacin alone was infused locally at a rate of 5.4 μg/min to inhibit prostaglandin synthesis. In preliminary experiments, we verified that bradykinin is indeed an endothelium-dependent vasodilator in human veins; in three subjects, bradykinin gave a maximal response of 90±22, which was reduced by infusion of NG-monomethyl-L-arginine (25 μg/min) to 39±15. The residual response could be completely abolished by coinfusion of indomethacin (5.4 μg/min) together with NG-monomethyl-L-arginine. We have also previously shown that responsiveness to bradykinin is highly reproducible. The dose response curves are separated in time by a 45-minute infusion of saline. The relaxation obtained during infusion of indomethacin alone was considered as the second baseline (100% relaxation). The vein was preconstricted again to 80% of the new baseline by adding phenytoinsine to the infusion of indomethacin, and a second dose-response curve to bradykinin was constructed. Bradykinin and indomethacin were then stopped, and phenytoinsine was infused at the same constant rate for about 40 minutes. A single dose of NTG...
The two groups of subjects were very similar in age and sex distribution. They significantly differed only in total cholesterol and low-density lipoprotein serum values. Values are given as mean±SD. Ranges are given in parentheses.

### Materials

All drugs were diluted in normal saline. The following drugs were used: phenylephrine hydrochloride (1% injection) (Winthrop Laboratories, New York, NY); bradykinin (Sigma F & D Division, St Louis, Mo) used under the IND #32261; indomethacin sodium trihydrate (Merck Sharp & Dohme, West Point, Pa), and NTG (Dupont Pharmaceuticals, Manati, Puerto Rico).

### Data Analysis

Results are expressed as mean±SD. Individual dose-response curves to bradykinin were fitted to a four-parameter logistic equation using the ALLFIT program. This iterative curve-fitting program provides an estimate of the maximal response (E\text{max}) and of the dose producing half-maximal response (ED\text{50}). When desensitization to bradykinin occurred before completion of the dose-response curve, the maximal dilatation obtained was considered as E\text{max}. Desensitization occurred in six subjects in each of the two groups. A log transformation was performed on individual ED\text{50} values since log values of doses have a more normal distribution, and the geometric mean was calculated as the anti-log of the mean of log values. A Student’s paired or unpaired two-tailed t test was used as appropriate to compare the individual values for ED\text{50} (after log transformation) and E\text{max}. A value of P<.05 was considered significant. All results are given as mean±SD. A x^2 test was performed to analyze the proportion of males and females in the two groups.

### Results

Subject characteristics are shown in Table 1. The two groups were very similar in age and sex distribution. The groups differed for serum total cholesterol and LDL values (P<0.001), whereas serum high-density lipoprotein (HDL) and triglycerides (TG) were not significantly different.

Endothelium-dependent venodilatation was evaluated by construction of a full dose-response curve to bradykinin. Typical dose-response curves to bradykinin in normocholesterolemic and hypercholesterolemic subjects are shown in Figs 1A and B. The mean E\text{max} to bradykinin was 80±38% in the normocholesterolemic group and 103±40% in the hypercholesterolemic subjects (P=.08). The bradykinin dose-response curve was repeated after exposure of the vein to indomethacin. Indomethacin did not change the baseline size of the vein; in 5 subjects, 3 hypercholesterolemic and 2 normocholesterolemic, a higher dose of phenylephrine was required to constrict the vein at 80% of baseline. In the presence of indomethacin maximal response to bradykinin was 112±41% in the hypercholesterolemic group and 81±31% in the normocholesterolemic group (P=.03) (Fig 2). Hypercholesterolemic subjects were more sensitive to bradykinin, with a log ED\text{50} value of 0.62±0.68 (geometric mean, 4.17 ng/min) versus 1.04±0.46 (geometric mean, 10.90 ng/min) in normocholesterolemic individuals (P=.05) (Fig 3). The same significant difference was maintained when the ED\text{50} for bradykinin in the presence of indomethacin was determined (0.51±0.61 versus 1.33±0.62; geometric means being, respectively, 3.24 and 21.38 ng/min) (P=.002).
Indomethacin did not change sensitivity to bradykinin in the hypercholesterolemic group, but in the normocholesterolemic group, indomethacin decreased sensitivity to bradykinin ($P=.03$) (Fig 3). The analysis of the data relative to bradykinin dose-response curves before and after indomethacin with the nonparametric Wilcoxon test confirmed the previous results obtained with the four-parameter logistic equation.

The two groups included subjects across a similar age range. Bradykinin-mediated vasodilation is not affected by aging. We confirmed this observation in our study, there being no correlation between age and $E_{\text{max}}$ to bradykinin ($r^2=.07$ in hypercholesterolemia and .02 in normocholesterolemia).

Endothelium-independent, NO-mediated vasodilation was tested by challenging the preconstricted veins with a single high dose of NTG. Venodilation to NTG was greater in the hypercholesterolemic subjects: $142\pm31\%$ versus $106\pm28\%$ in controls ($P=.007$). Results of these experiments are shown in Fig 4.

We also compared bradykinin and nitroglycerin responsiveness by calculating the ratio between their respective maximal responsiveness in each of the individual subjects. The ratio in the hypercholesterolemic group was $68\pm20\%$ and in the normocholesterolemic was $72\pm27\%$ ($P=.37$), confirming the conclusion that the bradykinin response is not changed when corrected for the response to NTG. We also performed a linear regression of the maximal bradykinin response versus NTG response and found a positive correlation with $r=.73$ and $P<.05$ for the hypercholesterolemic and with $r=.56$ and $P=.05$ for the normocholesterolemic; this
suggests that there may be some common underlying explanation for the enhanced responses in hypercholesterolemic subjects, such as cGMP-mediated effects. The vessels did not show a different sensitivity to the \( \alpha \)-adrenergic vasoconstrictor phenylephrine. Mean log \( ED_{50} \) values for phenylephrine (doses constricting the vessel to 80% of its baseline size) were 2.55±0.57 (geometric mean, 355 ng/min) in hypercholesterolemic versus 2.57±0.34 (geometric mean, 380 ng/min) in normocholesterolemic subjects (\( P = .46 \)).

Characteristics of the five hypercholesterolemic subjects who underwent treatment with lovastatin are shown in Table 2. Lovastatin significantly decreased total cholesterol and LDL, without changing concentrations of TG and HDL. The decrease in cholesterol level was 20%, as typically occurs after 1 month of treatment.\(^{24} \) \( ED_{50} \) to bradykinin decreased from 105±52% to 80±28% after treatment with lovastatin (\( P = .21 \); \( ED_{50} \) in the presence of indomethacin decreased from 130±46% to 85±28% (\( P = .10 \)).

**Discussion**

We tested the hypothesis that hypercholesterolemia impairs endothelium-dependent vasodilation in response to bradykinin in human veins in vivo. Veins do not develop atherosclerosis unless they are exposed to the same hemodynamic stress and oxygen tension as arteries, such as occurs in vein grafts.\(^{16,25} \) Therefore, the venous bed is appropriate for testing functional alterations caused by the interaction between elevated plasma lipoproteins and a normal endothelium. Surprisingly, we found that bradykinin-mediated venodilation not only was not impaired by hypercholesterolemia but was even greater than in normocholesterolemic controls.

The endothelium has an obligatory role in mediating relaxation to bradykinin and to other substances such as acetylcholine, histamine, ATP, and substance P.\(^{2} \) The
vasodilating substance released by the endothelium is termed EDRF and was identified as NO⁵ and is synthesized from L-arginine by endothelial NO-synthase.⁶,⁷ EDRF stimulates synthesis of cGMP in vascular smooth muscle leading to dilation of the vessel.⁸ In human veins, bradykinin-mediated vasodilation is greatly, but not completely, inhibited by both NO²-monomethyl-L-arginine,⁹ a specific inhibitor of NO-synthase,¹⁰ and methylene blue,¹¹ an inhibitor of soluble guanylyl cyclase. A component of bradykinin-mediated vasodilation is thought to be due to the release of prostaglandins.⁵¹ For this reason, each subject in the study had a second dose-response curve to bradykinin generated in the presence of indomethacin, a cyclooxygenase inhibitor, to investigate the component of the response that was due exclusively to EDRF release. The dose of indomethacin used, 5.4 μg/min, results in an estimated final concentration of 2.4 μg/mL in the vein, based on an average blood flow of 2.5 mL/min, typically found in dorsal hand veins (unpublished observations). This concentration (7·10⁻⁸ mol/L) is similar to that reported in the literature to be effective for blocking cyclooxygenase.⁶,⁷ Indomethacin caused a modest but significant twofold rightward shift of the bradykinin dose-response curve in normocholesterolemic subjects, suggesting that prostaglandin release contributes partially to bradykinin-mediated vasodilation in humans. Interestingly, indomethacin did not significantly change the response to bradykinin in hypercholesterolemic subjects; instead, there was a trend toward a greater E₅₀ and lower ED₃₀ of bradykinin after indomethacin infusion. This suggests that in the presence of hypercholesterolemia, production of dilating prostaglandins may be diminished after stimulation with bradykinin; it is also possible that vasoconstrictor prostaglandins are preferentially released. Human arteries have a reduced synthesis of PGH₂ in atherosclerosis.³² A similar abnormality has been shown in the aorta after a high-cholesterol diet for 4 weeks in rabbits.³³ In porcine coronary arteries with moderate atherosclerosis the endothelium-dependent relaxation to serotonin and ADP is enhanced after exposure of the vessels to indomethacin, probably due to an endoperoxide intermediate of the cyclooxygenase pathway.³¹ The synthesis of vasoconstricting endoperoxides, such as thromboxane B₂, is increased in aortas from cholesterol-fed rabbits.³⁴,³⁵ Atherosclerosis impairs EDRF-mediated relaxation because of the loss of endothelium and possibly because of impaired diffusion of EDRF through the thickened intima.⁴-⁶ Recent-onset, mild hypercholesterolemia impairs EDRF-mediated vasodilation in coronary arteries from swine fed for only 9 weeks with a high-cholesterol diet⁶⁰ and in rabbit abdominal aortas.¹¹ In humans with elevated LDL and angiographically normal coronary arteries, acetylcholine infused into the coronary vessels produces a modest coronary constriction compared with the vasodilation produced in normal subjects.³⁶ In hypercholesterolemic subjects, forearm arteries have a smaller increase in blood flow, as measured by venous occlusion plethysmography, after infusion of methacholine.¹² It is usually assumed that certain arterial beds, such as resistance vessels¹² and coronary microcirculation vessels,³⁷ do not develop atherosclerosis. However, there is some disagreement on the definition of absence of lesions; using more sophisticated techniques, such as transmission electron microscopy, alteration in the intima and in the elastic of vessels can be found after only 2 weeks of hypercholesterolemia.³⁸ A number of possible mechanisms may be involved in the blunted efficacy of endothelium-dependent vasodilators to relax arterial smooth muscle. EDRF (NO) is synthesized from L-arginine.⁶,⁷ Administration of L-arginine restored acetylcholine-mediated vasodilation in vivo in hindlimbs of hypercholesterolemic rabbits,³⁹ suggesting a reduction of synthesis and release of EDRF. However, in another study, EDRF, bioassayed after stimulation by acetylcholine, was not decreased even if vasodilation was impaired.⁴⁰ Indeed, it has been suggested that NO production was markedly increased after acetylcholine challenge in thoracic aortas from hypercholesterolemic rabbits, even though relaxation was diminished.⁴¹ The authors hypothesized that the receptor-mediated signal-transduction pathway that leads to the activation of the NO synthase is preserved. A subsequent step in the pathway leading to vasodilation is impaired, possibly leading to upregulated release of EDRF because of decreased response of the smooth muscle.⁴¹ Atherosclerotic lesions are rich in ox-LDLs,⁴² and ox-LDLs may directly inactivate EDRF.¹³,³¹ Furthermore, superoxide anions and hyperoxia inactivate EDRF.⁴³,⁴⁴

Our results demonstrate that hypercholesterolemia does not affect endothelium-dependent vasodilation in veins, in contrast to what has been reported in arteries. Given the low oxygen tension in veins, the oxidative damage and depletion of certain chemical groups associated with lipoprotein metabolism and oxidation and the release of free radicals by the macrophages may be lacking. EDRF is believed to be a nitrosylate compound in which NO is incorporated, most likely a nitrosothiol molecule.⁴⁵,⁴⁶ If thiol groups are needed for synthesis of EDRF, the oxidative damage associated with macrophage activation during accumulation of lipids⁷ could deplete cells of reduced sulfhydryl groups and impair incorporation of NO in the more potent nitrosylate molecule.

Other factors could explain the difference between veins and arteries; different endothelium-derived dilating factors have been identified, among which is endothelium-dependent hyperpolarizing factor (EDHF).¹ The relation between EDRF and EDHF is not clear yet, but arteries may lose endothelium-dependent mechanism of dilation earlier than veins.

The increased response to NTG in subjects with hypercholesterolemia is an unexpected and interesting finding. Since these subjects were also more sensitive to bradykinin, the results raise the hypothesis that there may be enhanced sensitivity to cGMP-dependent vasodilation in veins of hypercholesterolemic humans. However, to address the biochemical mechanism of the enhanced responsiveness to NTG, it would be necessary to measure directly the different components of the vasodilating cascade in isolated blood vessels.

While in some studies response to bradykinin in hypercholesterolemia is affected,⁵ there are reports of conserved response to bradykinin³¹ and to A23187⁷⁴ in hypercholesterolemic porcine coronary arteries. In studies conducted in humans in vivo,¹²,³⁶ acetylcholine...
has been used to test endothelium-dependent vasodilation. Acetylcholine acts as a vasoconstrictor on smooth muscle cells and a vasodilator through promoting release of EDRF, and a minimal impairment of endothelium function could therefore be unmasked by the prevailing vasoconstriction.

The results with lowering serum cholesterol with lovastatin support the overall conclusions of this study. We observed a clear trend, although not statistically significant at the P<.05 level, toward a decreased responsiveness to bradykinin after plasma cholesterol concentrations were decreased with lovastatin.

Our study demonstrates that hypercholesterolemia by itself is not sufficient to cause impaired EDRF-dependent vasodilation in blood vessels. Further investigation of the difference in interaction of lipoproteins with venous and arterial blood vessels will be important to determine the actual mechanism by which hypercholesterolemia affects endothelial function.

Acknowledgments
This work was supported by National Institutes of Health grant AG-05627. E.B. was supported by the Stanford Medical Scholars Program. W.E.H. was supported by a grant from the Swiss National Research Foundation. We gratefully acknowledge the technical assistance from the nurses and the Staff of the Geriatric Research Education and Clinical Center of the VA Hospital, Palo Alto. We thank the staff of the Stanford Blood Center for their help in recruiting volunteers. We thank Dr B. Efron for assistance with the statistical analysis.

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_Circulation_. 1993;88:2754-2761
doi: 10.1161/01.CIR.88.6.2754
_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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