Enhanced Constriction of the Peripheral Large Artery in Response to Acute Induction of a Low-Flow State in Human Hypercholesterolemia

Vincenzo Filitti, Philippe Giral, Alain Simon, Isabelle Merli, Muriel Del Pino, and Jaime Levenson

The diameter, blood velocity, and blood flow of the brachial artery were evaluated with a pulsed-Doppler apparatus before and after wrist occlusion in 16 normocholesterolemic and 27 hypercholesterolemic male subjects of similar age and body mass index. Before occlusion, no hemodynamic differences were observed between the two groups. Occlusion significantly reduced blood velocity and blood flow in the two groups (p<0.001), but such reductions were not different between hypercholesterolemic and normocholesterolemic groups. Occlusion decreased the arterial diameter in the hypercholesterolemic group only (p<0.001), and absolute diameter changes after occlusion were significantly different between the two groups (p<0.001). No correlation was found between the change in arterial diameter after occlusion and the baseline diameter before occlusion in the normocholesterolemic and hypercholesterolemic population overall. Absolute and percent diameter changes after occlusion were correlated with total cholesterol (r=-0.73, r=-0.72; p<0.001) and with low density lipoprotein (LDL) cholesterol (r=-0.68, r=-0.69; p<0.001) in the normocholesterolemic and hypercholesterolemic population overall, respectively. These findings indicate that the low-flow state induces a reduction in large-artery diameter in the hypercholesterolemic but not in the normocholesterolemic state and is closely related to the degree of elevation of blood cholesterol and of its LDL fraction. (Arteriosclerosis and Thrombosis 1991;11:161-166)

Hypercholesterolemia is recognized as a major cardiovascular risk factor, but the determination of its dominant hemodynamic changes associated with early lesion development remains unclear. Recent observations of aortas isolated from animals fed cholesterol-rich diets have shown several alterations in vascular reactivity, such as an augmented contractile responsiveness to certain vasoactive substances. These alterations were reversible with a switch to cholesterol-poor diets, and it has been speculated that changes in endothelial function might play an important role in their mechanisms. The objective of our study was to investigate in vivo the influence of hypercholesterolemia on large-artery reactivity in humans. The brachial artery was chosen as a model of the peripheral large artery, and its reactivity was tested in response to an acute reduction in blood flow because it has been recently shown that flow variations are capable of changing the arterial caliber, possibly via endothelium-mediated vasomotion. To this end, brachial artery diameter, blood velocity, and blood flow were determined with a pulsed-Doppler apparatus before and during mechanical occlusion of the wrist in 16 normocholesterolemic and 27 hypercholesterolemic subjects of similar age and body mass index.

Methods

Patients

Twenty-seven hypercholesterolemic male subjects, preliminarily selected at a work site by a group of occupational health physicians ("Prévention Cardiovasculaire en Médecine du Travail" [PCVMETRA]) and 16 normocholesterolemic, healthy, male controls of similar age and body mass index (weight/height²) were included in the study after they had given their informed consent for the procedure (Table 1). The

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lipid measurements were performed in the biochemistry laboratory of the hospital after a 14-hour fast (Table 1). Serum total cholesterol, triglycerides, and high density lipoprotein (HDL) cholesterol (after precipitation of low density [LDL] and very low density [VLDL] lipoproteins by phosphotungstic acid) were measured by an enzymatic method, and LDL cholesterol (mmol/l) was calculated according to the classical formula

\[
LDL\text{ cholesterol} = (\text{Total cholesterol} - \text{HDL cholesterol} - \text{triglycerides})/2.2
\]

Cholesterol measurement enabled us to classify the population into a normocholesterolemic group (16 controls with cholesterol <5.2 mmol/l) and a hypercholesterolemic group (27 subjects, including seven subjects with borderline-high cholesterol from 5.20 to 6.19 mmol/l and 20 subjects with high cholesterol ≥6.2 mmol/l). Triglycerides and HDL cholesterol were measured in 11 of the 16 normocholesterolemic subjects and in the 27 hypercholesterolemic subjects. All subjects had fasting plasma triglyceride levels below 3 mmol/l. Primary hypercholesterolemia was confirmed by exclusion of diseases or factors causing secondary hypercholesterolemia. None of the subjects was taking lipid-lowering drugs for at least 6 weeks before the study, and none had hypertension, coronary heart disease, or clinical evidence of raised atherosclerotic lesions of the abdominal aorta, of the cervical arteries, or of the lower-limb arteries. This latter point was confirmed by Doppler echographic examination of carotid, abdominal aortic, and femoral arterial sites.

### Hemodynamic Measurements

The subjects were hospitalized for 1 day and were referred to the hemodynamic laboratory to allow us to perform the noninvasive arterial measurements in the morning. The study was performed with the patients in a supine position in a warm, quiet room with a controlled temperature of 20±1°C. The right arm was supported at the midthoracic level. After 10 minutes of rest, systemic blood pressure was determined in the left arm with a semi-automatic device. The brachial artery circulation was studied by means of a bidimensional pulsed-Doppler velocimeter (Echovar Doppler pulsé, Montreuil, France) with a frequency of 8 MHz pulsed at 15 kHz and with two important features that have been previously described in detail: 1) a double-transducer probe permitting adjustment of the incident angle of the ultrasonic beam at 60±1° to the arterial axis and 2) a range-gated time system of reception enabling us to focus the sample volume of Doppler signals at a length of 0.04 cm and to advance it in 0.04-cm steps across the artery; this technique yielded a velocity profile within the artery. Advance of the sample volume was synchronized with the electrocardiogram, so that the step advance was started by the QRS complex over every cardiac pulse. Thus, the number of pairs of velocity peaks of the profile (N) permitted us to calculate the brachial artery diameter (D) as N × 0.04 × 0.866, 0.866 being the sine of 60°, the angle at which the beam crossed the arterial axis. Mean blood velocity (V_m) was determined by increasing the sample volume to the value of D and by superimposing the former on the lumen of the artery. The mean blood flow (Q) was deduced according to the formula

\[
Q = (\pi D^2/4) \times V_m \times 60
\]

Measurements derived from the pulsed-Doppler Echovar have been validated in vitro and in vivo: D was expressed in centimeters, V_m in centimeters per second, and Q in milliliters per minute. The variability of measurements was 7±2% for D and 5±2% for V_m.

### Acute induction of the low-flow state in the brachial artery by wrist occlusion

Blood flow through the brachial artery was acutely decreased with an occluding cuff placed around the wrist. By inflating the cuff to suprasystolic levels (200 mm Hg), the distal circulation to the hand was arrested and flow was reduced. Pulsed-Doppler measurements of brachial artery D, V_m, and Q were performed after 5 minutes of wrist occlusion. At the same time, systemic blood pressure was measured in the left arm during wrist occlusion.

### Statistical analysis

Group data were expressed as mean±SEM. Parameters and their changes after occlusion were compared between the two groups by
Results

Table 2 shows no significant differences in blood pressure, heart rate, brachial artery D, \( V_m \), and Q before and after wrist occlusion between normocholesterolemic and hypercholesterolemic groups, but arterial D was, on average, 0.011 cm larger in the hypercholesterolemic than in the control group before occlusion, whereas on average, D became 0.023 cm smaller in the hypercholesterolemic than in the control group after occlusion.

The results of our study indicate that the effect of hypercholesterolemia on peripheral large artery reactivity is relatively homogeneous. Selection was performed at the work site by cholesterol screening of employees, and diagnosis was based on total cholesterol elevation without a concomitant triglyceride elevation above 3 mmol/l. The brachial artery was chosen as a model of the peripheral large artery. Indeed, it is more accessible to our noninvasive pulsed-Doppler investigation than are other arteries, such as the femoral or popliteal arteries where atherosclerosis occurs more frequently than in the brachial artery. However, we found in a previous report that brachial artery compliance was reduced in patients with arteritis of the lower limbs, which is persuasive evidence of an effect of atherosclerosis on brachial arterial vessels. Arterial investigation was based on measurements made with a pulsed-Doppler device, as previously described and validated. It provides precise and quantitative measurements of D, \( V_m \), and Q of superficial large arteries. In addition to baseline hemodynamic measurements, we tested the response of the brachial artery caliber to acute induction of a low-flow state by arresting the distal circulation by means of mechanical occlusion of the wrist. It has recently been shown in animals that large arteries demonstrate vasoactivity in response to variations in Q. Thus, the reduction in brachial artery Q by exclusion of a part of the forearm and whole-hand circulation induces a reduction in brachial artery D in normal humans. This reduction in lumen D was consecutive to the distal circulatory arrest causing the local low-flow stimulus responsible for brachial artery vasoconstriction via possible endothelium-independent mechanisms. Moreover, several experimental studies in animals have pointed to hypercholesterolemic subjects, but no similar correlations were found between D change and either HDL cholesterol or triglycerides.

Discussion

The objective of the present study was to investigate the hemodynamic status of a peripheral large artery in primary uncomplicated human hypercholesterolemia. Any data in the literature on this disease have not yet concerned this topic. The group of hypercholesterolemic subjects included in this study is relatively homogeneous. Selection was performed at the work site by cholesterol screening of employees, and diagnosis was based on total cholesterol elevation without a concomitant triglyceride elevation above 3 mmol/l. The brachial artery was chosen as a model of the peripheral large artery. Indeed, it is more accessible to our noninvasive pulsed-Doppler investigation than are other arteries, such as the femoral or popliteal arteries where atherosclerosis occurs more frequently than in the brachial artery. However, we found in a previous report that brachial artery compliance was reduced in patients with arteritis of the lower limbs, which is persuasive evidence of an effect of atherosclerosis on brachial arterial vessels. Arterial investigation was based on measurements made with a pulsed-Doppler device, as previously described and validated. It provides precise and quantitative measurements of D, \( V_m \), and Q of superficial large arteries. In addition to baseline hemodynamic measurements, we tested the response of the brachial artery caliber to acute induction of a low-flow state by arresting the distal circulation by means of mechanical occlusion of the wrist. It has recently been shown in animals that large arteries demonstrate vasoactivity in response to variations in Q. Thus, the reduction in brachial artery Q by exclusion of a part of the forearm and whole-hand circulation induces a reduction in brachial artery D in normal humans. This reduction in lumen D was consecutive to the distal circulatory arrest causing the local low-flow stimulus responsible for brachial artery vasoconstriction via possible endothelium-independent mechanisms. Moreover, several experimental studies in animals have pointed to

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normocholesterolemic group (n=16)</th>
<th>Hypercholesterolemic group (n=27)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>130±2</td>
<td>129±2</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>78±1</td>
<td>78±1</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>65±2</td>
<td>65±2</td>
</tr>
<tr>
<td>Brachial artery diameter (cm)</td>
<td>0.437±0.014</td>
<td>0.434±0.014</td>
</tr>
<tr>
<td>Brachial artery blood velocity (cm/sec)</td>
<td>5.91±0.46</td>
<td>2.67±0.17*</td>
</tr>
<tr>
<td>Brachial artery blood flow (ml/min)</td>
<td>53±5</td>
<td>24±2*</td>
</tr>
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Values are mean±SEM. *p<0.001 when comparing variable before vs. after occlusion. †p<0.001 when comparing hypercholesterolemic vs. normocholesterolemic group.
the existence of alterations of large-artery reactivity induced by hypercholesterolemia.\textsuperscript{18,19}

The two dominant findings of our work were, first, the lack of difference in brachial artery hemodynamics in the basal state, and second, the different response of the brachial artery D to wrist occlusion between normocholesterolemic and hypercholesterolemic subjects. The absence of difference in brachial artery hemodynamics between the two groups before occlusion is undeniable for blood pressure, pulse rate, \( V_m \), and Q. The lumen D was 0.011 cm larger in the hypercholesterolemic than in the control group, but this difference was not statistically significant. These data suggest that blood cholesterol level did not influence the arterial circulation of the upper limb in basal conditions. These data are difficult to compare with other reports in the literature because only a few studies of the hemodynamic effects of hypercholesterolemia have been performed. They concerned animal experiments and arteries severely affected by atherosclerosis.\textsuperscript{18,19} This is not the case in the present study because no subject presented evidence of atherosclerosis of coronary or extracoronary arteries. However, these experimental animal studies did not show any significant changes in the lumen size of conducting large arteries of the limbs,\textsuperscript{17,18} which is in agreement with our finding on brachial artery D. The different responses of brachial artery D to wrist occlusion observed between normocholesterolemic and hypercholesterolemic groups constitutes a very intriguing finding and a major point of discussion. This difference was due to an unchanged artery D in normocholesterolemic controls contrasting with a significantly decreased D in the hypercholesterolemic group. A first possible limitation of this result is relevant to the technical accuracy of the Doppler device for measuring brachial artery D. It depends on the precision of the location of the proximal and distal arterial walls, with a sample volume that has a 0.04-cm size and is displaced by 0.04-cm gradations.\textsuperscript{9} This problem was previously tested in vitro in our laboratory by correlating the actual diameter of calibrated tubes and their calculated apparent echo-Doppler D.\textsuperscript{9} The intercept of this correlation gives a quantitative idea of the error of measurement of D, which was 0.035 cm, that is, 7\% of the brachial artery D.\textsuperscript{9} This error is only one percentage point below the percent D change after occlusion in the hypercholesterolemic group. However, the correlation obtained from calibrated tubes shows that the error of D measurement is systematic and represents the overestimation of D due to the sample volume size. When comparing the effects of occlusion on arterial D in the same patient, the systematic error is mathematically eliminated and does not affect the statistical evaluation of the difference in Ds before and during occlusion.

Thus, the fact that hypercholesterolemic subjects exhibit vasoconstriction of the brachial artery during wrist occlusion compared with normocholesterolemic controls seems relevant, and its interpretation raises several alternative hypotheses. An observation preliminary to discussion is that the larger decrease in the artery D in the hypercholesterolemia group is associated with a proportionally larger decrease in Q. While the \( V_m \) decreased equally (55\%) in the two groups, Q decreased 55\% in the control group in contrast to 63\% in the hypercholesterolemia group. This larger decrease in Q in hypercholesterolemic patients fits very well mathematically with the 9\% reduction in inner artery D of these patients compared with the lack of D change in the control group. The question is open whether the larger decrease in arterial D in the hypercholesterolemia group is a primary decrease, and hence, the decrease in Q is secondary, or whether the decrease in D is secondary to the decrease in Q. A first possibility for explaining a decrease in D is that the mechanical exclusion of the hand circulatory bed from the brachial circulation may change the systemic cardiovascular reflexes.
This is unlikely since distal circulatory arrest in the hand did not modify blood pressure and heart rate in the two groups as previously reported. Another reason could be the different baseline lumen diameters before occlusion between the two groups. The slightly larger lumen D observed in the hypercholesterolemic group compared with controls before occlusion represents approximately 30% of the reduction in D that was recorded in this group. It does not seem responsible for the higher D reduction in the hypercholesterolemic than in the control group since no correlation was found in the overall population between baseline artery D before occlusion and D change after occlusion.

Therefore, it could also be that the larger decrease in arterial D in hypercholesterolemic subjects could be secondary to a larger decrease in Q. It has been reported that the tone of large arteries responds to changes in Q in experimental animals and in humans. A first remark is that the Q reduction in normocholesterolemic subjects did not induce a change in lumen D, in agreement with previous reports in normotensive and hypertensive subjects submitted to the same wrist occlusion. It may be that reduction in Q during wrist occlusion in the normocholesterolemic group was not sufficient to produce a flow-mediated decrease in D. It has been shown that when brachial artery Q is more strongly reduced in normal humans by excluding the hand circulation and the major part of the circulatory bed, brachial artery D has been significantly decreased. It ensues that the D decrease observed in the hypercholesterolemic group could be due to the larger reduction in Q induced by wrist occlusion than in the normocholesterolemic group. The reason for this larger Q reduction is unclear. It might be due to a higher resting hand Q in the hypercholesterolemic group. However, no data actually exist in this study about Q measurements in the hand. It is also possible that the relatively slight difference (5 ml/min) in Q reduction between the two groups cannot entirely explain the lumen D decrease in the hypercholesterolemic group. The possibility remains that the decreased artery caliber after wrist occlusion in hypercholesterolemic patients was due to an increased reactivity of the artery tone to the low-flow state. The mechanisms of possible increased large-artery reactivity in the hypercholesterolemic group cannot be elucidated from the present work. On the basis of the considerable arguments for the role of the endothelium in flow-mediated vasodilation, it can be speculated that the endothelium may participate in the low-flow–dependent arterial vasoconstriction. The impairment of the flow-mediated vasoreactivity in large arteries, especially endothelium-dependent relaxation, has been demonstrated for the isolated aorta of the hypercholesterolemic rabbit. Whatever its precise mechanism, the large-artery lumen D decrease observed in the hypercholesterolemic group seems to be dependent on the blood cholesterol level as shown by the correlation between brachial artery D change and cholesterol level. Moreover, the existence of a similar correlation between arterial D change and LDL cholesterol level suggests that this fraction of serum lipids probably has a greater influence on large-artery reactivity than other fractions such as HDL cholesterol or triglycerides. Furthermore, analysis of Figure 2 indicates that the cholesterol change related to brachial artery D seems to exist more clearly in hypercholesterolemic than in normocholesterolemic subjects, suggesting that brachial vasoconstriction mainly relates to a high blood cholesterol state.

In conclusion, this study provides an original contribution in the important field of arterial reactivity of...
hypercholesterolemic subjects. Further investigations concerning arteries more exposed to atherosclerosis, such as carotid and femoral arteries, are required to analyze the influence of high blood cholesterol on the functional response of large arteries in the presence of the atherosclerotic process of arterial walls.

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References


Key Words: • large-artery diameter • blood velocity • pulsed Doppler flow meter • blood flow • hypercholesterolemia • flow-mediated vasomotion
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