Impaired Brachial Artery Endothelial Function Is Not Predicted by Elevated Triglycerides
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OBJECTIVES
The purpose of this study was to determine if patients with modest hyperlipidemia, and no other risk factors for coronary artery disease (CAD), have impaired endothelium-dependent (ED) vasoactivity.

BACKGROUND
Hypercholesterolemia impairs ED vasodilation, but the impact of elevated triglycerides on endothelial function is not as well established.

METHODS
High-resolution ultrasound was used to determine flow-mediated dilation (FMD) in the brachial artery (BA) after a 5-min arterial occlusion (endothelium-dependent stimulus) and nitroglycerin-induced dilation (endothelium-independent stimulus). We studied 40 healthy controls (Group 1), 38 patients with elevated low-density lipoprotein (LDL) cholesterol (Group 2) and 35 patients with elevated triglycerides (Group 3). Patients were excluded if they had known CAD or other risk factors for CAD, or if they were receiving lipid-lowering or vasoactive medications.

RESULTS
Control patients (Group 1) had normal LDL cholesterol (2.6 ± 0.8 mmol/liter) and triglyceride levels (1.0 ± 0.5 mmol/liter) compared with Group 2 (5.2 ± 1.2 mmol/liter, 3.8 ± 0.6 mmol/liter) and Group 3 (3.5 ± 0.9 mmol/liter, 4.2 ± 2.5 mmol/liter) subjects (p < 0.001). Baseline BA diameters were the same across the three groups. There was no significant attenuation of flow-mediated vasodilation (FMD) in either of the hyperlipidemic groups (Group 1: 10.9 ± 5.0% vs. Group 2: 8.6 ± 6.1% vs. Group 3: 9.4 ± 3.9%; p = 0.14). However, nitroglycerin-induced vasodilation was mildly reduced (Group 1: 21.0 ± 5.0% vs. 16.9 ± 7.6% vs. 17.3 ± 7.7%; p = 0.01). By multivariate analysis, after controlling for baseline diameters, only the ratio of LDL/high-density lipoprotein predicted a minor impairment in FMD.

CONCLUSIONS
In patients free from other cardiac risk factors, modest elevations of triglycerides or LDL cholesterol do not significantly attenuate BA endothelial-dependent vasodilation. Synergism with other cardiac risk factors may be required to significantly impair endothelial function in these patients. (J Am Coll Cardiol 1999;33:2038–43) © 1999 by the American College of Cardiology

Endothelial dysfunction represents one of the earliest events in the process of atherosclerosis. The endothelial cell is important in maintaining vascular integrity through a variety of mechanisms (1). These protective methods include vasodilation (2), as well as prevention of platelet aggregation (3) and smooth muscle proliferation (4). Endothelium-dependent vasodilation is mediated through the release of vasodilators such as nitric oxide (NO) (5). In response to physiologic stimuli, such as shear stress from flowing blood, the endothelium releases NO, which causes smooth muscle cell relaxation via an increased concentration of cGMP (6). Impaired activity of NO is associated with atherosclerosis and the subsequent development of vascular disease (7).

Impaired endothelium-dependent vasodilation has been associated with increased cholesterol (8,9), but much less is known about the role of other lipid subfractions. Hypertriglyceridemia is probably an independent risk factor for coronary artery disease (CAD) (10). Furthermore, elevated levels of triglycerides are important in modifying the effects of other lipid subfractions (11,12). Recent data suggest that low-density lipoprotein (LDL) cholesterol itself is not as injurious as oxidized LDL in producing impaired endothelial function (13). This effect is mediated through a number of mechanisms, including impaired synthesis and release of NO, as well as increased oxidative degradation of NO (14–16). Elevated triglycerides predict the presence of small dense LDL particles, which are more susceptible to oxidation, and are particularly prone to produce endothelial dysfunction (17,18). Thus, we hypothesized that patients with elevated triglycerides may have impaired endothelial function.
The purpose of this study was to examine brachial artery flow-mediated vasodilation (FMD) in patients with modest hyperlipidemia and without confounding risk factors for CAD, in order to determine the effect of triglycerides on endothelial function.

METHODS

Patients. Patients were prospectively assigned to one of three groups: Group 1, normal fasting lipid profile (n = 40), which was defined as an LDL cholesterol <3.4 mmol/liter, and a triglyceride level <2.2 mmol/liter; Group 2, elevated LDL cholesterol (n = 38) which was defined as LDL cholesterol >4.3 mmol/liter, with a triglyceride level <2.2 mmol/liter (n = 38); and Group 3, elevated triglycerides (n = 35), which was defined as triglycerides >2.2 mmol/liter with an LDL cholesterol <4.3 mmol/liter (n = 35). Groups 2 and 3 were combined to form a hyperlipidemic group to compare with controls. The study patients were all recruited from the University of Calgary Lipid Clinic. Group 1, which formed the control group, consisted of volunteers and staff. Exclusion criteria for all groups included any other risk factor for CAD, such as: hypertension, postmenopausal status, smoking, diabetes mellitus, family history of CAD, prior history of CAD, peripheral vascular disease, stroke or family history of a lipid abnormality in greater than two first-degree relatives. Patients and controls were also excluded if they were on any vasoactive or lipid-lowering medications.

Study protocol. Written, informed consent was obtained from all patients in accordance with the guidelines established by the Committee for the Protection of Human Subjects at our institution. A 7.5-MHz linear phased array ultrasound transducer (Hewlett-Packard, Andover, Massachusetts) was used to image the dominant arm brachial artery longitudinally just above the antecubital fossa. After an overnight fast, and measurement of a lipid profile, all patients rested for 10 to 15 min in a quiet room at room temperature. After a baseline image was obtained, a blood pressure cuff was inflated to 200 mm Hg on the proximal portion of the arm for 5 min, and then released. The increased flow in the artery after removal of the blood pressure cuff is termed reactive hyperemia and results in FMD (19). Images were obtained for the first 2 min after cuff deflation. This FMD was used as a measure of endothelium-dependent vasodilation (20–22). The brachial artery was then allowed to return to normal (5 min), and repeat baseline images were obtained. Then, 0.3 mg of sublingual nitroglycerin was administered, and the brachial artery was imaged for the ensuing 4 min. The response to nitroglycerin is a measure of endothelium-independent vasodilation (23). Blood pressure and heart rate were recorded during each stage of the investigation. Pulsed-wave Doppler was used to determine the flow velocity integral at each intervention. We have previously used this technique to detect impaired endothelial function in patients with known CAD (24).

Analysis. Images were recorded on VHS videotape. Three sequential systolic frames (taken at the end of the T wave on the electrocardiogram [ECG]) for each intervention (baseline, reactive hyperemia, repeat baseline and nitroglycerin) were digitized and saved to a computer. Previous studies have shown that maximal arterial dilatation occurs 1 min after cuff deflation, and 3 min after administration of nitroglycerin (25). Arterial diameter was determined over a 1-cm straight segment by locally developed software. The average diameter from each of the three frames was used to calculate the end point of interest, which was the percent diameter change of the brachial artery in response to reactive hyperemia or nitroglycerin. In our laboratory, the intraobserver and interobserver variability for repeated measurements are $0 \pm 0.02$ and $0.03 \pm 0.11$ mm, respectively. When reactive hyperemia studies are performed on two separate days, the mean difference in brachial vasodilator response in absolute terms is $3.1 \pm 2.9\%$.

Flow. Brachial artery blood flow was calculated as:

$$\text{Flow (ml/min)} = \text{velocity } \times \text{HR} \times \left(\frac{\text{diameter}^2}{4}\right) \times \pi \times 0.006$$

Statistics. Differences between clinical characteristics, and brachial artery vasodilator responses, were evaluated and analyzed by unpaired $t$ tests for two-group comparisons and one-way analysis of variance with a Bonferroni correction for multiple group comparisons. Predictors of brachial artery vasodilator responses to reactive hyperemia were obtained by univariate analysis. Those with a $p$ value $<0.05$ were entered into a multivariate regression model using the Systat software program (Evanston, Illinois). Previous studies have demonstrated that the percent brachial artery diameter change to reactive hyperemia is inversely correlated with the baseline diameter and, as such, this variable was included in the step-wise model (25). Statistical significance was defined as a two-sided $p$ value $< 0.05$. All data are expressed as the mean value $\pm$ SD. We calculated that by using 35 patients, our study had an 80% power to detect a statistically significant 2.5% decrease in FMD, assuming a standard deviation of 4%.

RESULTS

Subjects. The clinical characteristics and lipid profiles of the study patients are summarized in Table 1. The control

<table>
<thead>
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<th>Abbreviations and Acronyms</th>
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<tr>
<td>CAD = coronary artery disease</td>
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<td>ECG = electrocardiogram</td>
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<td>FMD = flow-mediated vasodilation</td>
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<tr>
<td>HDL = high-density lipoprotein</td>
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<tr>
<td>LDL = low-density lipoprotein</td>
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<td>NO = nitric oxide</td>
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group was younger than the study population, and the triglyceride group had more men than the other two groups; otherwise, because of the rigid exclusion criteria, the groups were well matched. The differences in the primary lipid subfractions between each group were statistically significant.

Brachial artery vasodilation. The change in brachial artery diameter in response to reactive hyperemia in all patients ranged from $-1\%$ to $25\%$. As has been reported before, there is a significant inverse relation between the degree of dilation and the baseline brachial artery diameter ($r = 0.29; p = 0.002$). There was no difference in baseline vessel diameter between the three groups in our study.

There was a trend for mild attenuation of endothelial-mediated vasodilation in the combined hyperlipidemic group when compared with controls ($9.0 \pm 5.1\%$ vs. $10.9 \pm 5.0\%, p = 0.06$) (Fig. 2). When evaluated by primary lipid abnormality, there was no difference in brachial artery vasodilator response to reactive hyperemia between the three groups ($10.9 \pm 5.0\%$ vs. $8.6 \pm 6.1\%$ vs. $9.4 \pm 3.9\%$, respectively, $p = 0.14$) (Fig. 1). There was a similar trend for attenuation of the brachial artery response to nitroglycerin ($p = 0.01$) (Fig. 1). These results suggest that there was no significant impairment of endothelium-dependent vaso-motor function in this population of patients, but there may be some mild disruption of endothelium-independent vasodilation.

Flow data. There was no difference in brachial artery flow at baseline or in response to reactive hyperemia between the three groups (Fig. 3).

Predictors of FMD. Since neither LDL cholesterol nor triglycerides predicted impaired endothelial function, the roles of other lipid subfractions were evaluated using a univariate linear regression analysis. The factors predicting an attenuated brachial artery response to reactive hyperemia were a larger baseline diameter, an increased ratio of either LDL/high-density lipoprotein (HDL) or total cholesterol/HDL and a reduced level of HDL. Because there was a difference between the groups with respect to age, we assessed the impact of age on FMD. No relationship was found. Triglycerides did not predict impaired endothelial response. In a multivariate linear regression analysis, when controlling for baseline brachial artery diameter, only the ratio of LDL/HDL cholesterol remained a statistically significant predictor of impaired endothelium function ($p = 0.04$) (Table 2).

DISCUSSION

Endothelium-dependent FMD is not significantly impaired in the brachial artery of patients with modest elevations of

**Table 1. Patient Characteristics**

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (n = 40)</th>
<th>Group 2 (n = 38)</th>
<th>Group 3 (n = 35)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (male/female)</td>
<td>28:12</td>
<td>24:14</td>
<td>29:6</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>32.5 ± 7.9</td>
<td>40.4 ± 11.2</td>
<td>44.9 ± 10.7</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Baseline SBP (mm Hg)</td>
<td>114 ± 10</td>
<td>117 ± 9</td>
<td>125 ± 13</td>
<td>NS</td>
</tr>
<tr>
<td>Baseline DIA (mm)</td>
<td>3.5 ± 0.7</td>
<td>3.6 ± 0.7</td>
<td>3.7 ± 0.6</td>
<td>NS</td>
</tr>
<tr>
<td>Cholesterol (mmol/liter)</td>
<td>4.4 ± 0.9</td>
<td>7.2 ± 1.3</td>
<td>6.3 ± 0.9</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>LDL (mmol/liter)</td>
<td>2.6 ± 0.8</td>
<td>5.2 ± 1.2</td>
<td>3.5 ± 0.9</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Triglycerides (mmol/liter)</td>
<td>1.0 ± 0.5</td>
<td>1.8 ± 0.6</td>
<td>4.2 ± 2.5</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>HDL (mmol/liter)</td>
<td>1.3 ± 0.3</td>
<td>1.1 ± 0.3</td>
<td>0.9 ± 0.2</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Cholesterol: HDL (mmol/liter)</td>
<td>3.6 ± 1.2</td>
<td>6.7 ± 1.7</td>
<td>7.6 ± 2.4</td>
<td>&lt; 0.001</td>
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DIA = diameter; HDL = high-density lipoprotein; LDL = low-density lipoprotein; SBP = systolic blood pressure.

**Figure 1.** Percent change in brachial artery diameter in response to reactive hyperemia (RH) and sublingual nitroglycerin (NTG) in Group 1 (solid bars), and in the combination of Groups 2 and 3 (hatched bars). *p = 0.06, **p = 0.01.

**Figure 2.** Percent change in brachial artery diameter in response to reactive hyperemia (RH), and sublingual nitroglycerin (NTG) in Group 1 (solid bars), Group 2 (open bars) and Group 3 (hatched bars). *p = 0.14; **p = 0.05.
triglycerides or LDL cholesterol without other risk factors for CAD.

**Triglycerides.** Controversy exists as to the role of hypertriglyceridemia as an independent risk factor for CAD. Triglycerides enhance the catabolism of HDL particles. Furthermore, they promote the formation of small dense LDL particles, and facilitate their oxidation and migration into the arterial wall (26). Much of the association between triglycerides and CAD in large population studies disappears in a multivariate analysis when controlling for HDL cholesterol (27). Recently, however, a large study has shown triglycerides to be an independent risk factor for CAD, even when controlling for HDL (10). Furthermore, two large studies have shown a synergistic effect of triglycerides on the ratio of LDL/HDL cholesterol to predict the incidence of CAD (11,12).

The effect of hypertriglyceridemia on endothelial function has been previously assessed in the brachial artery in small studies with conflicting results (28–30). Vogel et al. (30) and Lundman et al. (28) used a high-fat meal and an infusion of a triglyceride emulsion, respectively, to induce transient hypertriglyceridemia. Both of these studies found an impaired endothelial response in the brachial artery. Our study, and that of Chowienczyk et al. (29), used fasting lipid samples to determine triglyceride levels, and did not find impaired endothelial function. Also, patients were studied in the fasting state. Thus, postprandial triglyceride levels may be important for determining risk of impaired endothelial function. It is interesting to note that, in our study, Lundman et al. (28) also found some impairment of endothelium-independent vasomotion, suggesting that triglyceridemia may interfere with brachial artery smooth muscle function.

**LDL cholesterol.** There was no significant deleterious effect of elevated LDL cholesterol on endothelial function in the brachial artery in our study, which evaluated patients with modest hyperlipidemia and no other risk factors for CAD. This is in contrast to previous studies using this technique to assess the effect of elevated cholesterol on endothelial function (31,32). Sorensen et al. (31) found that FMD was impaired in hypercholesterolemic children when compared with controls (1.2 ± 0.4% vs. 7.5 ± 0.7%, respectively). However, this relationship disappeared when controlling for lipoprotein(a) (31). In another study, Clarkson et al. (32) found that FMD was impaired at baseline in 27 subjects with hypercholesterolemia, which improved after four weeks of treatment with l-arginine (1.7 ± 1.3% vs. 5.6 ± 3.0%, respectively). However, 17 of 27 subjects in the latter study had familial hypercholesterolemia and marked elevations in total or LDL cholesterol. Other studies have found oxidized LDL to be a better predictor of impaired endothelial function (17). In patients with hypercholesterolemia, conditions such as smoking and diabetes, which increase oxidative stress, are associated with greater endothelial dysfunction (33,34). For example, Celemajer et al. (35) found, in a large study, that cholesterol alone did not predict impaired endothelial function, but became significant when smoking status was taken into account. Furthermore, in a study evaluating the effects of cholesterol-lowering and antioxidant therapy in patients with elevated cholesterol and CAD, the greatest improvement in endothelial function occurred in patients treated with both a cholesterol-lowering drug and an antioxidant (36).

Hypercholesterolemia has been shown in large epidemiological studies to increase the risk for CAD (37,38). Although LDL lowering has been shown to decrease cardiac morbidity and mortality in primary prevention trials, the benefits are relatively modest in patients with hypercholesterolemia and no other cardiac risk factors (39). When combined, these results and our findings suggest that hypercholesterolemia alone has only a mild detrimental effect on vascular function and the subsequent development of atherosclerosis.

**HDL.** Low levels of HDL cholesterol have been associated with impaired endothelial function in other studies (40). Although we found that HDL cholesterol was inversely related to endothelial function in a univariate model, this relationship disappeared in a multivariate model. Using a multivariate model, we found that the ratio of LDL/HDL cholesterol was a statistically significant, but minor, predictor of impaired endothelial function (p = 0.04, r² = 0.121). It is likely that lipid subfractions, other than LDL, are important predictors of endothelial function.

**Study limitations.** Triglycerides were not correlated with impaired endothelial function in our study. However, we
used fasting lipid samples to determine triglyceride levels in our study population. Postprandial triglyceride levels may be a better predictor of risk for CAD, and thus, may also be associated with altered endothelial vasoactivity (41). Likewise, we did not assess particle size or the susceptibility of LDL oxidation, both of which are prone to induce endothelial dysfunction and may have altered our findings (18). In addition, LDL was calculated using the Friedwald equation. This is inaccurate when triglyceride levels are above 5 mmol/liter (6/3/5 patients). This may have introduced a small systematic error in the hypertriglyceridemic group.

The effects of impaired endothelial function are most clinically significant in the coronary circulation. We studied FMD in the brachial artery, a large peripheral conduit artery. A close relation between endothelial function in the coronary and peripheral circulations has been shown to exist (42). Nonetheless, although endothelial dysfunction is a systemic process, it may affect conduit and resistance arteries to a different degree.

Finally, there was a trend toward an impaired response of the brachial artery to nitroglycerin in Groups 2 and 3, suggesting that some of the deleterious effects of hyperlipidemia may be mediated by altered smooth muscle activity, rather than entirely by endothelial dysfunction. This has been demonstrated in two previous studies of hyperlipidemia and vascular reactivity (8,28). Regardless, we were unable to demonstrate a major impairment of either endothelium-dependent, or endothelium-independent, vasomotor function in this population of patients.

Conclusions. Modest elevations of triglycerides or LDL cholesterol are not associated with impaired endothelial function in the brachial artery of patients free from other risk factors for CAD. Synergism with other risk factors may be required to significantly impair vascular function.

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