Endothelial Vasodilator Function Is Related to Low-Density Lipoprotein Particle Size and Low-Density Lipoprotein Vitamin E Content in Type 1 Diabetes

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OBJECTIVES
We sought to determine whether endothelial vasodilator function (EVF) in patients with type 1 diabetes was related to low-density lipoprotein (LDL) particle size (LDLPS), LDL vitamin E content (LDLVE) or the susceptibility of LDL to oxidation (OxLDL).

BACKGROUND
Impaired EVF is an early feature of diabetic vascular disease and may be related to oxidant stress. Although small, dense LDL and oxidized LDL are features of type 2 diabetes and predict the development of coronary artery disease, their role in type 1 diabetes is less clear.

METHODS
Endothelium-dependent vasodilation was assessed in the brachial artery (flow-mediated vasodilation [FMD]) and in the forearm resistance circulation using venous occlusion plethysmography in response to graded doses of intrabrachial acetylcholine (ACh). Thirty-seven patients with type 1 diabetes mellitus (DM) and 45 matched controls underwent flow-mediated dilation, while a subset of 19 DM and 20 controls underwent plethysmography.

RESULTS
Total, LDL and high-density lipoprotein cholesterol or triglycerides were not different in DM compared with controls, but LDLPS was smaller (25.6 ± 0.06 vs. 26.1 ± 0.1 nm, p < 0.05) and LDLVE was reduced (2.0 ± 0.25 vs. 2.6 ± 0.18 μmol/mmol LDL, p < 0.05). Oxidative susceptibility of LDL was not different. Flow-mediated vasodilation was impaired in DM compared with controls (3.6 ± 0.25 vs. 7.1 ± 0.5%, p < 0.005), as was the vasodilator response to ACh (p < 0.05). Flow-mediated vasodilation was directly related to LDLPS and LDLVE in both the entire study cohort and DM alone (p < 0.05), but not to other parameters of the standard lipid profile. Similarly, endothelium-dependent vasodilation in the resistance circulation was directly related to LDLPS and LDLVE, but not to OxLDL.

CONCLUSION
These results suggest, but do not prove, that LDL particle size and LDL vitamin E may be determinants of conduit and resistance vessel endothelial vasodilator function in type 1 diabetes. Further work will be required to prove cause and effect. (J Am Coll Cardiol 2000; 35:292–9) © 2000 by the American College of Cardiology
endothelial vasodilator dysfunction in this condition (3). However, LDL particle size is not well characterized in type 1 diabetes. It is possible that the elevated peripheral insulin levels, which are a feature of type 1 diabetes (19), might influence LDL particle size (16,17).

Together with β-carotene, lycopene and ubiquinol 10, vitamin E is the principal antioxidant in LDL (20) and has been shown to correlate with the angiographic extent of atherosclerosis (13) in nondiabetic individuals. Vitamin E levels are decreased in type 2 diabetes (21), and the available data in type 1 diabetes suggest platelet levels of vitamin E are reduced (22). Whether LDLVE is reduced in young subjects with type 1 diabetes is unclear, as is the relationship of this antioxidant to endothelial vasodilator function.

We therefore sought to determine whether qualitative changes in LDL including LDLVE, LDLPS and OxLDL might contribute to endothelial vasodilator dysfunction in a group of young patients with type 1 diabetes.

**METHODS**

**Subjects.** Thirty-seven young type 1 diabetic subjects (DM) and 45 healthy control subjects were recruited for this study, approved by the Human Research Ethics Committee of Monash Medical Centre. All control subjects and diabetic patients provided written, informed consent. Control subjects were excluded if there were any signs of cardiovascular risk factors or disease. Diabetic subjects were all treated with insulin. The average daily total insulin dose was 67 ± 26 U (range 31 to 174 U). Duration of disease was on average 97 ± 52 months (mean ± SD). None of the diabetic subjects had clinical evidence of retinopathy, neuropathy or nephropathy (albumin excretion rate < 20 μg/min).

**General procedure.** Conduit artery function was assessed in all control and diabetic subjects using brachial artery ultrasound. Resistance vessel arterial function was assessed in a subgroup of 19 DM and 20 control subjects using venous occlusion plethysmography.

Subjects attended the laboratory fasted, having refrained from aspirin and nonsteroidal antiinflammatory drugs for at least five days before the study and caffeine containing beverages for at least 12 h. Each subject was given a standardized light breakfast, before which DM received their usual insulin dose. In DM, blood glucose levels were documented at the time of brachial artery ultrasound and twice during venous occlusion plethysmography studies. In view of the possibility of significant metabolic derangement influencing vascular measurements, a prospective decision to exclude patients with blood glucose levels > 18 mmol/liter was made. No patients required exclusion. All studies were performed in the morning in a dedicated quiet climate-controlled laboratory (22°C to 23°C) with dimmed lighting.

**Brachial artery ultrasound.** Endothelium-dependent and -independent vasodilation was assessed in the brachial artery using transcutaneous ultrasound. The method used has been described in detail previously (23,24). In brief, ultrasound images of the right brachial artery were obtained above the elbow using a high-resolution machine (HDI Ultramark 9; ATL, Seattle, Washington) with a 7- to 10-MHz linear-array transducer. Endothelium-dependent flow-mediated vasodilation was assessed as the percent change in arterial diameter in response to reactive hyperemia associated with 5 min of ischemia. Endothelium-independent vasodilation was assessed in response to a single tablet of sublingual nitroglycerin (GTN). Recording and analysis of images were performed, as described previously, by our group (24).

**Venous occlusion plethysmography.** Forearm blood flow (ml/100 ml forearm tissue/min) measurement was achieved by the well validated technique (25) of venous occlusion plethysmography using a calibrated mercury in silastic strain gauge (D.E. Hokanson, Bellevue, Washington) as described by this group previously (26). A 20-gauge, 5-cm polyethylene catheter (Cook; Brisbane, Australia) was introduced into the brachial artery of the nondominant forearm under local anesthesia utilizing aseptic conditions. The arterial line was used for on-line measurement of blood pressure and for direct intraarterial drug infusions. The catheter was connected via a minimum-dead-space saline-filled line to a pressure transducer (Biosensors International; Singapore). Physiological saline was infused at a rate of 0.4 ml/min through the catheter into the brachial artery to maintain patency. Measurement of resting blood flow was carried out at least 30 min after insertion of the brachial artery line and was repeated until a stable recording was obtained. Forearm blood flow responses were measured continuously for 2 min after infusion of each dose of drug. An average forearm blood flow was calculated from at least five venous occlusion cycles. Forearm vascular resistance (FVR) was calculated by dividing mean arterial pressure by forearm blood flow and was expressed in arbitrary units.

**Drug infusion protocol.** Nondominant forearm volume was measured in each subject by water displacement to

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**Abbreviations and Acronyms**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>ACh</td>
<td>acetylcholine</td>
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<tr>
<td>ANOVA</td>
<td>analysis of variance</td>
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<td>DM</td>
<td>study patients with type 1 diabetes mellitus</td>
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<td>EDTA</td>
<td>ethylenediamine tetra-acetic acid</td>
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<td>FMD</td>
<td>flow-mediated dilation</td>
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<td>FVR</td>
<td>forearm vascular resistance</td>
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<td>GTN</td>
<td>nitroglycerin</td>
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<td>HBA1c</td>
<td>glycosylated hemoglobin</td>
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<td>HDL</td>
<td>high-density lipoprotein</td>
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<td>HPLC</td>
<td>high-performance liquid chromatography</td>
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<td>LDL</td>
<td>low-density lipoprotein</td>
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<td>LDLP5</td>
<td>LDL particle size</td>
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<td>LDLVE</td>
<td>LDL vitamin E content</td>
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<td>OxLDL</td>
<td>oxidative susceptibility of LDL</td>
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LDLPS, LDLVE and Endothelial Function

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ensure no difference in this parameter between groups. All drugs were infused for 3 min at a rate of 0.4 ml/min before commencing measurement of forearm blood flow. Forearm blood flow responses were then measured continuously for 2 min while continuing infusion of each dose of drug. Endothelium-dependent vasodilation was assessed in response to acetylcholine chloride (ACH) (Miochol; Iolab Pharmaceuticals; Sydney, Australia) at doses of 2.7, 9 and 27 μg/min cumulatively. Endothelium-independent vasodilation was assessed in response to sodium nitroprusside (Faulding; Melbourne, Australia) at a dose of 9 μg/min.

Biochemical techniques. ISOLATION OF LDL AND ASSESSMENT OF LDL PARTICLE SIZE. Using density gradient ultracentrifugation, LDL cholesterol was isolated from ethylenediamine tetra-acetic acid (EDTA) plasma as previously described (27). After ultracentrifugation, the LDL formed a visible band. This was then isolated from the centrifuge tube by aspiration, and EDTA was removed by passing the sample through a sephadex G25 column (Pharmacia, Sydney, Australia). The LDL particle diameter was assessed, using the LDL fraction isolated by ultracentrifugation, by nondenaturing polyacrylamide gradient gel electrophoresis (Gradipore, Sydney, Australia) as previously described (28). The gels were scanned to determine the distance of the LDL peak from the origin. Particle diameter was calculated using a regression equation derived from the position of standards of known diameter (Cat. no: 17-0445-01; Pharmacia Biotech, Milwaukee, Wisconsin) by plotting the log of the standards against their positions on the scanned gel. The coefficient of variation on a 26.1-nm quality control sample run on every gel was 0.7%.

ASSESSMENT OF PLASMA AND LDL VITAMIN E CONTENT. Plasma vitamin E measurement was performed using reverse-phase high-performance liquid chromatography (HPLC) using an ultraviolet absorption detector as described previously (29). The vitamin E content of LDL was measured on LDL isolated by ultracentrifugation, using HPLC with a Spherisorb ODS–2, 5-μm analytical column (Alltech Associates, Baulkham Hills, Australia) and with standards purchased from Sigma Chemicals (St. Louis, Missouri) and expressed as a ratio of LDL vitamin E to LDL cholesterol (in μmol/mmol LDL).

ASSESSMENT OF LDL OXIDATIVE SUSCEPTIBILITY. Oxidative susceptibility of LDL was assessed using the Esterbauer technique (20) as modified by McDowell et al. (30). Copper (5 μmol) was added to isolated LDL diluted to 150 μmol/liter cholesterol in phosphate-buffered saline. The generation of products resulting from the oxidation of lipids was followed by measuring absorbance at 234 nm. The lag time was calculated as the time intercept between the line of maximum slope of the propagation phase of this reaction and the base-line absorbance at time = 0.

Statistical Analysis. Clinical characteristics are expressed as mean value ± standard deviation. Data are expressed as mean value ± standard error. Student’s t-test was utilized in the comparison of paired data (baseline characteristics, endothelium-dependent and -independent vasodilation). Differences between groups in the vasodilator responses to ACh were evaluated using a two-way repeated-measures analysis of variance (ANOVA). Simple and multiple linear regression analyses were performed to determine the best individual and combination of predictor variables. Statistical significance was accepted when the p < 0.05.

RESULTS

There were no significant differences in age (24 ± 5 vs. 22 ± 5 years), gender distribution or body mass index (24.7 ± 0.5 vs. 23.8 ± 0.5 kg/m²) between the diabetic (DM) and control groups, respectively. Glycosylated hemoglobin (HBA1c) levels of 8.5% indicated at least fair glycemic control in DM, whereas duration of diabetes was 65 ± 22 vs. 0.5 ± 2.6 years, gender distribution or body mass index (24.7 ± 0.5 vs. 23.8 ± 0.5 kg/m²) between the diabetic (DM) and control groups, respectively. Glycosylated hemoglobin (HBA1c) levels of 8.5% indicated at least fair glycemic control in DM, whereas duration of diabetes was 65 ± 22 months.

Lipid profile. There was no difference in fasting total cholesterol, LDL, HDL or triglyceride levels in DM compared with controls (Table 1). However, LDLPS was 0.5 nm smaller and LDLVE was 23% less in DM compared with control subjects (Table 1). There was no difference however in OxLDL between DM and control subjects.

The LDLPS was directly related to HDL (r = 0.26, p < 0.05) and inversely related to TG levels (r = 0.4, p < 0.05) in the diabetic group, while LDLPS was correlated with waist/hip ratio in the study cohort as a whole (r = 0.34, p < 0.005).

The lag time to oxidation of LDL was directly related to LDLPS (r = 0.36, p < 0.005) but not to LDLVE.

Insulin dose and levels. The average daily dose of insulin in DM was 65 ± 19 U (mean ± SD). Peripheral insulin levels were elevated in DM compared with controls (49.9 ± 4.8 vs. 12.5 ± 0.9 μU/ml, p < 0.005). There was no
relationship between insulin dose or peripheral insulin levels and LDLVE, endothelium-dependent vasodilation or parameters of the standard lipid profile. However, LDLPS was directly related to the average daily insulin dose \(r = 0.35, p < 0.05\) but not to ambient insulin levels.

**Endothelium-dependent flow-mediated vasodilation.** Base-line brachial artery diameter was not different between the diabetic or control groups \(4.2 \pm 0.08\) vs. \(4.0 \pm 0.09\) mm, \(p = \text{NS}\), respectively). Flow-mediated dilation (FMD) with reactive hyperemia was significantly reduced in DM compared with control subjects \(3.6 \pm 0.6\%\) vs. \(7.1 \pm 0.5\%\), \(p < 0.005\) (Fig. 1).

There was no relationship between FMD and total cholesterol, LDL, HDL or TG in the study cohort or DM alone. However, FMD was a function of LDL particle size in both the study cohort and DM alone \(r = 0.39, p < 0.0005\) and \(r = 0.48, p < 0.05\), respectively) (Fig. 2). FMD was also related to LDLVE in the study cohort as a whole \(r = 0.26, p < 0.05\) and within DM alone \(r = 0.39, p < 0.05\) (Fig. 3). Despite a relationship between FMD and LDLPS and LDLVE, there was no relationship between FMD and the lag time to oxidation of LDL.

Within DM, FMD was inversely related to HBA1c \(r = 0.36, p < 0.05\), but there was no relationship between FMD and ambient glucose or insulin levels at the time of measurement of FMD. Notably, FMD was directly related to baseline arterial diameter across both groups \(r = 0.32, p < 0.005\) and correlated directly with GTN-induced vasodilation in both the study cohort and DM alone \(r = 0.39, p < 0.0005\) and \(r = 0.34, p < 0.005\), respectively).

**GTN-induced endothelium-independent vasodilation.** The vasodilator response to sublingual GTN was impaired in DM compared with controls \(15.4 \pm 1.1\%\) vs. \(18.5 \pm 0.8\%\), \(p < 0.05\) (Fig. 1). This response was directly related to HDL in the study cohort as a whole \(r = 0.27, p < 0.05\) and inversely related to duration of diabetes \(r = 0.35, p < 0.05\). There was no relationship between GTN-induced dilation and LDLPS, LDLVE or lag time to oxidation of LDL.

**Endothelium-dependent vasodilation in response to ACh.** Resting forearm blood flow was 50% higher in DM compared with control subjects \(3.3 \pm 0.3\) vs. \(2.0 \pm 0.2\) ml/min/100 ml forearm, \(p < 0.005\) and resting FVR was lower \(48.5 \pm 4.2\) vs. \(29.7 \pm 2.8\) arbitrary units, \(p < 0.005\). In view of this difference in basal flow and resistance, the absolute change in FVR (ΔFVR) to ACh was calculated. The vasodilator response to ACh was impaired in DM (ANOVA, \(p < 0.05\), with the greatest difference between the groups occurring at the highest dose of ACh \(30 \mu\text{g/min}\) (Fig. 4). At the maximum dose of ACh, the absolute change in FVR was \(-22.3 \pm 3.3\) vs. \(-39.3 \pm 3.7\) arbitrary units, \(p < 0.001\) in DM compared with control subjects. The difference in vasodilator response to ACh remained even if a subgroup of diabetic \(n = 7\) and control...
subjects (n = 11) with equivalent resting blood flow were analyzed. There was a significant relationship between this change in FVR and both LDLPS and LDLVE in the study cohort as a whole ($r = 0.46$, $p < 0.05$ and $r = 0.34$, $p < 0.05$), respectively, and DM alone ($r = 0.35$, $p < 0.05$ and $r = 0.29$, $p < 0.05$), respectively (Fig. 5). In addition, the slope of the dose-resistance curve was directly related to both LDLPS and LDLVE in the study cohort ($r = -0.32$, $p < 0.05$ and $r = -0.32$, $p < 0.05$, respectively, Fig. 5). However, there was no relationship between either the slope of the dose-resistance curve or the absolute change in FVR and the lag time to oxidation of LDL.

Endothelium-independent vasodilation in response to sodium nitroprusside. In view of the differences in resting forearm blood flow between groups, the absolute change in blood flow and vascular resistance from baseline was again calculated. Sodium nitroprusside increased blood flow in both groups. There was, however, no difference in the magnitude of this response between DM and controls ($23.7 \pm 3.2$ vs. $22.6 \pm 2.5$ arbitrary units, $p = NS$). Similarly, there was no difference in mean arterial pressure between the two groups after the infusion of sodium nitroprusside. LDLPS, LDLVE or the oxidative susceptibility of LDL were not related to the vasodilator response to sodium nitroprusside.

DISCUSSION

This study demonstrates impaired endothelium-dependent vasodilator function of both the conduit and resistance circulation in young subjects with type 1 diabetes, fair glycemic control and a normal lipid profile. This vasodilator function was related to LDLPS and LDLVE but was not related to TC, LDL, HDL or TG. Despite demonstrating reduced LDLPS and lower LDLVE, differences in the oxidative susceptibility of LDL were not observed, at least when measured using a modified Esterbauer technique (20).

Our finding of impaired endothelial function in subjects with type 1 diabetes is consistent with a number of previous studies in both the conduit (2,31) and resistance (1,32,33) circulation, although some studies have shown no impairment of the response to muscarinic agonists in diabetic subjects (34–36). In this study, an impaired response to ACh in the diabetic group persisted even when subjects with similar resting flow to controls were analyzed, suggesting that this finding was not an artefact of resting hyperemia. The decreased response to a nitrovasodilators in the conduit artery but not in the resistance circulation in this study is in agreement with most (1,2,31–34,36) but not all previous investigations (35).

In this group of diabetics with relatively good glycemic control, there was no difference in the routine lipid profile compared with the control subjects. This is consistent with previous data showing that lipid abnormalities develop in type 1 diabetes as glycemic control deteriorates (37). We found that endothelial function in the conduit artery was inversely related to long-term glycemic control, but that there was no relationship between FMD and ambient glucose or insulin levels at the time of measurement of FMD. Moreover, there was no relationship between FMD and parameters of the standard lipid profile. Previous investigators have demonstrated a relationship between FMD and LDL in diabetic patients with suboptimal glycemic control (2). This perhaps suggests that the potential

![Figure 4](image-url)  
**Figure 4.** Absolute increase in forearm blood flow (A) and absolute reduction in forearm vascular resistance (B) in diabetic subjects (filled circles) and control subjects (open circles) during graded intraarterial infusion of acetylcholine (ACh). The changes in forearm blood flow and vascular resistance were attenuated in the diabetic group compared with the control group ($p < 0.05$).

![Figure 5](image-url)  
**Figure 5.** (A and B) The relationship between the absolute change in forearm vascular resistance ($\Delta FVR$) at peak dose of acetylcholine (ACh) and LDLPS (A) and LDLVE (B) in diabetic (filled circles) and control subjects (open circles). (C and D) The relationship between the slope of the $\Delta FVR$ ACh dose-response curve and LDLPS (C) and LDLVE (D).
for atherogenic factors to modify endothelial function in subjects with diabetes varies with glycemic control.

The atherogenic potential of small, dense LDL is well recognized and has been ascribed to an increased susceptibility to oxidation (38), lower binding affinity for the hepatic LDL apo B/E receptor reflected in a prolonged residence time in plasma (39), more efficient penetration of the arterial intima (40) and increased capacity for binding to intimal proteoglycans (41). Thus, reduced LDL particle size appears to represent a key modification of this lipoprotein. Small, dense LDL is well described in type 2 diabetes (42), but LDLPS in type 1 diabetes has not been well characterized. In this study, we observed LDLPS was reduced in young patients with type 1 diabetes (glycosylated hemoglobin [HBA1c] 8.5%). This contrasts with a previous observation in an older group of diabetics with poor glycemic control (HBA1c 12.2%) (43). The LDL subclass phenotype is influenced by genetic factors as well as external influences including drugs, menopausal status and diet (15), and also possibly by insulin levels and insulin sensitivity (16,17). In keeping with this latter possibility, we observed a relationship between LDLPS and the total daily insulin dose, although there was no correlation with peripheral insulin levels. The daily insulin dose is more likely to reflect total insulin exposure than a single insulin level. Furthermore, there was a correlation between LDLPS and waist/hip ratio, a surrogate marker for insulin sensitivity (44).

We observed reduced LDLPS to be a function of both high triglyceride levels and low HDL levels in agreement with previous findings (45). However, it appears from older (46) and more recent (12) data that small, dense LDL is a risk factor for vascular disease independent of HDL and triglyceride levels. The data on endothelial function from this and a previous study in patients with type 2 diabetes (3) would support this.

Epidemiological (47,48) and clinical (49) studies have suggested a role for dietary and supplemental vitamin E, respectively, in the prevention of coronary artery disease. The potential mechanisms by which this chain-breaking antioxidant exerts its effect are multiple and related not only to its antioxidant potential. At the endothelial level, vitamin E sequesters free radicals, responsible for the inactivation of nitric oxide (50), while exerting beneficial influences on leukocyte adhesion to endothelial cells (51), monocyte transmigration (52), oxidant-mediated cytotoxicity (53) and activated protein kinase C (54). Furthermore, LDLVE levels may be relevant to the oxidation of LDL, which occurs in the subintimal space (55).

Reduced antioxidant defenses, including glutathione, vitamin E and vitamin C, have been observed in type 2 diabetes (21). In type 1 diabetes, platelet but not plasma vitamin E (22) as well as vitamin C (56) are decreased. This study is the first demonstration that LDLVE is reduced in type 1 diabetes, as far as we are aware, and may reflect a dietary deficit, increased consumption of this antioxidant or abnormal metabolism (as occurs with vitamin C [57]). In addition, elevated insulin levels, as occur in type 1 diabetes (19), have been associated with reduced vitamin E levels (58), although in this study we could not demonstrate a relationship between LDLVE and ambient insulin levels or daily insulin dose. Causation aside, we observed a relationship between LDLVE and endothelial vasodilator function in both the conduit and resistance circulation. In keeping with this relationship, LDLVE has been shown to be related to the severity of atherosclerosis in patients with coronary disease (13). There was, however, no relationship between endothelial vasodilator function and plasma vitamin E emphasizing the importance of antioxidant localized in LDL itself.

Although we observed reduced LDLPS and LDLVE in DM, and a correlation between the lag phase of LDL oxidation and LDLPS, we could not demonstrate any difference in the oxidative susceptibility of LDL between DM and control subjects. Moreover, there was no relationship between endothelial function and oxidative susceptibility. This may be due to the sensitivity of the technique we employed to measure oxidative susceptibility, which determines the lag phase of conjugated diene formation resulting from copper-induced oxidation of LDL. Any change in oxidative susceptibility produced by the alterations in LDLPS and LDLVE may have been too small or subtle to be detected by this method. Studies exploring the change in lag phase of oxidation of LDL with supplemental vitamin E have shown that approximately doubling LDLVE will produce a significant change in this measurement (59). The 30% difference in LDLVE between DM and controls in this study is relatively modest by comparison.

A further possibility is that LDL in this diabetic group was already partly oxidized during isolation, as a consequence of reduced antioxidant defenses (LDLVE) and reduced particle size. This would tend to minimize any real differences in lag time between the two groups. Measurement of the oxidation status of LDL at baseline (e.g., thiobarbituric acid reactive substances) would provide this information.

The fact that endothelial vasodilator function is related to LDLVE independent of LDL oxidation is interesting. It is possible that vitamin E in LDL is being delivered to the endothelium, where it exerts a beneficial influence independent of its effect on LDL by affecting activated protein kinase C (54) and sequestering free radicals (50).

Studies that have examined endothelial function (14) and burden of disease (60–62) in patients with clinically evident atherosclerosis have shown a correlation between these parameters and the oxidative susceptibility of LDL (measured using the Esterbauer technique). No study, however, has shown a relationship between oxidative susceptibility of LDL as measured by this technique and endothelial function in patients without overt vascular disease (63–65). Thus, the oxidative susceptibility of LDL may correlate better with advanced atherosclerotic disease.
Potential limitations. While we have demonstrated an association between LDLPS and LDLVE and endothelial vasodilator function, we have not proved that there is a cause-and-effect relationship. Further work manipulating particle size and vitamin E content of LDL will be required to take these observations further.

We cannot conclude from this study that the oxidative susceptibility of LDL is not an important determinant of endothelial vasodilator function. The biological relevance of the modified Esterbauer technique to in vivo oxidation of LDL within the arterial wall is not clear (66). Oxidative modification of LDL may be better characterized by other methods (67–69).

In conclusion, we have shown that reduced LDLVE and small, dense LDL are important qualitative modifications of LDL that contribute to impaired endothelium–dependent vasodilation of the conduit and resistance circulation in type 1 diabetes. The in vitro oxidative susceptibility of LDL as measured by the lag phase of conjugated diene formation is not related to endothelial function in this group of subjects. These data add to the growing body of evidence indicating that qualitative modifications of LDL have an important atherogenic effect and may indeed be independent risk factors for vascular disease.

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REFERENCES
