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Atorvastatin But Not L-Arginine Improves Endothelial Function in Type I Diabetes Mellitus: A Double-Blind Study

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OBJECTIVES We sought to determine the effects of oral L-arginine and the hexamethylglutaryl coenzyme A reductase inhibitor atorvastatin on endothelial function in young patients with type I diabetes mellitus (DM).

BACKGROUND Endothelial dysfunction, a key early event in atherosclerosis, occurs in young patients with type I DM, and its reversal may benefit the progression of vascular disease. Cholesterol reduction in L-arginine improve endothelial function in nondiabetic subjects, but their effect in patients with type I DM is unknown.

METHODS In a double-blind, 2 × 2 factorial study, we investigated the effect of L-arginine (7 g twice daily) and atorvastatin (40 mg/day) on conduit artery vascular function in 84 normocholesterolemic young adults (mean ± SD: age 34 years [range 18 to 46], low density lipoprotein [LDL] cholesterol 2.96 ± 0.89 mmol/liter) with type I DM. Brachial artery dilation to flow (flow-mediated dilation [FMD]) and to the direct smooth muscle dilator glyceryl trinitrate (GTN) were assessed noninvasively using high resolution ultrasound at baseline and after six weeks of treatment.

RESULTS Atorvastatin resulted in a 48 ± 10% decrease in serum LDL cholesterol levels, whereas L-arginine levels increased by 247 ± 141% after L-arginine therapy. By analysis of covariance, treatment with atorvastatin resulted in a significant increase in FMD ($p = 0.018$. L-Arginine therapy had no significant effect on endothelial function, and there was no significant change in dilation to GTN after either intervention.

CONCLUSIONS In young patients with type I DM, improvement in endothelial dysfunction can be demonstrated after just six weeks of treatment with atorvastatin. In contrast to studies of hypercholesterolemia, however, L-arginine had no benefit. Treatment with atorvastatin at an early stage may have an impact on the progression of atherosclerosis in these high risk patients. (J Am Coll Cardiol 2000;36:410–6) © 2000 by the American College of Cardiology

Type I diabetes mellitus (DM) is a major risk factor for premature atherosclerosis, with a cumulative mortality rate from macrovascular disease as high as 30% by the age of 55 years (1). A key factor in the initiation of the atherosclerotic process is injury to the vascular endothelium, and endothelial dysfunction has been demonstrated in animal models of type I DM (2–4) and in young diabetic patients (5–10). Reversal of endothelial dysfunction, at this early stage, may therefore retard the development of cardiovascular disease.

An important aspect of endothelial dysfunction in diabetes is reduced bioavailability of endothelium-derived nitric oxide (NO) (4). The mechanisms that underlie this are complex and relate to both increased breakdown and decreased production of NO (11). Hyperglycemia, activation of protein kinase C and formation of advanced glycosylation end products are important sources of oxygen-derived free radicals (12–14), which accelerate the peroxidation of lipoproteins (15) and inactivate NO (16,17). In type I DM, increased susceptibility of low density lipoprotein (LDL) to oxidation has been reported (18), which may be related to poor control and duration of diabetes (18,19). Oxidized LDL has been shown to reduce NO synthase expression (20), enhance NO breakdown (21) and impair endothelial-dependent dilation (22). Our recent finding of an inverse correlation between conduit artery endothelial function and LDL cholesterol levels is consistent with an important role for LDL cholesterol in the initiation of diabetic vascular disease (23). Impaired NO synthesis may also occur in type I DM as a consequence of glycosylation or impaired metabolism of its substrate, L-arginine (24,25). A lack of essential cofactors (26) or alteration in the kinetics of NO synthase (27) may also decrease NO formation and even result in the production of superoxide anion (28,29).

In spontaneously diabetic rats, administration of L-arginine enhances endothelial-dependent dilation (30,31). L-Arginine therapy has also been shown to improve endothelial function in subjects with hypercholesterolemia

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Abbreviations and Acronyms

CI	=	confidence interval
DM	=	diabetes mellitus
FMD	=	flow-mediated dilation
GTN	=	glyceryl trinitrate
GTN-MD	=	glyceryl trinitrate–mediated dilation
HDL	=	high density lipoprotein
HMG-CoA	=	hexamethylglutaryl coenzyme A
LDL	=	low density lipoprotein
NO	=	nitric oxide

(8), and in cholesterol-fed animals, it retards the progression of atherosclerosis (32). However, few reports have been published on the effect of L-arginine in patients with type I DM. We have recently demonstrated that acute administration of L-arginine had no beneficial effect on endothelial function in patients with type I DM, in contrast to its effect in hypercholesterolemia (33). This suggests potential differences in the mechanism of endothelial dysfunction between these risk factor groups, or it may represent the more complex nature of endothelial function in type I DM. The benefit of longer term oral administration of L-arginine in type I DM, or its effect when given in conjunction with other therapies targeted at restoring endothelial function, has not been tested.

We hypothesized that cholesterol reduction and L-arginine therapy may have beneficial and potentially synergistic effects on endothelial function in patients with type I DM. We therefore studied the effect of the hexamethylglutaryl coenzyme A (HMG-CoA) reductase inhibitor atorvastatin, long-term oral L-arginine therapy and their combination on conduit artery endothelial-dependent dilation in young, clinically well patients with type I DM who had normal cholesterol levels.

METHODS

Study groups. Subjects aged 18 to 45 years with type I DM of >2 years duration (range 2 to 41 years) (type I DM defined as those requiring insulin treatment from diagnosis and a history of ketoacidosis) and a low density lipoprotein (LDL) cholesterol level <4.5 mmol/liter were recruited from diabetic clinics in London. They were nonsmokers who had either never smoked or not smoked for >2 years with a total exposure <1 pack-year, had a rest supine blood pressure <150/90 mm Hg, had no clinical evidence of large vessel atherosclerosis and were not taking a vasoactive or cholesterol-lowering medication. Eleven subjects had evidence of background diabetic retinopathy and five had received treatment for proliferative retinopathy. All subjects gave informed, written consent, and the local research Ethics Committee approved the protocol.

Study design. This was a randomized, double-blind, 2 × 2 factorial study. Subjects were randomized to take either L-arginine, 7 g twice daily, in the form of a lemon-flavored, sugar-free drink (Pharmacy Manufacturing Unit, Plymouth, United Kingdom) or placebo equivalent, and atorvastatin, 40 mg/day at night (Parke-Davis Ltd., Eastleigh, United Kingdom) or placebo. Subjects were thus classified into four treatment groups taking either two placebos, atorvastatin plus L-arginine/placebo, L-arginine plus atorvastatin/placebo or both L-arginine and atorvastatin therapy (Fig. 1). For both L-arginine and atorvastatin, the active drug and placebo were packaged identically. Randomization codes were generated independently (Parke-Davis Ltd.), and each container was labeled only with the subject's study number, which was allocated in a sequential order. The dose of L-arginine was chosen on the basis of animal experiments and clinical studies in subjects with hypercholesterolemia, in whom benefit on endothelial function has previously been demonstrated (8,34). The subjects were instructed to continue with their normal diabetic diet and insulin regimen throughout the study. Endothelium-dependent dilation was studied noninvasively at baseline and after six weeks of therapy. Seventy of the 84 subjects were studied in the morning after an overnight fast. The remaining 14 subjects were studied in the afternoon after a 6-h fast after their normal breakfast and morning insulin. All subjects were studied at the same time of day on both visits. No attempt was made to study subjects under conditions of euglycemia, as this would have been logistically difficult, and moreover would have introduced the potential confounding influence of a glucose and insulin infusion on endothelial function.

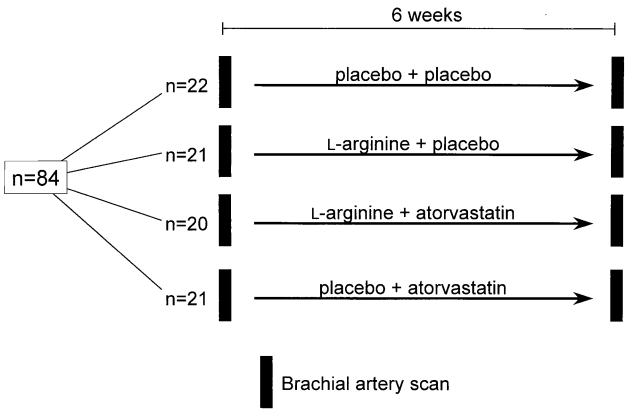

Figure 1. Study protocol. Subjects were randomized to either placebo, L-arginine (7 g twice daily), atorvastatin (40 mg/day) or combination therapy. Vascular function was assessed noninvasively at baseline and after six weeks of treatment.

At each visit, venous blood was taken without use of a tourniquet, for measurement of a full blood count (Bayer [Technicon] H1 system) and for assay of biochemical variables by reflectance spectrophotometry (Johnson & Johnson Vitros dry chemistry system, unless stated otherwise). Fructosamine (a time-averaged marker that reflects diabetic control over the preceding 30 days [35]) was determined from the rate of formation of formazan, using the Dimension AR system (Roche). Plasma levels of arginine, ornithine, glycine and citruline—amino acids closely linked to arginine metabolism—were determined after pre-

cipitation with sulfur salicylic acid, by use of a dedicated ion exchange amino acid analyzer (Amersham Pharmacia Biotech, Upsala, Sweden). Total and high density lipoprotein (HDL) cholesterol and triglyceride levels were measured by reflectance spectrophotometry, and LDL cholesterol was calculated using the Friedwald formula (36). Subjects were examined for the presence of microalbuminuria (albumin/creatinine ratio >2 mg/mmol) from two timed overnight urine collections.

Assessment of endothelial function. Brachial artery endothelium-dependent and -independent reactivities were assessed noninvasively using an accurate and reproducible high resolution ultrasound technique, as previously described (33,37). All scans were performed by the same operator (A.E.D.). Subjects lay at rest for at least 10 min before the first scan and remained supine throughout the procedure. Blood pressure and heart rate were monitored throughout the study. The right brachial artery was imaged in a longitudinal section 2 to 10 cm above the elbow, using a 7-MHz linear-array transducer supported by a stereotactic clamp and an Acuson 128XP/10 ultrasound system (Acuson Ltd., Mountain View, California). Blood flow velocity was measured by pulsed wave Doppler echocardiography, with the cursor set at 70° to the longitudinal axis of the artery and the range gate (1.5 mm) in the center of the artery. Brachial artery diameter was determined using an A-mode tracking device (Ingenious Systems, the Netherlands), at rest and 55 to 65 s after a brief period of reactive hyperemia induced by inflating a pneumatic tourniquet placed around the forearm to 300 mm Hg, with its rapid release after 4.5 min. All scans were recorded onto super VHS videotape. Hard copy images of the brachial artery were taken and notes made of the transducer and arm position, enabling precise reproduction of conditions and measurement of the same segment of the artery at subsequent visits. The peak blood flow velocity and the integral of blood flow velocity over the first 20 s of reactive hyperemia were determined by pulsed wave Doppler echocardiography. After a further 10 to 15 min at rest, sublingual glyceryl trinitrate (GTN) 400 μg, was administered, and the response to this endothelium-independent dilator was assessed after 3 min.

Data analysis. On completion of each study, the stored radio frequency data for each scan were analyzed by placement of volume sample cursors at the near and far vessel wall interfaces. Arterial distention was tracked and end-diastolic diameter determined on a beat-by-beat basis, with a spatial resolution of 50 μm. All scans were checked by an independent observer (M.J.M.) who had no knowledge of the origin of the scan. Scans in which 1) the image at both studies was not replicated; 2) a satisfactory distensibility waveform was not achieved; or 3) cursor placement was incorrect were rejected and excluded from the final analysis. Vessel dilation in response to flow (flow-mediated dilation [FMD]) and GTN were expressed as the percent increase in vessel diameter from baseline. Flow-mediated dilation is dependent on NO production (38) and correlates with measures of coronary endothelial function and atherosclerosis (39). Rest volumetric blood flow was calculated for each study by multiplying the velocity–time integral (corrected for angle) by the heart rate and vessel cross-sectional area. Although this method may lead to overestimation of blood flow, inaccuracies are consistent, allowing for a comparison between visits and individuals.

Statistical analysis. Data are presented as the mean value ± SD. The primary outcome measure for this study was change in FMD, and the secondary outcome measure was change in GTN–mediated dilation (GTN-MD) between the two visits. Two-way analysis of covariance was used to determine the effects of atorvastatin and L-arginine therapy and their interaction on vascular function. Covariates for age, gender, duration of diabetes, baseline LDL cholesterol and baseline FMD or GTN-MD were initially included, as previous studies have indicated that these variables may have a significant effect on endothelial function and the response to intervention. Subsequently, covariates for blood pressure, HDL cholesterol, triglycerides, glucose, fructosamine, total daily insulin dose, vessel size and the intensity of reactive hyperemia were added to the model to determine their effect. The effects of L-arginine and atorvastatin on plasma arginine and lipoprotein subfraction cholesterol levels were compared using the independent Student t test. Multiple regression analyses were used to explore the relations between baseline FMD and GTN-MD and subject characteristics and risk factor profile. Initial models were adjusted for age, gender and the intensity of reactive hyperemia. Subsequently, risk factor variables (blood pressure, total and LDL and HDL cholesterol levels and triglyceride levels) and variables related to diabetic control (glucose level, fructosamine level, total daily insulin dose, presence of microalbuminuria) were added one by one to determine whether there was any additional effect. Statistical significance was inferred at $p < 0.05$.

RESULTS

Baseline subject characteristics. Eighty-four subjects were randomized (53 men and 31 women). Age, gender distribution, levels of lipid subfractions, blood pressure and diabetic characteristics were comparable between the treatment groups and their respective placebo arms (Table 1). Ten subjects (12%) had microalbuminuria. Seventy-seven subjects (88%) completed the study, and reasons for withdrawal are outlined in Table 2. Compliance with the study drugs was determined from the volume of L-arginine and the number of tablets of atorvastatin unused on completion of the study—the rates of which were 87% and 96%, respectively. Atorvastatin was generally well tolerated, with no effect on measures of diabetic control. There was no incidence of muscle pains, increases in creatinine kinase or transaminase levels. Three subjects taking atorvastatin withdrew from the study. One subject had an episode of diabetic ketoacidosis shortly after randomization, related to a routine

Table 1. Baseline Characteristics of 84 Subjects With Type I Diabetes Mellitus

	Treatment Groups			
	Placebo	L-Arginine	Atorvastatin	L-Arginine Plus Atorvastatin
Male	22 (77%)	21 (72%)	21 (53%)	20 (55%)
Age (yrs)	34.9 ± 7.9	35.0 ± 6.4	33.7 ± 6.1	33.3 ± 6.8
Microalbuminuria	4	0	4	2
Duration of diabetes (years)	16.5 ± 9.6	15.0 ± 9.2	15.1 ± 8.5	16.2 ± 10.2
Fructosamine (μmol/liter)	408 ± 73	374 ± 75	427 ± 64	414 ± 64
Glucose (mmol/liter)	10.8 ± 6.2	9.9 ± 6.1	10.6 ± 5.1	11.4 ± 5.1
Daily insulin (IU)	52.8 ± 17.4	64.2 ± 22.5	53.3 ± 25.6	50.5 ± 15.6
Arginine (μmol/liter)	57.5 ± 15.6	68.9 ± 17.6	70.1 ± 28.6	65.2 ± 12.6
Total cholesterol (mmol/liter)	4.70 ± 1.07	4.78 ± 0.80	4.92 ± 1.07	5.08 ± 0.86
LDL cholesterol (mmol/liter)	2.82 ± 1.0	2.95 ± 0.81	3.08 ± 0.92	3.02 ± 0.82
HDL cholesterol (mmol/liter)	1.45 ± 0.33	1.34 ± 0.46	1.47 ± 0.37	1.62 ± 0.62
Triglycerides (mmol/liter)	0.93 ± 0.51	1.09 ± 1.08	0.83 ± 0.44	0.97 ± 0.34
SBP (mm Hg)	122 ± 13	127 ± 11	122 ± 14	120 ± 12
DBP (mm Hg)	73 ± 7	75 ± 8	75 ± 9	72 ± 6
Rest vessel size (mm)	3.93 ± 0.47	4.05 ± 0.65	3.69 ± 0.70	3.77 ± 0.73
FMD (%)	3.49 ± 2.99	3.33 ± 3.03	3.65 ± 3.20	3.01 ± 3.32
GTN-MD (%)	17.12 ± 8.49	16.44 ± 7.49	19.58 ± 8.00	16.07 ± 7.49
Rest blood flow (ml/min)	83.0 ± 48.0	110.6 ± 69.7	65.9 ± 53.0	86.0 ± 61.1
Hyperemic blood flow (AUC)*	75.7 ± 26.5	89.3 ± 41.9	69.8 ± 29.0	78.6 ± 35.7

*AUC = area under the time–flow curve for hyperemic blood flow over the first 20 s after release of the tourniquet. Data are presented as the number (%) of patients or mean value ± SD.

DBP = diastolic blood pressure; FMD = flow-mediated dilatation; GTN-MD = glyceryl trinitrate mediated dilatation; HDL = high-density lipoprotein; LDL = low-density lipoprotein; SBP = systolic blood pressure.

menisectomy; one subject complained of dizziness; and another subject complained of increased frequency of hypoglycemia. Two scans from two subjects were rejected, as they were technically unsatisfactory (as discussed earlier).

Biochemical effects. Neither L-arginine nor atorvastatin therapy had a significant effect on fasting glucose concentration, insulin requirements or overall diabetic control, as determined by fructosamine levels. In the subjects randomized to L-arginine, plasma arginine increased from 67 ± 15 to 159 ± 84 μmol/liter ($p < 0.001$). L-arginine had no significant effect on plasma lipid levels or the levels of any of the other measured amino acids. In subjects randomized to receive atorvastatin, total, LDL and HDL cholesterol and triglyceride levels decreased by (mean value ± SD) 33.3 ± 9.4% ($p < 0.001$), 48.3 ± 10% ($p < 0.001$), 6.1 ± 21.3% ($p = 0.045$) and 12.1 ± 25.6% ($p = 0.028$), respectively.

Effects on vascular function. Baseline vessel size, rest blood flow, reactive hyperemic blood flow, FMD and GTN-MD were comparable between the treatment groups (Table 1). For the whole cohort, FMD and GTN-MD were 3.38 ± 3.09% and 17.31 ± 7.87%, respectively. The values for FMD were significantly less than those seen in a comparable group of nondiabetic subjects previously studied in our laboratory (9.3 ± 3.8%) (23). Baseline FMD was higher in women than in men. (4.11 ± 3.79% vs. 2.97 ± 2.56%, $p = 0.1$). In the 10 subjects with microalbuminuria, there was a trend toward a reduction in FMD (3.51 ± 3.14% vs. 2.43 ± 2.55%, $p = 0.11$), as compared with normoalbuminuric subjects, and a significant reduction in GTN-MD (17.79 ± 8.0% vs. 12.75 ± 4.76%, $p = 0.02$). There was a significant correlation between baseline FMD and hyperemic blood flow velocity over the first 20 s after release of the tourniquet ($r = 0.23$, $p = 0.039$), but not with peak hyperemic blood flow, any of the measured variables of diabetic control, insulin levels, blood pressure or LDL or HDL cholesterol or triglyceride levels.

There was no significant change in rest vessel size, blood flow or reactive hyperemia in response to L-arginine or atorvastatin therapy. In subjects treated with placebo, L-arginine alone, atorvastatin alone and combination therapy, FMD changed by −0.34 ± 2.11%, −0.33 ± 2.49%, 1.75 ± 2.88% and 0.68 ± 2.45%, respectively. There was no

Table 2. Withdrawals After Randomization

Ref. No.	Gender	Age (yrs)	Treatment Group	Reason for Withdrawal
1	F	22	Atorvastatin	Ketoacidosis after routine menisectomy
40	F	33	L-arginine	Lost to follow-up
42	F	36	Atorvastatin plus L-arginine	Lost to follow-up
49	M	41	Placebo	Lost to follow-up
53	M	35	Atorvastatin	Dizziness
55	F	34	Atorvastatin	Episodes of hypoglycemia
78	F	38	Atorvastatin	Lost to follow-up

F = female; M = male.

Table 3. Determinants of Change in FMD

Source	*df*	Sum of Squares	Mean Square	F	p Value
Atorvastatin	1	28.678	28.678	5.84	0.018
L-Arginine	1	4.942	4.942	1.01	0.319
Interaction	1	7.575	7.575	1.54	0.219
Duration of diabetes	1	33.764	33.764	6.87	0.011
Baseline FMD	1	73.312	73.312	14.92	< 0.001
Error	69	338.949	4.912		
Total	74	487.448			

Term	Effect Size	95% Confidence Interval
Atorvastatin	1.26	0.22 to 2.30
L-Arginine	−0.51	−1.54 to 0.51
Interaction	−1.28	−3.35 to 0.78
Duration of diabetes	−0.07	0.02 to 0.13
Baseline FMD	−0.37	−0.56 to −0.18
Constant	0.37	−0.72 to 1.47

The upper table is the analysis of covariance matrix for the change in FMD over the six-week duration of the study. The lower table gives the regression coefficients (effect size). Improvement in FMD was significantly associated with atorvastatin therapy, even after taking into account the effect of baseline FMD and duration of diabetes. L-Arginine therapy had no significant benefit on FMD and attenuated the effect of atorvastatin when given in combination.
df = degrees of freedom; FMD = flow-mediated dilation.

evidence of a significant interaction between the effects of L-arginine and atorvastatin (p = 0.22), and therefore the effects of these interventions could be analyzed independently. Atorvastatin therapy was associated with a significant increase in FMD of 1.26% (95% confidence interval [CI] 0.22 to 2.30, p = 0.018), even after allowing for the effects of baseline FMD and duration of diabetes, which were the only prespecified baseline covariates that were significantly associated with a change in FMD (Table 3). There was no significant change after L-arginine −0.51% (95% CI: −1.54 to 0.51, p = 0.32). Other variables, including total, LDL and HDL cholesterol levels, presence of microalbuminuria, plasma glucose level, fructosamine level, total daily insulin dose and blood pressure, were added to the model, together with interactions between variables already in the model, but none was found to have a significant effect. To determine whether changes in other physiologic variables had influenced the improvement in endothelial function seen after atorvastatin, the changes in rest blood flow, reactive hyperemic blood flow and vessel size were added to the model, but similarly, none had a significant effect.

No significant association was found between any of the treatments and GTN-MD (atorvastatin −1.35% [95% CI −7.23 to 4.52]; L-arginine −3.50% [95% CI −9.43 to 2.43]; interaction −5.22% [95% CI −6.58 to 0.51]), indicating that the improvement in FMD seen in the atorvastatin group is likely to reflect enhanced endothelial-derived NO bioavailability.

Simple correlation coefficients were used to examine the relation between improvement in FMD and the change in cholesterol levels. There were borderline significant correlations between FMD and the change in triglyceride (r = −0.24, p = 0.04) and total cholesterol (r = −0.21, p = 0.066) levels, but not with the change in HDL (p = 0.19) or LDL (p = 0.11) cholesterol levels.

DISCUSSION

In this study, we have demonstrated that six weeks of treatment with the HMG-CoA reductase inhibitor atorvastatin improves endothelial dysfunction in young subjects with type I DM who had normal cholesterol levels. Dietary administration of L-arginine did not have a beneficial effect on endothelial function, in contrast to experimental models of type I DM and to studies in hypercholesterolemic subjects. Improvement in endothelial function in response to atorvastatin therapy may have a favorable effect on the progression of vascular disease in type I DM.

Role of cholesterol reduction in type I DM. The important role of lipids in the etiology of diabetic vascular disease, as well as a potential beneficial role for cholesterol reduction, was proposed by Joslin more than 70 years ago (40). Since then, epidemiologic studies have confirmed the link between cholesterol levels and cardiovascular morbidity and mortality (41), and recent clinical trials have demonstrated an improved outcome when lipid-lowering therapy is commenced in diabetic patients with established atherosclerosis (42). In this study, we purposefully selected subjects without grossly elevated cholesterol levels, who are likely to be representative of the majority of young patients with type I DM, to examine the impact of potentially complementary therapeutic approaches on diabetic vascular disease. Atorvastatin improved endothelial function after just six weeks of treatment. This rapid response is in keeping with the improvement in resistance vessel endothelial function reported in hypercholesterolemic subjects after two weeks of treatment with simvastatin (43). Improvement in FMD was relatively small as compared with responses seen in other

risk factor groups studied previously (8). However, the wide range of baseline FMD results seen in this study implies that not all subjects had endothelial dysfunction, reflecting the heterogeneous nature of endothelial dysfunction in type I DM. Baseline FMD was significantly associated with improvement in FMD, such that subjects with the lowest initial FMD had substantially greater improvement after six weeks of treatment. This may partly reflect regression to the mean, as baseline FMD also predicted improvement in endothelial function in the atorvastatin/placebo group (data not shown); however, these associations may also represent a biologically relevant effect in those subjects with the worst vascular disease. Further studies will be needed to determine 1) whether the benefit seen after atorvastatin therapy is restricted to specific subgroups of patients with type I DM; 2) whether the benefit is maintained or enhanced after longer periods of treatment; and 3) the significance of these effects on clinical cardiovascular disease.

The dose of atorvastatin used in this study was relatively high, as we sought to determine whether the reversal of endothelial dysfunction may be achieved within a relatively short time frame. Interestingly, improvement in endothelial function was not determined by baseline cholesterol level, and there was only a borderline significant association between the degree of cholesterol reduction and change in FMD. This may reflect additional actions of HMG-CoA reductase inhibitors that are independent of LDL cholesterol reduction, per se. Recognized effects include reduction in triglyceride levels, upregulation of endothelial NO synthase expression and effects on inflammatory cell activity (44,45), and these might be important mechanisms by which atorvastatin improved endothelial function in our subjects. Further studies will be needed to determine the effective dose of atorvastatin and its mechanism of action in restoring endothelial function in diabetes.

Role of L-arginine therapy in type I DM. After L-arginine therapy, there was a significant increase in plasma levels, but there was no benefit from L-arginine therapy on endothelial function. These data extend the results of our recent study, in which acute administration of L-arginine had no effect on endothelial function in a similar cohort of subjects with type I DM, and differs from the significant improvements seen in subjects with hypercholesterolemia (8,33). These data are consistent with fundamental differences in the pathogenesis of endothelial dysfunction between these risk factor groups. The mechanism by which L-arginine improves endothelial function remains controversial (46). In hypercholesterolemia, the effects of increased levels of endogenous competitive antagonists of NO synthase might be overcome by exogenous L-arginine (47). Few data have been published on levels of these antagonists in type I DM, but our results suggest L-arginine levels are not the rate-limiting factor in the synthesis of NO. An alternative explanation may be that additional abnormalities of the endothelium or vascular smooth muscle limited a beneficial effect of L-arginine. However, L-arginine therapy was not able to enhance endothelial function in our subjects, even when other vascular abnormalities were improved after atorvastatin therapy. Finally, recent data have indicated that endothelium-dependent dilation in response to L-arginine may partly be a humoral response mediated by release of insulin (48). This would preclude benefit in patients with type I DM, in whom endogenous insulin secretion is absent. There was a trend toward a reduction of the effect of atorvastatin in subjects who also received L-arginine. Although this interaction did not reach statistical significance, the power to detect such an effect is reduced in this small group, and it is possible that L-arginine has a deleterious effect on endothelial function in diabetes. This will have implications for its generalized use as an antiatherogenic agent (49) and highlights the need to specifically identify which patient subgroups are likely to benefit from such novel therapies.

Conclusions. Cardiovascular risk in type I DM remains high, despite improvements in the management of insulin deficiency and glycemic control (1,50). The process of atherosclerosis, manifested by abnormal conduit artery endothelial function, begins early in type I DM, even in the absence of clinical evidence of nephropathy. We have shown that, at this early stage, endothelial dysfunction in young patients with type I DM can be ameliorated by treatment with atorvastatin. In contrast, treatment with L-arginine had no significant benefit, and further studies will be required to determine its role in preventing the progression of vascular disease. Our results suggest that treatment with HMG-CoA-reductase inhibitors from an early age and at lower levels of cholesterol than presently recommended in the general population may be indicated in order to slow the progression of atherosclerosis to clinical cardiovascular disease.

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