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Oral L-arginine and vitamins E and C improve endothelial function in women with type 2 diabetes

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Abstract: Endothelial dilator function is impaired in people with type 2 diabetes mellitus (T2DM). Prior research indicates that this can be improved with intravenous administration of ascorbate or L-arginine, but whether these agents have this effect when administered by the clinically practical oral route is unknown. To investigate this question, 10 premenopausal women with T2DM and 10 healthy, premenopausal, non-diabetic women received, in random sequence, a 1-week administration of oral L-arginine (8 g daily) or vitamins E (1800 mg) and C (1000 mg) with an intervening 1-week washout period. Flow-mediated brachial artery dilation (FMD) was measured by ultrasonography and forearm blood flow was measured by plethysmography before and following blood pressure cuff-induced forearm ischemia before and after each week of treatment. At baseline, the women with T2DM had lesser FMD responses (0.028 ± 0.006 cm vs 0.056 ± 0.008 cm, p < 0.05). Post-ischemic forearm hyperemia was reduced at baseline in T2DM compared with controls (16.4 ± 1.8 vs 26.0 ± 1.4 ml 100 ml⁻¹ min⁻¹, p < 0.05). Administration of L-arginine caused a 50 ± 12% increase in FMD in T2DM (p < 0.05) and raised post-ischemic forearm blood flow by 29 ± 8% (p < 0.05). No significant changes were seen in controls. Administration of vitamins E and C in women with T2DM produced an increase in the brachial artery diameter response of 79 ± 15% (p < 0.05), but did not significantly increase the hyperemic blood flow response (p = NS). No significant changes in the responses of controls from pre to post vitamin administration were observed. We concluded that administration of two types of oral agents improved measures of endothelial function in people with T2DM.

Key words: antioxidant vitamins; diabetes; endothelial function; L-arginine

Introduction

Decreased endothelial nitric oxide activity is a common and early feature of type 2 diabetes and may contribute to the development of diabetic vascular complications. This defect can be improved by intravenous administration of L-arginine, the substrate for nitric oxide (NO) generation, and by intra-arterial administration of the antioxidant vitamin C. However, whether the clinically practical approach of oral administration of these agents can produce these effects is unclear.

Accordingly, we measured the influence of orally administered oral L-arginine and vitamins C and E on endothelial dilator function and forearm hyperemic responses in women with type 2 diabetes mellitus (T2DM) and non-diabetic controls.

Methods

Subjects

Ten women with T2DM and 10 non-diabetic women of similar body weight and age were recruited for the study (demographic data are provided in Table 1). The women ranged in age from 30 to 50 years. All the women in the study were premenopausal to avoid the potential effects of postmenopausal hormone status on endothelial function.

Hormonal status was evaluated in all women by history of regular menstrual cycles and by measurements of serum follicle stimulating hormone levels. All women were sedentary (defined as not participating in a regular exercise program) to minimize the effects of higher levels of physical activity on endothelial function. All the women were also non-smokers since smoking can impair endothelial function. In addition, no patient was exposed significantly to second-hand smoke (i.e. in the home or workplace).

The study was approved by the Colorado Multiple Institutional Review Board and all participants gave informed consent to participate in the study.

All the subjects were moderately overweight but none had a body mass index greater than 35. The presence of T2DM was documented by chart review, confirming the diagnosis of diabetes and/or presence of drug treatment for diabetes. T2DM women treated by diet or oral agents were included, but those treated with insulin were not included because this may represent more advanced disease. No patient was included if taking a lipid-lowering drug, a thiazolidenedione drug, metformin or an antihypertensive agent since these drugs can influence endothelial function. Women with T2DM were accepted for study only if they had total HbA1C levels < 9% (adequate control) on therapy.

We excluded people with diabetes who had clinically evident cardiovascular abnormalities since any fixed abnor-
mality might potentially limit endothelial dilator function. Using resting echocardiographic criteria, women were excluded if (1) regional wall motion abnormalities were present; (2) left ventricular wall thickness was >1.1 cm (LV hypertrophy); and (3) there was decreased contractility, i.e. fractional shortening <30%. People were also excluded if they had evidence of ischemic heart disease by history or an abnormal resting or exercise electrocardiogram (ECG). Those with pulmonary symptoms or systolic blood pressure >140 mmHg or diastolic pressure >90 mmHg were also excluded.

Additional exclusions were autonomic insufficiency assessed by measuring variation in RR intervals with cycled breathing and by the presence of a >20 mm fall in upright blood pressure without a change in heart rate, proteinuria (urine protein >200 mg/dl), a creatinine ≥2 mg/dl and clinically evident distal symmetrical neuropathy.

No patient was studied who had taken vitamins within the last 3 months. In addition, participants were asked to not take decongestants and non-steroidal anti-inflammatory drugs for the course of the study. Participants were also asked to refrain from caffeine, alcohol and exercise for 24 hours prior to testing.

Control subjects were screened identically to participants with T2DM. The control subjects were taking no medications and had a normal medical history.

**Study protocol**

The first visit consisted of a history and physical examination. On the second visit, participants were assigned randomly to take either 9 g of L-arginine daily or daily dosages of vitamins E (1800 mg) and C (1000 mg) for 7 days. Patients were not told which agent(s) they were taking. Blood was drawn for assay of the L-arginine level.

Subsequently, measures of endothelial-dependent vaso-dilation were assessed (at the same time of day for each subject and after an overnight fast). No patient took her assigned agent on the day on which testing occurred.

Measurements were made of brachial artery diameter response to hyperemic flow by vascular ultrasound and of hyperemic peripheral blood flow by plethysmograph. On each test day, repeated measurements were made before and after 5 min of cuff occlusion. There were rest periods of 30 min between measurements. After the test day, participants took their assigned treatment for 1 week. At the end of the week, the measurements described above were repeated. Subsequently, there was a washout period of 1 week. At this point, all measurements described above were repeated as an additional baseline. Participants were then assigned to take the other agent for 1 week and measurements were repeated at the end of this time period. A pill count was used to evaluate whether participants had taken the appropriate number of pills.

**Flow-mediated dilation**

We used brachial artery flow-mediated dilation to estimate NO-mediated dilator activity. This approach is validated by studies indicating dependence of this response on NO demonstrated by inhibition with inhibitors of nitric oxide synthase (NOS) and within-subject correlation between the brachial artery measurement and measures of coronary endothelial function obtained using the acetylcholine challenge. These measurements were made by an experienced ultrasonographer (SP), who is an expert in the measurement of brachial artery diameter and was blinded to treatment and to whether participants were controls or had diabetes. Endothelial function assessed by the brachial artery dilator response to increased blood flow has been shown to be dependent on endothelial-derived NO activity. The ultrasonographer was trained and validated in her skills (for a prior study) by Charles Mangano from the laboratory of Dr Robert Vogel and the methods used were those of that laboratory.

The brachial artery diameter was measured on B-mode ultrasound images, with the use of a 1–3 MHz linear-array transducer (Agilent Sonos 5500). Scans were obtained with the subject supine before and during reactive hyperemia that was induced by cuff inflation. Patients had a three-lead ECG attached. A narrow blood pressure cuff was placed securely on the upper right arm of all patients. The subjects lay quietly for at least 10 min before the first scan. The brachial artery was scanned in longitudinal section 2–15 cm above the antecubital fossa. The transmit (focus) zone was set to the depth of the anterior ‘m’ line (the interface between media and adventitia) because of the greater difficulty of evaluating the ‘m’ line of the anterior wall compared with that of the posterior wall. Depth and gain settings were set to optimize images of the interface between the lumen and endothelial borders.

The arterial diameter from ultrasonic images was measured at a fixed distance from the anatomical marker (such as a fascial plane or a vein seen in cross section) with the use of ultrasonic calipers by the sonographer. Measurements were taken from the anterior to the posterior ‘m’ line at end-diastole, coincidental with the R wave on a continuously recorded electrocardiogram. Baseline measurements were made first. A cuff was then inflated to a pressure of 50 mmHg over systolic pressure for 4 min, followed by release. During inflation, color Doppler was used to ensure that blood flow was completely occluded. The sonographer kept a probe on the arm to ensure that the probe site did not vary during the course of measurement. Measurements of brachial artery diameter were taken 50–70 s after deflation of the cuff. Four cardiac cycles were analyzed for each scan, and the measurements recorded. The vessel diameter in scans obtained after reactive hyperemia was expressed as a percentage of the average diameter of the artery in the two resting (or baseline) scans (considered as 100%).

**Plethysmographic measurements**

Forearm blood flow was determined in supine participants by venous occlusion strain gauge plethysmography (D.E. Hokanson Inc. Issaquah, WA, USA). Using calibrated mercury-in-silastic strain gauges positioned at the point of greatest forearm circumference and with the arm supported above the heart level, multiple measurements (five to 10 per patient) were made during resting conditions and following 5 min of supra-systolic cuff inflation (i.e. hyperemia). Rest periods of 10–15 min occurred between measurements. During all measurements, a supra-systolic wrist cuff excluded hand circulation and venous cuff pressure did not exceed 35 mmHg. The hyperemic blood flow response was defined as the peak blood flow during reactive hyperemia minus the average resting blood flow determined...
over several minutes. Forearm blood flow was calculated as ml 100 ml⁻¹ min⁻¹.

Assays

Measurement of L-arginine

These measurements were done by gas chromatography/mass spectrometry as follows. To 250 μl aliquots of sera labeled arginine was added as an internal standard (50 nM[¹⁵N₂]-arginine). This was followed by the addition of 300 μl of 50% acetic acid, and the sample was transferred to a DOWEX-50 hydrogen form column. The columns containing the extracts were washed with 2 ml water, and an amino-acid-rich fraction eluted with 1000 μl 15% NH₄). A standard curve was included which ranged from 1 to 100 nM arginine plus 50 nM internal standard. Fractions and curve were dried by (SAVANT) centrifugation under vacuum and the dried extracts and curve derivatised with 200 μl trifluoroacetic anhydride (TFAA), and heating for 20 min at 100°C.

Samples were allowed to cool and the TFAA evaporated under a stream of nitrogen at room temperature and dissolved in a solution of TFAA:acetonitrile (1:1); 1–3 μl were then analyzed by gas chromatography-mass spectrometry (GCMS). The GC column was a 15 M x 0.25 mm x 0.25 μm DB1 fused silica capillary column. The initial column temperature was 100°C, programmed to 200°C at a rate of 10°C per min. Ions monitored consisted of m/z 375 and 377 for arginine and [¹⁵N₂]-arginine TFAA respectively and m/z 03 for dimethylarginine TFA (DMA). Arginine concentration was calculated by isotope dilution standards and common test-serum samples to ensure batch-to-batch comparability.

Blood handling

All subjects’ blood was drawn in tubes free from any additives and allowed to sit at room temperature for a minimum of 2 h. The retracted clot was removed and the blood then spun for 15 min at 2100 RPM in an International Equipment Company Centra MP4. The sera was removed and the sample frozen at -70°C.

Statistical analysis

The two groups were compared using an unpaired t-test. An analysis of variance was used for comparisons within each group. Where data were skewed, the Mann–Whitney U, Wilcoxon tests or the Kruskal–Wallis test were used. Correlations were made using a Pearson’s product moment correlation coefficient.

Results

Women with T2DM and control women were of similar age (43 ± 7 years and 40 ± 6 years, respectively (p = NS)) and had similar body mass indices (30.6 ± 5.4 vs 29.2 ± 4.3, respectively (p = NS)). Women with T2DM and control women did not differ with regard to blood pressure or total cholesterol, but did differ in terms of HbA₁C as would be expected (Table 1). Nine patients were taking an oral agent for their diabetes and one was treated by diet and exercise.

Baseline measurements

There were significant differences at baseline before treatment in vascular responses between groups. T2DM participants had smaller post-ischemic hyperemic increases in both brachial artery diameter and forearm blood flow than controls (Table 2). Specifically, participants with T2DM had lesser hyperemic brachial artery dilator (change in DM=0.028±0.006 cm vs change in control = 0.056±0.008 cm, p < 0.05) and hyperemic blood flow (change in DM = 16 ml 100 ml⁻¹ min⁻¹ vs change in control = 26 ml 100 ml⁻¹ min⁻¹, p < 0.05) responses.

Response to L-arginine

Oral administration of L-arginine increased the brachial artery diameter response to cuff inflation by 50 ± 12% (p < 0.05) over the baseline response in participants with DM (Table 2, Figure 1), while no significant increase was seen in the response of controls. L-Arginine increased the brachial artery responses of participants with DM to levels no longer significantly different from baseline values in controls. Hyperemic forearm blood flow response increased after L-arginine administration by 29 ± 8% in participants with T2DM (p < 0.05) (Table 2, Figure 2), whereas no significant change in the response was seen in controls after a week of L-arginine. Although the blood flow pre–post stress response was greater in the patients with T2DM than in controls after L-arginine administration, the absolute value for hyperemic blood flow remained significantly higher for controls (p < 0.05 comparison between DM and control participants).

Response to vitamins E and C

Oral administration of vitamins E and C for 1 week increased the brachial artery diameter response to hyperemia in participants with T2DM by 79 ± 15% (p < 0.05) (Table 2, Figure 1), whereas no significant change was found in controls. The forearm hyperemic blood flow response to cuff inflation did not increase significantly in the T2DM participants (p = NS) (Table 2, Figure 2). Blood flow response post cuff inflation showed a trend toward an increase which achieved borderline significance in controls (21 ± 15%, p = 0.09) after vitamins E and C.

Table 1 Characteristics of women with T2DM and controls.

<table>
<thead>
<tr>
<th>Age</th>
<th>SBP</th>
<th>DBP</th>
<th>HbA₁C</th>
<th>Total cholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td>T2DM</td>
<td>43 ± 7</td>
<td>115 ± 13</td>
<td>78 ± 5</td>
<td>7.9 ± 1.5</td>
</tr>
<tr>
<td>Controls</td>
<td>40 ± 6</td>
<td>121 ± 13</td>
<td>75 ± 5</td>
<td>4.8 ± 0.5*</td>
</tr>
</tbody>
</table>

Data are mean ± SD. *p < 0.05 difference between persons with T2DM and controls. SBP, systolic blood pressure; DBP, diastolic blood pressure; HbA₁C, hemoglobin A₁C. No significant differences in values, other than the HbA₁C, were found between women with T2DM and controls.
Table 2  Effects of L-arginine or vitamins E and C on endothelial dilator function in T2DM compared with controls.

<table>
<thead>
<tr>
<th></th>
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<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>Brachial artery diameter (cm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T2DM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>0.412 ± 0.011</td>
<td>0.411 ± 0.019</td>
<td>0.420 ± 0.014</td>
<td>0.416 ± 0.010</td>
</tr>
<tr>
<td>Post</td>
<td>0.440 ± 0.018(\ast)(\ast)(\ast)</td>
<td>0.453 ± 0.017(\ast)(\ast)(\ast)</td>
<td>0.444 ± 0.015(\ast)(\ast)(\ast)</td>
<td>0.459 ± 0.038(\ast)(\ast)(\ast)</td>
</tr>
<tr>
<td>% change over pre-hyperemic state</td>
<td>6 ± 5%</td>
<td>11 ± 8%(\ast)</td>
<td>6 ± 5%</td>
<td>11 ± 9%(\ast)</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>0.402 ± 0.011</td>
<td>0.412 ± 0.014</td>
<td>0.402 ± 0.011</td>
<td>0.407 ± 0.012</td>
</tr>
<tr>
<td>Post</td>
<td>0.458 ± 0.011(\ast)</td>
<td>0.453 ± 0.011(\ast)</td>
<td>0.465 ± 0.005(\ast)</td>
<td>0.453 ± 0.013(\ast)</td>
</tr>
<tr>
<td>% change over pre-hyperemic state</td>
<td>16 ± 9%</td>
<td>10 ± 12%</td>
<td>16 ± 9%</td>
<td>12 ± 10%</td>
</tr>
<tr>
<td>Blood flow by plethysmography (ml 100 ml(^-1) min(^{-1}))</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T2DM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>3.1 ± 0.3</td>
<td>3.7 ± 0.3</td>
<td>3.2 ± 0.3</td>
<td>3.4 ± 0.4</td>
</tr>
<tr>
<td>Post</td>
<td>19.5 ± 1.8(\ast)(\ast)(\ast)</td>
<td>23.8 ± 1.2(\ast)(\ast)(\ast)</td>
<td>21.5 ± 2.3(\ast)(\ast)(\ast)</td>
<td>24.8 ± 2.7(\ast)(\ast)(\ast)</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>3.4 ± 0.2</td>
<td>3.9 ± 0.4</td>
<td>3.6 ± 0.3</td>
<td>3.7 ± 0.3</td>
</tr>
<tr>
<td>Post</td>
<td>29.4 ± 1.6(\ast)</td>
<td>29.7 ± 2.1(\ast)</td>
<td>27.7 ± 2.1(\ast)</td>
<td>32.8 ± 1.5(\ast)</td>
</tr>
</tbody>
</table>

Data are mean ± SEM.\(\ast\)\(p < 0.05\) difference between pre- and post-stress values within groups.\(\ast\)\(\ast\)\(p < 0.05\) difference between values of women with T2DM and non-diabetic controls.\(\ast\)\(\ast\)\(\ast\)\(p < 0.05\) difference between T2DM and non-diabetic controls of the change in pre–post cuff inflation response after treatment with either L-arginine or vitamins E and C compared with the change in pre–post cuff inflation of the respective pre-treatment values.

Pre-stress, before cuff inflation; Post-stress, after cuff inflation; T2DM, type 2 diabetes mellitus; Control, non-diabetic control.

![Figure 1](image_url) Change in hyperemic brachial artery dilation in response to cuff inflation before and after administration of L-arginine (left panel) and antioxidant vitamins (right panel) to women with T2DM (dashed line) compared with control subjects (solid line). The percentage change of the response from baseline to post-treatment is shown on the figure for both women with T2DM and controls.

**Assay values**

Oral administration of L-arginine for 1 week increased L-arginine levels significantly in the group with T2DM by 64\%(p < 0.05) (Table 3) and tended to increase the control value as well \(p = 0.09\).

**Discussion**

We found that at baseline, subjects with T2DM had depressed brachial artery dilator responses to post-ischemic hyperemia when compared with non-diabetic control sub-
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Figure 2 Change in forearm blood flow in response to cuff inflation measured by plethysmography. Changes are shown before and after administration of l-arginine (left panel) and antioxidant vitamins (right panel) to women with T2DM (dashed line) compared with control subjects (solid line). The percentage change of the response from baseline to post-treatment is shown on the figure for both women with T2DM and controls.

Table 3 l-Arginine level differences after administration of arginine or vitamins E and C.

<table>
<thead>
<tr>
<th></th>
<th>l-Arginine level at baseline pre-arginine</th>
<th>l-Arginine level after arginine administration</th>
<th>l-Arginine level at baseline pre-vitamin E and C</th>
<th>l-Arginine level after vitamin E and C administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>T2DM</td>
<td>77.8 ± 7.2</td>
<td>127.3 ± 9.6</td>
<td>90.6 ± 10.3</td>
<td>81.3 ± 7.4</td>
</tr>
<tr>
<td>Control</td>
<td>83.6 ± 13.0</td>
<td>115.6 ± 16.0</td>
<td>86.9 ± 7.8</td>
<td>95.2 ± 10.4</td>
</tr>
</tbody>
</table>

Data are mean ± SEM.

*p < 0.05 difference between pre- and post-arginine administration. Note that values for the two separate baselines did not differ within groups.

jects. Oral administration of l-arginine or vitamins E and C increased these responses to levels no longer different from those in non-diabetic subjects. The subjects with T2DM also had depressed forearm hyperemic blood flow responses following cuff-induced ischemia that increased after l-arginine treatment. Neither l-arginine nor vitamins E and C produced a significant change in brachial artery diameter or hyperemic blood flow responses in control subjects. The present findings suggest that oral administration of either l-arginine or vitamins E and C leads to augmentation of endothelial function in subjects with T2DM, with l-arginine having a stronger effect.

The study reaffirmed the presence of impaired endothelial dilator function in participants with T2DM who are free of apparent cardiovascular complications. Since body mass indices were similar between the groups, the findings cannot be accounted for by obesity but likely relate to the presence of T2DM. In addition, all subjects were sedentary such that differing degrees of inter-group habitual physical activity could also not account for the differences.

The present study also showed that in subjects with T2DM at baseline there was a reduction of hyperemic flow following forearm ischemia, suggesting an impairment of the regulation of blood flow in relation to metabolic demand. Post-ischemic hyperemia is mechanistically more complex than the brachial artery dilator response and is dependant on several factors including tissue hypoxia, hypercarbia and increased extracellular potassium. However, our finding of increased hyperemia following measures that improved endothelial NO function in subjects with T2DM suggests a significant role for NO. The finding that administration of l-arginine or vitamins E and C increases the blood flow response to metabolic demand may be of considerable importance in T2DM, which is characterized by under-perfusion of heart, brain and skeletal muscle at rest and during exercise.

That oral l-arginine administration improved endothelial dilator function in subjects with T2DM is in accord with findings that intravenous administration of l-arginine corrects deficient NO-mediated dilator function in a wide array of cardiovascular disorders and risk conditions. In people with T2DM, Chowienczyk et al reported that intravenous administration of l-arginine improved NO-mediated vasodilation, but had no such effect in people with type 1 DM. We could find no prior reports of the finding in the present study of enhanced endothelial dilator function following oral administration of l-arginine.

The mechanism by which l-arginine enhances endothelial dilator function remains unclear. Usual circulating levels of l-arginine in healthy people are thought to provide sufficient substrate for NO generation under normal circumstances, but in disorders associated with endothelial dysfunction there may be defects in active arginine cellular uptake, inhibitory effects of other amino acids and the gen-
eration of endogenous competitive inhibitors of NO synthase. All of these might be corrected by competitive reversal by exogenous L-arginine administration. Interestingly, in addition to its potential effects on NO generation, L-arginine may also act as an antioxidant.

Several mechanisms have been proposed to explain the endothelial dysfunction of T2DM. These include decreased NO production due to diabetes-related endothelial injury, increased degradation of NO by oxygen-derived free radicals, or oxidant-induced attenuation of endothelial NO synthase activity. Evidence supporting a role for oxidants in the endothelial dysfunction of T2DM include several studies showing that antioxidants reverse endothelial NO activity in diabetic animals and in patients with T2DM. Specifically, intra-arterial administration of vitamin C is found to rapidly increase endothelium-dependent vasodilation in patients with T2DM. A newer oral antioxidant, raxofeostal, was also shown to improve endothelial function in men with T2DM. However, in a study where oral vitamin E alone was administered to people with T2DM, no effect on endothelial function was observed. Rats rendered insulin-resistant, but not diabetic, by fructose feeding develop deficient endothelial NO dilator activity related to oxidant-mediated degradation and deficiency of the NO synthase cofactor, tetrahydrobiopterin. Since insulin resistance is a central feature of T2DM, these findings raise the possibility that antioxidants improve endothelial NO activity by preservation of tetrahydrobiopterin levels. Ascorbate also promotes release of NO from nitrosothiols and regeneration of NO from its breakdown product, nitrite.

An additional potential mechanism by which improvement in endothelial function may have occurred in the present study relates to the increased concentrations of the endogenous NOS antagonist asymmetric dimethylarginine (ADMA) found in patients with insulin resistance and T2DM and in in vitro studies of hyperglycemia. Importantly, ADMA acts as a competitive inhibitor of NOS and therefore the effect of ADMA on vascular function is reversed by L-arginine. In addition, ADMA accumulation in the condition of hyperglycemia can be blocked by antioxidant enzymes. These findings may help explain the beneficial effects of L-arginine and the antioxidant vitamins on endothelial dysfunction observed in this study.

Unanticipated observations were the decreases in brachial artery flow-mediated dilatation in normal subjects following administration of both L-arginine and vitamins C and E. In an attempt to further analyze this effect we sought to determine whether increased brachial artery dilatation during baseline measurements in control subjects might have decreased the observed increment following the ischemia induced by cuff inflation. We did indeed find small non-significant increases in the post-treatment resting brachial artery diameters in normal but not T2DM subjects. However, we also found similar decrements in the post-ischemic diameters in the normal subjects in the post-treatment phase (Table 2). Thus, the trend towards decreased flow-mediated dilatation in controls reflected changes both in baseline and post-ischemic components. We have no explanation for this.

Potential weaknesses of our study included the presence of a 1-week washout period separating the two random sequence treatment phases of the study. This is a particular problem with respect to the likely persistence of the fat-soluble vitamin E for which levels likely remained elevated for longer than 7 days following its discontinuation in subjects treated with vitamins in the first experimental period. However, this washout interval was sufficient to restore measurements of endothelial function to values similar to those measured at the initial baseline. Although we did not compare the separate contributions of vitamins E and C, the prompt recurrence of endothelial dysfunction following such a brief washout suggests a potentially greater role for vitamin C than E, a suggestion supported by the reported failure of vitamin E alone to reverse endothelial NO function in patients with T2DM.

We also did not include tests to determine whether the treatments were specific to endothelial-mediated dilatation or also enhanced endothelium-independent dilator responses. Evidence cited above has demonstrated that the hyperemic brachial artery dilator response is a reasonably specific indicator of endothelial, NO-mediated dilator function. However, although the findings indicate that endothelial dilator responses were enhanced, we cannot exclude an additional augmentation of non-endothelial dilator function.

It should be emphasized that the findings are most directly pertinent to people with T2DM who have neither signs nor symptoms of cardiovascular complications. It is possible that patients with advanced and complicated disease may be less responsive to the agents used in this study. Further research will evaluate the treatment of endothelial dysfunction in the case of advanced disease.

In conclusion, we found that oral administration of L-arginine and of antioxidant vitamins caused significant improvements in endothelial function and hyperemic blood flow in T2DM. Because endothelial dysfunction is thought to be an early factor in the progression of abnormalities of vascular structure and function, it seems possible that these relatively safe, inexpensive and readily available agents might attenuate the development of vascular complications of T2DM.

Acknowledgements

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