Ascorbic Acid Reduces Blood Pressure and Arterial Stiffness in Type 2 Diabetes

Brian A. Mullan, Ian S. Young, Howard Fee, David R. McCance

Abstract—Experimental evidence suggests that acute parenteral administration of high-dose ascorbic acid has beneficial vascular effects in type 2 diabetes. We studied the hemodynamic effects of chronic oral supplementation in this condition. Thirty patients, 45 to 70 years of age, with type 2 diabetes, were randomly assigned in a double-blind manner to receive 500 mg ascorbic acid daily by mouth or placebo. Patients were studied at baseline and after 4 weeks of assigned treatment. The central aortic augmentation index (AgIx) and the time to wave reflection (Tr) were derived from radial artery pulse wave analysis data. AgIx and Tr were used as measures of systemic arterial stiffness and aortic stiffness, respectively. Ascorbic acid decreased brachial systolic blood pressure from 142.1 ± 12.6 (SD) to 132.3 ± 12.1 mm Hg (difference [95% CI] 9.9 [4.7, 15.0]; \( P < 0.01 \)), brachial diastolic pressure from 83.9 ± 4.8 to 79.5 ± 6.0 mm Hg (4.4 [1.8, 7.0]; \( P < 0.01 \)), and AgIx from 26.8 ± 5.5% to 22.5 ± 6.8% (4.3 [1.5, 7.1]; \( P < 0.01 \)). Tr increased from 137.1 ± 12.6 to 143.4 ± 9.2 ms (6.3 [−10.1, −2.5]; \( P < 0.01 \)). Placebo had no hemodynamic effects, and this difference between treatments was significant (\( P < 0.01 \) for blood pressure and Tr, \( P = 0.03 \) for AgIx). We have therefore shown that after 1 month, oral ascorbic acid lowered arterial blood pressure and improved arterial stiffness in patients with type 2 diabetes. As strict control of blood pressure reduces cardiovascular risk in diabetes, ascorbic acid supplementation may potentially be a useful and inexpensive adjunctive therapy. Larger and longer studies now need to be performed. (Hypertension. 2002;40:804-809.)

Key Words: blood pressure • antioxidants • arteries • vitamins • diabetes mellitus

Type 2 diabetes mellitus is one of the most common chronic illnesses in the world.\(^1\) The major cause of death in individuals with this condition is macrovascular disease. Impaired endothelial function, characterized by reduced nitric oxide bioactivity, is an early feature of diabetes.\(^2\) and endothelial dysfunction is now thought to have an important role in the pathophysiology of atherosclerosis.\(^3\) Deficiency of endothelium-derived nitric oxide affects vascular tone, leukocyte adhesion to the endothelium, platelet aggregation, and vascular smooth muscle proliferation. Endothelial dysfunction may also contribute to the increased arterial stiffness observed in type 2 diabetes.\(^4\) Increasing evidence suggests that arterial stiffness may be an important additional and independent risk factor for cardiovascular disease.\(^5\) Stiffening of the arterial tree increases the velocity and amplitude of pulse waves reflected from the periphery back to the heart.\(^6\) This results in larger reflected waves reaching the ascending aorta earlier and augmenting the central systolic pressure. Augmentation of central systolic pressure increases left ventricular workload and therefore myocardial oxygen demand.

Endothelial dysfunction in type 2 diabetes may be secondary to a number of factors such as hyperglycemia, insulin resistance, hypertension, or dyslipidemia. Ascorbic acid deficiency may also be important.\(^7,8\) Plasma ascorbate concentrations are reduced in type 2 diabetes, and this does not appear to be due to inadequate dietary intake.\(^9\) In addition, the reductive enzymatic recycling of ascorbic acid may be attenuated.\(^10\) Ascorbate has a number of important biological functions, including enhancement of endothelial nitric oxide bioactivity.\(^11\) Diminished tissue concentrations and impaired recycling of ascorbic acid may therefore exacerbate diabetic endothelial dysfunction.

Intra-arterial administration of ascorbic acid has been reported to reverse impaired endothelium-dependent vasodilation in a number of conditions associated with increased cardiovascular risk, including diabetes.\(^12,13\) Ceriello et al\(^14\) have shown that a large bolus of intravenous ascorbate can reduce blood pressure in type 1 diabetes. Although the observed hypotensive effect was acute, it was short-lived. Pilot data of our own from patients with type 2 diabetes suggest that high-dose intravenous ascorbic acid can also acutely decrease systemic arterial stiffness, as assessed by the aortic augmentation index.\(^15\) We have therefore performed a randomized, double-blind, placebo-controlled study to investigate the hypothesis that chronic supplementation with oral ascorbic acid may help to reduce systemic arterial stiffness in type 2 diabetes.
Methods

Subjects

Patients with type 2 diabetes, 45 to 70 years of age and controlled with diet or oral hypoglycemic agents, were recruited from local outpatient clinics. The duration of illness was <10 years, and treatment was unchanged for 3 months before and during the study. Antihypertensive therapy was permitted, but diastolic blood pressure had to be <95 mm Hg. Other exclusion criteria included macrovascular disease, proliferative diabetic retinopathy, urinary albumin/creatinine ratio >3 mg/mmol, smoking, total cholesterol >8 mmol/L, triglycerides >5 mmol/L, and the use of antioxidants or hormone replacement therapy. The study had approval from the Research Ethics Committee of Queen’s University Belfast, and all patients gave written informed consent.

Study Design

Study patients were randomly assigned in a double-blind manner to receive either oral ascorbic acid (500 mg daily) or matched placebo. The Pharmacy Department of the Royal Group of Hospitals, Belfast, was responsible for the supply and randomization of the medication. Patients were examined at baseline and after 4 weeks of the assigned treatment. Existing antihypertensive therapies were continued throughout the study period. The examinations were conducted in the morning after an overnight fast and involved the collection of blood and urine samples, the measurement of brachial arterial blood pressure, and biochemical indexes. All medications were withheld on the morning of testing. Compliance was assessed by tablet count and measurement of plasma ascorbate concentration.

Blood Pressure Measurement and Pulse Wave Analysis

After 30 minutes of supine rest, brachial blood pressure was measured with the validated Omron HEM-705CP oscillometric sphygmomanometer. Measurements were made in triplicate and averaged. Central (ascending aortic) pressure waveforms were derived and analyzed using the technique of pulse wave analysis (SphygmoCor-P\textsuperscript{\textcircled{C}}; PWV Medical), as previously described. The features of the central aortic pressure waveform are illustrated in Figure 1. Augmentation was defined as the difference between the second systolic peak (P\textsubscript{2}; caused by wave reflection) and the first systolic peak (P\textsubscript{1}; caused by left ventricular contraction). The augmentation index (AgIx) was this difference expressed as a percentage of the central pulse pressure. Because the AgIx is partly dependent on the heart rate, all the values reported in this study were corrected for a heart rate of 72 beats/min. The AgIx was used to estimate overall systemic arterial stiffness. Pilot data from our unit allowed us to estimate reproducibility. In 54 diabetic and nondiabetic subjects, 21 to 66 years of age, the mean±SD of the difference between repeated measurements was 0.2±1.7%. This compares favorably with other published studies.

The time to wave reflection (Tr) was the time between the foot of the wave pressure and the inflection point on the central pressure waveform and provided a measure of the transit time between the ascending aorta and the first main reflectance site (the aortic bifurcation). Tr was therefore used to assess aortic pulse wave velocity and hence aortic stiffness. The subendocardial viability ratio (SEVR) was calculated as the ratio of the area under the central pressure time curve during diastole to that during systole. SEVR has been shown to be a measure of the propensity for myocardial ischemia on the basis of altered hemodynamic forces.

Biochemical and Hematologic Evaluation

At each visit, blood was sent to the hospital’s biochemistry and hematology laboratories for measurement of plasma glucose, insulin, HbA\textsubscript{1c}, and lipids. Plasma ascorbic acid concentrations were measured from samples of EDTA-anticoagulated blood, by means of the technique described by Vuillermier and Keck.

Statistical Analysis

Results were analyzed with the SPSS statistical package (SPSS Inc). Paired and unpaired 2-tailed Student t tests were used to compare characteristics within or between groups as appropriate. Two-way ANOVA for repeated measures was used to compare the effects of ascorbic acid with placebo. Data were reported as mean±SD unless otherwise stated.

Sample size calculations were based on pilot data from our unit, which showed that the standard deviation of the aortic augmentation index, in patients with type 2 diabetes, was 4.6%. Therefore, it was estimated that 15 patients per group would give 80% power, at the 5% level of significance, to detect an absolute difference in augmentation index of 5%.

Results

The baseline characteristics of the placebo and ascorbic acid treated groups are shown in Table 1. Fifteen patients were recruited to placebo and 15 to ascorbic acid. The groups were matched for age, gender, physical characteristics, glycemic control, dyslipidemia, and fasting plasma insulin levels. Physical and biochemical indexes did not change in either group.
Effect of Placebo and Ascorbic Acid Treatment on the Central Aortic Hemodynamics Derived From Peripheral Pulse Wave Analysis

Ascorbic acid (500 mg) by mouth daily for 4 weeks decreased plasma levels increased from 43.3±19.3 to 78.1±19.5 μmol/L (P<0.001). As expected, no change was observed with placebo, and the difference between treatments was significant (P<0.01). There was an inverse correlation between mean arterial blood pressure and plasma ascorbic acid concentration (r=−0.39; P=0.002; Pearson correlation coefficient).

Discussion
Ascorbic acid (500 mg) by mouth daily for 4 weeks decreased blood pressure, reduced systemic arterial stiffness (as as-

### Table 2: Effect of Placebo and Ascorbic Acid Treatment on the Central Aortic Hemodynamics Derived From Peripheral Pulse Wave Analysis

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Ascorbic Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline</strong></td>
<td>HR, beats/min</td>
<td>75.1±10.1</td>
</tr>
<tr>
<td></td>
<td>ASP, mm Hg</td>
<td>129.7±11.7</td>
</tr>
<tr>
<td></td>
<td>ADP, mm Hg</td>
<td>85.1±6.4</td>
</tr>
<tr>
<td></td>
<td>Ag, mm Hg</td>
<td>11.9±4.7</td>
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<tr>
<td></td>
<td>AgIx, %</td>
<td>28.0±7.4</td>
</tr>
<tr>
<td></td>
<td>Tr, ms</td>
<td>138.5±10.2</td>
</tr>
<tr>
<td></td>
<td>SEVR, %</td>
<td>147.3±20.2</td>
</tr>
<tr>
<td><strong>Difference</strong></td>
<td>HR, beats/min</td>
<td>1.7 (−0.6, 4.1)</td>
</tr>
<tr>
<td></td>
<td>ASP, mm Hg</td>
<td>1.0 (−3.5, 5.4)</td>
</tr>
<tr>
<td></td>
<td>ADP, mm Hg</td>
<td>−0.6 (−2.8, 1.7)</td>
</tr>
<tr>
<td></td>
<td>Ag, mm Hg</td>
<td>0.2 (−1.8, 2.2)</td>
</tr>
<tr>
<td></td>
<td>AgIx, %</td>
<td>0.5 (−1.6, 2.5)</td>
</tr>
<tr>
<td></td>
<td>Tr, ms</td>
<td>0.4 (−2.1, 2.9)</td>
</tr>
<tr>
<td></td>
<td>SEVR, %</td>
<td>0.5 (−12.9, 13.9)</td>
</tr>
</tbody>
</table>

Hemodynamic data are presented as mean±SD. Final column shows the P value for 2-way repeated measures analysis of variance. HR indicates heart rate; ASP, aortic systolic pressure; ADP, aortic diastolic pressure; Ag, augmentation; AgIx, augmentation index (corrected for heart rate); Tr, time to wave reflection; and SEVR, subendocardial viability ratio.

*P<0.05; †P<0.01; ‡P<0.001 vs baseline (paired t test).
sessed by the augmentation index and pulse pressure), and increased aortic compliance (as assessed by the timing of the reflected pressure wave). The improvement in arterial stiffness after a relatively short treatment time suggests a functional rather than structural change in the vasculature. There is increasing evidence to suggest that nitric oxide plays an important role in the functional regulation of large-artery stiffness. Enhanced endothelial nitric oxide bioactivity may explain our observed changes in blood pressure and arterial stiffness.

**Ascorbic Acid and Endothelial Nitric Oxide Bioactivity**

There are a number of mechanisms whereby ascorbic acid may increase nitric oxide bioactivity. Oxidative stress is increased in diabetes. Free radicals such as the superoxide anion can degrade nitric oxide. Ascorbic acid is an extremely potent free radical scavenger and may thus protect nitric oxide from excessive degradation. However, it has recently been reported that supraphysiological concentrations of ascorbate are required to prevent the interaction of superoxide and nitric oxide.

LDL particles are small and dense in type 2 diabetes and are susceptible to oxidation. Oxidized LDL is directly toxic to endothelial cells and can impair the endothelial production of nitric oxide. Ascorbic acid is a lipid-soluble antioxidant and protects LDL particles from oxidative attack. Ascorbate is required for the regeneration of α-tocopherol. Ascorbic acid may thus prevent LDL oxidation, either through the recycling of α-tocopherol or by scavenging free radicals directly.

Ascorbic acid may also enhance endothelial nitric oxide synthase activity. This may be secondary to the regulation of redox state but may also be due to an increase in the intracellular content of tetrahydrobiopterin.

Vascular smooth muscle is another potential site for the action of ascorbic acid. In vitro experiments have shown that guanylate cyclase sensitivity to nitric oxide may be enhanced after ascorbic acid administration.

Finally, ascorbic acid may reduce insulin resistance. Insulin can cause endothelium-dependent, nitric oxide–mediated vasodilation. By improving insulin sensitivity, ascorbic acid may increase nitric oxide release from the endothelium. It should be noted, however, that we observed no changes in glycemia or in fasting plasma insulin values.

Despite the possible mechanisms proposed above, to date only one small in vivo study in humans has provided direct evidence for oral ascorbic acid enhancement of nitric oxide bioactivity and corresponding reduction in arterial blood pressure. A recent publication by Duffy et al reaffirmed the findings of a previous report by the authors showing that chronic treatment of hypertensive patients with oral ascorbic acid, in a dose sufficient to double plasma levels, resulted in a reduction in mean arterial blood pressure of $\approx 10$ mm Hg. However, they were unable to show any beneficial effect of ascorbic acid on conduit vessel vasomotor function. Perhaps the plasma levels achieved with oral dosing have a greater effect on resistance vessels.

**Ascorbic Acid and Cardiovascular Risk**

A recent large, prospective population study confirmed previous epidemiologic evidence for an inverse relation between plasma ascorbic acid concentration and cardiovascular death. This study showed that increasing plasma ascorbic acid concentration was strongly and independently associated with reduction in risk of death from all causes, cardiovascular disease, and ischemic heart disease, with a dose-response relation across the whole population distribution. We found in subjects with type 2 diabetes that a $34.8 \mu$mol/L increase in plasma ascorbate reduced systolic blood pressure by 9.9 mm Hg. This is of similar magnitude to that reported by Duffy et al in hypertensive patients. Interestingly, a controlled diet study has also shown an inverse relation between plasma ascorbate and blood pressure. Further studies to elucidate the dose-response effect are warranted.

Unfortunately, results from randomized trials to test the influence of antioxidants on coronary event rates and prognosis have been disappointing. The HOPE and GISSI-Prevenzione trials failed to demonstrate any improvement in patients with coronary artery disease. One explanation for the negative results may be the use of vitamin E (α-tocopherol) as the sole antioxidant supplement. When α-tocopherol reacts with a radical, it becomes the α-tocopheroxyl radical, which itself may participate in pro-oxidative events. As discussed earlier, adequate concentrations of ascorbate are required for the regeneration of α-tocopherol. Therapeutic use of vitamin E, without additional vitamin C, may therefore be scientifically unsound. A recent randomized controlled trial by Brown et al has investigated the combined effects of vitamins E and C on cardiovascular risk reduction. This study examined the effect of simvastatin-niacin and antioxidant therapy (vitamin E, vitamin C, β-carotene, and selenium), alone and together, on secondary prevention. Significant beneficial clinical effects were, however, only observed in the simvastatin-niacin group, and it was concluded that there was little justification for the use of antioxidant vitamins for the prevention of cardiovascular events. This study did have a number of limitations. The study patients were not a uniform group and included diabetics (types 1 and 2) and nondiabetics, normotensive and hypertensives, and smokers and non-smokers. The simvastatin-niacin group was smaller than the other groups and had a significantly lower prevalence of diabetes. Because diabetes is associated with a greater risk of cardiovascular complications, this difference between the groups may have affected the findings.

Not all randomized, controlled trials with ascorbic acid have shown a blood pressure–lowering effect. Two negative studies by Lovat et al and Ghosh et al used doses of ascorbic acid similar to those used in our study. However, the patients in these studies were older than our patients, and they had much higher baseline blood pressures. The study by Lovat et al was also difficult to interpret because of possible interaction between the crossover periods. Ghosh et al did show a significant reduction in both systolic and diastolic blood pressures with ascorbic acid, but these became nonsignificant when the reductions were compared with the placebo responses. In addition, the placebo and ascorbic acid groups
in this study were not evenly matched for baseline plasma ascorbate concentration.

Why, in our patient group, have we observed beneficial cardiovascular effects with ascorbic acid supplementation? There are a number of possibilities. Type 2 diabetic subjects have multiple risk factors for cardiovascular disease, for example, hyperglycemia, insulin resistance, dyslipidemia, and hypertension. Each of these factors, separately, may be associated with increased free radical generation.47 Diabetic patients may therefore have greater levels of oxidative stress than other groups of patients at risk of cardiovascular disease and may respond more favorably to antioxidant supplementation. These patients may also be more deficient in ascorbate than the general population. Previous studies in type 2 diabetes have revealed diminished tissue levels and impaired recycling mechanisms for ascorbic acid.9,10 The individuals in the study all came from Northern Ireland. This region has a high prevalence of cardiovascular disease.48 It is therefore possible that genetic and/or environmental factors may also have influenced the response of our patients to treatment with ascorbic acid.

Study Limitations
It might be argued that we should only have studied normotensive type 2 diabetic patients and that the beneficial hemodynamic effects of ascorbic acid may only occur in diabetic subjects with raised blood pressure. However, hypertension is a common manifestation of type 2 diabetes and insulin resistance.49 Inclusion of these subjects gives our study greater clinical relevance. Evidence that ascorbic acid had a beneficial effect in type 2 diabetes, independent of existing hypertension, comes from the fact that there was no correlation between baseline blood pressure and the fall in blood pressure after ascorbic acid supplementation. Also, blood pressure values in the diabetic patients were similar to those obtained in a group of healthy, older subjects studied by Fotherby et al.50 This latter study reported no change in clinic blood pressure but a small decrease in daytime ambulatory systolic blood pressure (2.0±0.5 mm Hg) after 3 months of 500 mg per day ascorbic acid supplementation. Therefore, baseline blood pressure cannot explain the significant and clinically relevant blood pressure–lowering effects of ascorbic acid in type 2 diabetes. The reduction in systemic arterial stiffness and aortic stiffness could have been a consequence of the fall in blood pressure.6 However, previous experiments have shown that ascorbic acid can decrease arterial stiffness independent of blood pressure.15,51

Perspectives
The incidence of type 2 diabetes is increasing worldwide, and patients with diabetes have a greatly increased risk of death from cardiovascular disease. This risk may be secondary to associated factors such as hyperglycemia, insulin resistance, dyslipidemia, and hypertension. Increased arterial stiffness has also been observed in type 2 diabetes. Stiffening of the arterial tree is now thought to be an important additional and independent risk factor for cardiovascular disease. Plasma ascorbate concentrations are known to be diminished in diabetes, and the recycling of ascorbic acid may be impaired. Experimental evidence suggests that acute parenteral administration of high-dose ascorbic acid may have beneficial vascular effects in diabetes. We have shown in this pilot study that chronic daily supplementation with 500 mg oral ascorbic acid can lower blood pressure and improve arterial stiffness in patients with type 2 diabetes. Strict control of blood pressure is known to reduce the incidence of macrovascular complications and death in these patients.52 Ascorbic acid supplementation may therefore be a useful and inexpensive adjunctive therapy. Priority funding should now be given to the performance of larger and longer studies.

Acknowledgments
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