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Vitamin C Improves Endothelium-Dependent Vasodilation in Patients With Insulin-Dependent Diabetes Mellitus

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Objectives. **We sought to determine whether the antioxidant vitamin C improves endothelium-dependent vasodilation of forearm resistance vessels in patients with insulin-dependent diabetes mellitus.**

Background. **Endothelium-dependent vasodilation is impaired in patients with diabetes mellitus. Oxidatively mediated degradation of endothelium-derived nitric oxide contributes to abnormal endothelium-dependent vasodilation in animal models of diabetes mellitus.**

Methods. **The study group included 10 patients with insulin-dependent diabetes mellitus and 10 age-matched control subjects. Forearm blood flow was determined by venous occlusion plethysmography. Endothelium-dependent vasodilation was assessed by intraarterial infusion of methacholine (0.3 to 10 μg/min). Endothelium-independent vasodilation was assessed by intraarterial infusion of nitroprusside (0.3 to 10 μg/min). Forearm blood flow dose–response curves were determined for each drug infusion before and during concomitant infusion of vitamin C (24 mg/min).**

Results. **In diabetic subjects, endothelium-dependent vasodilation was augmented by the concomitant infusion of vitamin C ($p = 0.001$). Endothelium-independent vasodilation was not affected by the concomitant infusion of vitamin C (p = NS). In control subjects, vitamin C infusion did not affect endothelium-dependent vasodilation (p = NS).**

Conclusions. **Vitamin C selectively restores the impaired endothelium-dependent vasodilation in the forearm resistance vessels of patients with insulin-dependent diabetes mellitus. These findings indicate that nitric oxide degradation by oxygen-derived free radicals contributes to abnormal vascular reactivity in humans with insulin-dependent diabetes mellitus.**

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Patients with diabetes mellitus develop atherosclerotic vascular disease earlier and with greater severity than nondiabetic subjects (1–7). Approximately 35% of patients with insulin-dependent diabetes mellitus die of coronary artery disease by the age of 55 years (1). Endothelial dysfunction may occur early in the process of atherogenesis and has been observed before the development of overt atherosclerosis in patients with diabetes (8–13).

The endothelium plays a critical role in regulating vascular function, in part by the elaboration and release of nitric oxide. Endothelium-derived nitric oxide reduces vascular resistance, inhibits platelet and leukocyte adhesion to the vascular wall and attenuates vascular smooth muscle proliferation (14–18). Among the potential mechanisms contributing to endothelial dysfunction in diabetic patients is inactivation of nitric oxide by oxygen-derived free radicals. Indeed, studies in animal models have demonstrated that administration of antioxidants restores normal endothelial function (19–23).

The intent of the present study was to assess whether oxygen-derived free radicals impair endothelial function in humans with insulin-dependent diabetes mellitus. Vitamin C is a water-soluble antioxidant capable of scavenging free radicals and sparing other endogenous antioxidants from consumption (24–27). Therefore, we tested the hypothesis that short-term intraarterial administration of vitamin C could improve endothelium-dependent vasodilation in patients with insulin-dependent diabetes mellitus.

Methods

Subjects. The study group included 10 patients (4 men, 6 women; mean [±SD] age 34 ± 8 years, range 22 to 48) with insulin-dependent diabetes mellitus, as defined by the National Diabetes Data Group criteria (28). The duration of diabetes mellitus ranged from 1.7 to 31.0 years (mean 12.4 ± 3.3). All diabetic patients had a history of ketoacidosis and were currently receiving insulin therapy. The study group also

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included 10 healthy volunteers (5 men, 5 women; mean age 34 ± 10 years, range 20 to 48).

All subjects were recruited from the Boston area by means of print advertisements. Each subject was screened by history, physical examination and laboratory evaluation. Criteria for exclusion for all subjects included hypertension (defined as a blood pressure >140/90 mm Hg), history of tobacco use, hypercholesterolemia (defined as low density lipoprotein cholesterol >75th percentile for age and gender), cardiac or pulmonary disease, serologic evidence of hepatic, hematologic or renal dysfunction and any use of antihypertensive, cardiac, vasoactive or antioxidant medications. The subjects had neither evidence of atherosclerotic disease as manifested by angina or claudication nor physical findings of decreased pulses, bruits or asymmetric blood pressures. All diabetic patients had a fundoscopic examination and were screened for proteinuria. No diabetic patient had evidence of neuropathy, orthostatic hypotension, rest tachycardia or poor glycemic control (defined as a glycosylated hemoglobin >9%). This study was approved by the Human Research Committee of Brigham and Women's Hospital, and each subject gave written informed consent.

Drug protocol. Methacholine chloride (Roche Laboratory, Division of Hoffman-La Roche Inc.), a congener of acetylcholine, was infused through the brachial artery to assess endothelium-dependent vasodilation. Forearm blood flow was measured during escalating doses of methacholine at 0.3, 1.0, 3.0 and 10 μg/min. Sodium nitroprusside (Elkins-Sinn Inc.) was infused through the brachial artery to assess the vasodilator response to an exogenous nitric oxide donor. Forearm blood flow was measured during increasing doses of sodium nitroprusside at 0.3, 1.0, 3.0 and 10 μg/min. All drug infusions were administered for 3 min at each dose at a rate of 0.4 ml/min. The drug doses were chosen to achieve a maximal increase in local forearm blood flow without significant alterations in systemic hemodynamic variables.

Vitamin C (sodium ascorbate, Abbott Laboratories) was infused through the brachial artery to assess the effect of this antioxidant on the vasodilator response to methacholine and sodium nitroprusside. Vitamin C was infused at a constant dose of 24 mg/min and a constant rate of 0.4 ml/min to limit the cumulative dose of vitamin C to <1,000 mg and to achieve a local forearm concentration of 1 to 10 mmol/liter. This concentration of vitamin C has been shown to completely protect human plasma from lipid peroxidation caused by free radical–mediated peroxidation in vitro and to improve endothelium-dependent vasodilation in non–insulin-dependent diabetic patients and smokers (26,29,30).

Experimental protocol. Each subject was studied in the morning in a temperature-controlled room at 23°C in the postabsorptive state. Alcohol and caffeine were withheld 12 h before the study. Aspirin and nonsteroidal medications were withheld 7 days before the study. All diabetic patients were receiving conventional insulin therapy twice a day. Insulin was held for the 12 h before the study; longer periods were avoided because of the risk of ketoacidosis. Under local anesthesia and using sterile technique, a 20-gauge polyethylene catheter was inserted into the brachial artery for determination of blood pressure and for administration of drugs.

At the beginning of each study protocol, normal saline (0.9% sodium chloride) was infused intraarterially at a rate of 0.4 ml/min, and measurements of forearm blood flow and blood pressure were obtained at 10-min intervals for a minimum of 30 min until a baseline stable state was achieved. All subjects underwent the following protocol: 1) intraarterial administration of escalating doses of methacholine to define the forearm blood flow dose–response relation; 2) a rest period of at least 60 min to reestablish baseline forearm blood flow; 3) infusion of vitamin C intraarterially for 10 min, with measurement of forearm blood flow; and 4) a repeat forearm blood flow dose–response curve to methacholine during concomitant infusion of vitamin C. Using a similar protocol, five diabetic patients underwent an additional protocol on a separate date to determine the forearm blood flow dose–response relation for sodium nitroprusside, before and during concomitant vitamin C infusion. All drugs were administered at a constant infusion rate of 0.4 ml/min.

Hemodynamic assessment. Bilateral forearm blood flow was assessed by venous occlusion strain-gauge plethysmography, using calibrated mercury-in-Silastic strain gauges, and expressed as ml/100 ml tissue per min (D. E. Hokanson). Both arms were supported above the level of the heart. Venous occlusion pressure averaged 34 ± 1 mm Hg. A wrist cuff was inflated to suprasystolic pressures before each forearm blood flow determination to prevent circulation to the hand. Each forearm blood flow measurement comprised a minimum of five separate determinations at 10- to 15-s intervals. The vascular response to each drug was assessed by the forearm blood flow in the drug infusion arm. To exclude a systemic response during each drug infusion, forearm blood flow was simultaneously assessed in the contralateral arm. Forearm vascular resistance was calculated as the ratio of mean blood pressure to forearm blood flow and expressed as mm Hg/ml per 100 ml tissue per min (U).

Blood pressure was assessed with the arterial cannula attached to a Statham P23 pressure transducer that was aligned to an amplifier on a physiologic recorder (Gould Inc.). Heart rate determination was made from a simultaneous electrocardiographic signal and calculated from the RR interval.

Statistical analysis. Results are presented as mean value ± SE. Unpaired and two-tailed *t* tests were used to assess the differences in clinical characteristics between the diabetic and nondiabetic subjects. Analysis of the dose–response curves for each drug infusion before and during concomitant infusion of vitamin C was performed using two-way analysis of variance (ANOVA) for repeated measures. Comparisons of forearm blood flow before and during concomitant infusion of vitamin C for each drug dose were made using two-tailed *t* tests, unpaired or paired as appropriate, and adjusted with a Bonferonni correction for multiple comparisons. Statistical significance was accepted at the 95% confidence level ($p < 0.05$).

Table 1. Clinical Characteristics of 20 Study Subjects

	Diabetic Patients (n = 10)	Nondiabetic Subjects (n = 10)
Age (years)	34 ± 2.7	34 ± 3.2
Male/female	6/4	5/5
Body mass index (kg/m^2)	25 ± 1	24 ± 1
Mean blood pressure (mm Hg)	80 ± 4	78 ± 3
Creatinine (mg/dl)	1 ± 0.1	1 ± 0.1
Total cholesterol (mg/dl)	173 ± 6	168 ± 8
HDL cholesterol (mg/dl)	43 ± 2	42 ± 2
LDL cholesterol (mg/dl)	115 ± 6	109 ± 7
Triglycerides (mg/dl)	70 ± 9	92 ± 12
Glucose (mg/dl)	185 ± 20	83 ± 4*
Glycosylated hemoglobin (%)	7.1 ± 0.5	4.4 ± 0.2*
Insulin (U/ml)	35.1 ± 12.8	5.2 ± 0.9†

*p = 0.01 and †p = 0.04 versus diabetic patients. Data presented are mean value ± SE. HDL = high density lipoprotein; LDL = low density lipoprotein.

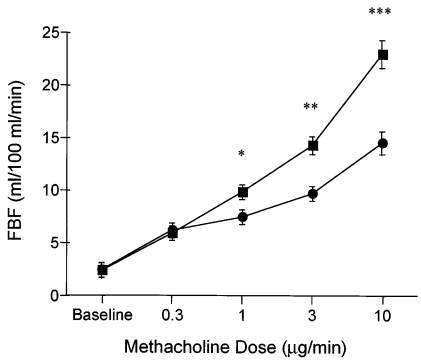


Figure 1. Forearm blood flow (FBF) dose–response curves to methacholine in insulin-dependent diabetic patients (**circles**) and control subjects (**squares**). The endothelium-dependent vasodilator response was attenuated in diabetic patients compared with that in control subjects (p = 0.001 by ANOVA). *p = 0.03, **p = 0.04 and ***p = 0.001, significant differences in forearm blood flow at each methacholine dose for diabetic patients versus nondiabetic subjects.

Results

Subjects. The clinical characteristics of the study and control groups are presented in Table 1. Both groups were well matched for age. No subject in either group had any evidence of hypertension, hypercholesterolemia, tobacco use or cardiovascular disease. The mean blood pressure, blood urea nitrogen and serum creatinine were similar between the two groups. The diabetic patients had significantly higher glucose, glycosylated hemoglobin and insulin levels. No diabetic patients had evidence of neuropathy. Three diabetic patients had evidence of nonproliferative retinopathy by fundoscopic examination.

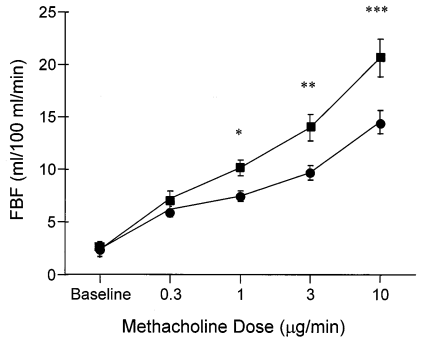
Basal forearm blood flow and vitamin C. Basal forearm blood flow was comparable between diabetic patients and control subjects (2.4 ± 0.1 vs. 2.4 ± 0.3 ml/100 ml tissue per min, p = NS). Baseline forearm vascular resistance was 34.0 ± 2.0 U in the patients with diabetes and 38.1 ± 5.8 U in the control subjects (p = NS). Intraarterial infusion of vitamin C did not alter baseline forearm blood flow in either group (2.4 ± 0.2 ml/100 ml tissue per min in diabetic patients vs. 2.1 ± 0.2 ml/100 ml tissue per min in control subjects, p = NS).

Effect of vitamin C on endothelium-dependent vasodilation. Methacholine increased forearm blood flow and decreased forearm vascular resistance in both groups (Fig. 1). However, the response to methacholine was significantly blunted in diabetic patients compared with control subjects (p = 0.001 by ANOVA). At the peak dose of methacholine (10 μg/min), forearm blood flow was 14.5 ± 1.1 ml/100 ml tissue per min in the diabetic patients compared with 23.0 ± 1.3 ml/100 ml tissue per min in the control subjects (p = 0.005). Similarly, forearm vascular resistance at the peak dose was higher in diabetic patients (6.0 ± 0.8 U) than in control subjects (3.5 ± 0.2 U, p = 0.03). Methacholine infusion did not alter forearm blood flow or forearm vascular resistance in the contralateral arm and did not affect the systemic blood pressure or heart rate in either group.

In patients with insulin-dependent diabetes mellitus, the forearm blood flow response to methacholine was augmented by concomitant infusion of vitamin C (p = 0.001 by ANOVA) (Fig. 2). At the peak dose of methacholine (10 μg/min), forearm blood flow increased from 14.5 ± 1.1 ml/100 ml tissue per min before infusion of vitamin C to 20.6 ± 1.8 ml/100 ml tissue per min during vitamin C administration (p = 0.005). At the peak dose of methacholine (10 μg/min), forearm vascular resistance decreased from 6.0 ± 0.8 U before vitamin C to 4.2 ± 0.5 U after it (p = 0.04).

The forearm blood flow response to methacholine in control subjects was not changed by concomitant infusion of vitamin C (p = NS by ANOVA) (Fig. 3). At the peak dose of methacholine (10 μg/min), forearm blood flow before and during infusion of vitamin C was 23.0 ± 1.3 and 23.4 ± 1.8 ml/100 ml tissue per min, respectively (p = NS). Similarly, forearm vascular resistance was not altered by vitamin C (3.5 ± 0.2 U before vs. 3.5 ± 0.3 U during the infusion, p = NS).

Figure 2. Forearm blood flow (FBF) dose–response curves to methacholine in insulin-dependent diabetic patients before (**circles**) and during (**squares**) the infusion of vitamin C. The endothelium-dependent vasodilatory response was augmented during the concomitant infusion of vitamin C (p = 0.001 by ANOVA). *p = 0.02, **p = 0.04 and ***p = 0.005, significant differences in forearm blood flow at each methacholine dose before versus during vitamin C administration.



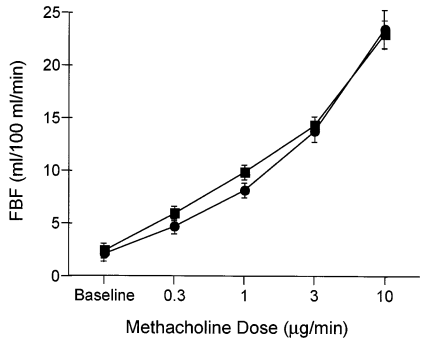


Figure 3. Forearm blood flow (FBF) dose–response curves to methacholine in control subjects before **(circles)** and during **(squares)** the infusion of vitamin C. Endothelium-dependent vasodilation was not different during the concomitant infusion of vitamin C (p = NS by ANOVA).

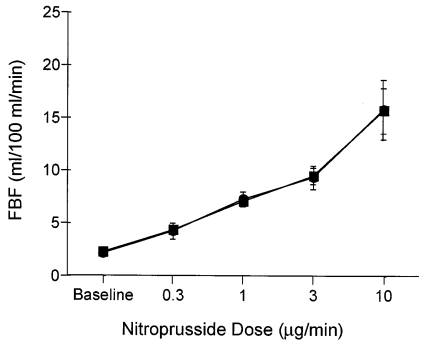


Figure 4. Forearm blood flow (FBF) dose–response curves to sodium nitroprusside in diabetic patients before **(circles)** and during **(squares)** the infusion of vitamin C. Endothelium-independent vasodilation was not different during the concomitant infusion of vitamin C (p = NS by ANOVA).

Effect of vitamin C on endothelium-independent vasodilation. In patients with insulin-dependent diabetes mellitus, the forearm blood flow dose–response curve to sodium nitroprusside was not affected by the concomitant infusion of vitamin C (p = NS by ANOVA) (Fig. 4). At the peak dose of sodium nitroprusside (10 μg/min), forearm blood flow was 15.7 ± 2.2 before and 15.8 ± 2.8 ml/100 ml tissue per min during infusion of vitamin C (p = NS). Forearm vascular resistance was 5.5 ± 1.0 U before and 5.6 ± 0.9 U during vitamin C infusion (p = NS). Sodium nitroprusside infusion did not alter forearm blood flow or forearm vascular resistance in the contralateral arm and did not affect systemic blood pressure or heart rate in either group.

Discussion

The salient finding of this study is that short-term intraarterial administration of the antioxidant vitamin C restores endothelium-dependent vasodilation in patients with insulin-dependent diabetes mellitus. Endothelium-independent vasodilation was not affected by the infusion of vitamin C. In nondiabetic control subjects, vitamin C had no effect on the endothelium-dependent vasodilator response. These findings suggest that oxygen-derived free radicals may decrease the bioavailability of endothelium-derived nitric oxide and impair endothelium-dependent vasodilation in patients with insulin-dependent diabetes mellitus.

Endothelial dysfunction in diabetes mellitus. The vascular endothelium in healthy subjects synthesizes and releases a variety of substances that regulate vascular tone, including nitric oxide (14–17). Endothelium-dependent vasodilation is reduced in experimental models of diabetes mellitus (31–34). Also, endothelium-dependent relaxation is reduced in arteries isolated from nondiabetic animals exposed to a hyperglycemic environment (20,35). Moreover, studies conducted in humans with insulin-dependent as well as non–insulin-dependent diabetes mellitus have found impaired endothelium-dependent vasodilation (9–13). Our study also confirms the observation that endothelium-dependent vasodilation is impaired in patients with insulin-dependent diabetes mellitus. Potential factors contributing to this dysfunction include decreased synthesis or release of endothelium-derived nitric oxide and increased inactivation of endothelium-derived nitric oxide by oxygen-derived free radicals.

Oxidant stress and endothelial dysfunction. Oxygen-derived free radicals, particularly superoxide anions, inactivate endothelium-derived nitric oxide (36–40). In animal models of hypercholesterolemia, vascular production of superoxide anions is increased and is causally related to impaired endothelium-dependent relaxation (41–43). Treatment with antioxidants restores endothelial function in these models (40–42,44–46). Similarly, superoxide anions may inactivate nitric oxide in diabetes mellitus (19–23). In animal models of diabetes mellitus, the impairment in endothelium-dependent relaxation can be improved by treatment with superoxide dismutase (19–21,23).

There are several potential etiologies of oxidative stress in diabetes mellitus. Oxidant production may be increased or antioxidant defenses impaired, each leading to a greater concentration of oxygen-derived free radicals. Production of oxygen-derived free radicals is augmented in circulating granulocytes and monocytes from patients with diabetes, as well as from nondiabetic subjects exposed to a hyperglycemic environment (47,48). Experimental hyperglycemia increases arachidonic acid metabolism and eicosanoid synthesis, which augment oxygen-derived free radical production (31,49,50). In porcine vascular smooth muscle cells cultured in a hyperglycemic environment, formation of F_2-isoprostane (generated as a product of peroxidation of arachidonic acid by free radicals) is enhanced (51). Auto-oxidation of glucose, as catalyzed by transition metals and glycosylation of proteins, can generate oxygen-derived free radicals (52–54). Moreover, the activity of the superoxide anion scavenger, superoxide dismutase, is decreased in rats with diabetes mellitus induced by streptozotocin or alloxan monohydrate (55,56). In addition, studies have shown decreased levels of vitamin C in diabetic patients, despite adequate vitamin C intake (26,27,57–62), and in-

creased levels of oxidized metabolic products of ascorbic acid, such as dehydroascorbic acid (63–65). Vitamin C is a potent endogenous antioxidant in human plasma, capable of scavenging of oxygen-derived free radicals (24–27). In this manner, vitamin C not only serves as an antioxidant, but also spares other endogenous antioxidants from consumption.

We previously demonstrated (29) that vitamin C improves endothelium-dependent vasodilation in patients with non–insulin-dependent diabetes mellitus. Insulin-dependent diabetes mellitus is clinically and pathologically different from non–insulin-dependent diabetes mellitus. Insulin-dependent diabetes mellitus is characteristically a disease of younger patients and is typically associated with a lower body mass index, ketotic prone metabolism and strict insulin requirement. Non–insulin-dependent diabetes mellitus, in contrast, is the result of insulin resistance. Non–insulin-dependent diabetes mellitus typically affects older patients with a higher body mass index and a higher incidence of hypertension and hypertriglyceridemia than age-matched nondiabetic subjects. Although both subsets of diabetes are characterized by premature atherosclerosis, the prevalence of vascular disease varies considerably between the two groups, with the risk of proliferative retinopathy being twofold higher in insulin-dependent diabetic patients at 20 years after diagnosis (66). Despite these metabolic, clinical and pathologic differences, we found a common reduction in endothelium-dependent vasodilation, as well as a remarkably similar response to the early administration of the antioxidant vitamin C (29). This finding suggests that the imbalance between oxidative stress and antioxidant defenses may be a common link in causing endothelial dysfunction in these diverse patient groups.

Conclusions. In the current study, intraarterial administration of the antioxidant vitamin C restored endothelium-dependent vasodilation in patients with insulin-dependent diabetes mellitus. This result supports the notion that oxygen-derived free radicals may contribute to abnormal vascular function in patients with diabetes mellitus. It is not certain that comparable effects would be observed after oral intake of vitamin C, because comparable plasma concentrations would be difficult to achieve. Further studies of the effects of long-term oral antioxidant therapy on vascular function are warranted as a means of reducing vascular disease in patients with diabetes.

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