Poor vitamin C status is associated with increased carotid intima-media thickness, decreased microvascular function, and delayed myocardial repolarization in young patients with type 1 diabetes\textsuperscript{1–3}

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ABSTRACT
Background: Vascular endothelial dysfunction, accelerated thickening of arterial intima, and changes in ventricular repolarization contribute to increased cardiovascular morbidity in type 1 diabetes (T1D). Although vitamin C has important antioxidant functions and increased oxidative stress is a central mechanism of cardiovascular dysfunction in T1D, the relation between vitamin C and the cardiovascular system in young diabetic patients has not been investigated.

Objective: In a cohort of young patients with T1D, we investigated the relation of plasma concentrations of vitamin C with indexes of vascular function and structure and duration of the QT interval corrected for heart rate (QT\textsubscript{c}).

Design: Carotid artery intima-media thickness, cutaneous microvascular function, and duration of the QT\textsubscript{c} interval were measured in 59 patients (mean age: 17 y; range: 10–22 y) with T1D (diabetes duration: 3–20 y). Plasma vitamin C was analyzed by HPLC with coulometric detection.

Results: Carotid artery intima-media thickness and duration of the QT\textsubscript{c} interval were higher in patients in the lowest tertile of vitamin C than in those in the highest tertile (P < 0.05 for both). The cutaneous microvascular response to acetylcholine was lower (P = 0.003) in the lowest tertile group than in the highest tertile group, but the response to sodium nitroprusside was not significantly different between these 2 groups. All differences remained significant after adjustment for age, sex, diabetes duration, body mass index, and glycated hemoglobin.

Conclusions: In this relatively small-scale cross-sectional study of young patients with T1D, lower plasma concentrations of vitamin C seem to be associated with adverse changes in the microcirculation, peripheral arteries, and ventricular repolarization. Large-scale prospective studies are needed to confirm these results and to clarify the underlying mechanisms. \textit{Am J Clin Nutr} 2009;90:447–52.

INTRODUCTION

Changes in vascular function and structure and in myocardial depolarization and repolarization, expressed as the QT interval corrected for heart rate (QT\textsubscript{c}) on an electrocardiogram, occur early in patients with type 1 diabetes (T1D) and appear to predict an increased risk of cardiovascular morbidity and mortality later in life (1, 2). Enhanced oxidative stress is a central mechanism of vascular complications in T1D, also being evoked as a pathogenic link to QT\textsubscript{c} interval prolongation (3, 4). In some, but not in all studies, dietary supplementation with vitamin C, an important component of the antioxidant system, has been suggested to reverse endothelial dysfunction in the coronary or peripheral arteries of patients with overt atherosclerosis or in those with conditions that predispose to atherosclerosis (5, 6). To date, there have been no studies of the possible association between plasma vitamin C status and markers of cardiovascular disease in T1D.

In a cohort of young patients with T1D, we investigated the relation of plasma concentrations of vitamin C with indexes of vascular function (eg, cutaneous microvascular endothelial vasomotor function) and structure (eg, carotid artery intima-media thickness; cIMT) and with QT\textsubscript{c} interval duration.

SUBJECTS AND METHODS

Subjects

Fifty-nine children and adolescents (33 males and 26 females) with a mean age of 17 y (range: 10–22 y) and a mean duration of diabetes of 9 y (range: 3–20 y) were recruited from the diabetes outpatient clinic at the Lund University Hospital. Patients were recruited between April 2007 and June 2008. Exclusion criteria were a family history of other major cardiovascular disease risk factors, such as primary hypercholesterolemia, hypertension, premature coronary and cerebrovascular disease, current smoking, and systemic hypertension. Body weight, height, and arterial blood pressure (systolic and diastolic) were measured. Data on diabetes duration were obtained from the registry of outpatient diabetes clinic. The protocol of the study was approved by the

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Ethical Committee for Human Research at the Lund University. Written informed consent was obtained from all participants aged ≥18 y or from the guardians of those aged <18 y. All participants gave oral consent.

Assessment of cIMT

A high-resolution ultrasound system (Acuson Sequoia C512; Siemens AG, Erlangen, Germany) equipped with a 15-MHz probe was used. The imaging protocol was described in detail previously (7). In short, longitudinal scans in the bidimensional mode of the 1-cm long distal end of the left common carotid artery were imaged so that the lumen-intima and intima-media interfaces were distinguishable. All images corresponded to the R-wave on an electrocardiogram. Four scans obtained from each individual were recorded on videotape for offline analysis of the cIMT. The ultrasound scans were taken by 2 sonographers (MO and AM) experienced with carotid imaging. The mean cIMT was used for the statistical analyses.

Assessment of cutaneous microvascular function

Cutaneous microvascular responses were assessed by 2 observers (MO and AM) using a laser Doppler multifiber probe (481-1; Perimed AB, Järfa¨lla, Sweden) during transdermal iontophoresis of acetylcholine [endothelium-dependent agonist (Ach)] and sodium nitroprusside [endothelium-independent agonist (SNP)]. The volar side of the forearm of the nondominant upper extremity was chosen in all patients. Anodal iontophoresis was used for Ach, whereas SNP was delivered via cathodal iontophoresis. The current was set at 100 μA for 20 s for both drugs, and 5 doses were applied to obtain the dose-response curves (8). The response to Ach was expressed as the percentage change in the maximal response after the fifth dose to baseline.

QT and QTc interval measurement

Participants were asked to not drink caffeine-containing beverages ≥2 h before the study visit. A resting period of ≥20 min was used before the first investigation. A single-lead ECG was recorded for 5 min with a handheld HRV device (DailyCare BioMedical Inc, Chungli, Taiwan). The recordings were transferred to a computer, and the data were automatically analyzed by HRV analysis software (DailyCare Biomedical Inc). The mean QT and QTc intervals over 5 min were used in the statistical analysis.

Biochemical analysis

Blood for ascorbate measurements was immediately centrifuged for 1 min at 16000 × g, and an aliquot of plasma was immediately stabilized with an equal amount of 10% (wt:vol) meta-phosphoric acid containing 2 mmol disodium–EDTA/L. The precipitate was removed by centrifugation at 16000 × g for 1 min (4°C), and the supernatant fluid was stored at −80°C for <3 mo until analyzed. The concentrations of ascorbate (the reduced form of vitamin C) and vitamin C [ie, ascorbate + dehydroascorbic acid (DHA)] measured after reduction with Tris(2-carboxyethyl)phosphine hydrochloride (9)] were measured by HPLC with the use of uric acid as endogenous standard (10). DHA (ie, the oxidized form of vitamin C) was calculated by subtracting ascorbate from total vitamin C and was expressed as the fraction oxidized to that of total vitamin C as a percentage (11). HDL cholesterol, LDL cholesterol, and total cholesterol (TC) were analyzed with an enzymatic method (Roche/Hitachi 912; with an Roche Diagnostics, Mannheim, Germany). Plasma high-sensitivity C-reactive protein (hsCRP) was measured by enzyme-linked immunosassay with the use of polyclonal antibodies (DACO Diagnostics, Glostrup, Denmark). Plasma fibrinogen was assessed by using an automated coagulation analyzer (Sysmex CA-7000; Sysmex Corporation, Mundelein, IL). Serum orosomucoid was assessed by immunonephelometry (IMMAGE Immunochemistry System, Beckman Coulter Inc, Fullerton, CA).

Statistical analyses

Because of its skewed distribution, the hsCRP data were log transformed. All other data were normally distributed. Between-group (based on vitamin C tertiles) comparisons were performed by ANOVA. An analysis of covariance (ANCOVA) and a Tukey-Kramer post hoc test were used to adjust for possible confounding effects of age, sex, diabetes duration, BMI, and glycated hemoglobin (Hb A1c). Eventual correlations between the hypothesized predictor variables and the dependent variable were assessed by simple regression. A multiple regression model was used to identify independent factors affecting IMT, cutaneous microvascular function, and QTc. A P value ≤0.05 was considered statistically significant. Data are presented as means ± SDs or as medians with interquartile ranges (IQRs) when appropriate. All analyses were performed by using SPSS for Windows (version 16; SPSS Inc, Chicago, IL).

RESULTS

There were no significant difference in vitamin C concentrations between male and female patients (49.66 ± 16.79 and 58.98 ± 21.09 μmol/L, respectively; P = 0.064). There was a significant inverse correlation between DHA and vitamin C concentrations (P = 0.0001, r = −0.60; Figure 1). In the whole cohort, there was no significant correlation between hsCRP and DHA (P = 0.09, r = 0.22). However, when the patients were categorized in relation to sex, DHA correlated with hsCRP in females (P = 0.03, r = 0.43), but not in males (P = 0.56, r = 0.10).

On the basis of vitamin C tertiles, there were no significant differences in demographic, metabolic (ie, lipids and Hb A1c), and inflammatory (CRP, fibrinogen, and orosomucoid) indexes between groups (Table 1).

CIMT and plasma vitamin C

CIMT was significantly greater in the lowest vitamin C tertile than in the highest tertile (0.41 ± 0.07 mm compared with 0.37 ± 0.06 mm; P = 0.04; Figure 2A). After adjustment for age, sex, diabetes duration, BMI, and Hb A1c, the difference between the lowest and the highest tertiles of vitamin C remained significant (P < 0.05, ANCOVA). A weak, yet significant, inverse correlation between cIMT and plasma vitamin C was observed (P = 0.02, r = −0.29; Figure 2B).
Cutaneous microvascular function and plasma vitamin C

The maximum response of cutaneous microcirculation to Ach (% change from baseline) was significantly lower in the lowest vitamin C tertile than in the highest tertile (344 ± 21 ms compared with 398 ± 36 ms; P = 0.04; Figure 4A). After adjustment for age, sex, diabetes duration, BMI, and Hb A1c (P < 0.05, ANCOVA). Also, the maximum response to Ach showed a moderate, yet significant, correlation with vitamin C concentrations (P = 0.005, r = 0.41; Figure 3B). There was no difference in the response to SNP between vitamin C tertiles (P = 0.62).

QTc interval and plasma vitamin C

The QTc interval was greater in the lowest vitamin C tertile than in the highest tertile (421 ± 21 ms compared with 393 ± 36 ms; P = 0.04; Figure 4A). After adjustment for age, sex, diabetes duration, BMI, and Hb A1c, the difference between these groups remained significant (P < 0.05). The QTc interval was negatively correlated with vitamin C (P = 0.04, r = -0.27) and showed a moderate association with DHA concentrations (P = 0.004, r = 0.38).

DISCUSSION

The findings of the present study suggest that young patients with T1D with poor plasma vitamin C status may be more prone to microvascular dysfunction, thickening of arterial intima-media, and QTc prolongation than are patients with adequate

![FIGURE 1. Correlation between plasma concentrations of vitamin C and dehydroascorbic acid (DHA) in 58 children and adolescents with type I diabetes (P = 0.0001, r = -0.60).](https://academic.oup.com/ajcn/article-abstract/90/2/447/4597024/fig-1?target=19)

![FIGURE 2. A: Box plot illustrating the differences in carotid artery intima-media thickness between vitamin C tertiles. The box plot shows the 25th percentile, median, and 75th percentile and the 10th and 90th percentiles as horizontal lines outside the box. n = 15, 19, and 25 for the lowest, middle, and highest tertiles, respectively. *Significantly different from the highest tertile, P = 0.04 (ANCOVA after adjustment for age, sex, diabetes duration, BMI, and glycated hemoglobin with Tukey-Kramer post hoc test for multiple comparisons). B: Association of carotid artery intima-media thickness with plasma vitamin C in 59 children and adolescents with type I diabetes (P = 0.02, r = -0.29).](https://academic.oup.com/ajcn/article-abstract/90/2/447/4597024/fig-2?target=19)
plasma vitamin C concentrations, independent of demographic and diabetes-related variables known to influence these cardiovascular indexes.

To our knowledge, the relation between plasma vitamin C status and cIMT, an index of early atherosclerosis, has not yet been studied in patients with T1D. The Atherosclerosis Risk in Communities Study, involving adults aged >55 y without symptomatic or prevalent coronary heart disease, showed a significant inverse relation between vitamin C intake and cIMT (12). Several epidemiologic studies have found and inverse relation between vitamin C status and risk of cardiovascular disease (13–18). However, thus far, most of the major intervention studies have not been able to support a link between vitamin supplementation and health. However, in the Atherosclerosis Prevention Study, combined vitamin C and E supplementation did slow down atherosclerotic progression in high-risk men (19). In contrast, the Carotid Ultrasound Disease Assessment Study found no association between dietary intake or plasma concentrations of antioxidant vitamins (vitamin A, C, and E) and intimal thickening in men or women (20).

It is not yet known whether vitamin C supplementation at a younger age could decelerate intimal thickening or retard the onset of cardiovascular complications in later life. On the basis of the epidemiologic evidence, this might be an important task for future studies given the predictive value of childhood cIMT in atherosclerosis progression and complications in the adult life (21).

The decrease in microvascular endothelium-dependent function in patients with lower concentrations of vitamin C does lend additional support to the hypothesis that, at least in T1D, vitamin C could be an important factor protecting against the development of cardiovascular disease. Holowatz et al (22) showed that vitamin C supplementation augments cutaneous vasodilation in human skin. In hypertensive patients, intradermal microdialysis of vitamin C improved reflex cutaneous vasodilation via both nitric oxide– and non-NO–dependent mechanisms (23). Vitamin C may increase nitric oxide bioavailability via a general lowering of the oxidative stress level and a subsequent decrease in superoxide-related nitric oxide inactivation, or, more specifically, by improving the activity of nitric oxide synthase through enhanced bioavailability of its cofactor tetrahydrobiopterin (24, 25).

In our cohort of young patients with T1D, poor plasma vitamin C status was also associated with a prolonged QTc interval. Prolongation of the QTc interval is a significant predictor of adverse cardiovascular prognosis in patients with T1D (26) and may be detected already in young patients with T1D (27). In nondiabetic patients, vitamin C supplementation improved cardiac and autonomic responses (28). However, studies in rats lacking L-gulonolactone oxidase, the final enzyme in vitamin C
biosynthesis, showed no difference in heart rate, arterial blood pressure, and occurrence or persistency of rhythm abnormalities in relation to vitamin C supplementation (29). The reason for this discrepancy is not clear.

In experimental studies, the reactive oxygen species pathway was implicated in hERG (ether-a-go-go related gene) dysfunction because of diabetes-related metabolic disturbances such as hyperglycemia, increased lipid peroxidation, and protein oxidation (30). hERG potassium channels are essential for normal electrical activity in the heart, and different factors influencing hERG could lead to prolongation of the QTc interval (31). Vitamin C, because of its excellent oxidant scavenging properties, might affect hERG potassium channels, which would explain the lower QTc interval in the highest vitamin C tertile. A positive correlation between the QTc interval and DHA concentrations strengthens the suggested involvement of oxidative stress in QTc prolongation. However, this hypothesis needs further verification.

In the present study, a significant inverse correlation between DHA and vitamin C was observed. The presence of DHA suggests that the oxidation rate of ascorbate supersedes the capacity of dehydroascorbic acid reductases to keep vitamin C reduced, i.e., ascorbate recycling (32). A similar inverse correlation was previously found in smokers, but not in nonsmokers, and was suggested to mirror the increased oxidative stress related to smoking per se (11). The increase in oxidation ratio (DHA to vitamin C) in T1D patients with poor vitamin C status is apparently more pronounced than that in smokers and may indicate an even higher level of oxidative stress in these individuals.

In conclusion, our findings suggest that young patients with T1D with low plasma vitamin C concentrations could be more prone to microvascular dysfunction, thickening of arterial intima-media, and QTc interval prolongation. These findings need to be confirmed in large-scale prospective studies, which should also address the possible interaction with other factors such as sex, smoke exposure, physical activity, and dietary intake. Also, it is not clear whether the observed effects were related to increased oxidative stress in these individuals or whether they were the result of impaired homeostatic mechanisms with a specific requirement for vitamin C. Future studies are therefore needed to establish the exact mechanisms by which these changes may occur, and, perhaps, should our findings be confirmed and the mechanisms clarified, to investigate whether vitamin C supplementation in young patients with T1D can decelerate the development of subclinical stages of cardiovascular disease and delay the onset of related complications.

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