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Lack of effect of acute oral ingestion of vitamin C on oxidative stress, arterial stiffness or blood pressure in healthy subjects

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Abstract
Vitamin C is a potent antioxidant *in vitro* and has been reported to act as a vasodilator, possibly by increasing nitric oxide bioavailability. This study examined the antioxidant and vascular effects of a single large oral dose of vitamin C in 26 healthy human volunteers. Haemodynamic and oxidative DNA and lipid damage markers were measured for 8 h following an oral dose of 2 g vitamin C or placebo. Vitamin C had no effect on vasodilation (measured by augmentation index (mean change = 0.04%, 90% CI = −2.20% to 2.28%) or forearm blood flow (−0.19%/min (−0.68, 0.30)), in comparison to placebo) or on several markers of oxidative stress including DNA base oxidation products in blood cells, 8-hydroxy-2′-deoxyguanosine (8O HdG) in urine (0.068 (−0.009, 0.144)) or urinary or plasma total F_2-isoprostanes (−0.005 ng/ml (−0.021, 0.010), −0.153 ng/mg (−0.319, 0.014), respectively).

Keywords: *Vitamin C, arterial stiffness, oxidative stress, isoprostanes*

Introduction

There is considerable evidence that oxidative stress contributes to endothelial dysfunction and the consequent increase in adverse cardiovascular outcomes in a variety of conditions such as smoking, diabetes or atherosclerosis [1,2]. It is also widely believed that antioxidant preparations should reduce the degree of oxidative damage and restore normal endothelial function.

Vitamin C is a potent antioxidant, at least *in vitro* [1,2], and is commonly thought to play a major part in human antioxidant defence systems. Several epidemiological studies have associated higher vitamin C intake with improved cardiovascular outcomes [3–5], although large-scale intervention studies have found no cardiovascular protective effect of supplemental vitamin C [6]. In contrast to the community-based chronic supplementation studies, acute studies in which vitamin C was administered by infusion into the brachial artery have reported a reversal of endothelial dysfunction in diabetes [7] and hypercholesterolaemia [8]. Similarly, a study with acute administration of oral vitamin C has reported an acute vasodilatory effect in healthy volunteers [9], while chronic oral dosing of vitamin C has been reported to have no effect or various effects on vascular and oxidative markers in healthy and diabetic

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subjects [10,11]. This current study was performed to test the hypothesis that an acute oral administration of a large dose of vitamin C (2 g) would exert antioxidant effects and produce vasodilatory effects. We used measures of lipid peroxidation and DNA damage to assess the effect on oxidative stress and assessed blood pressure and wave reflection to examine acute vascular effects of vitamin C. We also compared the vascular effects of vitamin C with the positive controls glyceryl trinitrate (GTN), a non-endothelial dependent vasodilator and salbutamol, an endothelial-dependent vasodilator [12].

Subjects and methods

The study was approved by the Ethics Committee of the National University Hospital, Singapore and all subjects gave written informed consent prior to any study procedure.

Study population

Twenty-six healthy subjects (eight female and 18 male) were enrolled to target 24 completers. Based on individual subjects' intra-day standard deviation of 6.5 percentage points, 24 completers would provide ~80% power to detect a 5-percentage point decrease in augmentation index (AIx) when comparing the vitamin C arm to the placebo arm, in a one-sided test with 5% Type I error rate. None of the subjects smoked, none was taking medication or health supplements, none had a history of any cardiovascular disease or a first-degree relative with diabetes.

Study design

The subjects underwent three study periods on different days, with each period separated by at least 3 days. Sub-lingual GTN and salbutamol were used as positive controls in a fixed sequence in period 1. In this period, a sub-lingual GTN tablet (500 μg, Glaxo Wellcome) was placed under the tongue of the subject. The tablet was removed after 3 min. Salbutamol (400 μg, Glaxo Wellcome) was given by inhalation using a spacer device at least 30 min after the administration of GTN. In periods 2 and 3, subjects received either vitamin C (2 g) (ascorbic acid, Sunward Pharmaceutical Pte Ltd, Singapore) or vitamin C placebo as single doses, in a cross-over, random order. The subjects and investigators were blinded to the administration of vitamin C or placebo (water flavoured with lemon juice).

Vitamin C plasma concentrations were measured at baseline and at 2, 4, 6 and 8 h after dosing by high-performance liquid chromatography (HPLC) with ultraviolet (245 nm) and electrochemical detection (+0.70 V) [13].

Vascular measurements

In the positive control period, the pulse waveform was measured at baseline and at 3, 5, 10, 15 and 20 min after the administration of the GTN tablet. The pulse waveform was measured again prior to dosing of salbutamol and at 5, 10, 15 and 20 min after the inhalation of salbutamol. Forearm blood flow (FBF), pulse wave velocity (PWV) and systemic vascular resistance (SVR) were also measured at baseline and at 20 min after the inhalation of salbutamol.

Vascular measurements were obtained at baseline and hourly for 8 h in vitamin C and placebo periods. All vascular measurements were performed prior to blood sample collection at each time point.

Blood pressure (BP) was measured in the right brachial artery using an automated oscillometric device (Critikon Dinamap Pro 100, Critikon Company LLC, FL). The technique of pulse wave analysis (SphygmoCor 2000 v6.1, PWV Medical, Sydney, Australia) was used to determine aortic pressure and augmentation index (AIx), using a previously validated transfer function [14]. The augmentation provides a measure of the contribution to afterload of the pulse pressure wave reflected from the periphery and is an indirect measure of arterial tone [15,16]. The SphygmoCor machine was also used to calculate aortic and conduit artery (brachial) PWV, a measure of arterial stiffness, from pressure recordings of pulse waveforms at the carotid and radial arteries (brachial PWV or CR-PWV) or carotid and femoral arteries (aortic PWV or CF-PWV). One tonometer was used to acquire sequential recordings of the pressure waveforms at the carotid and then distal arteries. The timing of these pressure waveforms was then compared with that of the R wave on a simultaneously recorded electrocardiogram (ECG) and the PWV was calculated as the arterial path length divided by the difference in R wave to pulse waveform foot (foot of the wave) timings.

FBF was measured by strain gauge plethysmography in the non-dominant arm (Hokanson EC6, Washington) [17]. Unless otherwise stated, FBF results are expressed as the change from baseline values. Forearm vascular resistance (FVR) was determined as brachial mean blood pressure (MBP) divided by FBF. A non-invasive bioimpedence method (BioZ.com Hemodynamic Monitoring system, BZ-4110) was used to estimate systemic vascular resistance, cardiac output (CO) and cardiac index (CI).

Oxidative stress markers

In the vitamin C and placebo periods for healthy volunteers, blood samples were taken at baseline and hourly over 8 h for measurement of plasma total (free + esterified) F_2-isoprostane and DNA oxidative damage in blood cells. Urine samples were collected

at baseline and at 6 h in the vitamin C and placebo periods to measure F_2-isoprostanes and 8-hydroxy-2′-deoxyguanosine (8OHdG). Plasma and urinary F_2-isoprostanes were extracted using an anionic solid phase extraction method and measured by gas chromatography-mass spectrometry coupled with negative chemical ionization (GC-MS-NCI) after sequential derivatization [18]. Urinary 8OHdG was extracted by solid phase extraction and measured by gas chromatography-mass spectrometry-electron ionization (GC-MS) after derivatization [19]. Oxidative DNA damage was also assessed by a panel of DNA base oxidation products by extracting the cellular fractions of blood samples and analysing using GC-MS [20].

Statistical analysis

Demographics are summarized by means and standard deviations, vascular and biochemical parameters by means and standard error of the mean.

The change from baseline values for all vascular parameters at each time point was evaluated using the analysis of covariance technique with baseline as a covariate and treatment, period and sequence as fixed effects in the model. Subject was also used as a random factor. Least square means, mean differences with 90% confidence intervals and *p*-values were estimated to compare between vitamin C and placebo treatments or between GTN and salbutamol treatments.

The oxidative markers for secondary analyses were plasma (and urinary) F_2-isoprostanes, blood cell DNA damage and urinary 8OHdG. As most parameters, except for plasma F_2-isoprostanes, were measured at pre-dose and at the 6 h time point (or at 8 h for DNA damage), changes from baseline values were derived. For plasma F_2-isoprostanes, the maximum drop from baseline for each subject was derived. The oxidative markers were analysed using the same statistical methods as the vascular parameters.

Results

Subjects

A total of 26 healthy subjects (eight females and 18 males) were enrolled into the study. The age of the subjects ranged from 21–26 years. The body mass index (BMI) ranged between 18.7–26.8 kg/m^2. Twenty-five subjects completed the study. One subject discontinued the study prior to completion. Table I summarizes the baseline subject characteristics.

Table I. Baseline subject characteristics.

Baseline subject characteristics	*M* (SD)
Age (years)	23.5 (1.4)
Height (cm)	170.9 (8.6)
Weight (kg)	65.2 (11.5)
Waist circumference (cm)	81.5 (8.2)
Total Fat (%)	26.0 (5.8)
BMI (kg/m^2)	22.2 (2.4)
Fasting Insulin (μM/mL)	8.7 (3.4)
Fasting Glucose (mmol/L)	4.6 (0.4)
Homocysteine (μmol/L)	13.4 (7.6)
HbA1c (mmol/L)	5.1 (0.3)
Total cholesterol (mmol/L)	4.6 (0.7)
LDL-Cholesterol (mmol/L)	2.7 (0.7)
HDL-Cholesterol (mmol/L)	3.2 (0.7)
Triglycerides (mmol/L)	0.9 (0.4)
Supine SBP (mmHg)	112.0 (9.7)
Supine DBP (mmHg)	70.1 (7.1)
Supine HR (bpm)	62.3 (10.3)
SV (ml)	84.4 (15.8)
SVR (dyn sec)	1243.3 (322.3)
CO (1/min)	5.2 (1.3)
CI (1/min/m^2)	3.0 (0.6)
Carotid-radial PWV (m/s)	7.1 (1.1)
Carotid-femoral PWV (m/s)	6.0 (0.8)
Aortic augmentation index (AIx) (%)	1.4 (12.0)
Forearm blood flow (ml/100ml/min)	2.4 (0.8)

Results in healthy volunteers

Vitamin C concentrations. Vitamin C levels rose from 40 μM to ~120 μM after vitamin C administration in healthy controls, with C_{max} at 4 h, while remaining constant during placebo administration (Figure 1).

Vascular effects

Positive control data. GTN produced a mean maximum decrease of $17.26 \pm 1.64\%$ ($p < 0.001$) and salbutamol a maximum decrease of $10.43 \pm 1.67\%$ ($p < 0.001$) in AIx. These drugs increased heart rate (HR) by 9.6 ± 1.19 bpm and 9.3 ± 1.19 bpm, respectively.

Effects of vitamin C. No difference was found in the AIx between vitamin C and placebo treatments (Figure 2). There was no evidence of any time effect of vitamin C. The estimated difference in maximum aortic AIx drop between treatments was 0.04%, with 90% CI of -2.20% to 2.28%. Vitamin C did not change diastolic BP (DBP) or HR at any time point (not shown). The mean maximum decrease in systolic BP (SBP) during the vitamin C period was 5.6 ± 0.8 and during the placebo period 7.6 ± 0.8 ($p = 0.03$). No differences were found in CF-PWV and CR-PWV between vitamin C and placebo (Figure 3). No differences in FBF, SVR or CO were found between treatments (Table II).

The intra-subject coefficients of variation (calculated from the baseline measurements of the three periods in healthy subjects) were: SBP 2.5%,

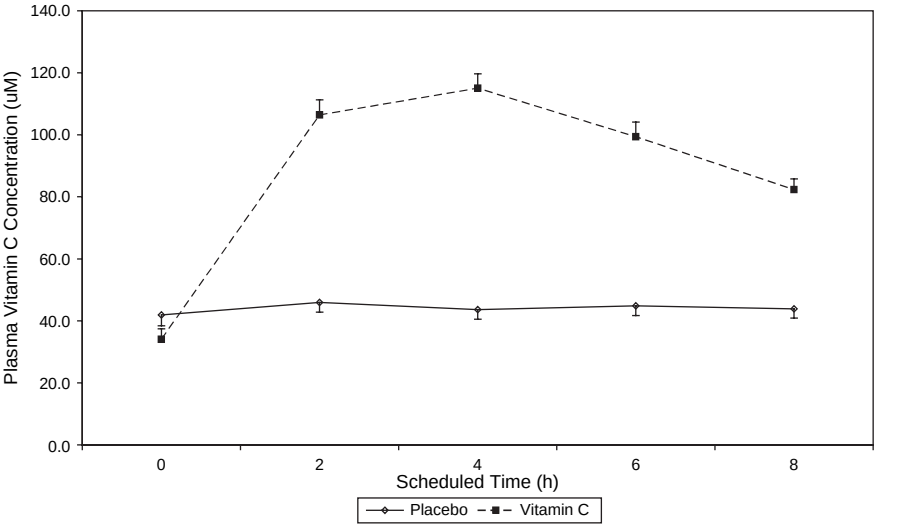

Figure 1. Mean plasma vitamin C concentration profile for placebo and vitamin C. The plasma vitamin C rose to 120 μM 4 h after vitamin C administration (filled symbols) and remained constant during placebo administration (open symbols).

CF-PWV 5.5%, CR-PWV 7.8%, FBF 13.1% and the SD for AIx, 6%.

Biomarkers of oxidative damage. No difference was found in plasma F_2-isoprostane levels between vitamin C and placebo (Figure 4). The maximum drop in plasma F_2-isoprostane levels during the vitamin C period was 0.035 ± 0.011 ng/ml and during the placebo period 0.040 ± 0.011 (90% CI for the difference, -0.021 to 0.010 ng/ml). The changes in urinary F_2-isoprostane levels were also similar between vitamin C and placebo periods (0.118 ± 0.081 and 0.271 ± 0.080 at 6 h, with 90% CI for the difference, -0.319 to 0.014 ng/mg creatinine).

No difference was found in any of 11 oxidized base products of blood cell DNA or in urinary 8OHdG concentrations between vitamin C and placebo (Table III).

Discussion

Previous work on this topic has indicated that intra-arterial infusions of vitamin C are able to overcome the endothelial impairment found in association with diabetes, hypertension or hypercholesterolaemia [7,8,21], while the literature on the effects of oral administration of vitamin C has been conflicting and confusing, with some studies finding vasodilating effects of oral vitamin C in healthy subjects [9], some finding none [22], with some groups reporting beneficial effects of chronic supplementation with vitamin C in patients with type 2 diabetes mellitus

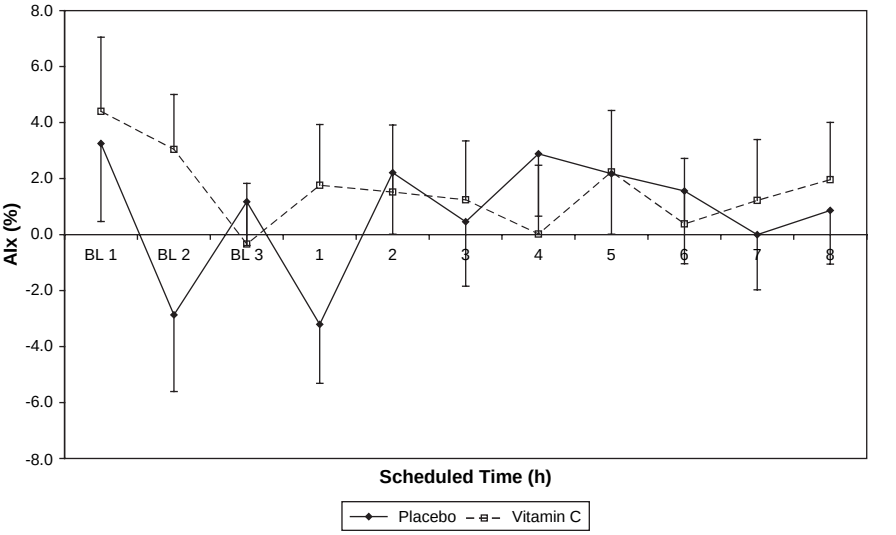

Figure 2. Mean (±SE) AIx (%) profiles for placebo and vitamin C. Vitamin C administration has no significant effect in the augmentation index (AIx), an indirect measure of arterial tone (closed symbols: mean AIx after placebo, open symbols: mean AIx after vitamin C administration).

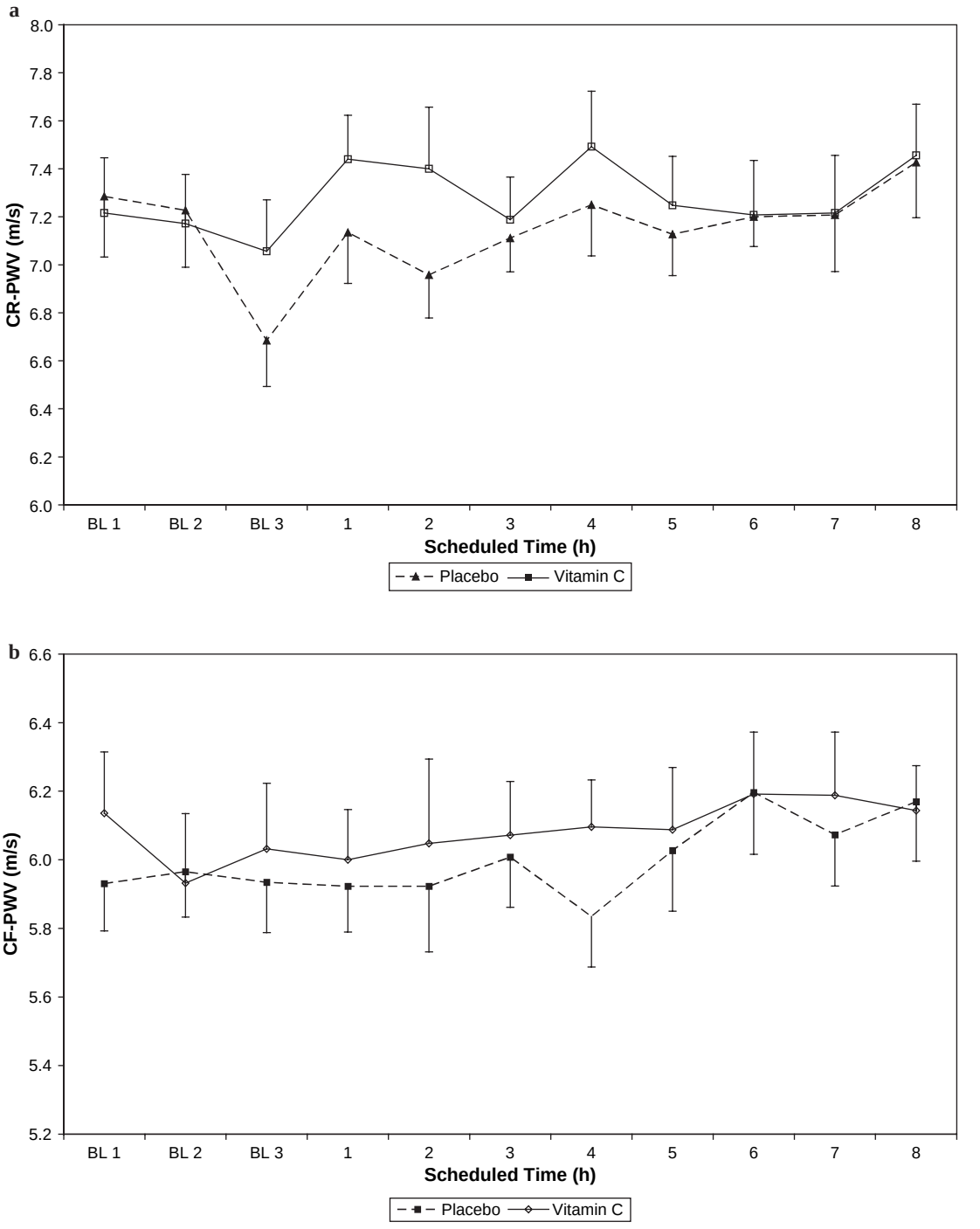

Figure 3. (A) Mean (±SE) CR-PWV (m/s) (conduit artery stiffness) profiles for placebo and vitamin C. Conduit arterial stiffness tended to increase after administration of vitamin C (open symbols) but this effect is not different from the change after placebo administration (closed symbols). (B) Mean (±SE) CF-PWV (m/s) (aortic stiffness) profiles for placebo and vitamin C. Aortic stiffness also increased after vitamin C administration (open symbols) but this effect is not different from the change after placebo (closed symbols).

[10], some reporting no benefit [11]. Various measures of vascular function were used by the various groups and few reported any measures of oxidative stress. The primary aim of this study was to establish whether single large doses of vitamin C have any effects on a range of measures of vascular function, while simultaneously employing the best measures available to examine whole-body oxidative stress. The results clearly exclude any significant effect of very large oral doses of vitamin C on several vascular parameters and on the best available markers of oxidative stress.

In this study, absorption of vitamin C is confirmed by measuring the plasma concentrations. The baseline levels of vitamin C (38 ± 18 μmol/l) are similar to those seen in other studies (42 ± 8 μmol/l [9], 58 ± 6 μmol/l [11], 46.6 ± 17.6 μmol/l [23]) and the rise in vitamin C is similar to the levels achieved with the

Table II. Comparison of forearm blood flow (FBF), systemic vascular resistance (SVR) and cardiac output (CO) between vitamin C and placebo treatments.

Parameter	Treatment	Least squares (LS) mean	LS mean difference (90% CI)	*p*-value
Max increase in FBF (%/min)	Vitamin C	1.18	−0.19 (−0.68, 0.30)	0.516
	Placebo	1.37		
Max drop in SVR (dyn sec)	Vitamin C	138.4	−27.6 (−76.9, 21.7)	0.346
	Placebo	166.0		
Max increase in CO (1/min)	Vitamin C	0.50	−0.07 (−0.27, 0.13)	0.553
	Placebo	0.57		

same dose of vitamin C (2 g) by Wilkinson et al. [9] (C_{max} 120 ± 26 vs 104 ± 8 μmol/l) and is similar to or higher than those achieved by longer-term dosing of smaller amounts of vitamin C (e.g. treatment level of 122 ± 10 μmol/l in Darko et al [11], vitamin C dose 500 mg tds for 21 days).

Vascular effects of vitamin C

The results demonstrate that orally administered vitamin C (2 g) had no effect (beneficial or otherwise) on haemodynamic parameters in healthy volunteer subjects. The data exclude an effect on augmentation index of > 2.28% (the 90% confidence interval for the effect of vitamin C on AIx in these subjects was − 2.20 to 2.28%). They also exclude clinically significant effects of vitamin C on HR, BP, SVR, CO, FBF or arterial stiffness (as measured by arterial PWV [16]).

The positive control data confirm the sensitivity of the vascular measurements. The sizes of the maximum drops in AIx with GTN and salbutamol in the control period (17.3% and 10.4%, respectively) were very similar to those previously reported in healthy subjects (13.0% and 11.6%, respectively [24]).

The vascular results differ from those reported by Wilkinson et al. [9], who reported that oral vitamin C lowered AIx at a single time point (6 h) in a small number of subjects ($n = 8$) and they did not find any difference in HR, CI or SVR between baseline and placebo treatments; it may be that their finding resulted from the play of chance in a small sample. Our subjects and theirs were different in race (Chinese, presumed Caucasian), but had similar baseline augmentation indices and similar baseline and peak vitamin C concentrations. There may have been other unknown differences between the study subjects. As opposed to the exploratory study of Wilkinson et al. [9], the current study was sufficiently powered to exclude an effect size of 5 percentage points in AIx.

Effects of vitamin C on biomarkers of oxidative damage

Several groups have suggested that vitamin C may affect the vasculature by exerting an antioxidant effect

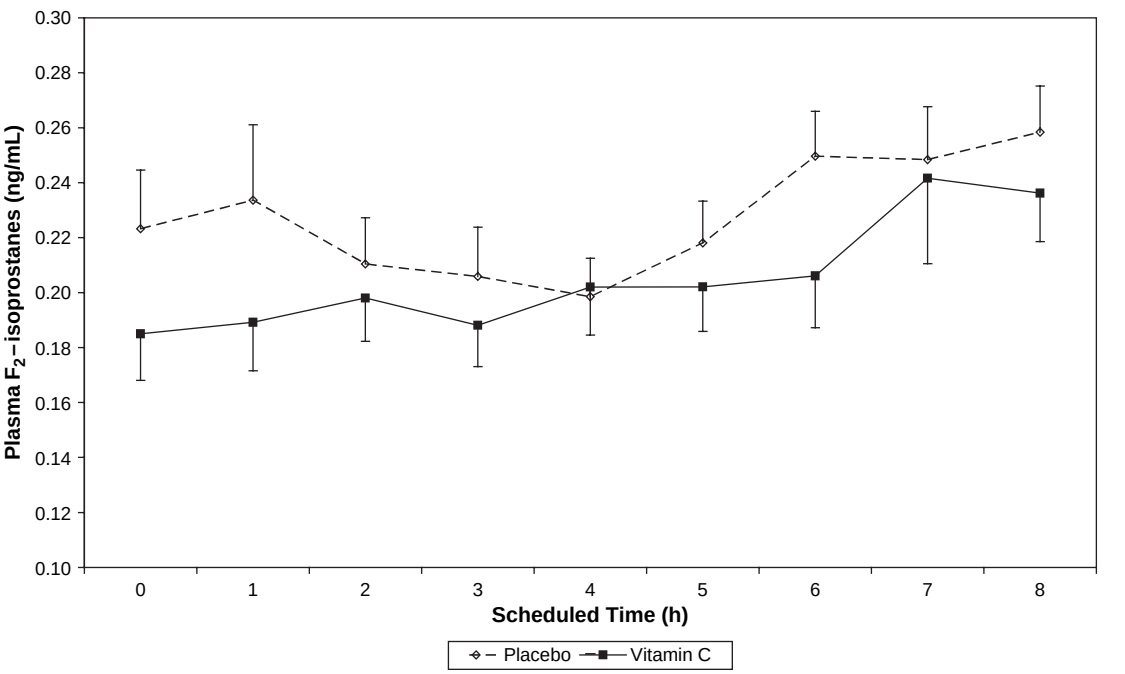

Figure 4. Mean (±SE) plasma F_2-isoprostanes levels (ng/mL) for placebo and vitamin C in healthy subjects. Vitamin C has no effect on plasma F_2-isoprostane, a marker of oxidative stress (closed symbols: total plasma F_2-isoprostane concentrations after vitamin C administration, open symbols: isoprostane concentrations after placebo administration).

Table III. Change from baseline in cellular DNA base products (nmol/mg) for vitamin C and placebo at 8 h.

Base products	Treatment	Least square mean	Least squares mean difference	*p*-value
2-OH Adenine	Vitamin C	0.010	0.009 (−0.008, 0.027)	0.360
	Placebo	0.001		
5-(OH, Me) Uracil	Vitamin C	−0.018	−0.017 (−0.045, 0.011)	0.311
	Placebo	−0.002		
5-Formyl Uracil	Vitamin C	0.007	0.011 (−0.008, 0.030)	0.316
	Placebo	−0.004		
5-OH Cytosine	Vitamin C	0.009	0.013 (−0.004, 0.030)	0.207
	Placebo	−0.004		
5-OH Uracil	Vitamin C	−0.002	−0.004 (−0.008, 0.001)	0.218
	Placebo	0.001		
5-OH, Me Hydantoin	Vitamin C	0.007	0.004 (−0.003, 0.011)	0.361
	Placebo	0.003		
8-OH Adenine	Vitamin C	0.050	0.039 (−0.006, 0.084)	0.156
	Placebo	0.012		
8-OH Guanine	Vitamin C	0.057	0.068 (−0.009, 0.144)	0.142
	Placebo	−0.011		
FAPy Adenine	Vitamin C	0.016	0.023 (−0.008, 0.054)	0.211
	Placebo	−0.008		
FAPy Guanine	Vitamin C	−0.003	0.002 (−0.012, 0.017)	0.783
	Placebo	−0.006		
Thymine Glycol (cis)	Vitamin C	0.031	0.028 (−0.108, 0.164)	0.728
	Placebo	0.003		
8OHdG (urinary)	Vitamin C	0.212	−0.002 (−0.213, 0.208)	0.985
	Placebo	0.215		

at the level of the endothelium [9]. This current study demonstrates that vitamin C did not affect biomarkers of oxidative stress, in blood cells, plasma or urine in our subjects. As the markers used are generally considered to represent the best available measures of oxidative damage [25–28] and as F_2-isoprostanes in particular turn over very rapidly, with a plasma half-life of only 16 min [29], it seems unlikely that there is any clinically relevant effect on oxidative damage which we have missed. Although it is not yet clear what magnitude of change in F_2-isoprostanes is significant, subjects with impaired glucose tolerance (IGT), newly diagnosed DM or established DM have been reported to have plasma F_2-isoprostanes values 1.5-fold, 1.7-fold and 2–3-fold higher than controls [30,31]. Plasma F_2-isoprostanes are reported to decrease by 32% after improved glycaemic control in diabetic patients [32], by 32% after weight loss in obese women [33] and by 21–32% after smoking cessation [34]. Similarly, urinary levels of the 8-iso-$PGF_{2\alpha}$ are reported to be increased up to 2-fold in diabetic patients [32] and to decrease by 32% after improved glycaemic control in these patients. With 26 subjects and intra-subject CVs of 31% and 46%, we had 90% power to detect a 22% decrease in plasma F_2-isoprostanes or a 30% decrease in urinary F_2-isoprostanes, i.e. changes considerably smaller than the difference reported between diabetics and controls or similar to those seen after therapeutic interventions. Although we had low power to detect smaller differences, F_2-isoprostanes levels did not show any tendency to decrease after this large single dose of vitamin C. It remains possible that higher concentrations of vitamin C (for example those obtained by intra-arterial infusion) may exert an anti-oxidant effect, that changes in markers of oxidative damage might take longer to occur, that there might be localized changes in oxidative stress not reflected in levels of circulating F_2-isoprostanes or even that high-dose vitamin C could be deleterious in diabetes [35].

In summary, an oral dose of 2 g vitamin C does not have an acute vasodilatory effect in young healthy volunteers or in patients with type 2 diabetes (data not shown) and has no measurable effect on biomarkers of oxidative damage in healthy subjects. It seems unlikely that the relatively short exposure of the vascular endothelium was insufficient to allow vitamin C to act, since intra-arterial or intravenous administration of vitamin C has been report to act in less than 30 min [10,21]. It is more likely that plasma concentrations of vitamin C achieved with oral dosing are not sufficient to affect markers of oxidative stress or interfere with the endothelial interaction of superoxide and nitric oxide [36]. The role of vitamin C intake on the human vasculature remains to be clarified, but this study and others of longer duration [6,11,35,37] suggest that there is no significant beneficial effect of oral vitamin C supplementation on vascular wave reflection, the function of the vascular endothelium or on vascular outcomes.

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