Effects of a ‘healthy’ diet and of acute and long-term vitamin C on vascular function in healthy older subjects

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Abstract

Objective: Aging is associated with endothelial dysfunction. We studied the acute and longer-term effects of vitamin C compared to a ‘Mediterranean-type’ diet on endothelial function in healthy older subjects. Methods: Bilateral venous occlusion plethysmography was used to measure forearm blood flow in subjects aged 57–80 years. Responses to cumulative intra-arterial doses of the endothelium-dependent dilator bradykinin (BK; \(n=56\); 20, 40, 80 pmol/min) and the nitric oxide donor glyceryl trinitrate (GTN; \(n=54\); 4, 8, 16 nmol/min), were determined alone and in the presence of vitamin C (25 mg/min). We then randomised 54 subjects to a ‘healthy’ diet (\(n=18\)), vitamin C (1 g/day; \(n=18\)) or placebo for 6 weeks and reassessed endothelial and smooth muscle function. Results: Acute intra-arterial vitamin C did not alter dilatation to BK or GTN. Similar increases in plasma vitamin C occurred on oral vitamin C (83±64 to 135±88 pmol/l) and ‘healthy’ diet (84±5 to 135±27 μmol/l; \(P<0.01\) for both), with no change seen on placebo. Treatment with a ‘healthy’ diet but not oral vitamin C improved endothelium-dependent (\(P=0.043\)) and endothelium-independent dilatation (\(P=0.011\)). Conclusions: A ‘Mediterranean-type’ diet rich in vitamin C improves vascular function. Neither acute intra-arterial nor sustained administration of oral vitamin C improves vascular function in healthy older subjects.

Keywords: Aging; Endothelial function; Nitric oxide

1. Introduction

Aging is an important independent risk factor for cardiovascular disease. Endothelial dysfunction associated with reduced bioactivity of nitric oxide (NO) occurs in patients with cardiovascular risk, and might be a common pathway [1] by which risk factors may contribute to progression of atherosclerosis and cardiovascular disease [2–6]. In humans, aging is associated with reduced endothelium-dependent vasodilatation in the coronary [7] and forearm vasculature [8–10] even in the absence of other cardiovascular risk factors.

A key mechanism for age-related endothelial dysfunction and increased cardiovascular risk is increased oxidant stress, with increased concentrations of reactive oxygen species leading to reduced activity of NO [3]. Plasma concentrations of endogenous anti-oxidants, including vitamin C are reduced in elderly subjects and are inversely associated with the incidence of cardiovascular events [11]. These data suggest that reduced vitamin C (or other
dietary antioxidants) in the elderly might contribute to increased oxidative stress, reduced NO activity and cardiovascular disease.

Studies of administration of intra-arterial vitamin C in the forearm in subjects with cardiovascular risk factors other than age have reported increased endothelium-dependent relaxation in forearm resistance vessels [2–6]. In addition, Taddei et al. [12] have reported that intra-arterial vitamin C improves endothelium-dependent vasodilatation in elderly subjects (n = 12). However, these studies investigated the effects of single doses of vitamin C to achieve supraphysiological concentrations. Whether improved endothelial function can be achieved using clinically useful oral doses of vitamin C remains to be determined. Moreover, the role of other dietary anti-oxidants has not been investigated. We therefore studied whether sustained (6 weeks) vitamin C supplementation as tablets or as part of a ‘Mediterranean-type’ diet improves endothelial function in healthy older subjects.

2. Methods

2.1. Subjects

A total of 56 healthy older subjects (26 males; 53 Caucasian, one South Asian, two Afro-Caribbean; mean age 67 ± 1 (S.E.M.) years, range 57–80 years; average sitting blood pressure: 134/78 ± 3/1 mmHg) were recruited. The study conforms to the principles outlined in the Declaration of Helsinki (Cardiovascular Research 1997;35:2–3). All subjects gave written informed consent to the studies, which were approved by the local hospital ethics committee.

Subjects were screened by medical history, physical examination, electrocardiograph and laboratory analysis. They were excluded if any of the following were present: cardiovascular disease, diabetes mellitus, transient ischaemic attack or cerebrovascular accident, current smoking (26 subjects had never smoked; three subjects stopped smoking 6–12 months before the study; the rest had been ex-smokers for 18 ± 2 years), treatment for hyperlipidaemia, hypertension or use of other vasoactive medication. Subjects taking antioxidant supplements were asked to omit these for at least 6 weeks prior to study.

Height and weight were measured with subjects in light clothing and shoes removed. Sitting arterial blood pressure was measured after 10 min rest on two separate occasions using an automatic oscillometric device (Omron HEM 705 CP) [13]. Blood pressure was taken as the mean of three readings from the second visit. Blood samples were taken for biochemical analysis (renal and liver function, fasting glucose and fasting lipid profile) and determination of the plasma vitamin C levels [14].

2.2. Study protocol

Forearm endothelial and smooth muscle function was assessed at baseline. Subjects were randomised to 6 weeks of a healthy ‘Mediterranean-type’ diet (n = 18) or to a double-blind study of either vitamin C supplements (n = 18; 1 g/day as a single dose) or matched placebo tablets (n = 18). The subjects randomised to the ‘healthy’ diet were asked to make two main changes in their diet. Firstly, they were asked to increase their intake of fruit and vegetables by three portions a day. A portion was a helping that was standard for each particular subject. Secondly, they were asked to follow other aspects of a generally healthy ‘Mediterranean diet’ such as red meat to be replaced by poultry and fish, butter or cream to be replaced by margarine and use of olive oil for cooking, based on the De Lorgeril Mediterranean diet [15]. The effect on plasma vitamin C of these dietary changes in healthy older subjects was measured in a pilot study in nine older subjects (mean age 67 ± 1 (S.E.M.) years, range 57–75 years). After dietary intervention vitamin C intake increased from 141 ± 25 to 351 ± 22 mg/day (P < 0.01). Plasma ascorbic acid levels showed a significant increase from 68 ± 16 to 90 ± 16 μmol/l (P < 0.01). Based on this pilot data we estimated that the above dietary intervention would increase dietary vitamin C intake by approximately 150% above usual dietary vitamin C intake in healthy older subjects. Compliance was confirmed by tablet count and by food diaries kept by the subjects. After 6 weeks the weight and blood pressure were re-measured. Blood samples were taken for determination of the plasma vitamin C levels, and forearm endothelial and smooth muscle function was re-assessed.

2.3. Assessment of vascular function

Mercury-in-rubber strain gauge plethysmography [16] was used to measure bilateral forearm blood flow. We assessed the influence of acute and chronic administration of vitamin C, a ‘healthy’ diet and placebo on endothelium-dependent and -independent relaxation. Bradykinin (BK) or acetylcholine (ACh) were used to probe endothelium-dependent dilatation, and GTN was used to assess endothelium-independent dilatation.

Alcohol and caffeine were withheld for 12 h before the studies, which were performed in a temperature-controlled laboratory (26–28 °C) in the post-absorptive state. Drugs or physiological saline [0.9% (w/v) NaCl] were infused continuously at 0.5 ml/min into the brachial artery of the non-dominant arm through a 27 SWG needle introduced under local anaesthetic (1% lignocaine). During recording periods, the hands were occluded from the circulation by inflating a cuff placed around each wrist to supra-systolic pressure. Blood flow was derived by measuring the rate of
increase in forearm volume caused by inflating upper arm congesting cuffs to 40 mmHg for 10 s in every 15-s cycle. After a baseline period of 15 min following brachial artery cannulation, cumulative dose–response curves were constructed to BK (20, 40 and 80 pmol/min; each dose for 3 min), followed after 15 min by GTN (4, 8 and 16 nmol/min; each dose for 3 min). After a further 15 min to allow return to baseline blood flow, vitamin C (25 mg/min) was infused intra-arterially and dose–response curves to BK and GTN in the presence of vitamin C constructed as above. The dose of vitamin C was based on that found in previous studies to increase endothelium-dependent relaxation in the forearm in subjects at increased cardiovascular risk [2,4–6]. This dose of vitamin C leads to a local forearm concentration reported to protect human plasma from free radical-mediated lipid peroxidation [17]. Six weeks after randomisation to either ‘healthy’ diet, vitamin C supplements or placebo, the above protocol was repeated. As there is evidence for selective impairment of specific endothelium-dependent relaxant pathways by other cardiovascular risk factors [18], in 16 of the above subjects (mean age 66±1 years; range 57–78 years) the above protocol was repeated using ACh (25, 50 100 nmol/min) instead of BK as the endothelium-dependent dilator.

2.4. Drugs

BK (Calbiochem–Novabiochem, Nottingham, UK), GTN (Lipa Pharmaceuticals, Middlesex, UK), ACh (Sigma Chemical Co., Dorset, UK), ascorbic acid (Evans Medical Ltd., Leatherhead, UK) were obtained from commercially available sources. They were diluted to the desired concentration using normal saline. Fresh solutions were made for each study. Vitamin C tablets and matching placebo tablets were generously supplied by Wellbeing (Birmingham, UK).

2.5. Data analysis

Forearm blood flow, expressed as ml/min/100 ml forearm volume, was calculated according to the method of Whitney [16]. The ratio of blood flow in infused to control arm was calculated for each measurement period. Dilatation in response to the infused drugs was expressed as percentage increase in this blood flow ratio during control (saline) infusion. Responses were compared by analysis of the area under the dose–response curve and expressed in arbitrary units. Results are expressed as mean±S.E.M. Data were compared by Wilcoxon signed ranks test, where \( P<0.05 \) was considered significant. Differences in area under the curve (AUC) for dose–response curves for the three limbs of the 6-week treatment study were compared using Kruskal–Wallis (KW) tests. Where the KW test showed a significant difference, post-hoc comparisons between pairs of data were compared by the Mann–Whitney test.

3. Results

The baseline clinical and biochemical characteristics of the study population are shown in Tables 1 and 2. All subjects had normal renal function.

3.1. Effects of acutely administered vitamin C on endothelial function

Baseline forearm blood flow was 4.64±0.35 ml/min/100 ml forearm volume during saline infusion and did not alter significantly during intra-arterial administration of vitamin C (4.85±0.36 ml/min/100 ml forearm volume, \( P=0.42 \)). The BK response was unaffected by co-administration of vitamin C \( (n=56; \text{AUC } 1050±90 \text{ before and } 1014±95 \text{ in the presence of vitamin C, } P=0.549; \text{ differ-}

<table>
<thead>
<tr>
<th>Bradykinin study</th>
<th>Acetylcholine study</th>
<th>Placebo</th>
<th>Vitamin C tablets</th>
<th>‘Healthy’ diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>56</td>
<td>16</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>Age [median, interquartile range (years)]</td>
<td>67 (64–70)</td>
<td>65 (61–71)</td>
<td>69 (65–71)</td>
<td>66 (63–72)</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>26/30</td>
<td>5/11</td>
<td>9/9</td>
<td>9/9</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>25.4±0.5</td>
<td>25.7±0.6</td>
<td>25.4±0.9</td>
<td>26.1±0.9</td>
</tr>
<tr>
<td>Blood pressure (mmHg)</td>
<td>134/78±3/1</td>
<td>123/75±4/3</td>
<td>136/80±4/2</td>
<td>134/78±5/3</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>5.7±0.1</td>
<td>5.7±0.2</td>
<td>5.8±0.2</td>
<td>5.9±0.2</td>
</tr>
<tr>
<td>LDL (mmol/l)</td>
<td>3.6±0.1</td>
<td>3.5±0.2</td>
<td>3.8±0.2</td>
<td>3.6±0.2</td>
</tr>
<tr>
<td>HDL (mmol/l)</td>
<td>1.6±0.1</td>
<td>1.6±0.1</td>
<td>1.5±0.08</td>
<td>1.6±0.1</td>
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<tr>
<td>Triglycerides (mmol/l)</td>
<td>3.9±0.2</td>
<td>3.7±0.3</td>
<td>4.1±0.3</td>
<td>3.9±0.3</td>
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<tr>
<td>Glucose (mmol/l)</td>
<td>5.3±0.1</td>
<td>5.0±0.2</td>
<td>5.2±0.1</td>
<td>5.4±0.1</td>
</tr>
<tr>
<td>Vitamin C (μmol/l)</td>
<td>85±3</td>
<td>80±5</td>
<td>83±5</td>
<td>83±4</td>
</tr>
</tbody>
</table>

Values are mean±S.E.M.
Table 2
Clinical characteristics of study populations after 6 weeks intervention

<table>
<thead>
<tr>
<th></th>
<th>Pre-randomisation</th>
<th>After 6 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Placebo</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>25.4±0.9</td>
<td>24.3±0.7</td>
</tr>
<tr>
<td>Blood pressure (mmHg)</td>
<td>136/80±4/2</td>
<td>129/77±4/2*</td>
</tr>
<tr>
<td>Vitamin C (µmol/l)</td>
<td>83±5</td>
<td>88±9</td>
</tr>
<tr>
<td><strong>Vitamin C tablets</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>26.1±0.9</td>
<td>26.1±1.0</td>
</tr>
<tr>
<td>Blood pressure (mmHg)</td>
<td>134/78±5/3</td>
<td>134/78±4/2</td>
</tr>
<tr>
<td>Vitamin C (µmol/l)</td>
<td>83±4</td>
<td>135±8*</td>
</tr>
<tr>
<td><strong>‘Healthy’ diet</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
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<tr>
<td>Blood pressure (mmHg)</td>
<td>131/77±5/2</td>
<td>132/75±5/3</td>
</tr>
<tr>
<td>Vitamin C (µmol/l)</td>
<td>84±5</td>
<td>135±27*</td>
</tr>
</tbody>
</table>

Values are mean±S.E.M.
* P<0.05 vs. baseline pre-randomisation values.

ence in AUC for BK response: −36 (95% confidence interval (CI) −145 to 216; Fig. 1a). Vitamin C did not alter the response to ACh (n=16: AUC 1820±226 before and 1724±204 in the presence of vitamin C, P=0.569; difference in AUC for ACh response: −96 (95% CI −324 to 518; Fig. 2). Co-administration of vitamin C did not alter the vasodilator response to GTN in either study (n=54: AUC 751±70 before and 758±58 in the presence of vitamin C, P=0.949; difference in AUC for GTN response: +7 (95% CI −134 to 122; Fig. 1b; n=16 AUC 543±75 before and 574±91 in the presence of vitamin C, P=0.605; difference in AUC for GTN response: +31 (95% CI −244 to 180).

3.2. Effects of 6 weeks intervention

3.2.1. Plasma vitamin C

With 6 weeks intervention the increase in plasma vitamin C was similar with supplements (83±4–135±8 µmol/l) compared with a ‘healthy’ diet (84±5–135±27 µmol/l) but vitamin C levels were unchanged with placebo (83±5–88±9 µmol/l); KW for change in vitamin C, P=0.008.

3.2.2. Bradykinin-dependent responses

After 6 weeks of treatment baseline forearm blood flow did not change significantly in any of the three groups. However, there were significant differences between the three study groups in BK-dependent vasodilatation (mean change in BK AUC was: ‘healthy’ diet: +249±148, vitamin C tablets: −91±182, placebo: −355±172; KW: P=0.043; Fig. 3). Post-hoc tests showed a significant increase in BK-dependent vasodilatation with a ‘healthy’ diet (P=0.011) versus placebo. There was a trend for increased BK-dependent vasodilatation with vitamin C as tablets versus matched placebo but this did not achieve statistical significance (P=0.29; 95% CI −245 to 773).

3.2.3. GTN-dependent responses

After 6 weeks treatment, there were significant differences between the three study groups in GTN-dependent relaxation (mean change in GTN AUC: ‘healthy’ diet: +354±123, vitamin C tablets: −10±104, placebo: −197±175. KW: P=0.011; Fig. 4). This increase in GTN AUC was highly significant with a ‘healthy’ diet (P=0.01).
0.003) versus matched placebo but did not achieve statistical significance with vitamin C tablets versus placebo ($P=0.18; 95\% \text{ CI } -231 \text{ to } 604$).

### 3.3. Other measurements

There was no significant change in blood pressure or body mass index following 6 weeks supplementation with either vitamin C tablets or a ‘healthy’ diet (Table 2). Systolic and diastolic blood pressures were significantly lower after 6 weeks on placebo. Pre-randomisation values were 136/80±4/2 mmHg and after 6 weeks were 129/77±4/2 mmHg ($P=0.003$ and $P=0.014$ for systolic and diastolic pressures).

### 4. Discussion

There are two principal findings of this study. Firstly, we found that acute intra-arterial vitamin C has no major effect on endothelial or smooth muscle reactivity in healthy older subjects. Secondly, a healthy ‘Mediterranean-type’ diet for 6 weeks improves vasodilator function in the forearm of healthy older subjects whereas supplementation of vitamin C as tablets has no major effect.

Decreased synthesis, reduced bioavailability and impaired effector pathways for nitric oxide and other endothelium-derived relaxant factors have been implicated in age-associated impairment of endothelium-dependent relaxation, and each of these facets of endothelial function can be impaired by oxidative stress. Reactive oxygen species including superoxide inactivate NO directly, and the product of the reaction between NO and superoxide (peroxynitrite) is directly toxic to endothelial cells and has other actions to impair vascular relaxation [19]. Contributory factors to increased oxidant stress with age include reduced intake of dietary antioxidants [20] and increased generation of reactive oxygen species by co-morbid diseases as well as cumulative exposure to environmental oxidative stress through diet and pollution. Anti-oxidant rich foods such as fresh fruit and vegetables are costlier, and may be difficult to eat if dentition is poor. Cheaper oxidant-rich foods such as saturated fats may be substituted. There may also be reduced activity of endogenous anti-oxidant defences, including glutathione and anti-oxidant quenching enzymes such as superoxide dismutase.
[21,22]. Vitamin C is an important water-soluble anti-
oxidant in human plasma and is capable of scavenging
oxygen-derived free radicals such as superoxide anion
[17]. In the present study, acute administration of vitamin
C did not significantly alter endothelium-dependent relaxa-
tion in forearm resistance vessels in older subjects. This is
in contrast to previous studies of administration of acute
vitamin C in the forearm in subjects with other car-
diovascular risk factors, in which increased endothelium-
dependent relaxation in forearm resistance vessels has been
consistently reported [2–6]. Of note, this was despite our
use of the standard protocol for acute vitamin C adminis-
tration in the above positive published studies [2–6] and
assessment of two different endothelium-dependent relax-
ants, BK and ACh, in separate studies. Our study with 56
subjects for the BK responses and 54 subjects for the GTN
responses had a much higher power than previous reports
thereby making a type II error less likely. One explanation
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tary older subjects. However, their findings were likely to
have been confounded by the presence of smokers in their
study group as smoking is well recognised to cause
vitamin C reversible endothelial dysfunction in the forearm
[2].

In several previous studies, the endothelium-dependent
probe used was methacholine, which acts largely by nitric
oxide independent pathways [23]. Thus, vitamin C re-
versibility of endothelial dysfunction in those studies was
likely to have tested oxidant stress-mediated impairment of
NO-independent endothelial relaxant pathways.

Bias in our study by selection of physically fit subjects
appears unlikely as only ten of our 56 volunteers took any
form of exercise and in none of them was this vigorous or
frequent. The UK has very high standardised mortality
rates for ischaemic heart disease and a ‘healthy’ UK
person is likely to be less healthy than subjects from most
other developed countries. Our subjects were recruited
from the local population and had a wide range of social
backgrounds and dietary habits. Of note, average fasting
cholesterol levels in our study were typical for the UK
population with a mean 10% above action levels rec-
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but not to the pertussis toxin-insensitive mediator BK was
noted in patients with hypercholesterolaemia [18]. One
small previous study by Taddei et al. [12] (n=12) ob-
served impaired endothelium-dependent relaxation in re-
sponse to ACh, reversible by vitamin C in healthy seden-
tary older subjects. However, their findings were likely to
have been confounded by the presence of smokers in their
study group as smoking is well recognised to cause
vitamin C reversible endothelial dysfunction in the forearm
[2].
younger people [38]. At present drugs and invasive procedures are mainstays for the management of coronary heart disease. These can be expensive and are associated with considerable morbidity and mortality. The 2000 American Heart Association dietary guidelines highlighted several dietary issues requiring further research [39], recommending that antioxidant vitamins and other nutrients be derived from foods rather than vitamin supplements. Our data support this advice to use a diet rather than supplements as tablets or as sources of increased nutrient intake.

In conclusion the present study suggests that a healthy ‘Mediterranean-type’ diet improves endothelial function in the forearm and provides a basis for recommending a healthier diet to all older people, regardless of their apparent cardiovascular health. Our study also supports caution in the indiscriminate use of vitamin supplements as tablets.

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References


