

Effect of orange juice intake on vitamin C concentrations and biomarkers of antioxidant status in humans¹⁻⁴

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ABSTRACT

Background: Consumption of fruit and vegetables is associated with improved health and a decreased prevalence of chronic degenerative processes.

Objectives: The objectives were to assess the bioavailability of vitamin C from orange juice and its influence on plasma vitamin C and 8-*epi*-prostaglandin F_{2α} (8-*epi*-PGF_{2α}) concentrations in a healthy human population.

Design: Six men and 6 women consumed 500 mL commercial fresh-squeezed orange juice/d for 14 d, corresponding to an intake of 250 mg ascorbic acid/d. On the first day of the study, the subjects drank the juice in one dose (dose-response study), and on days 2–14 they consumed 250 mL in the morning and 250 mL in the afternoon. Blood was collected every hour for 6 h on the first day and again on days 7 and 14.

Results: Baseline plasma vitamin C concentrations were significantly higher ($P = 0.03$) among the women than among the men (56.4 ± 4.4 compared with 44.3 ± 3.5 $\mu\text{mol/L}$). In the dose-response study, the maximum increase in plasma vitamin C occurred 3 h postdose in both the men and the women. Vitamin C concentrations remained significantly higher on days 7 and 14 than at baseline. Baseline concentrations of 8-*epi*-PGF_{2α} were significantly higher ($P = 0.03$) among the men than among the women (249.6 ± 25.4 compared with 177.7 ± 6.2 pg/mL) but decreased significantly ($P = 0.04$) by day 14 of the intervention. A significant inverse correlation was observed between vitamin C and 8-*epi*-PGF_{2α} ($r = -0.791$, $P = 0.0022$). Among smokers, baseline vitamin C was lower and 8-*epi*-PGF_{2α} higher than among nonsmokers.

Conclusions: Drinking orange juice (500 mL/d) increases plasma concentrations of vitamin C and reduces concentrations of 8-*epi*-PGF_{2α} in humans. These effects were significantly more pronounced in smokers. *Am J Clin Nutr* 2003;78:454–60.

KEY WORDS Orange juice, vitamin C, bioavailability, F₂-isoprostanes, uric acid, food-frequency questionnaire, smoking

INTRODUCTION

An increased consumption of fruit and vegetables has been associated with beneficial effects on the risk of disease (1–5). Citrus juices, especially orange juice and grapefruit juice, are rich sources of vitamin C, but their health benefits remain poorly understood. Recent reports suggested that drinking generous amounts of a mixture of various juices improves the blood

lipid profile, reduces oxidative stress, prevents atherogenic modifications of LDL cholesterol and platelet aggregation (6–8), and improves HDL-cholesterol concentrations (9). Several studies have shown that vitamin C plays an important role in human health, including effects on the immune system (10), the risk of Alzheimer disease (11), and the efficiency with which lysosomes in brain glial cells degrade modified proteins (12). However, no study has examined the bioavailability of vitamin C in orange juice and its possible beneficial effects on reducing isoprostane concentrations.

Isoprostanes are a family of eicosanoids of nonenzymatic origin that are produced by the random oxidation of phospholipids by oxygen radicals and that are elevated by oxidative stress (13, 14). One of the isoprostanes, 8-*epi*-prostaglandin F_{2α} (8-*epi*-PGF_{2α}), has been shown to act as a vasoconstrictor (15) and to be associated with the hepatorenal syndrome and pulmonary oxygen toxicity (16). Previous studies showed decreased concentrations of antioxidants and high concentrations of 8-isoprostanes among smokers (17, 18), which may be due to a low intake of antioxidants (19) or a greater utilization of vitamin C by free radicals produced during smoking (20).

High serum uric acid may be an important predicting risk factor for cardiovascular events, such as myocardial infarction (21). Multivariate analysis of data from the MONICA cohort of 1044

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males showed a significant association between serum uric acid concentrations and cardiovascular mortality (22). Uric acid may have a direct injurious effect on the endothelium, altering endothelial cell function and reducing nitric oxide bioavailability, which is relevant to cardiovascular disease risk.

Antioxidants have important roles in cell function and have been implicated in processes associated with aging, including vascular processes, inflammatory damage, and cancer (23–25). Vitamin C may also contribute to the maintenance of a healthy vasculature and to a reduction in atherogenesis through the regulation of collagen synthesis, prostacyclin production, and nitric oxide (26–28). In addition to its antioxidant role, vitamin C has roles at the molecular level, including acting as a cofactor for dopamine β -hydroxylase (EC 1.14.17.1), influencing neurotransmitter concentrations, improving lysosomal protein degradation, and mediating glutamate uptake (5, 29, 30). The second National Health and Nutrition Examination Survey reported that a low intake of vitamin C is associated with blood concentrations of vitamin C ≤ 0.3 mg/dL (17 μ mol/L) (31), whereas blood concentrations in well-nourished persons fluctuate between 0.8 and 1.3 mg/dL (45 and 74 μ mol/L). Fruit and vegetables are the main sources of vitamin C, but 25% of women and $\approx 33\%$ men eat < 2.5 servings of fruit and vegetables daily, which provides ≈ 80 mg vitamin C/d. Therefore, the objectives of this study were to assess the bioavailability of vitamin C in orange juice and its effect on concentrations of 8-*epi*-PGF_{2 α} (F₂-isoprostanes) in a healthy human population.

SUBJECTS AND METHODS

Subjects

Twelve healthy volunteers (6 men and 6 women) were enrolled in this study. The subjects were aged between 20 and 32 y ($\bar{x} \pm$ SD: 22 ± 3 y), and their mean body mass index (in kg/m²) was 22.2 ± 1.6 and did not change significantly during the study. All the subjects continued their habitual diets during the study. Subjects were taking no vitamin or mineral supplements and no medications. None of the subjects were pregnant, lactating, or had any chronic illness. All study participants were in good health on the basis of a medical history, a physical examination, and normal results on clinical laboratory tests, including measurement of triacylglycerol (0.9 ± 0.3 mmol/L), cholesterol (4.4 ± 1.1 mmol/L), glucose (4.8 ± 0.4 mmol/L), and hematocrit (0.44 ± 0.03). Subjects received oral and written information about the study and gave their written consent. The study was approved by the Clinic Research Ethics Committee of the Hospital Universitario Clínica Puerta de Hierro, Madrid.

Collection of plasma samples and experimental protocol

The vitamin C bioavailability study was divided into 2 components: a dose-response test and a multiple-dose-response study. For the dose-response test, an intravenous catheter was inserted into each subject's forearm after he or she had fasted for ≥ 12 h. After blood samples were collected at baseline, the volunteers consumed 500 mL orange juice and blood samples were taken every 60 min up to 6 h. Blood samples were collected in heparin-coated tubes and were centrifuged at $2000 \times g$ for 15 min at 4 °C. After the plasma was collected, aliquots in triplicate were immediately mixed with an equal volume of cold 6% (wt:vol) metaphosphoric acid containing 1 mmol/L of the metal

TABLE 1

Composition of commercial fresh-squeezed orange juice per 100 mL¹

Energy (kJ)	170
Protein (g)	0.6
Carbohydrates (g)	10
Fat (g)	< 1
Vitamin C (mg) ²	54.0 ± 10.5
Total flavanones (mg) ²	10.7 ± 0.4
Total carotenoids (μ g) ²	423.9 ± 27.3

¹ Don Simón Naranja Exprimida (J García Carrión, SA, Jumilla, Murcia, Spain).

² $\bar{x} \pm$ SD. Concentrations obtained from the analyses performed in our laboratory.

ion chelator diethylenetriaminepentaacetic acid for vitamin C and uric acid analysis. The remaining plasma was stored at -80 °C for analysis of 8-*epi*-PGF_{2 α} . For the multiple-dose-response study, the subjects were instructed to drink the juice at home in 2 doses, 250 mL in the morning and 250 mL in the afternoon, for 2 consecutive weeks. Blood samples were taken again during the intervention on days 7 and 14 of the study.

The composition of the commercial fresh-squeezed orange juice consumed by the participants is reported in **Table 1**. The vitamin C, flavanone, and carotenoid composition of the juice was measured by reversed-phase HPLC with methods currently used in our laboratory (32–34). Total energy, protein, carbohydrate, and fat contents were provided by J García Carrión, SA, Jumilla, Murcia, Spain.

Nutritional status assessment

Dietary history data were obtained through a food-frequency questionnaire (including 125 food items) administered to the subjects by a nutritionist. The reference period for the food-frequency questionnaire was the preceding 3 mo. The food-frequency questionnaires were then scanned (OPSCAN5; National Computer Systems, St Paul), transferred into electronic files, and converted into statistical datasets for analysis. The Minnesota NUTRIENT DATA SYSTEM (program 2.8, version 25; University of Minnesota, Minneapolis) was used to calculate the nutrient intake profiles (35). In general, as previously reported, a simple self-administered dietary questionnaire can provide useful information about individual nutrient intakes over several months (36).

Measurement of vitamin C

Ascorbate was analyzed by paired-ion, reversed-phase HPLC coupled with electrochemical detection. Briefly, a 100- μ L plasma sample was mixed with an equal volume of cold 6% (wt:vol) metaphosphoric acid containing 1 mmol/L of the metal ion chelator diethylenetriaminepentaacetic acid (Sigma, St Louis). A portion of the sample was analyzed on an LC8 column (150 mm \times 4.6 mm internal diameter, 3- μ m particle size; Supelco, Bellefonte, PA) with 99% deionized water and 1% methanol containing 40 mmol sodium acetate/L and 1.5 mmol dodecyltriethylammonium phosphate/L (Q12 ion pair cocktail; Regis, Morton Grove, IL) as the mobile phase delivered at a flow rate of 1 mL/min. Samples were injected with an autosampler (1100 series; Hewlett Packard Co, Wilmington, DE). Ascorbate was detected at an applied potential of 0.6 V, with the gain set at 100 nA by an LC 4B amperometric electrochemical detector (Bioanalytical Systems, West Lafayette, IN). Ascorbate was

TABLE 2

Plasma vitamin C and 8-*epi*-prostaglandin F_{2α} (8-*epi*-PGF_{2α}) concentrations at baseline and on days 7 and 14 of the study in men and women¹

	Men (n = 6)			Women (n = 6)		
	Baseline	Day 7	Day 14	Baseline	Day 7	Day 14
Vitamin C (μmol/L) ²	44.3 ± 3.5 ³	64.2 ± 4.4	67.2 ± 3.3	56.4 ± 4.4	68.7 ± 4.8	68.8 ± 6.4
8- <i>epi</i> -PGF _{2α} (pg/mL)	249.6 ± 25.4 ³	214.2 ± 19.0	182.9 ± 17.2 ⁴	177.7 ± 6.2	170.9 ± 10.7	180.3 ± 15.1

¹ $\bar{x} \pm \text{SEM}$. There were significant sex-by-time interactions for 8-*epi*-PGF_{2α} ($P = 0.02$) and for vitamin C ($P = 0.001$) by repeated-measures ANOVA.²Significantly different on days 7 and 14 than at baseline for men and women combined (time effect), $P < 0.05$ (repeated-measures ANOVA and Tukey's test).³Significantly different than at baseline in women, $P = 0.03$ (Student's t test).⁴Significantly different than at baseline (time effect), $P = 0.04$ (repeated-measures ANOVA and Tukey's test).

eluted as a single peak with a retention time of 5.5 min. Peaks were integrated with a ChemStation (Hewlett Packard). Ascorbate concentrations were calculated on the basis of a calibration curve and are expressed in μmol/L (33).

Measurement of uric acid

Uric acid was analyzed by paired-ion, reversed-phase HPLC coupled with electrochemical detection by following the same procedure described for vitamin C; an electrode potential of 0.6 V was used, but the gain was set at 1 μA. Uric acid was eluted as a single peak with a retention time of 3.5 min. Peaks were integrated with a ChemStation (Hewlett Packard). Uric acid concentrations were calculated on the basis of a calibration curve and are expressed as μmol/L (33).

Measurement of 8-isoprostane

8-*epi*-PGF_{2α}, which has been proposed as a marker of oxidative stress, was measured by using an enzyme immunoassay kit (37). First, samples (0.5–1 mL) were mixed gently with 50 μL affinity sorbent (mouse anti-8-isoprostane covalently bound to Sepharose 4B) (Cayman Chemical Co, Ann Arbor, MI) for 60 min at room temperature to purify the samples. Next, the samples were briefly centrifuged at $11\,750 \times g$ (4°C) so that the sorbent would form a sediment (Cayman Chemical Co). The sorbent, which contains the bound 8-isoprostane, was rinsed with washing solution, and, after the supernatant fluid was discarded, the sorbent pellet was resuspended in 300 μL of an ethanol elution solution. The ethanol washes were evaporated to dryness in a vacuum centrifuge and were immediately dissolved in 125 μL enzyme immunoassay buffer. Then, samples were analyzed in duplicate by using the enzyme immunoassay kit. This assay is based on the competition between 8-isoprostane and an 8-isoprostane-acetylcholinesterase conjugate (8-isoprostane tracer) for a limited number of 8-isoprostane-specific rabbit antiserum binding sites. The rabbit antiserum 8-isoprostane (either free or tracer) complex binds to the rabbit immunoglobulin G mouse monoclonal antibody that was previously attached to the well. The plate was washed to remove any unbound reagents and then Ellman's reagent (which contains the substrate to 8-isoprostane-acetylcholinesterase; Cayman Chemical Co) was added to the well. The product of this enzymatic reaction has a distinct yellow color and absorbs strongly at 412 nm. The intensity of this color, determined spectrophotometrically, is proportional to the amount of 8-isoprostane tracer bound to the well, which is inversely proportional to the amount of free 8-isoprostane present in the well during the incubation. The concentration of 8-isoprostane in the test samples was interpolated from the standard curve by using log transformation (16).

Statistical analysis

Descriptive statistics (including means and SDs) were used to summarize the characteristics of the subjects. Values are presented as means ± SDs or ± SEMs when appropriate. Repeated-measures analysis of variance comparing the concentrations of vitamin C, uric acid, and 8-*epi*-PGF_{2α} between the sexes and at different times of the intervention were performed by using SYSTAT 10 (SPSS Inc, Chicago) to test for statistical significance at the $P \leq 0.05$ level. When sex-by-time interactions or sex effects were observed, Tukey's honestly significant difference test was used to determine differences at different time points. The correlations within variables were examined by linear regressions or by Spearman's correlation as appropriate, also by using the SYSTAT program (38).

RESULTS

The food-frequency questionnaire data showed that energy intake tended to be higher ($P = 0.06$) among the men (2562.0 ± 598.2 kcal/d) than among the women (1994.8 ± 773.9 kcal/d). The main contributor to the difference in energy intake observed may have been the higher ($P = 0.02$) intake of carbohydrates among the men (275.8 ± 67.3 g) than among the women (201.6 ± 64.8 g). No significant differences in micronutrient intake were observed between the men and women. There was also no significant difference ($P = 0.3$) in daily vitamin C intake between the men (136.1 ± 53.0 mg) and the women (111.9 ± 39.3 mg).

Baseline plasma vitamin C concentrations were significantly higher ($P = 0.03$) in the women (56.4 ± 4.4 μmol/L) than in the men (44.3 ± 3.5 μmol/L; **Table 2**). On the first day of the intervention, the maximum increase in vitamin C occurred 3 h after consumption of the orange juice (500 mL) containing 250 mg vitamin C in both the men and the women (**Figure 1**). At 3 h (maximum peak), plasma concentrations had increased over baseline by 64% in the men (72.6 ± 4.0 compared with 44.3 ± 3.5 μmol/L; $P = 0.001$) and by 40% in the women (78.8 ± 4.6 compared with 56.4 ± 4.4 μmol/L; $P = 0.009$).

Plasma vitamin C concentrations were also analyzed on days 7 and 14. Plasma vitamin C concentrations remained elevated ($P < 0.05$) during the study (Table 2). However, no significant differences were observed between the men and the women except at baseline. There was a significant sex-by-time interaction for bioavailability of vitamin C ($P = 0.001$). Interestingly, although the concentration of vitamin C at the end of the study (day 14) remained significantly higher than at baseline, it tended to be lower (although not significantly so) than the concentration reached by drinking the juice all in one dose (500-mL dose compared with two 250-mL doses).

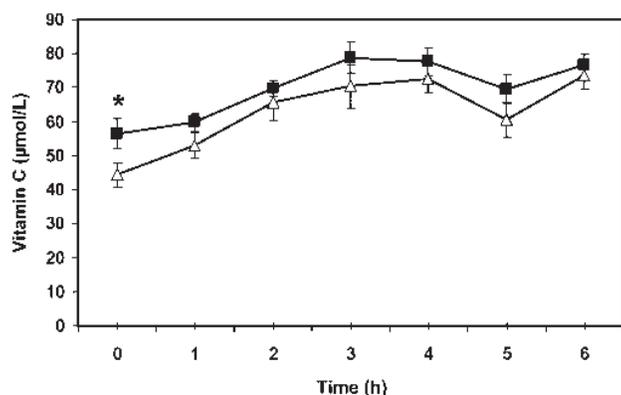


FIGURE 1. Mean (\pm SEM) plasma vitamin C concentrations in men (Δ ; $n = 6$) and women (\blacksquare ; $n = 6$) at baseline (0 h) and every hour after drinking orange juice. *Significant difference between women and men, $P = 0.03$. There was no significant main effect of sex; however, there was a significant sex-by-time interaction by ANOVA. For both sexes combined, values at 0 and 1 h were significantly lower than values at 2, 3, 4, and 6 h, $P < 0.005$, and values at 2 and 5 h were significantly lower than values at 3, 4, and 6 h, $P < 0.005$ (repeated-measures ANOVA and Tukey's honestly significant difference test).

Uric acid concentrations were significantly higher ($P < 0.01$) among the men than among the women (baseline: 342.0 ± 16.7 compared with 216.1 ± 16.9 $\mu\text{mol/L}$; **Table 3**). In general, uric acid concentrations were lower when the vitamin C concentration was higher, and an inverse association was observed between uric acid concentrations and plasma vitamin C concentrations across time in both men and women ($r = -0.19$, $P = 0.05$).

Baseline plasma concentrations of 8-*epi*-PGF_{2 α} were significantly higher ($P = 0.03$) among the men (249.6 ± 25.4 pg/mL) than among the women (177.7 ± 6.2 pg/mL; **Table 2**). Interestingly, we observed a trend toward a decrease in plasma concentrations of 8-*epi*-PGF_{2 α} among the men on day 7 of the intervention, which became significant on day 14 ($P = 0.04$). No significant changes were observed by time among the women.

An inverse correlation was observed between vitamin C concentrations and 8-*epi*-PGF_{2 α} concentrations among both the men and the women at baseline ($r = -0.60$, $P = 0.05$) and on day 14 of the study ($r = -0.55$, $P = 0.06$). Although the 8-*epi*-PGF_{2 α} concentration decreased significantly on day 14 in the men, the vitamin C concentration was more stable, which affected the correlation. However, because changes in 8-*epi*-PGF_{2 α} and the association between vitamin C and 8-*epi*-PGF_{2 α} may be highly relevant to health status, we plotted concentrations of vitamin C and 8-*epi*-PGF_{2 α} at baseline and at the end of the study (day 14)

TABLE 3

Plasma uric acid concentrations at baseline, 6 h postdose, and on day 14 of the intervention in men and women¹

	Men ($n = 6$)	Women ($n = 6$)
	$\mu\text{mol/L}$	
Baseline	342.0 ± 16.7^2	216.1 ± 16.9
6 h	341.0 ± 13.3^2	198.9 ± 16.0
14 d	299.4 ± 27.6^2	202.0 ± 17.7

¹ $\bar{x} \pm \text{SEM}$.

²Significantly different from women, $P < 0.01$ (repeated-measures ANOVA and Tukey's test).

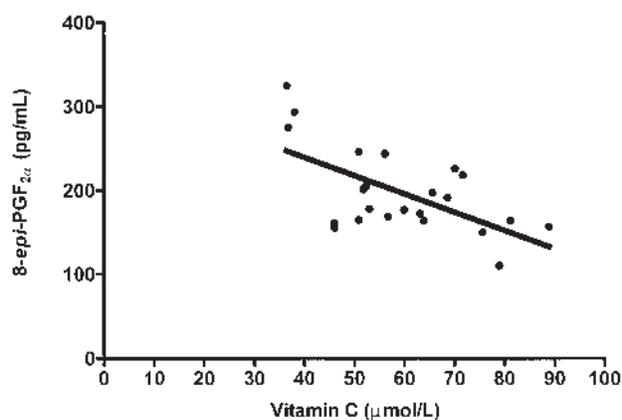


FIGURE 2. Correlation between plasma concentrations of 8-*epi*-prostaglandin F_{2 α} (8-*epi*-PGF_{2 α}) and vitamin C at baseline and at the end of the intervention (day 14) combined. $r = -0.791$, $P = 0.0022$; $n = 23$.

combined as more representative of the association of vitamin C with 8-*epi*-PGF_{2 α} ($r = -0.791$, $P = 0.0022$; **Figure 2**). The baseline concentration of vitamin C in one subject was extremely low (outlier) and we were unable to confirm whether this was a true measurement or an artifact; thus, that concentration was excluded from the correlation.

Among the men, the baseline plasma vitamin C concentration was significantly higher ($P = 0.04$) among nonsmokers than among smokers, and the plasma 8-*epi*-PGF_{2 α} concentration was significantly higher ($P = 0.045$) among smokers than among nonsmokers (**Table 4**). After daily consumption of orange juice, vitamin C concentrations increased significantly among smokers ($P = 0.04$) at the end of the first week, and, at the end of study, the concentration of vitamin C was 93% higher than at baseline (71.6 ± 3.9 compared with 37.1 ± 0.5 $\mu\text{mol/L}$; $P = 0.02$). Nonsmokers also showed higher vitamin C concentrations ($P = 0.045$) on days 7 and 14 than at baseline. On days 7 and 14, plasma 8-*epi*-PGF_{2 α} concentrations among smokers were significantly ($P \leq 0.04$) lower than at baseline.

DISCUSSION

In the present study, we showed for the first time that drinking 2 glasses of orange juice (500 mL) daily increases plasma vitamin C concentrations by 40–64%. Interestingly, changes in vitamin C were significantly inversely correlated with concentrations of 8-*epi*-PGF_{2 α} , suggesting that vitamin C may play a critical role in reducing the formation of compounds produced by the random oxidation of phospholipids by oxygen radicals.

Vitamin C intake as assessed by the food-frequency questionnaire was not significantly different between the men and the women in the present study. However, plasma vitamin C concentrations were lower in the men than in the women. These differences in basal plasma vitamin C concentrations may have been mediated by an augmented consumption of this nutrient as a result of increased metabolic requirements or because of an excess of reactive oxygen species formed in men compared with women. In fact, we noticed that the concentration of 8-*epi*-PGF_{2 α} was significantly higher among smokers than among nonsmokers. Therefore, if vitamin C is the most important antioxidant present in the aqueous solution, an increased formation of free radicals or byproducts derived from free radical-mediated reactions would be associated with an enhanced consumption of vitamin C (39).

TABLE 4

Plasma vitamin C and 8-*epi*-prostaglandin F_{2α} (8-*epi*-PGF_{2α}) concentrations at baseline and on days 7 and 14 of the study in male nonsmokers and smokers¹

	Nonsmokers (n = 3)			Smokers (n = 3)		
	Baseline	Day 7	Day 14	Baseline	Day 7	Day 14
Vitamin C (μmol/L) ²	51.5 ± 2.9 ³	66.7 ± 8.9	62.8 ± 4.5	37.1 ± 0.5	61.7 ± 3.7	71.6 ± 3.9
8- <i>epi</i> -PGF _{2α} (pg/mL)	201.1 ± 25.7	191.7 ± 45.2	188.0 ± 15.3	298.0 ± 14.5 ⁴	236.6 ± 24.7 ⁵	177.9 ± 34.9 ⁵

¹ $\bar{x} \pm$ SEM. There were significant smoking-by-time interactions for 8-*epi*-PGF_{2α} ($P = 0.025$) and for vitamin C ($P = 0.01$) by repeated-measures ANOVA.

²Significantly different on days 7 and 14 than at baseline for nonsmokers and smokers combined (time effect), $P < 0.05$ (repeated-measures ANOVA and Tukey's test).

³Significantly different than at baseline in smokers, $P = 0.04$ (Student's t test).

⁴Significantly different than at baseline in nonsmokers, $P = 0.045$ (Student's t test).

⁵Significantly different than at baseline (time effect), $P \leq 0.04$ (repeated-measures ANOVA and Tukey's test).

Vitamin C is an essential nutrient for humans; unlike most mammals, we cannot synthesize vitamin C and therefore must acquire it from the diet. Some studies have investigated the bioavailability of chemically identical natural and synthetic ascorbic acid and found no clinically significant difference in bioavailability or bioactivity (40). However, no studies documenting the bioavailability of vitamin C from orange juice have been reported. This is surprising because orange juice is one of the most important sources of vitamin C in the human diet. Recently, the plasma kinetics of flavanones in orange and grapefruit juice were examined, but not their effects on plasma vitamin C concentrations (41). Intake of a mix of fruit and vegetables (500 g) for 4 wk was associated with a 64% increase in vitamin C concentration (42). At low concentrations, the absorption of ascorbic acid occurs through an active transport process, whereas at high concentrations, absorption is mediated by a combination of both active and passive diffusion in the gastrointestinal tract (43, 44). Thus, consumption of foods rich in vitamin C may favor absorption by slowing the interaction of the juice with the gastric wall. In fact, in the present study, consumption of 250 mg vitamin C, contained in 2 glasses of orange juice (500 mL), significantly increased plasma vitamin C from 30–50 to 60–90 μmol/L in just 3 h. The increased concentration was maintained as long as the subjects were drinking the orange juice, which suggests that this is an efficient means of increasing vitamin C concentrations in the body. In other studies, blood concentrations of vitamin C were manipulated through the use of high vitamin C intake in the form of tablets (2000 mg), and vitamin C concentrations reached 116 μmol/L 2 h after ingestion and 95 μmol/L after taking 500 mg (45), which suggests that high-dose supplements might not be the most efficient way of increasing the body's pool of vitamin C.

A high consumption of fruit and vegetables is associated with a decreased risk of chronic diseases, and an increased intake of vitamin C is associated with health status (11, 30). Vitamin C is an essential cofactor in neurotransmitter synthesis (46) and is important in vascular function (45). In addition, ascorbic acid scavenges superoxide anions and plays an important role in the control of the intracellular redox state (47). Therefore, in the present study, we evaluated the effect of vitamin C on the formation of F₂-isoprostanes.

Isoprostanes are a family of eicosanoids of nonenzymatic origin produced by the random oxidation of phospholipids by oxygen radicals. 8-*epi*-PGF_{2α} has received significant attention as the result of its vasoconstrictive, platelet activation, and mitogenic properties (16, 17). In 1990 PGF₂-like compounds were discovered to be produced in abundance in vivo through the peroxidation

of arachidonic acid from phospholipids, a reaction catalyzed by free radicals independent of the cyclooxygenase enzyme (EC 1.14.99.1). These compounds are isomeric to cyclooxygenase-derived PGF_{2α} and are named F₂-isoprostanes, which increase with oxidant injury (48). In fact, F₂-isoprostanes provide an accurate measure of oxidant stress in vivo. Several studies have assessed changes in F₂-isoprostane concentrations in relation to health status and different pathologic processes (13, 49, 50). Interestingly, we observed a significant inverse correlation between the concentration of vitamin C and concentrations of 8-*epi*-PGF_{2α} at baseline. Furthermore, after subjects drank the orange juice daily for 14 d, concentrations of 8-*epi*-PGF_{2α} were reduced by 27%, and the correlation remained the same, even though concentrations of 8-*epi*-PGF_{2α} and vitamin C changed significantly. We believe that the lack of further reduction in 8-*epi*-PGF_{2α} concentrations in women after orange juice consumption might have been because concentrations of 8-*epi*-PGF_{2α} were significantly lower and vitamin C concentrations significantly higher in women than in men throughout the study. Other studies also reported changes in 8-isoprostane concentrations after supplementation with vitamin C, vitamin E, or both (51–53).

Cigarette smoke contains numerous oxidants, and it is believed that smoking may cause oxidant injury. In this study, we also found that among those men who reported smoking (50%), concentrations of 8-*epi*-PGF_{2α} were significantly higher than in nonsmokers. Furthermore, the highest concentrations of 8-*epi*-PGF_{2α} were detected among the subjects with the lowest vitamin C concentrations. Interestingly, these subjects experienced major reductions in 8-*epi*-PGF_{2α} after drinking orange juice. Similar observations were previously reported by others, who showed that plasma ascorbic acid is depleted in smokers compared with nonsmokers and is repleted by moderate supplementation (51, 54). Moreover, in human studies of cigarette smokers and nonsmokers, a higher amount of F₂-isoprostanes was found in smokers' urine and plasma (51).

Another finding of the present study was the inverse association observed, predominantly in men, between high plasma concentrations of vitamin C and lower concentrations of uric acid, which has recently been considered of great interest (55). The mechanisms involved in this association remain unknown. Epidemiologic studies have indicated the presence of relevant associations between uric acid and cardiovascular disease (55, 56). In addition, several observational studies have shown that serum uric acid concentrations are higher among patients with established coronary heart disease (57). About one-quarter of hypertensive patients have a coexistent hyperuricemia (57, 58). A multivariate

analysis of data from the MONICA cohort of 1044 men indicated a significant association between high serum uric acid concentrations and cardiovascular mortality (22). Several mechanisms appear to be involved in this association, including increased platelet adhesiveness and platelet lysis, formation of free radicals, and oxidative stress (59–61).

Some limitations of the present study should be acknowledged. Perhaps the most important of these is the small number of subjects enrolled in the study. However, the relatively small variability in the analysis performed (both intra- and interassay) contributed to the differences observed. Another limitation of the study is the lack of outcomes associated with plasma concentrations after the intake of orange juice. Because this was a healthy population, the main objective was to assess the bioavailability of vitamin C in the juice and its association with oxidative stress.

In conclusion, drinking orange juice increased vitamin C concentrations and reduced oxidative stress in vivo by lowering the concentration of F₂-isoprostanes, a finding that provides new evidence of the health benefits of eating fruit. Drinking 2 glasses of orange juice (500 mL/d) containing 250 mg vitamin C increased vitamin C concentrations in plasma and reduced the concentrations of 8-*epi*-PGF_{2α} and uric acid. Total vitamin C was significantly and inversely correlated with 8-*epi*-PGF_{2α}. These effects were more accentuated among smokers than among nonsmokers. These findings suggest that the protective effect of vitamin C is greater in persons with higher stress and that higher concentrations of vitamin C in tissues can be gained by drinking orange juice daily. Evaluation of the health-promoting properties of vitamin C in fruit deserves further attention. 

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CS-M and AM were responsible for study design, sample and data analyses, and writing of the manuscript, and MPC, BdA, LP, BO, and FG were responsible for study design and sample collection. None of the authors had any financial or personal interest, including advisory board affiliations, in any company or organization sponsoring the research.

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