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Pulsed electric fields–processed orange juice consumption increases plasma vitamin C and decreases F2-isoprostanes in healthy humans

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Abstract

Orange juice, a rich source of vitamin C, accounts for 60% of all fruit juices and juice-based drinks consumed in western Europe. Orange juice preservation is currently accomplished by traditional pasteurization. Pulsed electric fields (PEF) have been studied as a nonthermal food preservation method. Food technology needs in the area of processing are driven by nutrition. Therefore, the objectives of this study were to assess the bioavailability of vitamin C from pulsed electric fields–treated orange juice in comparison with freshly squeezed orange juice and its impact on 8-*epi*$PGF_{2\alpha}$ concentrations (biomarker of lipid peroxidation) in a healthy human population. Six subjects consumed 500 mL/day of pulsed electric fields–treated orange juice and six subjects consumed 500 mL/day of freshly squeezed orange juice for 14 days, corresponding to an intake of about 185 mg/day of ascorbic acid. On the first day of the study, subjects drank the juice in one dose, 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 hours on the first day and again on days 7 and 14. In the dose-response study, the maximum increase in plasma vitamin C occurred 4 hours postdose. Vitamin C remained significantly higher on days 7 and 14 in both orange juice groups. Plasma 8-*epi*$PGF_{2\alpha}$ concentrations was lower at the end of the study ($P < 0.001$) in both groups. Plasma levels of vitamin C and 8-*epi*$PGF_{2\alpha}$ were inversely correlated. Pulsed electric fields–preservation of orange juice retains the vitamin C bioavailability and antioxidant properties of fresh juice with a longer shelf-life.

Keywords: Orange juice; Pulsed electric fields; Vitamin C; F2-isoprostanes; Uric acid

1. Introduction

Epidemiological evidence suggests the protective effects of plant-based diets on certain cardiovascular diseases and other chronic diseases [1,2]. Two large cross-sectional studies in the United States, the National Health and Nutrition Examination Survey (NHANES) II and III, showed an association between high serum vitamin C concentration and a decreased prevalence of stroke [3,4]. Recent studies suggest that intake of orange juice, a rich source of vitamin C, improve HDL-cholesterol plasma levels in subjects with moderate hypercholesterolemia [5].

Orange juice accounts for 60% of all fruit juice and juice-based drinks consumed in western Europe [6]. Hydrosoluble nutrients such as vitamin C that are present in orange juice are susceptible to degradation by heat [7]. Thus, preservation of food quality with little depletion in the hydrosoluble vitamin contain is a main concern [8,9]. New approaches involve nonthermal processing technologies that offer full or partial alternatives to heat for the inactivation of micro-organisms and enzymes [10,11]. Pulsed electric fields (PEF) treatment involves applying very short electric pulses (μs) at high electric field intensities (typically pulse duration within the interval of 10–1000 μs; electric field

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strength within 20–80 kV/cm). During the PEF procedure, energy loss due to heating of foods is minimized, thereby reducing nutritional depletion and changes in the physical properties of foods [12,13].

Recent studies have confirmed that PEF-processed orange juice retains many of the characteristics of fresh juice. PEF-treated orange juice retains all the physical properties, along with a 97.5% of vitamin C, and a reduction of 92.7% in the pectinmethylesterase activity required to prevent cloudiness in the juice. In addition, PEF treatment improves the microbial shelf-life and browning index during storage at 4°C compared with heat pasteurization (96.4°C for 30 s) [6,14–16]. Until now, research has been focused on the sensory attributes and shelf-life of PEF-treated juices, but there are no studies assessing the impact of PEF technology on the health benefits of orange juice. Specifically, it is known that PEF application to vegetable foods results in effective permeabilization of cellular membranes and in some cases disruption of their structural integrity [17]. However, whether these structural changes in the cell walls of the orange juice sacs affect the intestinal absorption of the bioactive compounds contained in PEF-treated products remains to be elucidated.

Antioxidant vitamins are important for the cardiovascular system [18]. Vitamin C contributes to preserving a healthy vasculature through the regulation of collagen synthesis, prostacyclin production, and maintenance of nitric oxide levels [19–21]. Fruits and vegetables are the main sources of vitamin C, but 25% of women and about 33% of men in US eat less than 2.5 servings of fruits and vegetables daily to provide about 80 mg/day of vitamin C [22,23]. Data retrieved from the Data Food Networking (DAFNE) databank concluded that, on average, more than 64% of the households are likely to consume less than the recommended daily fruit and vegetable intake of five servings per day [24].

A substantial body of evidence indicates that the measure of isoprostanes provides a reliable noninvasive approach to assess lipid oxidation in vivo in humans [25]. One of the isoprostanes, the 8-*epi*$PGF_{2\alpha}$, has been shown to act as a vasoconstrictor [26] and to be associated with the hepatorenal syndrome [27] and pulmonary oxygen toxicity [28]. Previous studies showed high concentrations of F2-isoprostanes in presence of low levels of antioxidants and chronic diseases [29].

Elevated serum uric acid concentration appears to be an important risk factor predicting myocardial infarction [30]. In addition, hyperuricemia may have a direct injurious effect on the endothelium, altering endothelial cell function and reducing nitric oxide bioavailability, relevant in the development of vascular dysfunction and cardiovascular risk.

Therefore, the objectives of this study were to assess the bioavailability of vitamin C and levels of 8-*epi*$PGF_{2\alpha}$ after the consumption of PEF-treated in comparison with freshly squeezed orange juice in a healthy human population.

2. Methods and materials

2.1. Subjects

Twelve healthy volunteers (six men and six women) were enrolled in this study. The subjects' age was between 21 and 31 years, and their body mass index was (in kg/m^2) 23.6 ± 1.2 and did not change during the study. All the subjects continued their habitual diets during the study. Subjects were taking no vitamin/mineral supplements and no medications. No subject was 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 from clinical laboratory tests. 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 Hospital Universitario Clínica Puerta de Hierro (Madrid, Spain).

2.2. Study design

The vitamin C bioavailability study was divided into two components: a dose-response test and a multiple-dose–response study. For the dose-response test, an intravenous catheter was inserted into the subject's forearm and blood was drawn before and every 60 minutes for 6 hours after the subject drank the juice. After blood samples were collected at baseline, subjects were assigned into two groups: 1) six volunteers who consumed 500 mL of PEF-treated orange juice, and 2) six volunteers who consumed 500 mL of FS orange juice. Blood samples were collected in heparin-coated tubes and were centrifuged at 2000 × *g* for 15 minutes at 4°C. After 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 ion chelator diethylenetriaminepentaacetic acid for vitamin C and uric acid analysis. The remaining plasma was stored at −80°C for analysis of 8-*epi*$PGF_{2\alpha}$. 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 PEF-treated orange juice and the FS orange juice consumed by the participants was analyzed by reverse-phase high-performance liquid chromatography (HPLC) with methods currently used in our laboratory [31–33].

2.3. PEF-treatment of orange juice

Oranges (*Citrus sinensis* L.) of the Navel Late variety (Spain) were purchased from a local supermarket and kept at 4°C before being processed. The orange juice was obtained using a squeezer (Lomi model 4, Madrid, Spain) and then was filtered using a 2-mm steel sieve. The fresh orange

juice was designated as freshly squeezed (FS) orange juice. PEF treatment was carried out in a continuous flow bench scale system (OSU-4F, Ohio State University, Columbus, OH) using square-wave pulses. The treatment system consisted of eight colinear chambers in a series, each with two stainless steel electrodes separated by a gap of 0.29 cm. The flow rate of the process was adjusted to 200 mL/min and was controlled by a variable speed pump (model 75210-25, Cole Palmer, Vernon Hills, IL). The product was refrigerated in the space provided between the chambers by means of an ice-water bath with shaking. The PEF processing conditions for orange juice were 35 kV/cm electrical field applied in a bipolar mode, 800 Hz pulse frequency, 4-μs pulse width, and 750-μs total treatment time. Temperature was never more than 50°C. After treatment, orange juice was kept at 4°C until its consumption.

2.4. Plasma vitamin C determination

Ascorbate was analyzed by paired-ion, reverse-phase HPLC coupled with electrochemical detection as previously described by Martin and Frei [34]. Ascorbate concentration was calculated based on a calibration curve and its concentration expressed in μmol/L.

2.5. Plasma 8-isoprostane determination

We used an enzyme immunoassay kit to determine the concentration of plasma 8-isoprostane (8-*epi*$PGF_{2\alpha}$) (Cayman Chemical, Ann Arbor, MI) in plasma [35]. 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 IgG mouse monoclonal antibody that has been previously attached to the well.

2.6. Plasma uric acid determination

We analyzed uric acid by paired-ion, reverse-phase HPLC coupled with electrochemical detection using the same procedure described for vitamin C determination with the electrode potential of +0.6 V but with the gain set at 1 μamp [34].

2.7. Statistical analysis

All values are presented as mean ± SEM. Repeated measures analysis of variance comparing the concentrations of vitamin C, 8-*epi*$PGF_{2\alpha}$, and UA between type of juices and at different times of intervention were performed by using Systat 10 software (SPSS Inc., Chicago, IL) to test for statistical significance at the $P < 0.05$ level. When type of juice by time or type of juice differences were detected, Tukey's Honestly Significant Difference (HSD) test was run to determine differences at various time points between and within groups.

Table 1
Composition of pulsed electric fields (PEF)–treated and freshly squeezed (FS) orange juices per 100 mL

	PEF Treated	FS
Energy (kJ)*	175 ± 3.8	174 ± 3.6
Protein (g)*	0.7 ± 0.03	0.6 ± 0.02
Carbohydrates (g)*	10.2 ± 0.7	10.5 ± 0.9
Fat (g)*	<1	<1
Vitamin C (mg)†	36.1 ± 3.0	38.1 ± 2.3
Total flavanones (mg)†	11.1 ± 0.8	12.5 ± 0.9
Total carotenoids (μg)†	802.0 ± 30.3	869.3 ± 35.5

Values are means ± SEM, (PEF, $n = 6$; FS, $n = 6$). There was not significant difference between both orange juices, $P < 0.05$ (Student t test).
* Concentrations were measured by conventional AOAC methods [33].
† Concentrations were measured by HPLC analysis [31,32].

3. Results

The composition of the PEF-treated and the FS orange juices consumed by the participants is reported in Table 1. No significant differences were observed between the orange juice types consumed.

No significant differences on baseline plasma vitamin C concentrations were observed between the subjects who drank the PEF-treated orange juice (43.7 ± 3.2 μmol/L) and those who drank the FS orange juice (45.8 ± 3.4 μmol/L) (Table 2). On the first day of the intervention, the maximum increase in vitamin C occurred 3–4 hours after consumption of the orange juice (500 mL) in both groups. The PEF-treated and the FS orange juice contained approximately 185 mg of vitamin C. At 3–4 hours (maximum peak), plasma concentration increased over baseline by 50% in both the PEF group (66.1 ± 3.6 μmol/L vs 43.7 ± 3.2 μmol/L; $P = 0.001$) and the FS juice group (68.9 ± 6.8 μmol/L vs 45.8 ± 3.4 μmol/L; $P = 0.043$).

Plasma vitamin C concentrations were also analyzed on days 7 and 14 of the intervention. The increased plasma vitamin C concentration after drinking the two types of orange juices remained elevated during the study, with concentrations significantly higher than baseline in both groups. Vitamin C concentrations on days 7 and 14 were 57.0 ± 3.8 μmol/L ($P = 0.03$) and 56.8 ± 3.2 μmol/L ($P = 0.04$) in subjects who consumed PEF orange juice; and 61.4 ± 4.7 μmol/L ($P = 0.020$), and 60.7 ± 4.5 μmol/L ($P = 0.026$) in subjects who consumed FS orange juice (Table 2). However, the concentration of vitamin C on days 7 and 14, although significantly higher than baseline levels, was lower than the increase reached by drinking the juice in one dose.

Baseline 8-*epi*$PGF_{2\alpha}$ concentrations did not statistically differ among the subjects drinking the PEF-treated or the FS orange juice (Table 2). However, all the subjects consuming

Table 2
Plasma vitamin C, 8-*epi*$PGF_{2\alpha}$, and uric acid concentration at baseline and on days 7 and 14 of drinking pulsed electric fields (PEF)–treated and freshly squeezed (FS) orange juices

	PEF Treated			FS		
	Baseline	7 days	14 days	Baseline	7 days	14 days
Vitamin C (μmol/L)*	43.7 ± 3.2	57.0 ± 3.8	56.8 ± 3.2	45.8 ± 3.4	61.4 ± 4.7	60.7 ± 4.5
8-*epi*$PGF_{2\alpha}$ (pg/mL)†	219.4 ± 7.6	176.2 ± 9.7	162.2 ± 6.7	198.8 ± 9.5	185.1 ± 9.7	165.0 ± 11.7
Uric acid (μmol/L)	271.6 ± 33.7	254.0 ± 29.1	258.5 ± 30.5	259.2 ± 38.8	237.4 ± 36.5	235.1 ± 38.8

Values are means ± SEM (PEF, $n = 6$; FS, $n = 6$). There was no significant treatment × time interaction for vitamin C ($P = 0.933$), 8-*epi*$PGF_{2\alpha}$ ($P = 0.260$), and uric acid ($P = 0.987$), based on repeated-measures ANOVA.

* Vitamin C significantly higher than baseline for PEF and FS combined at both 7 and 14 days (time effect), $P < 0.05$, based on repeated-measures ANOVA (Tukey test).

† 8-*epi*$PGF_{2\alpha}$ significantly lower than baseline for PEF at both 7 and 14 days and for FS at 14 (time effect), $P < 0.05$, based on repeated-measures ANOVA (Tukey test).

PEF-treated or the FS orange juice showed a significant decrease in plasma concentrations of 8-*epi*$PGF_{2\alpha}$on day 14 of the intervention (162.2 ± 6.7 pg/mL, $P < 0.001$, and 165.0 ± 11.7 pg/mL, $P = 0.036$, respectively) (Table 2). Interestingly, plasma concentration of 8-*epi*$PGF_{2\alpha}$ decreased on day 7 among the subjects consuming the PEF-treated orange juice (176.2 ± 9.7 pg/mL, $P = 0.005$) (Table 2).

We also found an inverse correlation between vitamin C concentrations and 8-*epi*$PGF_{2\alpha}$ levels in subjects drinking the PEF-treated orange juice, and in subjects drinking the FS orange juice at baseline and at days 7 and 14 ($r = -0.707$, $P = 0.0010$, and $r = -0.733$, $P = 0.0005$, respectively) (Fig. 1).

Baseline UA concentrations did not differ between both groups (Table 2). By day 14 of the intervention, plasma uric acid concentrations tended to be lower among the subjects who drank the PEF-treated or the FS orange juice (Table 2). In general, uric acid concentration was lower when vitamin C was higher; across individuals an inverse association ($r = -0.357$, $P = 0.033$) between uric acid concentration and plasma vitamin C levels was observed.

4. Discussion

In the present work, we have shown that drinking two glasses of PEF-treated orange juice (500 mL) daily increases plasma vitamin C concentration and decreases 8-*epi*$PGF_{2\alpha}$ levels. These effects were similar to those obtained by the consumption of FS orange juice. The impact of eating more fruits and vegetables on human health has been a subject of several studies. Supplementation with vitamin C was associated with a significant reduction in the oxidazibility of LDL [36]. The orange juice–mediated effects shown in this study suggest that the high concentration of vitamin C may play a critical role in reducing the formation of compounds produced by random oxidation of phospholipids relevant to reducing the incidence of chronic disease. In addition, other nutrients present in the orange juice may have also contributed to the beneficial effects observed in this study.

Several studies have investigated the effects of PEF processing on vitamin C in orange juice. The ascorbic acid loss after PEF treatment is significantly less than that for thermal pasteurization [16]. Other authors have reported

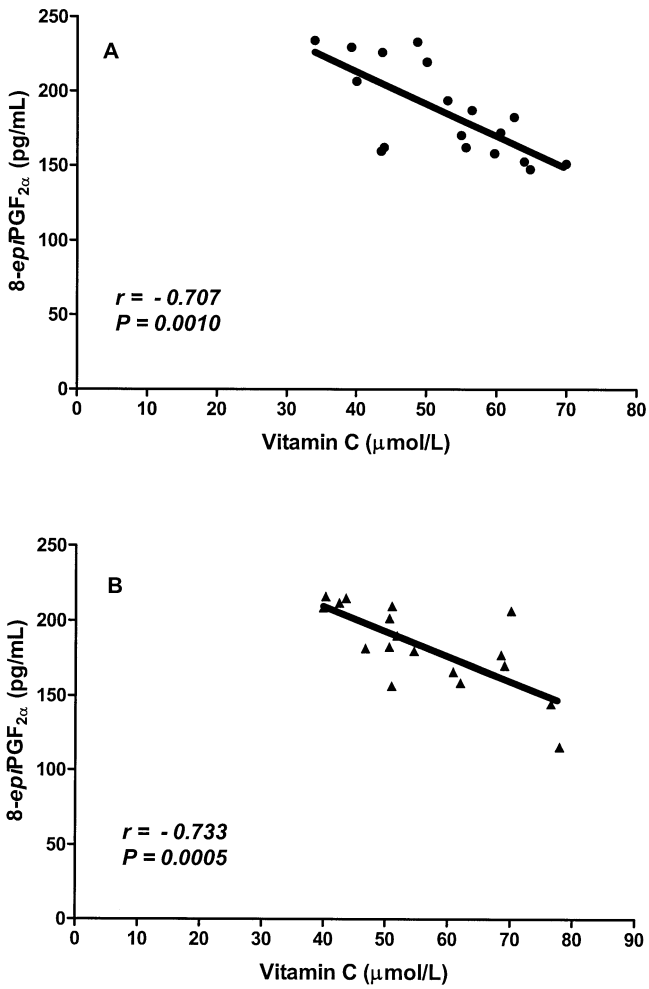

Fig. 1. Inverse correlation between plasma 8-*epi*$PGF_{2\alpha}$ and vitamin C concentrations at baseline and at days 7 and 14 of drinking pulsed electric field–treated orange juice ($n = 18$). (*A*) Study group given pulsed electric fields–treated orange juice. (*B*) Study group given freshly squeezed orange juice.

that heat-pasteurized orange juice had a significantly lower concentration of ascorbic acid than PEF-treated orange juice during storage at 4°C due to the higher processing temperature. Specifically, these authors have indicated that the concentration of ascorbic acid at 4°C after 47 days in PEF-treated (35 kV/cm, 59 μs) orange juice is significantly higher ($P < 0.05$) than in the heat-pasteurized (94.6°C, 30 s) orange juice. In the same way, as storage time increased, PEF-treated orange juice showed significantly higher content of flavor compounds than did the pasteurized orange juice during storage at 4°C ($P < 0.05$) [6]. However, the beneficial effect of drinking PEF-treated orange juice on human health has not been carefully evaluated. The vitamin C in PEF-treated orange juice was found in this study to be as bioavailable as in FS orange juice. These results are in agreement with the fact that the active form of vitamin C, L-ascorbic acid, was not significantly modified by high-voltage electric pulses. Thus, PEF processing retained the bioavailability characteristics of fresh juice. Consequently, along with the findings of quality PEF-treated orange juice, this treatment could be an alternative to traditional thermal processing. In addition, the findings reported here suggest that this technology may be important to preserve a more nutritious orange juice.

Vitamin C is an essential micronutrient required for normal metabolic functioning of body. Human and other primates have lost the ability to synthesize vitamin C and must therefore acquire it from diet. Vitamin C is especially plentiful in citrus fruit [37]. A recent study showed a 64% increase on plasma vitamin C concentration after ingesting a mixture of fruits and vegetables (500 g) for 4 weeks [38]. Our results show that intake of the ~185 mg of vitamin C contained in two glasses of PEF-treated orange juice or FS orange juice (500 mL) significantly increased plasma vitamin C concentration from a baseline of 44 μmol/L to 67 μmol/L in just 3–4 hours after drinking the juice, and remained elevated as long as subjects were drinking the orange juice. Interestingly, blood levels of vitamin C above 49 μmol/L have been associated with a 64% reduction in the risk of cataracts [39]. Vitamin C is an essential cofactor in neurotransmitter synthesis [40] and is important in preserving endothelial dependent vascular function [41].

There is substantial evidence for the antioxidant activity of vitamin C. Vitamin C effectively scavenges most aqueous reactive oxygen and nitrogen species before they can interact with and oxidize other substrates, including lipids [37]. Therefore, in this study we evaluated the effect of vitamin C on formation of F2-isoprostanes, a specific biomarker of lipid peroxidation, which are formed from nonenzymatic, radical-mediated oxidation of arachidonyl-containing lipids [42]. In fact, F2-isoprostanes provide an accurate way to measure oxidative stress in vivo. Increased concentrations of F2-isoprostanes have been detected in persons with diabetes [43], in persons with hypercholesterolemia [44], and in LDL exposed in vitro to various types of oxidative stress [45]. Interestingly, we observed a significant decrease in 8-*epi*$PGF_{2\alpha}$ levels after subjects drank the PEF-treated orange juice or the FS orange juice daily, and a significant inverse correlation between plasma vitamin C and 8-*epi*$PGF_{2\alpha}$ levels. These findings are in agreement with a previous study from our research group in which the subjects drank commercial orange juice [46]. A recent epidemiological study describing oxidative damage in 298 healthy adults and the nutritional factors that may be associated with this damage concludes that plasma ascorbic acid had a strong correlation with F2-isoprostanes plasma concentrations [2]. Other studies have also reported changes on F2-isoprostane levels after supplementation with vitamin C or E, or both [47,48].

Epidemiological studies have indicated the presence of relevant associations between uric acid and cardiovascular disease [49,50]. A multivariate analysis of data from the Monitoring of Trends and Determinants in Cardiovascular Disease (MONICA) cohort of 1044 men showed a significant association between high serum uric acid and cardiovascular mortality [51]. Several mechanisms, including increased platelet adhesiveness and platelet lysis, vascular endothelial cell injury, formation of free radicals, and oxidative stress appear to be involved in this association [52–54]. In this study, we found an inverse correlation between plasma vitamin C and uric acid concentrations.

Drinking PEF-treated orange juice increases vitamin C levels and reduces oxidative stress in vivo by lowering the concentration of F2-isoprostanes, which provides new evidence on the health benefits of consuming fruit juices. In fact, several studies have observed that high vitamin C intake is associated with important vascular benefits by improving endothelial cell function and lowering blood pressure.

Some limitations of this study should be acknowledged. One of them is the lack of outcomes associated with plasma levels after intake of orange juice. Another limitation is the small number of subjects enrolled in the study. Because this is a healthy population, the main objective was to assess the bioavailability of vitamin C in PEF-treated in comparison with FS orange juice and its association with concentrations of 8-*epi*$PGF_{2\alpha}$.

In conclusion, drinking two glasses of PEF-treated orange juice (500 mL/day) containing approximately 180 mg of vitamin C was associated with a significant increase in plasma vitamin C concentration and a decrease in plasma levels of 8-*epi*$PGF_{2\alpha}$. Vitamin C was significantly and inversely correlated with 8-*epi*$PGF_{2\alpha}$. These effects were similar to those obtained with FS orange juice. These findings support the use of PEF technology to preserve orange juice, because the juice retains the vitamin C bioavailability characteristics and the antioxidant properties of fresh juice with a longer shelf-life.

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