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Journal of Nutritional Biochemistry 17 (2006) 183–189

Journal of Nutritional Biochemistry

Mediterranean vegetable soup consumption increases plasma vitamin C and decreases F_2-isoprostanes, prostaglandin E_2 and monocyte chemotactic protein-1 in healthy humans☆

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Abstract

Consumption of fruits and vegetables is associated with a reduced risk of death from all causes including heart disease and stroke. In this work, the bioavailability of vitamin C from a Mediterranean vegetable soup (gazpacho) constituted mainly of tomato, pepper and cucumber, and its influence on plasma vitamin C, 8-*epi*-prostaglandin $F_{2\alpha}$ (8-*epi*-$PGF_{2\alpha}$), prostaglandin E_2 (PGE_2), monocyte chemotactic protein-1 (MCP-1), and the cytokines/tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and IL-6 concentrations in a healthy human population were assessed. Six men and six women consumed 500 ml of commercial gazpacho per day for 14 days, corresponding to an intake of 78 mg of ascorbic acid per day. There were no differences ($P=.22$) in baseline plasma vitamin C concentrations between the men and women. The maximum increase ($P<.05$) in plasma vitamin C occurred 4 h postdose in both men and women. Vitamin C concentrations were significantly higher ($P<.03$) on Days 7 and 14 of the intervention. Baseline concentrations of uric acid and 8-*epi*-$PGF_{2\alpha}$ were significantly higher ($P\leq.032$) in men than in women. Baseline concentrations of 8-*epi*-$PGF_{2\alpha}$ decreased significantly ($P\leq.05$) by Day 14 of the intervention. A significant inverse correlation was observed between vitamin C and 8-*epi*-$PGF_{2\alpha}$ ($r=-.415$, $P=.049$). Baseline concentrations of PGF_2 and MCP-1 were significantly higher ($P\leq.025$) in men than in women but decreased significantly ($P\leq.05$) by Day 14 of the intervention. No effect on TNF-α, IL-1β and IL-6 was observed at Day 14 of the intervention. Drinking gazpacho (500 ml/day) significantly increases plasma concentrations of vitamin C and significantly decreases 8-*epi*-$PGF_{2\alpha}$, PGE_2 and MCP-1 concentrations in healthy humans.

Keywords: Vegetable soup; Vitamin C; Bioavailability; F_2-Isoprostanes; Uric acid; Cytokines; Inflammatory markers

1. Introduction

Plant-based foods provide essential nutrients needed for life, health promotion and disease prevention. A number of epidemiological studies have demonstrated that diet plays a crucial role in the prevention of chronic diseases. The traditional Mediterranean diet rich in fruits, vegetables, legumes, whole grain, nuts and low-fat dairy products protects against the development and progression of cardiovascular diseases [1–3].

Gazpacho is a typical Mediterranean dish, defined as a ready-to-serve cold vegetable soup, which contains about 75% vegetables (tomato, cucumber, pepper), 2–10% olive oil and other minor components (onion, garlic, wine vinegar and sea salt) [4], and is rich in vitamin C. Consumption of this vegetable soup, with such a diverse composition, could help

☆ Supported in part by the Coordinated Grant CAM 07G/0040/2000-CAM 07G/0041/2000 (Comunidad Autónoma de Madrid), the Grant AGL2002-04059-C02-02 (Ministry of Science and Technology, Madrid, Spain) (MPC); a Fulbright/Ministry of Education, Culture and Sports Award for Postdoctoral Research in the United States of America, Visiting Scholar Program, Commission for Cultural, Educational and Scientific Exchange between the United States of America and Spain, and a Ramón y Cajal Research Contract (Ministry of Science and Technology, Madrid, Spain) (CS-M). An earlier version of this article was presented at Experimental Biology 2004, Washington DC, USA, April 2004.

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doi:10.1016/j.jnutbio.2005.07.001

fulfill the recommendation to eat more vegetables and achieve the health benefits derived from its bioactive compound combination [5,6].

Vitamin C interacts with damaging oxygen radicals [7] and contributes to preserving a healthy vasculature through the regulation of collagen synthesis, prostacyclin production and maintenance of nitric oxide levels [8–10]. In Western diets, over 85% of the daily dietary intake of ascorbic acid is derived from fruit and vegetables [11]. Tomato is a good source of vitamin C and is the second most highly consumed vegetable in the USA [12] and in Europe [7].

The strong association of vitamin C with fruit and vegetable intake found in numerous epidemiological studies may imply that vitamin C is an important component of the protective effect seen for these foods [13]. On the basis of these facts, several intervention studies have been conducted to investigate the protective role of fruit and vegetables rich in vitamin C in lowering the risk of chronic diseases [10,14]. However, the bioavailability of vitamin C from different sources is not completely known, and further work is needed in this area [15].

Oxidative stress is defined as an excess production of free radicals and reactive oxygen species that overwhelms the natural radical-blocking or scavenging mechanisms of the body [16]. F_2-Isoprostanes, which are specific products arising from the peroxidation of arachidonic residues in lipids, may be one measure of oxidative stress of lipid peroxidation in the human body that holds promise [17,18]. Increased levels of F_2-isoprostanes have been reported in diseases associated with oxidative stress [19] and are diminished following consumption of olive oil [20], soy-containing isoflavone [21], orange juice [14] and antioxidants such as vitamins E [22] and C [23].

Increasing evidence suggests that inflammation and oxidative stress play a pivotal role in the development of certain degenerative diseases associated with aging [24]. Inflammation could depress concentrations of some vitamins and carotenoids; thus, exogenous sources of antioxidants serve to counteract the reactive species produced during the inflammatory process, limiting or preventing the cellular damage [25]. A recent cross-sectional study, involving 1514 men and 1528 women from Greece, shows a higher reduction in the concentrations of pro-inflammatory markers [C-reactive protein (CRP) and cytokines interleukin-6 (IL-6) and tumor necrosis factor (TNF-α)] in those subjects showing a higher degree of adherence to the traditional Mediterranean diet. Specifically, among the various food groups evaluated, fruits and vegetables and moderate alcohol intake (negative) show the highest degree of independent association with the investigated inflammatory markers [26]. Therefore, the anti-inflammatory activity of phytochemicals may play an important role in the prevention of chronic diseases. Prostaglandin E_2 (PGE_2) is one of the major prostaglandins produced during inflammation [27]. Monocyte chemotactic protein-1 (MCP-1) is expressed at high levels in atherosclerotic plaques [28]. Cytokines such as TNF-α, interleukin-1β (IL-1β) and IL-6 are responsible for the progression of the inflammatory response. Associations between these inflammatory and oxidative stress biomarkers in relation to antioxidant concentrations after dietary interventions will provide valuable information regarding the health benefits of diets rich in fruits and vegetables.

Serum uric acid level is associated with cardiovascular events [29–31] and with elevation of inflammation markers [32]. Uric acid may have a direct injurious effect on the endothelium, altering endothelial cell function and reducing nitric oxide bioavailability, relevant to cardiovascular risk.

Therefore, the objectives of this study were to assess the bioavailability of vitamin C from commercial gazpacho and its effect on concentrations of biomarkers of oxidative stress and inflammation in a healthy human population.

2. Subjects and methods

2.1. Subjects

Twelve healthy volunteers (six men and six women) were enrolled in this study. The subjects' age was 22±0.5 years, and their body mass index (kg/m^2) was 22.5±0.9 and did not change during the study. All the subjects continued their habitual diets during the study. Subjects were not taking vitamin/mineral supplements and medications. None of the subjects was pregnant, lactating or had any chronic illness. Smokers and subjects with inflammatory disease or taking anti-inflammatory drugs were excluded from the study. All study participants were in good health on the basis of a medical history, a physical examination and normal results from clinical laboratory tests, including glucose (4.5±0.1 mmol/L), hematocrit (0.43±0.01), cholesterol (4.3±0.1 mmol/L) and triacylglycerol (0.7±0.1 mmol/L). Subjects received oral and written information about the study and gave their written consent. The Clinic Research Ethics Committee of Hospital Universitario Clínica Puerta de Hierro, Madrid, Spain, approved the study.

2.2. Collection of plasma samples and experimental protocol

The vitamin C bioavailability study was divided into two components: a dose–response test and a multiple-dose–response study. For the dose–response evaluation, after 12 h of fasting, an intravenous catheter was inserted into each subject's forearm, and blood was drawn before and every 60 min for 6 h after the subjects consumed the vegetable soup. Blood samples were collected in heparin-coated tubes and centrifuged at 2000×*g* for 15 min at 4°C. After plasma was collected, aliquots in triplicate were immediately mixed with an equal volume of cold 6% (w/v) 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*-prostaglandin $F_{2\alpha}$ (8-*epi*-$PGF_{2\alpha}$). After taking blood samples at baseline, defined as t0, volunteers consumed 500 ml of commercial gazpacho, and blood samples were

taken every 60 min, which corresponded with the times t1, t2, t3, t4, t5 up to 6 h or t6 (dose–response test evaluation). Then, the subjects were instructed to consume the gazpacho at home, in two 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 gazpacho consumed by the participants is reported in Table 1. The vitamin C and carotenoid composition of the vegetable soup were measured by reversed-phase HLPC with methods currently used in our laboratory [33]. Total energy, protein, carbohydrate and fat contents were provided by J García Carrión SA (Jumilla, Murcia, Spain).

2.3. Measurement of vitamin C

Ascorbate was analyzed by paired-ion, reversed-phase HPLC coupled with electrochemical detection. Mixed plasma sample was analyzed on a LC8 column (150×4.6 mm id, 3 μm particle size) (Supelco, Bellefonte, PA) using 99% deionized water and 1% methanol containing 40 mmol/L sodium acetate and 1.5 mmol/L dodecyltriathylammonium phosphate (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, Wilmington, DE). Ascorbate was detected at an applied potential of +0.6 V with the gain set at 100 nA by a LC 4B amperometric electrochemical detector (Bioanalytical Systems, West Lafayette, IN). Ascorbate was eluted as a single peak with a retention time of 5.5 min. Peaks were integrated with a ChemStation (Hewlett Packard). Ascorbate concentration was calculated based on a calibration curve, and its concentration expressed in micromoles per liter [34].

2.4. Measurement of 8-isoprostane

We used an enzyme immunoassay (EIA) kit (Cayman Chemical, Ann Harbor, MI) to determine the concentration of 8-isoprostane (8-*epi*-$PGF_{2\alpha}$) in plasma as we have previously described [14,35]. The intra- and interassay variability was low with a CV $\leq$10%.

2.5. Measurement of uric acid

Uric acid was analyzed by paired-ion, reversed-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 μA as we have previously described [34].

Table 1
Composition of the commercial gazpacho per 100 ml[a]

Energy (kJ)	146
Protein (g)	0.75
Carbohydrates (g)	2
Fat (g)	2.7
Vitamin C[b] (mg)	15.5±1.3
Total carotenoids[b] (μg)	2,629±144

[a] Gazpacho Don Simón (J García Carrión SA).
[b] Mean±S.E.M. Concentration obtained from the analysis performed in our laboratory.

2.6. Measurement of PGE_2

Prostaglandin E_2 was measured by a high-sensitivity immunoassay (R&D Systems, Minneapolis, MN) based on a competitive binding technique in which PGE_2 present in a sample competes with a fixed amount of alkaline phosphatase-labeled PGE_2 for sites on a mouse monoclonal antibody. [35,36]. The intra- and interassay variability was low with a CV $\leq$8%.

2.7. Measurement of MCP-1

MCP-1 was measured by the quantitative sandwich enzyme immunoassay technique (R&D Systems). A monoclonal antibody specific for MCP-1 has been precoated onto a microplate. Standards and samples were placed into the wells, and any MCP-1 present was bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for MCP-1 was added to the wells. Following a wash to remove any unbound antibody–enzyme reagent, a substrate solution was added to the wells, and color develops in proportion to the amount of MCP-1 bound in the initial step. The color development was stopped, and the intensity of the color was measured. The intra- and interassay variability was low with a CV $\leq$8%.

2.8. Measurement of cytokines TNF-α, IL-1β and IL-6

These assays employ the quantitative sandwich enzyme immunoassay technique (R&D Systems). A monoclonal antibody specific for TNF-α, IL-1β and IL-6 has been precoated onto a microplate. Standards and samples were placed into the wells, and any TNF-α, IL-1β and IL-6 present were bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for TNF-α, IL-1β and IL-6 was added to the wells. Following a wash to remove any unbound antibody–enzyme reagent, a substrate solution was added to the wells, and color develops in proportion to the amount of TNF-α, IL-1β and IL-6 bound in the initial step. The color development was stopped, and the intensity of the color was measured. The intra- and interassay variability was low with a CV $\leq$10%.

2.9. Statistical analysis

Descriptive statistics (including mean and standard deviation) were used to summarize the characteristics of subjects. All values are presented as means±S.D. Repeated-measures analysis of variance (ANOVA) comparing the concentrations of vitamin C, 8-*epi*-$PGF_{2\alpha}$, uric acid, PGE_2, MCP-1, TNF-α, IL-1β and IL-6 between sexes and at different times of intervention were performed by using Systat 10 (SPSS, Chicago, IL) to test for statistical

Table 2
Plasma vitamin C, 8-*epi*-$PGF_{2\alpha}$, uric acid, PGE_2, MCP-1, TNF-α, IL-1β and IL-6 concentrations at baseline and on Days 7 and 14 of the study in men and women[a]

	Men ($n=6$)			Women ($n=6$)		
	Baseline	Day 7	Day 14	Baseline	Day 7	Day 14
Vitamin C (μmol/L)[b]	46.0±2.2	56.0±2.5	57.9±1.6	50.2±2.3	59.8±2.2	61.2±1.5
8-*epi*-$PGF_{2\alpha}$ (pg/ml)[c]	203±20.4[d]	158±12.3[d]	126±7.0[d]	137±17.1	110±12.8	90.9±7.7
Uric acid (μmol/L)[e]	399±11.7[f]	359±20.2[f]	340±19.5[f]	287±13.5	255±17.7	265±16.1
PGE_2 (pg/ml)[g]	318±20.5[h]	ND	250±16.2[h]	241±27.2	ND	164±14.1
MCP-1 (pg/ml)[i]	604±51.0[j]	ND	448±40.3[j]	452±40.9	ND	329±48.2
TNF-α (pg/ml)	5.4±1.5	ND	5.3±1.8	4.0±1.4	ND	4.1±1.0
IL-1β (pg/ml)	0.8±0.1	ND	1.0±0.3	0.7±0.2	ND	0.8±0.2
IL-6 (pg/ml)	0.40±0.08	ND	0.28±0.06	0.22±0.05	ND	0.18±0.07

[a] Means±S.E.M. There were no significant sex-by-time interactions for vitamin C ($P=.979$), 8-*epi*-$PGF_{2\alpha}$ ($P=.540$), uric acid ($P=.523$), PGE_2 ($P=.732$), MCP-1 ($P=.521$), TNF-α ($P=.802$), IL-1β ($P=.352$) and IL-6 ($P=.863$) by repeated-measures ANOVA.
[b] Significantly different on Days 7 and 14 than at baseline for men and women (time effect); $P<.05$ (repeated-measures ANOVA and Tukey's test).
[c] Significantly different on Day 14 than at baseline for men and women (time effect); $P<.05$ (repeated-measures ANOVA and Tukey's test).
[d] Significantly different from women; $P<.05$ (repeated-measures ANOVA and Tukey's test).
[e] Significantly different on Day 14 than at baseline for men (time effect); $P<.05$ (repeated-measures ANOVA and Tukey's test).
[f] Significantly different from women; $P<.01$ (repeated-measures ANOVA and Tukey's test).
[g] Significantly different on Day 14 than at baseline for men and women (time effect); $P<.05$ (Student's *t*-test).
[h] Significantly different from women; $P<.05$ (repeated-measures ANOVA and Tukey's test).
[i] Significantly different on Day 14 than at baseline for men and women (time effect); $P<.05$ (Student's *t*-test).
[j] Significantly different from women; $P<.05$ (repeated-measures ANOVA and Tukey's test).

significance at the $P \leq .05$ 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 using the Systat program [37].

3. Results

There were no significant differences ($P=.22$) in baseline plasma vitamin C concentrations between men and women (Table 2). On the first day of the intervention, the maximum increase in vitamin C occurred 3–4 h after consumption of the vegetable soup (500 ml) containing about 78 mg of vitamin C in both men and women (Fig. 1). At 4 h (maximum peak), plasma concentrations had increased over baseline by 29% in men (59.5±2.5 μmol/L compared with 46.0±2.2 μmol/L; $P<.05$) and by 24% in women (62.3±2.3 μmol/L compared with 50.2±2.3 μmol/L; $P<.05$). Subjects continued taking the same amount of vegetable soup daily (500 ml) during the next 14 days. However, on the second day of the intervention, subjects started taking the vegetable soup in two servings, half in the morning and half in the afternoon. Plasma vitamin C concentrations were also analyzed on Days 7 and 14 of the intervention. At Day 14, vitamin C plasma concentrations increased by 26% in men (57.9±1.6 μmol/L compared with 46.0±2.2 μmol/L; $P=.02$) and by 22% in women (61.2±1.5 μmol/L compared with 50.2±2.3 μmol/L; $P=.03$) (Table 2). Plasma vitamin C concentrations remained elevated during the study (Table 2); however, no statistically significant differences were observed between Days 7 and 14 in men or women. There was no significant sex-by-time interaction for bioavailability of vitamin C ($P=.979$). Interestingly, although the concentration of vitamin C at the end of the study (Day 14) remained higher than at baseline, it tended to be lower (although not significantly so) than the concentration reached by drinking the vegetable soup all in one dose (500-ml dose compared with two 250-ml doses).

Baseline plasma concentrations of 8-*epi*-$PGF_{2\alpha}$ were significantly higher ($P=.032$) among men than among women (Table 2). Interestingly, we observed a trend toward a decrease in plasma concentrations of 8-*epi*-$PGF_{2\alpha}$ among men and women on Day 7 of the intervention, which became significant on Day 14 ($P \leq .05$). An inverse correlation was

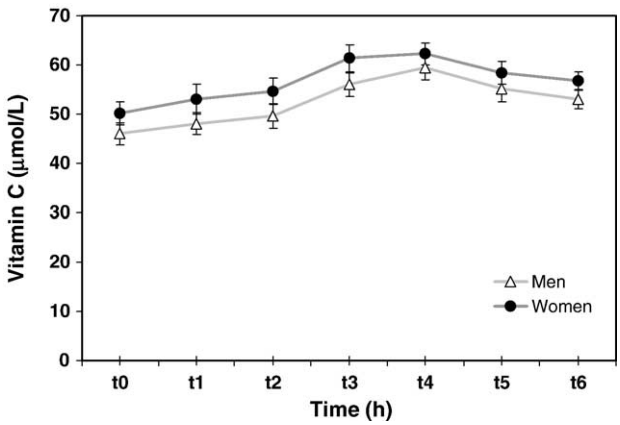

Fig. 1. Mean (±S.E.M.) plasma vitamin C concentrations in men (Δ, $n=6$) and women (●, $n=6$) at baseline (0 h) and every hour after drinking gazpacho. There was no significant main effect of sex and sex-by-time interaction by ANOVA.

observed between vitamin C concentrations and 8-*epi*-$PGF_{2\alpha}$ concentrations in both men and women at baseline and at the end of the study (Day 14) ($r=-.415$, $P=.049$). In addition, we observed a positive and strong correlation between 8-*epi*-$PGF_{2\alpha}$ concentrations and uric acid ($r=.794$, $P<.0001$), PGE_2 ($r=.758$, $P<.0001$) and MCP-1 ($r=.765$, $P<.0001$) concentrations in both men and women at baseline and at the end of the study (Day 14).

Uric acid concentrations were significantly higher ($P<.001$) among men than among women (Table 2). In general, uric acid concentration was 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 ($r=-.511$, $P=.0014$). A positive correlation was observed between uric acid concentrations and PGE_2 concentrations ($r=.603$, $P=.0009$), and MCP-1 concentrations ($r=.621$, $P=.002$) in both men and women at baseline and at the end of the study (Day 14).

Baseline PGE_2 plasma concentrations were significantly higher ($P=.024$) among men than among women (Table 2). We observed a significant ($P\leq.043$) decrease in plasma concentrations of PGE_2 among men and women on Day 14 of the intervention. In addition, we observed an inverse correlation between vitamin C concentrations and PGE_2 concentrations in both men and women at baseline and at the end of the study (Day 14) ($r=-.322$, $P=.063$). Also, a positive correlation was observed between PGE_2 concentrations and MCP-1 concentrations ($r=.572$, $P=.0017$), and IL-6 concentrations ($r=.401$, $P=.033$) in both men and women at baseline (t0) and on Day 14.

Baseline MCP-1 plasma concentrations were significantly higher ($P=.025$) among men than among women. We observed a significant ($P\leq.05$) decrease in plasma concentrations of MCP-1 among men and women on Day 14 of the intervention. In addition, we observed an inverse correlation between vitamin C concentrations and MCP-1 concentrations in both men and women at baseline and at the end of the study (Day 14) ($r=-.589$, $P=.0043$).

There were no significant differences ($P>.9$) in baseline plasma TNF-α, IL-1β and IL-6 concentrations between men and women (Table 2). No statistically significant differences were observed in plasma TNF-α, IL-1β and IL-6 concentrations between baseline and Days 7 and 14 among men or women.

4. Discussion

In this study, we have shown for the first time that drinking two servings of commercial gazpacho (about 500 ml) daily for 14 days increases plasma vitamin C concentrations by 26% in men and 22% in women. Changes in vitamin C were significantly inversely correlated with concentrations of 8-*epi*-$PGF_{2\alpha}$, uric acid, PGE_2 and MCP-1, suggesting that vitamin C may play a critical role in oxidative and inflammatory processes.

Most animals synthesized their own vitamin C, but humans must rely upon dietary sources. No studies documenting the bioavailability of vitamin C from this type of vegetable soup have been reported. In the present study, we have shown that drinking 500 ml (17 oz=two bowls) of vegetable soup daily increased the plasma vitamin C from 45–50 to 60–65 μmol/L in just 4 h. The increased vitamin C concentration was maintained as long as the subjects were drinking the vegetable soup, which suggests that this is one efficient way of increasing vitamin C concentrations in the body.

Evidence from prospective studies suggests that a high dietary intake of antioxidants is protective for cardiovascular disease [38–40]. It has been postulated that dietary antioxidants, particularly vitamin C, may protect from inflammation resulting from both endogenous and exogenous causes, like oxidation. Therefore, in this study, we evaluated the effect of vitamin C on the formation of F_2-isoprostanes, as biomarker of oxidative damage in vivo, and on PEG_2, MCP-1 and the cytokines TNF-α, IL-1β and IL-6. The vegetable soup studied is rich in several nutrients, in addition to vitamin C; however, the role played by vitamin C on oxidative stress and inflammatory biomarkers was evident based on the strong correlation found between plasma vitamin C concentrations and biomarkers of oxidation and inflammation. However, we do not discard the possibility that other nutrients present in the vegetable soup may have also contributed to the effects found. In fact, the plasma concentration of carotenoids was also increased (data not shown). Thus, we believe that the effects found are a result of the natural combination of bioactive compounds present in the vegetable soup with additive and synergistic effects.

Quantification of F_2-isoprostanes has proven to be a major advance in assessing oxidative stress status in vivo. In addition, F_2-isoprostanes can also exert potent biological activity and potentially mediate some of the adverse effects of oxidant injury [41]. Some studies have investigated the effects of antioxidant supplements on levels of F_2-isoprostane [22,23]; however, few studies have examined the effect of dietary interventions [14,20,21]. Recently, it has been reported that the overall tomato-based product intake may be associated with a reduced risk of cardiovascular diseases [42] and a decreased low-density lipoprotein oxidizability and urinary excretion of 8-iso-$PGF_{2\alpha}$ after tomato-product consumption [43]. Interestingly, we observed a reduction in the concentration of 8-*epi*-$PGF_{2\alpha}$ after subjects drank the vegetable soup daily for 14 days and a significant inverse correlation between the concentration of vitamin C and concentrations of 8-*epi*-$PGF_{2\alpha}$. Based on this, our research group found that plasma vitamin C was inversely correlated with 8-*epi*-$PGF_{2\alpha}$ concentrations among stroke patients [35].

Epidemiological studies have reported the association between serum uric acid and heart disease risk [44,45]. In the present study, after subjects consumed the vegetable soup daily for 14 days, concentrations of uric acid decreased, although they did not reach significance, among men and

women. In addition, we found a significant inverse correlation between the concentration of vitamin C and concentrations of uric acid. Observational studies have found that serum uric acid concentrations are higher among patients with established coronary heart disease [30]. This is in agreement with the positive correlation found between uric acid and 8-*epi*-$PGF_{2\alpha}$ concentrations, suggesting the possible contribution of both compounds to vascular injury.

Prostaglandins are known for their potent physiological properties and are among the many mediators of the inflammatory response. Prostaglandin E_2 is generated at sites of inflammation in substantial amounts and can mediate many of the pathologic features of inflammation [46]. In the present study, we found a decrease in PGE_2 concentrations in men and women at the end of the intervention. It is possible that this decrease in PGE_2 concentration was mediated in part by the improved antioxidant defenses, including ascorbate, tocopherol and carotenoids, in these subjects after the intervention. Based on this, we found in a previous study an inverse correlation between α-tocopherol concentration and levels of PGE_2 in a population aged 61 ± 10 years [35]. In addition, in the present study, a positive correlation between PGE_2 and 8-*epi*-$PGF_{2\alpha}$ concentrations was found.

MCP-1 is a chemoattractant for human monocytes and may play a role in the accumulation of monocytes after the interaction of antigen and sensitized lymphocytes. Experimental evidence for the role of MCP-1 in atherogenesis has been obtained using animal models of atherosclerosis [47]. In addition, MCP-1 has been shown to be expressed in human atherosclerotic lesions [48]. Consumption of the vegetable soup affected MCP-1 concentrations, which were lower in both men and women at the end of the intervention (Day 14). The main effect of sex, with higher MCP-1 (and 8-*epi*-$PGF_{2\alpha}$ and PGE_2) concentrations at baseline and throughout the study among men than among women, was observed, which may be compatible with the well-known sex difference in cardiovascular diseases. Our results (the main effect of sex and the correlation found) are in agreement with other studies, which also found higher oxidative stress in men than in women in healthy individuals [49,50]. A positive correlation between MCP-1 and 8-*epi*-$PGF_{2\alpha}$, uric acid and PGE_2 concentrations was also found, supporting the important association between oxidation and inflammation.

Cytokines (TNF-α, IL-1β and IL-6) are tightly regulated and are responsible for the progression of the inflammatory reaction to a systemic involvement, encompassing a wide range of organ systems and regulation of other molecules such as CRP, chemokines and adhesion molecules. No effect was found in the cytokine levels at the end of the intervention. In agreement with our results, Bruunsgaard et al. [51] showed that supplementation with vitamins E and C had no effect on the circulation levels of TNF-α, IL-6 or CRP in a healthy population of men.

There are some limitations in this study that should be acknowledged. Perhaps the most important of them 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 among men and women before and after consuming the vegetable soup. Another limitation of the study is the lack of outcomes associated with plasma levels following intake of the vegetable soup. Because this is a healthy population, the main objective was to assess the bioavailability of vitamin C from this vegetable soup and its association with biomarkers of oxidative stress and inflammation.

In conclusion, drinking gazpacho significantly increased vitamin C concentrations and significantly decreased oxidative stress and inflammation in vivo by lowering the concentration of F_2-isoprostanes and PGE_2 and MCP-1, which provides new evidence for the healthy benefits of eating vegetables. Total vitamin C was significantly and inversely correlated with 8-*epi*-$PGF_{2\alpha}$, uric acid, PGE_2 and MCP-1. In addition, 8-*epi*-$PGF_{2\alpha}$ was significantly and positively correlated with uric acid, PGE_2 and MCP-1. These results suggest that even small increases in the concentration of vitamin C in the blood could have a significant effect on oxidative and inflammatory status, supporting the important effect on health of small dietary changes.

Acknowledgments

We thank I. Blanco, C. Herrero, T. Motilla and P. Martínez and the volunteers who participated in the study for their time, patience and enthusiasm.

References

[1] Liu RH. Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. Am J Clin Nutr 2003; 78(Suppl):517S–20S.
[2] Cox BD, Whichelow MJ, Prevost AT. Seasonal consumption of salt vegetables and fresh fruit in relation to the development of cardiovascular disease and cancer. Public Health Nutr 2000;3:19–29.
[3] Joshipura K, Ascherio A, Manson JE, Stampfer MJ, Rimm EB, Speizer FE, et al. Fruit and vegetable intake in relation to risk of ischaemic stroke. JAMA 1999;282:1233–9.
[4] Aguilera A, Brotons M, Rodríguez M, Valverde A. Supercritical fluid extraction of pesticides from a table-ready food composite of plant origin (gazpacho). J Agric Food Chem 2003;51:5616–21.
[5] Sorensen G, Stoddard A, Peterson K, Cohen N, Hunt MK, Stein E, et al. Increasing fruit and vegetables consumption through work sites and families in the Treatwell 5-A-Day Study. Am J Public Health 1999;89:54–60.
[6] Davey MW, Van Montagu M, Inze D, Sanmartin M, Kanellis A, Smirnoff N, et al. Plant L-ascorbic acid: chemistry, function, metabolism, bioavailability and effects of processing. J Sci Food Agric 2000;80:825–60.
[7] Simon JA. Vitamin C and cardiovascular disease: a review. J Am Coll Nutr 1992;11:107–25.
[8] Ness AR, Khaw KT, Bingham S, Day NE. Vitamin C status and respiratory function. Eur J Clin Nutr 1996;50:573–9.
[9] Cullum A. Increasing fruit and vegetable consumption: the 5 a day programme. Nutr Bull 2003;28:159–63.
[10] Ness AR, Khaw KT, Bingham S, Day NE. Vitamin C status and serum lipids. Eur J Clin Nutr 1996;50:724–9.

[11] Taylor CA, Hampl JS, Johnston CS. Low intakes of vegetables and fruits, especially citrus fruits, lead to inadequate vitamin C intakes among adults. Eur J Clin Nutr 2000;54:573–8.
[12] Willcox JK, Catignani GL, Lazarus S. Tomatoes and cardiovascular health. Crit Rev Food Sci Nutr 2003;43:1–18.
[13] Block G, Dietrich M, Norkus E, Morrow JD, Hudes M, Caan B, et al. Factors associated with oxidative stress in human populations. Am J Epidemiol 2002;56:274–85.
[14] Sánchez-Moreno C, Cano MP, De Ancos B, Plaza L, Olmedilla B, Granado F, et al. Pulsed electric fields-processed orange juice consumption increases plasma vitamin C and decreases F_2-isoprostanes in healthy humans. J Nutr Biochem 2004;15:601–7.
[15] Szeto YT, Tomlinson B, Benzie IFF. Total antioxidant and ascorbic acid content of fresh fruits and vegetables: implications for dietary planning and food preservation. Br J Nutr 2002;87:55–9.
[16] Halliwell B. Establishing the significance and optimal intake of dietary antioxidants: the biomarker concept. Nutr Rev 1999;57:104–13.
[17] De Zwart LL, Meerman JHN, Commandeur JNM, Vermeulen NPE. Biomarkers of free radical damage. Applications in experimental animals and in humans. Free Radic Biol Med 1999;26:202–26.
[18] Basu S. Isoprostanes: novel bioactive products of lipid peroxidation. Free Radic Res 2004;38:105–22.
[19] Roberts L, Reckelhoff J. Measurement of F_2-isoprostanes unveils profound oxidative stress in aged rats. Biochem Biophys Res Commun 2001;287:254–6.
[20] Visioli F, Riso P, Grande S, Galli C, Porrini M. Protective activity of tomato products on in vivo markers of lipid oxidation. Eur J Nutr 2003;42:201–6.
[21] Wiseman H, O'Reilly JD, Adlercreutz H, Mallet AI, Bowey EA, Rowland IR, et al. Isoflavone phytoestrogens consumed in soy decrease F_2-isoprostane concentrations and increase resistance of low-density lipoprotein to oxidation in humans. Am J Clin Nutr 2002;72:395–400.
[22] Patrignani P, Panara MR, Tacconelli S, Seta F, Bucciarelli T, Ciabattoni G, et al. Effects of vitamin E supplementation on F_2-isoprostane and thromboxane biosynthesis in healthy cigarette smokers. Circulation 2000;102:539–45.
[23] Dietrich M, Block G, Benowitz NL, Morrow JD, Hudes M, Jacob III P, et al. Vitamin C supplementation decreases oxidative stress biomarker F_2-isoprostanes in plasma of nonsmokers exposed to environmental tobacco smoke. Nutr Cancer 2003;45:176–84.
[24] Ford ES, Liu S, Mannino DM, Giles WH, Smith SJ. C-reactive protein concentration and concentrations of blood vitamins, carotenoids, and selenium among United States adults. Eur J Clin Nutr 2003;57:1157–63.
[25] Pradhan AD, Ridker PM. Do atherosclerosis and type 2 diabetes share a common inflammatory basis? Eur Heart J 2002;23:831–4.
[26] Chrysohoou C, Panagiotakos DB, Pitsavos C, Das UN, Stefanadis C. Adherence to the Mediterranean diet attenuates inflammation and coagulation process in healthy adults. The ATTICA study. J Am Coll Cardiol 2004;44:152–8.
[27] Harris SG, Padilla J, Koumas L, Ray D, Phipps RP. Prostaglandins as modulators of immunity. Trends Immunol 2002;23:144–50.
[28] Reape TJ, Groot PHE. Chemokines and atherosclerosis. Atherosclerosis 1999;147:213–25.
[29] Alderman MH. Serum uric acid as a cardiovascular risk factor for heart disease. Curr Hypertens Rep 2001;3:184–9.
[30] Alderman MH. Uric acid and cardiovascular risk. Curr Opin Pharmacol 2002;2:126–30.
[31] Bickel C, Rupprecht HJ, Blankenberg S, Rippin G, Hafner G, Daunhauer A, et al. Serum uric acid as an independent predictor of mortality in patients with angiographically proven coronary artery disease. Am J Cardiol 2002;89:12–7.
[32] Leyva F, Anker S, Swan JW, Godsland IF, Wingrove CS, Chua TP, et al. Serum uric acid as an index of impaired oxidative metabolism in chronic heart failure. Eur Heart J 1997;18:858–65.
[33] Sánchez-Moreno C, Plaza L, De Ancos B, Cano MP. Quantitative bioactive compounds assessment and their relative contribution to the antioxidant capacity of commercial orange juices. J Sci Food Agric 2003;83:430–9.
[34] Martin A, Frei B. Both intracellular and extracellular vitamin C inhibit atherogenic modification of LDL by human vascular endothelial cells. Arterioscler Thromb Vasc Biol 1997;17:1583–90.
[35] Sánchez-Moreno C, Dashe JF, Scott T, Thaler D, Folstein MF, Martin A. Decreased levels of plasma vitamin C and increased concentrations of inflammatory and oxidative stress markers after stroke. Stroke 2004;35:163–8.
[36] Christman JW, Abdolrasulnia R, Shepherd VL, Rinaldo JE. Paradoxical regulation by PGE-2 on release of neutrophil chemoattractants by rat bone marrow macrophages. Prostaglandins 1991; 41:251–62.
[37] Bland JM, Altman DG. Calculating correlation coefficients with repeated observations: Part 1. Correlation within subjects. BMJ 1995; 310:446.
[38] Sesso HD, Buring JE, Norkus EP, Gaziano JM. Plasma lycopene, other carotenoids, and retinol and the risk of cardiovascular disease in women. Am J Clin Nutr 2004;79:47–53.
[39] Lonn EM, Yusuf S. Is there a role for antioxidant vitamins in the prevention of cardiovascular diseases? An update on epidemiological and clinical trials data. Can J Cardiol 1997;13: 957–65.
[40] Khaw KT, Bingham S, Welch A, Luben R, Wareham N, Oakes S, et al. Relation between plasma ascorbic acid and mortality in men and women in EPIC-Norfolk prospective study: a prospective population study. Lancet 2001;357:657–63.
[41] Roberts II LJ, Morrow JD. Products of the isoprostane pathway: unique bioactive compounds and markers of lipid peroxidation. Cell Mol Life Sci 2002;59:808–20.
[42] Sesso HD, Liu SM, Gaziano JM, Buring JE. Dietary lycopene, tomato-based food products and cardiovascular disease in women. J Nutr 2003;133:2336–41.
[43] Visioli F, Caruso D, Galli C, Viappiani S, Galli G, Sala A. Olive oils rich in natural catecholic phenols decrease isoprostane excretion in humans. Biochem Biophys Res Commun 2000;278:797–9.
[44] Fang J, Alderman MH. Serum uric acid and cardiovascular mortality–The NHANES I epidemiologic follow-up study, 1971–1992. JAMA 2000;283:2404–10.
[45] Culleton BF, Larson MG, Kannel WB, Levy D. Serum uric acid and risk for cardiovascular disease and death: the Framingham Heart Study. Ann Intern Med 1999;131:7–13.
[46] Serhan CN, Levy B. Success of prostaglandin E_2 in structure–function is a challenge for structure-based therapeutics. Proc Natl Acad Sci U S A 2003;100:8609–11.
[47] Ross R. Atherosclerosis — an inflammatory disease. N Engl J Med 1999;340:115–26.
[48] De Lemos JA, Morrow DA, Sabatine MS, Murphy SA, Gibson CM, Antman EM, et al. Association between plasma levels of monocyte chemoattractant protein-1 and long-term clinical outcomes in patients with acute coronary syndromes. Circulation 2003;107: 690–5.
[49] Proteggente AR, England TG, Rice-Evans CA, Halliwell B. Iron supplementation and oxidative damage to DNA in healthy individuals with high plasma ascorbate. Biochem Biophys Res Commun 2001; 288:245–51.
[50] Ide T, Tsutsui H, Ohashi N, Hayashidani S, Suematsu N, Tsuchihashi M, et al. Greater oxidative stress in healthy young men compared with premenopausal women. Arterioscler Thromb Vasc Biol 2002;22: 438–42.
[51] Bruunsgaard H, Poulsen HE, Pedersen BK, Nyyssönen K, Kaikkonen J, Salonen JT. Long-term combined supplementations with α-tocopherol and vitamin C have no detectable anti-inflammatory effects in healthy men. J Nutr 2003;133:1170–3.