Supplementation with cactus pear (*Opuntia ficus-indica*) fruit decreases oxidative stress in healthy humans: a comparative study with vitamin C[1–3]

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ABSTRACT

Background: Cactus pear (*Opuntia ficus-indica*) fruit contains vitamin C and characteristic betalain pigments, the radical-scavenging properties and antioxidant activities of which have been shown in vitro.

Objective: We investigated the effects of short-term supplementation with cactus pear fruit compared with vitamin C alone on total-body oxidative status in healthy humans.

Design: In a randomized, crossover, double-treatment study, 18 healthy volunteers received either 250 g fresh fruit pulp or 75 mg vitamin C twice daily for 2 wk, with a 6-wk washout period between the treatments. Before (baseline) and after each treatment, 8-epi-prostaglandin $F_{2\alpha}$ (8-epi-PGF$_{2\alpha}$) and malondialdehyde in plasma, the ratio of reduced to oxidized glutathione (GSH:GSSG) in erythrocytes, and lipid hydroperoxides in LDL were measured as biomarkers of oxidative stress; plasma Trolox-equivalent antioxidant activity (TEAC) and vitamins A, E, and C were evaluated as indexes of antioxidant status.

Results: Both treatments caused comparable increases compared with baseline in plasma concentrations of vitamin E and vitamin C ($P < 0.05$); vitamin A and TEAC did not change significantly. After supplementation with cactus pear fruit, 8-epi-PGF$_{2\alpha}$ and malondialdehyde decreased by ≈30% and 75%, respectively; GSH:GSSG shifted toward a higher value ($P < 0.05$); and LDL hydroperoxides were reduced by almost one-half. Supplementation with vitamin C did not significantly affect any marker of oxidative stress.

Conclusions: Consumption of cactus pear fruit positively affects the body's redox balance, decreases oxidative damage to lipids, and improves antioxidant status in healthy humans. Supplementation with vitamin C at a comparable dosage enhances overall antioxidant defense but does not significantly affect body oxidative stress. Components of cactus pear fruit other than antioxidant vitamins may play a role in the observed effects.   *Am J Clin Nutr* 2004;80:391–5.

INTRODUCTION

Aerobic life is characterized by a steady formation of prooxidants, including reactive oxygen species and their products, that is approximately balanced by antioxidant defense systems. The balance is not perfect, however, so that a certain degree of oxidative damage occurs even in healthy persons. When the generation of prooxidants greatly exceeds the cell's capacity to protect itself, serious oxidative stress occurs, and the accumulation of damage will result in pathophysiologic events. It has repeatedly been suggested that increased consumption of fruit and vegetables is associated with protection against various pathologies, including cancer and cardiovascular and cerebrovascular diseases, in which oxidative damage is an important etiologic factor (1–3). On the basis of the assumption that the antioxidant components of fruit and vegetables may be responsible for the effects of such food, many studies have focused on vitamin C and carotenoids. However, the results of supplementation studies with pure vitamins are not conclusive about the contribution of pure vitamins to the protection of health (4). Other antioxidant components, such as polyphenolic pigments, have received considerable attention in the past decade. Most fruit contain antioxidant vitamins and one or more polyphenolic compounds.

The cactus pear (*Opuntia ficus-indica* L. Mill) is a common vegetation in Mexico, much of Latin America, South Africa, and the Mediterranean. We recently investigated the antioxidant activity of the fruit of cactus pear and showed, for the first time, that the aqueous extracts possess a high total antioxidant capacity, expressed as trolox equivalents, and exhibit a marked antioxidant capacity in several in vitro assays, including the oxidation of red blood cell membrane lipids and the oxidation of human LDLs induced by copper and 2,2'-azobis(2-amidinopropane-hydrochloride) (5). When antioxidant components of the fruit have been evaluated, vitamin C and only negligible amounts of carotenoids and vitamin E were found (6), whereas polyphenols were absent (5). On the other hand, 2 betalain pigments, the purple-red betanin and the unique yellow indicaxanthin (7–10), the radical-scavenging and reducing properties of which were recently shown (5, 11, 12), occur in the fruit.

The aim of the present study was to determine the effects of short-term supplementation with cactus pear fruit on biomarkers of oxidative stress and plasma antioxidant status in healthy humans. To

investigate the potential contribution of fruit components other than vitamin C, a crossover study of a 2-wk supplementation with cactus pear fruit and vitamin C, at a dosage comparable with the amount ingested in the fruit, was carried out.

SUBJECTS AND METHODS

Fruit

Cactus pear fruit from yellow Sicilian cultivars were obtained from a local market at comparable ripening stages and were consumed within 48 h of collection. The antioxidant vitamin and phytochemical contents of the fruit were checked at the beginning of the study and once a week during the time of supplementation according to methods reported elsewhere (5).

Subjects and study design

Eighteen subjects [10 women and 8 men; aged ($\bar{x} \pm$ SD): 33.33 $\pm$ 11.27 y; body mass index (in kg/m^2): 23 $\pm$ 2.5] were recruited and provided written informed consent to participate in the study. All participants were in good health as determined by a medical history questionnaire, physical examination, and clinical laboratory tests. All subjects fulfilled the following eligibility criteria: *1*) no history of cardiovascular, hepatic, gastrointestinal, or renal disease; *2*) no antibiotic or supplemental vitamin or mineral use for $\geq$6 wk before the start of the study; and *3*) nonsmoking. Women were not using exogenous hormones. The study protocol was in accordance with the Helsinki Declaration of 1975, as revised in 1983.

This was a randomized, crossover, double-treatment study. The subjects were randomly divided into 2 groups, which were assigned to supplementation with either 250 g cactus pear pulp or 75 mg vitamin C at each of the 2 main daily meals for 2 wk. After a 6-wk washout period, each group received the other treatment. The subjects were allowed to continue their usual dietary habits, adhering to a Mediterranean diet (13). Fruit intake was limited to either the cactus pear fruit or vitamin C during the relevant supplementation periods.

Blood samples, which were collected in EDTA-coated tubes (1 mg/mL), were obtained before (baseline) and at the end of each supplementation period from the subjects after they had fasted overnight for $\geq$10 h. Plasma was separated from red blood cells by centrifugation at 4 °C and 2000 $\times$ *g* for 10 min. Suitable portions of plasma were immediately used to prepare LDL, and the remaining plasma was portioned and stored at $-$80 °C for the biochemical analysis described below and to measure total cholesterol (Roche Diagnostics; F Hoffmann-La Roche, Basel, Switzerland) within 2 wk. Red blood cells were washed with 5 mmol phosphate-buffered saline/L, pH 7.4, and were used immediately.

Oxidative variables

Total plasma F$_2$-isoprostanes (8-epi-prostaglandin F$_{2\alpha}$, or 8-epi-PGF$_{2\alpha}$) were measured by gas chromatography–mass spectrometry with negative chemical ionization as described elsewhere (14). The isoprostanes were converted to pentafluorobenzyl esters, sialylated, and separated on an SPB-1701 column (30 m $\times$ 0.25 mm internal diameter; 0.25 μm film thickness; Supelco, Bellefonte, PA) with the use of an Autospec Ultima o-TOF instrument (Micromass, Waters, United Kingdom) and methane as the reagent gas. Samples (2 μL) were injected into a

temperature-programmed injector (model 6890 GC; Hewlett-Packard, Palo Alto, CA). The gas chromatograph was programmed from 175 °C to 280 °C at a rate of 30 °C/min.

Malondialdehyde (MDA) was evaluated in 50-μL plasma samples by the use of a colorimetric reaction with thiobarbituric acid (15), which was followed by neutralization of the samples by equivalent volumes of a mixture consisting of 4.5 mL 1.0 mol NaOH/L and 45.5 mL methanol. Isocratic HPLC separation of the MDA adduct was performed with a Supelco Supelcosil LC-18 column as above, eluted with 40% methanol in 50 mmol potassium phosphate buffer/L, pH 6.8, at 1.5 mL/min. The MDA– thiobarbituric acid adduct was detected at 532 nm and quantified by reference to a calibration curve of tetrahydroxypropane, which was submitted to the thiobarbituric acid colorimetric procedure. Butylated hydroxytoluene (0.03%) was added to the thiobarbituric acid reagent to prevent artifactual lipid peroxidation during the assay procedure.

The ratio of reduced to oxidized glutathione (GSH:GSSG) was evaluated in red blood cells according to GSH measurement by the 5,5'-dithiobis(2-nitrobenzoic acid)–reductase recycling method (16) with a microtiter plate assay (Kayman Chemical Co, Ann Arbor, MI)

Plasma antioxidant status

Total antioxidant capacity in plasma was evaluated as trolox-equivalent antioxidant capacity (TEAC) according to Rice-Evans and Miller (17). *all-trans*-Retinol and α-tocopherol were extracted with 2 volumes of absolute ethanol and 8 volumes of petroleum ether from 200 μL plasma diluted to 1.0 mL with phosphate-buffered saline. The organic extracts were gathered, dried under nitrogen, resuspended in suitable solvent, and analyzed with the use of an LC-18 HPLC column (0.46 $\times$ 25 cm; Supelco) and 1.0 mL methanol/min. *all-trans*-Retinol and α-tocopherol were detected at wavelengths of 320 and 290 nm, respectively. Under the conditions described, *all-trans*-retinol eluted after 5.2 min and α-tocopherol after 13.4 min. An automatic wavelength change after 9 min allowed the detection of both compounds in the same sample. Ascorbic acid was determined in 500 μL plasma from blood collected in 1.0 mmol dithiothreitol/L. Extraction, HPLC separation, and spectrophotometry at 266 nm were as reported (15) with minor changes, which included the length of the HPLC column (25 $\times$ 0.46 cm) and isocratic elution with 10-mmol/L KH$_2$PO$_4$ buffer containing 10 mmol tetrabutylammonium bromide/L in 1% methanol in water, pH 7.0, at 1.2 mL/min. The retention time of ascorbate was 5.3 min. All procedures were performed under red light to avoid artifactual photooxidation of lipids by the low-energy quanta of visible light and to preserve light-sensitive vitamins.

LDL analysis

LDL was isolated by stepwise centrifugation at 4 °C, according to Kleinveld et al (18), with a model L8-70 ultracentrifuge (Beckman, Palo Alto, CA) fitted with a 50 Ti rotor and by using potassium bromide for density adjustments. EDTA and salts were removed from LDL by gel filtration on Sephadex G-25 medium (Pharmacia Biotech, Milan, Italy). Proteins were measured by the Bio-Rad (Hercules, CA) colorimetric method (19). In typical preparations, 0.6 mg apolipoprotein B-100 was obtained from 1 mL plasma. To prevent autoxidation reactions,

TABLE 1
Antioxidant vitamins and phytochemicals in the supplemented cactus pear pulp[1]

Compound	Value (per 100 g pulp)
Vitamin C (mg)	29 ± 2
α-Tocopherol (μg)	80 ± 5
β-Carotene (μg)	1.5 ± 0.2
Betanin (mg)	1.21 ± 0.15
Indicaxanthin (mg)	9.3 ± 0.68
Polyphenols	ND

[1] All values are the $\bar{x} \pm$ SD of 5 determinations performed in duplicate on 5 lots of fruit. Antioxidant vitamins and phytochemicals were measured as reported in references 5 and 6. ND, not detectable.

LDL was used immediately or after overnight storage at $-80\ °C$. The conjugated diene lipid hydroperoxides in the lipid fraction of LDL (LDL-CD hydroperoxides) were extracted from LDL samples (1.0 mg protein in 0.15 mol NaCl/L) with 2 volumes of $CHCl_3$:MeOH (2:1, by vol). The organic extract was dried under a nitrogen stream, resuspended in cyclohexane, and quantitated spectrophotometrically at 234 nm by using a molar absorption coefficient of 28 000 (20). The results are expressed as nmol/mg LDL protein.

Statistical analysis

Statistical calculations were carried out with INSTAT-3 statistical software (GraphPad Software Inc, San Diego). The data were analyzed by one-way analysis of variance with Bonferroni's correction for multiple comparisons. In all cases, significance was accepted when the null hypothesis was rejected at the $P < 0.05$ level.

RESULTS

The content of antioxidant vitamins and betalain pigments in the fruit used throughout the study was measured, and the results are shown in **Table 1**. The values did not vary substantially with respect to those reported previously for Sicilian cactus pear cultivars (5, 6).

Antioxidant status

The TEAC evaluated in plasma is usually considered to provide indications of the body's global antioxidant status. TEAC did not change significantly from baseline after cactus pear supplementation (**Table 2**). However, increases in vitamin C (1.35-fold) and vitamin E (1.13-fold) were observed. The latter was significant even when the value was lipid-standardized by dividing for total cholesterol. In addition, although the plasma concentration of vitamin A did not vary significantly, a trend toward higher values was evident. Supplementation with vitamin C at a dosage comparable with the amount of vitamin C provided by the cactus pear fruit resulted in no significant differences in the plasma antioxidant pattern (Table 2).

Markers of oxidative stress

Biomarkers of oxidative stress in plasma, erythrocytes, and LDL were measured. The concentrations of the biomarkers of oxidative damage to lipids appeared to be strongly modified by the 2-wk supplementation with cactus pear. MDA was $\approx$4-fold

TABLE 2
Trolox-equivalent antioxidant activity (TEAC) and concentrations of major antioxidant vitamins in the plasma of healthy humans before (baseline) and after 2 wk of supplementation with cactus pear fruit or vitamin C[1]

Variable	Baseline	After supplementation	
		Cactus pear	Vitamin C
TEAC (mmol/L)	1.38 ± 0.05	1.40 ± 0.06	1.41 ± 0.05
Vitamin C (μmol/L)	62.1 ± 10	84 ± 15^2	86 ± 12^2
Vitamin E (μmol/L)	18.3 ± 1.4	20.8 ± 2.0^2	21.5 ± 2.1^2
Lipid-standardized vitamin E[3] [(μmol/L)/(mmol/L)]	3.81 ± 0.30	4.30 ± 0.42^2	4.47 ± 0.43^2
Vitamin A (μmol/L)	2.0 ± 0.38	2.19 ± 0.35	2.1 ± 0.4

[1] All values are the $\bar{x} \pm$ SD of 18 determinations performed in duplicate on samples from different subjects; $n = 18$. All variables were measured as reported in Subjects and methods. The baseline values are the mean of the 2 baseline measurements obtained at the beginning of the study and after the 6-wk washout period. There were no significant differences between the 2 treatment groups.
[2] Significantly different from baseline, $P < 0.05$ (Bonferroni's multiple-comparison test).
[3] Vitamin E/cholesterol.

lower than at baseline, whereas 8-epi-PGF$_{2\alpha}$ concentrations decreased by about one-third (**Table 3**).

Glutathione is the most powerful intracellular antioxidant, and GSH:GSSG is a representative marker of the antioxidative capacity of the cell. Supplementation with cactus pear enhanced concentrations of GSH and decreased concentrations of GSSG in red blood cells, resulting in a higher GSH:GSSG (Table 3).

Quantitative evaluation of LDL oxidative status was carried out by measuring the resident LDL hydroperoxides. LDL isolated after supplementation with cactus pear was less oxidized than LDL isolated at baseline, as shown by the significantly decreased concentration of LDL-CD hydroperoxides (Table 3).

TABLE 3
Markers of oxidative stress in healthy humans before (baseline) and after 2 wk of supplementation with cactus pear fruit or vitamin C[1]

Variable	Baseline	After supplementation	
		Cactus pear	Vitamin C
MDA (μmol/L)	1.08 ± 0.26	0.28 ± 0.13^2	1.22 ± 0.34
8-epi-PGF$_{2\alpha}$ (ng/L)	45 ± 10	31 ± 9^2	43 ± 12
RBC-GSH (mmol/L)	2.0 ± 0.14	2.19 ± 0.12^2	2.01 ± 0.15
RBC-GSSG (mmol/L)	0.19 ± 0.03	0.14 ± 0.02^2	0.20 ± 0.04
GSH:GSSG	10.5 ± 1.5	15.6 ± 2^2	10.5 ± 1.4
LDL-CD hydroperoxides (nmol/mg LDL protein)	6.5 ± 0.7	3.9 ± 0.5^2	6.8 ± 0.88

[1] All values are the $\bar{x} \pm$ SD of 18 determinations performed in duplicate on samples from different subjects; $n = 18$. All variables were measured as reported in Subjects and methods. The baseline values are the mean of the 2 measurements obtained at the beginning of the study and after the 6-wk washout period. MDA, malondialdehyde; 8-epi-PGF$_{2\alpha}$, 8-epi-prostaglandin F$_{2\alpha}$; RBC, red blood cell; GSH, reduced glutathione; GSSG, oxidized glutathione; CD, conjugated diene. There were no significant differences between the 2 treatment groups at baseline.
[2] Significantly different from baseline and significantly different from vitamin C supplementation, $P < 0.05$ (Bonferroni's multiple-comparison test).

In contrast, after 2 wk of treatment with vitamin C, concentrations of 8-epi-PGF$_2\alpha$ did not change significantly compared with baseline and concentrations of MDA were slightly, although not significantly, higher. No variations in the oxidative status of LDL and red blood cells were found after vitamin C supplementation (Table 3). Therefore, the oxidative damage to lipids was not reduced by vitamin C alone, at least at a dosage comparable with that provided by the cactus pear fruit.

DISCUSSION

The results of the present study show that daily supplementation with 500 g cactus pear fruit pulp for 2 wk greatly improves the oxidative stress status of healthy subjects. The experimental evidence includes remarkable reductions in plasma markers of oxidative damage to lipids, such as isoprostanes and MDA; an improvement in the oxidative status of LDL; considerably higher concentrations of major plasma antioxidants; and improvement in the redox status of erythrocytes.

Free radical–driven oxidation of lipids is a central feature of oxidant stress, which is why quantification of the end products of lipid peroxidation is considered to be a measure of whole-body oxidative damage (21). F$_2$-isoprostanes have been described as prostaglandin F isomers produced by cyclooxygenase-independent oxidation of arachidonic acid (21). Their quantification in plasma is valued as the most sensitive and specific index of lipid peroxidation and has emerged as one of the most reliable biomarkers for assessing oxidative stress status in vivo. We found markedly reduced plasma concentrations of 8-epi-PGF$_2\alpha$ after supplementation with cactus pear fruit, thereby indicating a marked reduction in body oxidative damage. Plasma MDA is another marker of lipid oxidation (22, 23), and its measurement may provide further indication of oxidative injury in vivo. Consistent with the decrease in F$_2$-isoprostanes, a sharp decline in plasma MDA concentrations was observed.

Apart from being a cofactor of the GSH-peroxidase family, glutathione is involved in the prevention of oxidation and cross-linking of protein thiols. Therefore, the intracellular GSH concentration, as related to GSSG, is an important index of the body's oxidative status (24). The elevation of GSH:GSSG observed in red blood cells indicates that supplementation with cactus pear reduces oxidative damage and results in an enhancement of the reducing potential of the cells.

Our measurements also showed that circulating LDLs were less oxidized after supplementation with cactus pear fruit. The oxidation of LDL occurs to a large extent within blood vessel walls (25, 26), which means that the oxidative status of LDL reflects the oxidation status of the blood vessels (27). The remarkable reduction in plasma F$_2$-isoprostanes, the concentration of which correlates with the concentration of cellular lipid hydroperoxides (21) and which is a biomarker of in vivo LDL oxidation (28, 29), appears to be consistent with the observation that supplementation with cactus pear results in less oxidized particles.

Whereas body lipids appeared to be preserved, plasma concentrations of antioxidant vitamins, such as vitamin C and vitamin E, increased after supplementation with cactus pear fruit. The vitamin C concentration appears to be a reflection of the absorption of vitamin C from fruit. On the other hand, because the fruit is not a source of α-tocopherol, the increase in vitamin E may result from a sparing effect on it by vitamin C (30). Despite the increase in these antioxidants, plasma total antioxidant capacity did not appear to be significantly affected after supplementation. On the assumption that their TEAC is 1.0 (30), the contribution of vitamin E and vitamin C to the plasma TEAC value at baseline was only 1.3% and 4.4%, respectively, which agrees with other reports (31, 32). Therefore, micromolar variations in these compounds cannot significantly modify the TEAC value. The contribution to the plasma TEAC of small amounts of other antioxidant components absorbed from the fruit may have also escaped the calculation.

Vitamin C is the only well-characterized antioxidant in cactus pear fruit. It is for this reason that we compared the effects of supplementation with cactus pear fruit with those of supplementation with an equivalent dosage of vitamin C. Supplementation with vitamin C raised plasma concentrations of vitamins C and E to an extent comparable with that observed after fruit supplementation. However, supplementation with vitamin C did not bring about any significant changes in the markers of lipid oxidation. Thus, in accordance with other reports (33, 34), we showed that the balanced antioxidant mixture of fruit, which includes vitamin C and co-nutrients, is more helpful than are single vitamins in preventing oxidative damage in vivo.

Our data suggest that something in the cactus pear fruit other than vitamin C may help to decrease lipid oxidation. Polyphenols, which have been considered as important contributors to the antioxidant activity of fruit and vegetables (35–37), do not occur in cactus pear fruit (5, 38). Betanin and indicaxanthin, the characteristic pigments of the fruit, may be considered. The radical-scavenging and antioxidant activity of these compounds has been shown in several chemical and biological models (5, 11, 12), including the ex vivo oxidation of human LDL (39). More importantly, they are absorbed from red beet (11) and cactus pear (6) and are bioavailable in humans, with peak plasma concentrations on the micromolar order at 3 h. Thus, a potential contribution of these phytochemicals to the observed protective effects of the cactus pear fruit may be hypothesized. Antioxidant or other biological activities of betalains in vivo deserve to be more deeply investigated. Our findings of improved body redox status suggest major benefits from diets including cactus pear, which may reduce the risk of age-related and degenerative diseases in which the level of body oxidative stress may play a pathogenic role.

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