

Immunity and Antioxidant Capacity in Humans Is Enhanced by Consumption of a Dried, Encapsulated Fruit and Vegetable Juice Concentrate¹

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Abstract

The daily consumption of fruits and vegetables is a common dietary recommendation to support good health. We hypothesized that a commercially available encapsulated fruit and vegetable juice powder concentrate (FVJC) could support functional indices of health due to increased intake of various phytonutrients. This was a double-blind, randomized, placebo-controlled investigation of 59 healthy law students who consumed either FVJC or placebo capsules for 77 d. Blood was collected on d 1, 35, and 77 to examine the number of circulating $\alpha\beta$ - and $\gamma\delta$ -T cells, cytokine production, lymphocyte DNA damage, antioxidant status, and levels of carotenoids and vitamin C. A log of illnesses and symptoms was also kept. The FVJC group tended to have fewer total symptoms than the placebo group ($P < 0.076$). By d 77 there was a 30% increase in circulating $\gamma\delta$ -T cells and a 40% reduction in DNA damage in lymphocytes in the FVJC group relative to the placebo group. Plasma levels of vitamin C and of β -carotene, lycopene, and lutein increased significantly from baseline in the FVJC group as did plasma oxygen radical absorptive capacity (50%). Interferon- γ produced by phorbol-stimulated lymphocytes was reduced 70% in the FVJC group, whereas other cytokines (IL-4, IL-6, transforming growth factor β) were unchanged relative to treatment or time. FVJC consumption during this study period resulted in increased plasma nutrients and antioxidant capacity, reduction in DNA strand breaks, and an increase in circulating $\gamma\delta$ -T cells. J. Nutr. 136: 2606–2610, 2006.

Introduction

Components of fruits and vegetables, such as vitamins, minerals, and other phytochemicals are effective in maintaining health and immune function (1–3). The objective of this research was to evaluate the consumption of fruits and vegetables in the form of an encapsulated powder concentrate from a fruit and vegetable juice blend and to correlate ingestion of this capsule with serum antioxidant levels, immune biomarkers, and illness.

An encapsulated fruit and vegetable juice concentrate (FVJC)³ has been shown to increase proliferation of peripheral blood mononuclear cells (PBMC), improve natural killer (NK) cell cytotoxicity in elderly nonsmokers, and increase IL-2 production in both smokers and nonsmokers (4). We hypothesized that consumption of this FVJC might also impact markers of immune function in a younger population, particularly related to the

$\gamma\delta$ -T-cell population. $\gamma\delta$ -T cells, having characteristics of innate and acquired immunity, play a role in protecting epithelial linings, immunosurveillance against virally infected and tumor cells, and a role in wound healing (5–7). A previous study also found that FVJC consumption for 84 d reduced the amount of DNA damage in PBMC, as measured by the comet assay (4). Moreover, studies using this preparation showed that plasma carotenoids (4,8–12) and plasma vitamin C levels increase (9–11), whereas indicators of oxidative damage, such as serum lipid peroxide levels (12) and plasma malondialdehyde levels, decrease (11).

Our objective was to extend these studies and examine an otherwise healthy population that might be experiencing stress. Law students are reported to have reduced subjective well-being relative to the general population or to medical students (13). Subjective well-being refers to how people evaluate their lives. Law students have also been observed to acquire impairment in both psychological and physical health (14). Stress is known to have detrimental effects on immunity and may increase susceptibility to infectious agents, influence the severity of infections, reduce the response to vaccines, activate latent herpes or Epstein-Barr viruses, and reduce the rate of wound healing (15). The stress response resembles a pro-oxidative state with the potential for free radical damage to a number of cells and tissues.

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³ Abbreviations used: EBV Epstein-Barr virus; FVJC fruit and vegetable juice powder concentrate; NK natural killer; ORAC oxygen radical absorbance capacity; PBMC peripheral blood mononuclear cells; PMA phorbol 12-myristate 13-acetate.

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We hypothesized that consumption of FVJC capsules could prevent damage to specific immune cells in these students. We measured the number of circulating T cells, cytokine secretion after culture, DNA strand breaks in lymphocytes, antioxidant status, and the number and duration of illnesses. Also evaluated was a marker for stress, Epstein-Barr virus (EBV) titers (16,17).

Materials and Methods

Study design. Healthy men ($n = 23$) and women ($n = 36$), ranging in age from 21 to 53 y (mean 25 y), were recruited from the University of Florida's Levin College of Law in Gainesville, Florida, during August, 2004. Exclusion criteria included the use of tobacco products, chronic or excessive alcohol consumption, use of illegal pharmaceuticals, pregnant or lactating females, and recent surgery or illness. The study was conducted over an 11-wk period. The study was approved by the University of Florida's Institutional Review Board. Written informed consent was obtained from each subject.

On d 1, subjects were randomly assigned, in a double-blind fashion, to either the placebo group, who received capsules containing microcrystalline cellulose, or the FVJC group, who received capsules containing primarily fruit and vegetable juice powder concentrate derived from acerola cherry, apple, beet, broccoli, cabbage, carrot, cranberry, kale, orange, peach, papaya, parsley, pineapple, spinach, and tomato (Juice Plus+ NSA). The FVJC capsules provided ~ 7.5 mg β -carotene, 234 mg vitamin C, 45 IU vitamin E, 420 μ g folate, 60 mg calcium, and about 42 kJ (9–11). Subjects were instructed to consume 4 capsules/d (2 in the morning and 2 in the evening) with meals and to otherwise not alter their habitual diet.

On d 77, when study participants arrived for their final blood draw, a questionnaire was used to evaluate compliance, efficacy of blinding, and intercurrent illness. Compliance was also assessed by the returned capsule count. Subjects gave detailed specific symptom information on running or congested nose, stiffness or chills, headache, cough, fever, sore throat, and aches. They noted the date of occurrence, duration of each particular symptom, whether medical treatment was sought, and if medication was prescribed. Symptoms refer to the sum of all of the symptoms for each person for all of their reported illnesses. Participants also provided their semester grade-point average at the end of the term.

Plasma collection and analysis. Fasting blood was collected into 2 EDTA tubes on d 1, 35, and 77. One tube was placed at 4°C, one at room temperature, and both were processed within 1 h after collection. Plasma was removed from the 4°C blood and processed in relative darkness. After a 1:5 dilution was made in 6% *m*-phosphoric acid to preserve ascorbic acid, samples were frozen at -85°C . Aliquots of untreated plasma were frozen at -85°C for oxygen radical absorbance capacity (ORAC) assay, carotenoid profiling, and EBV antibody titer studies.

For PBMC separation, 7 mL of whole, anticoagulated blood held at room temperature was diluted 1:1 with 0.15 mol/L of NaCl. Six mL of the diluted blood was layered over 3 mL of Nycoprep 1.077 (Axis-Shield) and centrifuged ($800 \times g$, 20 min, 20°C). The mononuclear cell layer was removed and washed twice with RPMI 1640 (Cellgro; Mediatech) complete (100,000 U/L penicillin; 100 mg/L streptomycin; 0.25 mg/L fungizone; 50 mg/L gentamycin; 2 mmol/L *l*-glutamine; 25 mmol/L HEPES buffer) containing 10% fetal bovine serum (Cellgro). Cell pellets were resuspended in RPMI 1640 complete.

Flow cytometry. Percentages of $\alpha\beta$ - and $\gamma\delta$ -T-cell populations in whole PBMC suspensions were determined by 2-color flow cytometry on d 1 and 77. On the day of each blood draw, PBMC (5×10^5) was incubated with phycoerythrin-labeled anti-human CD3⁺ and 1 of 2 T-cell surface markers: fluorescein isothiocyanate-labeled anti-human $\alpha\beta$ -TCR or fluorescein isothiocyanate-labeled anti-human $\gamma\delta$ -TCR; or with the CD3⁺ isotype control phycoerythrin-labeled anti-human IgG2a_K (eBioscience) alone. Data collected was from gated CD3⁺ cells with fluorescence brighter than the nonspecific isotype control, and analyzed using WinMDI software (Scripps Institute).

DNA strand break assay. An aliquot of PBMC (1×10^9 /L) in complete RPMI 1640 medium with 10% autologous plasma, were placed into

2 wells of a 12-well Falcon plate from d 1, 35, and 77. Hydrogen peroxide (H_2O_2) was added to 1 of the wells to a final concentration of 10 mmol/L, with the untreated well serving as control. The cells were incubated at 37°C for 2 h. Cells were washed once and processed using the ApoAlert DNA Fragmentation Assay Kit (BD Biosciences Clontech). This assay is based on the terminal deoxynucleotidyl transferase-mediated dUTP nick-end-labeling (TUNEL) assay. Terminal deoxynucleotidyl transferase catalyzes the incorporation of fluorescein-dUTP at the free 3'-hydroxyl ends of fragmented DNA. The fluorescein-labeled DNA was quantified by flow cytometry. Data were analyzed using WinMDI software (Scripps Institute). Results are expressed as a ratio of DNA strand breaks in cells treated with H_2O_2 to untreated cells.

Cytokine level determination in supernatants from cell proliferation. On samples from d 1 and 77, PBMC in RPMI 1640 complete medium containing 50 mmol/L 2-mercaptoethanol, 10% autologous plasma, 10 μ g/L phorbol 12-myristate 13-acetate (PMA, Sigma) and 1,000,000 U/L recombinant human IL-2 (r-IL-2, BD Biosciences) was seeded ($3 \times 10^5/200\mu\text{L}$) into 8 wells of a 96-well U-bottom microtiter plate and incubated in a humidified 5% CO_2 atmosphere at 37°C . After 72 h, the media were removed, replaced with medium without PMA, and the cells were incubated for an additional 11 d. During this time, not only are $\alpha\beta$ -T cells stimulated to proliferate and produce cytokines, but the $\gamma\delta$ -T cells are also proliferating and stimulated. On d 14, supernatant fluids were frozen at -85°C . These conditions allow enough time for activation of both $\alpha\beta$ -T cells and $\gamma\delta$ -T cells. Accumulated levels of human INF- γ , IL-4, TGF- β , and IL-6 in PBMC supernatants were quantified using ELISA (BioSource International). Plates were read at 450 nm on a SPECTRAMax 340PC plate reader (Molecular Devices).

Plasma oxygen radical absorbance capacity. Antioxidant activity in protein-free plasma was determined using the ORAC assay, as previously described for a 96-well microplate reader (18). Briefly, protein was removed by centrifugation ($12,000 \times g$ for 5 min) and 200 μL of 6% *m*-phosphoric acid was added to an equal volume of plasma. Samples were diluted with phosphate buffer and fluorescence loss was monitored on a 96-well fluorescent microplate reader (Molecular Devices). Data are from d 1, 35, and 77 of treatment and is expressed in mmol Trolox equivalents/L of plasma.

Plasma vitamin C analysis. Total plasma vitamin C in samples collected from d 1, 35 and 77 was determined using a modification of the dinitrophenylhydrazine (DNPH) assay (19). Plasma was combined with DNPH-thiourea-copper sulfate reagent at a ratio of 3:1, vortexed, and incubated at 37°C for 2 h. After chilling for 10 min, 1.5 mL cold 65% H_2SO_4 was added and tubes were incubated in the dark for 30 min at room temperature. Duplicate samples (270 μL) of the final reaction were transferred from each tube to duplicate wells of a 96-well flat-bottom plate. L-ascorbic acid was used to prepare a standard curve, and the absorbance was measured at 520 nm on the SPECTRAMax 340PC plate reader. Vitamin C values are expressed as $\mu\text{mol/L}$ of plasma.

Epstein-Barr virus antibody titers. The level of EBV antibody in the plasma, as an indicator of stress, was determined using an ELISA kit for EBV Viral Capsid Antigen p18 IgG antibodies (DiaSorin), according to the manufacturer's directions.

Carotenoid analysis. Plasma samples were analyzed by Craft Technologies for carotenoids by HPLC, as previously described (20). Serum concentrations of vitamin A (retinol and retinyl palmitate) and carotenoids (lutein, zeaxanthin, α -cryptoxanthin, β -cryptoxanthin, *trans* lycopene, *cis* lycopene, α -carotene, *trans* β -carotene, *cis* β -carotene) were measured using HPLC with absorbance detection. A small volume (150 μL) of serum/plasma was mixed with an equal volume of buffer, then mixed with ethanol containing the internal standard (tocol). The analytes were extracted from the aqueous phase into hexane. The combined hexane extracts were dried completely under vacuum, and the extract was redissolved in ethyl acetate and diluted in mobile phase. An aliquot was injected onto a C18 reversed phase column and eluted isocratically. The analytes all possessed an absorbance proportional to their concentration in solution; therefore these properties were used for quantitative analysis. The mode of detection was chosen to provide the highest sensitivity and selectivity. Carotenoids were measured by

TABLE 1 Demographics and characteristics of subjects by treatment group¹

	FVJC, n = 31	Placebo, n = 28	P-value ²
Age, y	25.3 ± 3.4	27.4 ± 8.1	0.186
BMI, kg/m ²	24.0 ± 3.9	23.4 ± 3.1	0.571
Years in law school	1.6 ± 0.8	1.8 ± 0.9	0.476
Blinding ³	51.6	39.3	0.493
Semester GPA (4.0 scale)	3.4 ± 0.34	3.4 ± 0.38	0.992
Subjects reporting illness, n	17	17	
Illnesses reported, n/77d	0.65 ± 0.60	0.79 ± 0.63	0.387
Number of symptoms	8.3 ± 6.1	14.9 ± 2.7	0.076

¹ Values are means ± SD.

² P-value from *t* test except where noted.

³ Percentage of subjects guessing correct per group assignment; significance determined by *z*-test.

absorbance at 450 nm; retinol and retinyl esters were measured by UV absorbance at their absorption maxima of 325 nm. Chromatograms were recorded using a computer data system. Analytes were quantified by external standard quantitation using neat standards to calculate response factors based on the peak area of the analyte. The quantities of analytes were corrected for recovery post-run based upon the internal standard. Limits of detection were 3–9 nmol/L for carotenoids and retinoids. CV were <5% for retinol and α -tocopherol, <7% for β -carotene, and <11% for minor carotenoids.

Statistical analysis. All statistics were performed using SigmaStat, version 3.11, Systat software. Demographics and population characteristics of the 2 groups were compared by *t*-test. Proportions in each group of those guessing the treatment to which they were assigned were statistically compared using the *z*-test. Regression analysis was by linear regression. Two-way repeated measures ANOVA (treatment × time) was used on most data unless otherwise stated. Post hoc analysis on results with equal variance was done using the Student-Newman-Keuls test. When equal variance failed, results were compared by the Mann-Whitney Rank Sum test. Values are means ± SEM, differences are deemed significant when $P \leq 0.05$. Trends are suggested when $P < 0.051$ –0.10.

Results

Study attrition consisted of 1 subject due to an unrelated illness and another due to withdrawal from law school, both of which occurred within the first study week. Compliance was 87.3% ± 10.9 pills consumed in the FVJC group and 90.2% ± 9.5 in the placebo group.

The 2 groups did not differ in age, body mass index, or grade-point average at the end of the semester (Table 1). Of the individuals taking the FVJC capsule, 51.6% guessed correctly as to which capsule they took, whereas 39.3% of the placebo group guessed correctly ($P = 0.493$, Table 1).

Seventeen individuals in the FVJC group and 17 in the placebo group reported 1 or 2 illnesses. No one reported having

3 or more illnesses. The number of self-reported illnesses between the 2 groups did not differ, but there was a trend ($P = 0.076$) for the FVJC group to have fewer total symptoms than the placebo group (Table 1). High variability in the placebo group (CV = 85.2%) may be responsible for the inability to detect a significant difference with this number of individuals.

The percentage of circulating $\gamma\delta$ -CD3⁺ T cells in the FVJC group increased 30% after 77 d, whereas treatment did not affect the percentage of $\alpha\beta$ -CD3⁺ T cells in the peripheral blood (Table 2). The percentage of $\gamma\delta$ -CD3⁺ T cells in the FVJC group after 77d of treatment was 30% greater than at baseline ($P = 0.091$) and 30% greater than in the placebo ($P = 0.049$).

The level of INF- γ produced by PMA-stimulated PBMC (after culture with IL-2) was significantly decreased in the FVJC group (1.49 ± 0.19 ng/L on d 1 vs. 0.47 ± 0.19 ng/L on d 77). INF- γ levels in the placebo group did not significantly differ between d 1 and d 77 (1.14 ± 0.20 ng/L on d 1 vs. 0.67 ± 0.2 ng/L on d 77). Statistically, there was a time effect within the FVJC group ($P = 0.002$) but no time effect in the placebo ($P = 0.114$). There were no differences between time and/or treatment in the levels of IL-4, TGF- β , or IL-6 produced by PMA-stimulated PBMC from FVJC consumers or the placebo (data not shown).

Deproteinized plasma ORAC values significantly increased in the FVJC group over time (Table 3). Plasma ORAC values also significantly increased in the placebo group by the end of d 77, but not to the extent observed in the FVJC group. Plasma ORAC and β -carotene values correlated ($P = 0.002$, $r = 0.23$) as did ORAC and lycopene ($P = 0.026$, $r = 0.03$) but vitamin C did not correlate with ORAC ($P = 0.142$, $r = 0.40$). At baseline, DNA damage in lymphocytes was significantly greater in the FVJC group than in the placebo group (Table 3). By d 35, DNA damage was reduced in the FVJC group to the same level as the placebo group, and was statistically lower than at baseline, with d 77 remaining the same as d 35. DNA damage in lymphocytes remained the same for all samples from the placebo group. Baseline plasma vitamin C concentrations did not differ in the 2 groups. Plasma vitamin C significantly increased in the FVJC group, but did not change in the placebo group over time (Table 3). Plasma EBV titers did not differ between groups or change over time (Table 3).

Plasma levels of β -carotene, lycopene, and lutein was significantly greater in the FVJC group on d 35 and 77 than in the placebo group (Table 4). The 2 groups did not differ in plasma concentrations of zeaxanthin, α -carotene, α -cryptoxanthin, or β -cryptoxanthin at any of the time points (data not shown).

Discussion

In this study, the FVJC group had a change from baseline in $\gamma\delta$ -T cells and antioxidant status. Of the individuals that experienced an illness during the study, those on the placebo tended to

TABLE 2 Percentage of 2 types of peripheral blood T-lymphocytes in subjects consuming FVJC capsules or placebo¹

Cell type	FVJC, n = 31		Placebo, n = 28		2-Way ANOVA P-value		
	d 1	d 77	d 1	d 77	Time	Treatment	Time × treatment
	%						
$\alpha\beta$ -T cells	55.8 ± 1.5	56.9 ± 1.5	54.6 ± 1.6	56.9 ± 1.6	0.278	0.697	0.717
$\gamma\delta$ -T cells	5.5 ^b ± 0.4	7.2 ^a ± 0.4	5.6 ^b ± 0.4	5.4 ^b ± 0.5	0.091	0.049	0.038

¹ Values are means ± SEM using the mean of duplicate samples for each subject. Means in a row with superscripts without a common letter differ, $P < 0.05$.

TABLE 3 Plasma antioxidant values, lymphocyte DNA damage, and EBV titers in subjects consuming FVJC capsules or placebo¹

Parameter	FVJC, n = 31			Placebo, n = 28			2-Way ANOVA P-value		
	d 1	d 35	d 77	d 1	d 35	d 77	Time	Treatment	Time × treatment
ORAC ²	1.55 ^c ± 0.08	2.06 ^b ± 0.08	2.33 ^a ± 0.08	1.51 ^c ± 0.08	1.65 ^c ± 0.08	1.91 ^b ± 0.08	<0.001	<0.001	0.033
DNA damage ³	2.61 ^a ± 0.19	1.65 ^b ± 0.20	1.62 ^b ± 0.20	1.86 ^b ± 0.21	1.61 ^b ± 0.20	1.48 ^b ± 0.19	<0.001	0.055	0.152
Vitamin C, $\mu\text{mol/L}$	54 ^b ± 3	79 ^a ± 3	69 ^a ± 3	62 ^b ± 3	65 ^b ± 3	54 ^b ± 3	<0.001	0.019	0.001
EBV titers	140.9 ± 11.9	tnp ⁴	141.8 ± 11.9	155.2 ± 12.4	tnp	146.2 ± 12.4	0.737	0.441	0.686

¹ Values are means ± SEM. Means in a row with superscripts without a common letter differ.

² mmol Trolox equivalents/L plasma.

³ Ratio of fluorescence with and without H₂O₂ added to the cells.

⁴ Test not performed at this time point.

experience more days of symptoms than subjects taking FVJC. CV in both groups were high for this measure, although higher in the placebo group.

The FVJC group had an increase in the number of circulating $\gamma\delta$ -T cells compared with their baseline or with the placebo group. The subtle influence of the FVJC on these cells is appropriate because large increases in this cell type would not be expected. The number of $\alpha\beta$ -T cells did not differ in either study group.

EBV antibody titers, shown by some to be a marker for stress (16,17), did not change in either group over time. The students told us they felt stressed; however, their stress levels did not affect antibody titers. An explanation may be that people respond differently to different types of stress, e.g., stress from physical activity vs. psychological stress (21,22); thus, the type of stress experienced by this group may not affect viral titers. Lower INF- γ is associated with psychological stress (23); however, if this is a reflection of stress, other cytokines would be expected to change also. IL-4 decreases with stress (24) whereas IL-6 increases with stress (25). Because cytokines did not change in our subjects throughout the study, we cannot document a change in subject stress during the investigation.

The significant reduction in INF- γ secretion in the PBMC from FVJC consumers suggests amelioration or reorganization of immunity to a noninflamed state. INF- γ is a pleiotropic cytokine that is crucial for the regulation of immune responses. Lower INF- γ could be a result of less illness in the treatment group or potentially a response to nutrients and antioxidants in the supplement. This would have to be confirmed in another study.

The concentrations of the plasma carotenoids lutein, lycopene, and β -carotene, were all significantly greater after the consumption of FVJC, whereas the levels of other carotenoids measured (zeaxanthin, cryptoxanthin, and α -carotene) remained unchanged. Changes in immunity were shown to be influenced by carotenoids (26,27) as well as other nutrients and phytochemicals. β -Carotene supplementation of elderly men was associated with enhanced natural killer (NK) cell cyto-

toxicity without altering the number of NK cells in the peripheral circulation, or secretion of INF- α , INF- γ , or IL-12 in cultured PBMC (28). NK cells are not the only ones having cytotoxicity activity, the $\gamma\delta$ -T cells also have this capacity. Perhaps β -carotene protects or supports $\gamma\delta$ -T cells rather than NK cells. This theory would require additional investigation.

By chance, randomization resulted in groups with different baseline lymphocyte DNA damage with the placebo group having significantly less than the FVJC group. The lower amount of DNA damage after consumption of FVJC was likely due to a variety of nutritive and nonnutritive phytochemicals, including the carotenoids (29,30) and other flavonoids and phenolic acids.

ORAC measures the quenching of the peroxyl radical-induced oxidation to measure classical chain breaking antioxidant activity (31). This suggests that the increased antioxidant capacity in FVJC consumers measured by ORAC is derived from the carotenoids, but many substances are likely to be involved in protecting against DNA strand breaks. It is not known why subjects on the placebo had slightly higher ORAC by the end of the study, however, all subjects knew they were on a dietary supplement study and possibly some individuals changed eating habits during the trial.

Plasma vitamin C concentrations in the FVJC group appeared to reach a steady state by d 35, as described in the review by Padyatty et al. (32), where vitamin C levels reach a plateau at doses of 200 mg/d and higher.

The objective of this study was to determine whether a commercially available product could potentially provide measurable health-related benefits in the study population. Most people consume only 3 servings of fruits and vegetables a day, whereas the latest guidelines are ≥ 5 servings of fruits and vegetables a day, depending on energy intake (33).

Although many phytochemicals express beneficial activities as single compounds in in vitro and animal studies, a variety of phytochemicals such as those found in plant foods, is suspected to be more effective in the prevention of disease (34,35). The formulation of the FVJC was effective in raising carotenoid levels, ascorbic acid levels, and plasma antioxidant capacity and

TABLE 4 Plasma carotenoid profiles in subjects consuming FVJC capsules or placebo¹

Carotenoid	FVJC, n = 31			Placebo, n = 28			2-way ANOVA P-value		
	d 1	d 35	d 77	d 1	d 35	d 77	Time	Treatment	Time × treatment
	$\mu\text{mol/L}$								
Total β -carotene	0.43 ^c ± 0.15	1.61 ^b ± 0.05	2.10 ^a ± 0.14	0.42 ^c ± 0.15	0.39 ^c ± 0.15	0.39 ^c ± 0.15	<0.001	<0.001	<0.001
Lutein	0.18 ^b ± 0.01	0.21 ^{ab} ± 0.01	0.24 ^a ± 0.01	0.18 ^b ± 0.01	0.18 ^b ± 0.01	0.18 ^b ± 0.01	0.285	0.042	0.422
Total lycopene	0.93 ^b ± 0.07	1.10 ^{ab} ± 0.06	1.22 ^a ± 0.07	0.95 ^b ± 0.07	0.89 ^b ± 0.07	0.98 ^b ± 0.07	0.053	0.019	0.130

¹ Values are means ± SEM. Means in a row with superscripts without a common letter differ.

was associated with a greater percentage of $\gamma\delta$ -T cells and the tendency for a reduced number of symptoms and duration of illness.

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