

Several Indicators of Oxidative Stress, Immunity, and Illness Improved in Trained Men Consuming an Encapsulated Juice Powder Concentrate for 28 Weeks^{1–3}

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Abstract

Phytonutrients from plant foods provide numerous antioxidants. We hypothesized that supplementation for 28 wk with a commercially available encapsulated juice powder concentrate (JPC) could influence indicators of oxidative stress, immunity, and illness. Trained men ($n = 41$; 34 ± 5 y; maximum oxygen uptake = 55 ± 7 mL·kg⁻¹·min⁻¹) from a homogenous police Special Forces unit were randomly assigned in a double blind manner to either JPC ($n = 21$) or placebo ($n = 20$). We used multiple 7-d food records to assess dietary intake and found inadequate mean daily fruit and vegetable consumption (3.2 ± 1.2 servings). The group physician documented all duty days lost due to illness. We collected plasma at baseline and study wk 4, 8, 16, and 28 for analysis of carbonyl groups on protein (CP) and TNF α . Over the 28-wk investigation, CP was lower in the JPC group, with both a treatment and a time \times treatment interaction ($P < 0.05$). Concentrations of both CP and TNF α at 16 and 28 wk were lower in the JPC than in the placebo group ($P < 0.001$). TNF α increased during the first 8 wk followed by a decrease in both groups for the following 20 wk ($P < 0.001$). Over the final 20 wk of the study, the placebo group tended to have more days of illness than the JPC group ($P = 0.068$). These data suggest beneficial JPC effects with regard to reduction of duty days lost due to illness and reduction of CP and TNF α concentrations in this group of trained men over 28 wk. *J. Nutr.* 137: 2737–2741, 2007.

Introduction

In Europe, the WHO suggests adults consume 400 g/d of fruit and nonstarchy vegetables (1) and Denmark recommends a minimum of 600 g or 6 servings per day (2). In North America, the United States recommends 5–13 servings of fruits and vegetables each day, in proportion to total energy intake (3), and Canada recommends 8–10 servings for adult males under age 50 y (4). These variable recommendations show a minimum daily consumption of 5 or more servings of fruits and vegetables is common public health advice. Dietary produce provide an important source of phytochemicals and antioxidant nutrients, along with water, fiber, and carbohydrates. Epidemiology observations have suggested that increased consumption of fruits and vegetables is associated with a decreased risk of chronic

degenerative diseases, perhaps related to the increased intake of antioxidants (5). Oxidative stress-related exercise studies report supplementation with antioxidants can influence markers of oxidative stress such as carbonyl groups on protein (CP)⁹ (6). Antioxidant supplementation has also been reported to influence immune markers such as TNF α and IL-6 in trained and untrained subjects (7).

Some benefits of increased produce consumption may be obtained by adding a juice powder concentrate (JPC) to the daily diet. For example, a pilot study found that JPC reduced plasma CP and malondialdehyde concentrations in trained male cyclists (8) and others have reported reduced CP in aerobically trained men after controlled exercise periods (9). A study in untrained subjects found 11 wk of JPC intake positively influenced several markers of immune function and oxidative stress, specifically increasing $\gamma\delta$ -T cells and plasma oxygen radical absorbance capacity while decreasing the plasma cytokine, IFN- γ (10).

Stress likely has detrimental effects on immunity and may increase susceptibility to infectious agents (11). Regular exercise

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³ Supplemental Tables 1–3 and Supplemental Figure 1 are available with the online posting of this paper at *jn.nutrition.org*.

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training can adapt the endogenous antioxidant enzyme systems [e.g. increasing the activity of superoxide dismutase (SOD) and glutathione peroxidase (GPx)]. This could result in decreased oxidative damage and increased resistance to oxidative stress (12–14). The influence of antioxidant supplementation and exercise training on these enzyme activities is unclear (15).

The objectives of this study were to evaluate JPC effects, compared with placebo, on blood concentrations of oxidative stress and immunity biomarkers, in addition to the functional effects (monitored as duty days lost due to illness), in a homogeneous cohort of trained men over an extended period of time (28 wk). These subjects were a trained and disciplined cohort living in a group environment following a standardized diet and training regimen.

Materials and Methods

Study design. This was a randomized, double blind, placebo-controlled study. The study term was a 28-wk experimental period from November 2005 to June 2006 (Supplemental Table 1). We conducted anthropometric measurements and physical activity and dietary questionnaires, and determined maximum oxygen uptake ($\text{VO}_{2\text{max}}$) 4–6 wk prior to the start of this investigation, followed by a minimum 4-wk washout period from all dietary supplements. Dietary supplement use was prohibited during the study period. On study d 1, subjects were randomly assigned to receive either JPC or placebo capsules. Those randomized to JPC ($n = 21$) received capsules containing primarily blended fruit, vegetable, and berry JPC derived from: apple, beet, bilberry, blackberry, black currant, blueberry, broccoli, cabbage, carrot, cherry (acerola), Concord grape, cranberry, elderberry, kale, orange, peach, papaya, parsley, pineapple, raspberry, red currant, spinach, and tomato (Juice Plus+, NSA). Six JPC capsules provided ~7.5 mg β -carotene, 200 mg vitamin C, 60 mg vitamin E in the form of RRR- α -tocopherol, 600 μg folate, and ~63 kJ/d. The study subjects randomized to placebo ($n = 20$) received capsules identical in appearance containing microcrystalline cellulose. All subjects took 6 capsules daily with meals for 28 wk (3 capsules in the morning and evening, except on training days: 3 capsules with the last meal before training and 3 capsules with the first meal after training). Capsule compliance was >85% in both groups, estimated from returned capsule counts and questionnaires.

Blood was collected 3 h after a standardized breakfast (providing ~4222 kJ: 32–34 g protein, 144–150 g carbohydrate, and 28–30 g fat) for biochemical analysis. Duty and training obligations did not allow for a meal after the laboratory visits for blood collection. The standardized meal limited nutrient variation due to self-selection on the mornings scheduled for blood draws. Prospective subjects were healthy, nonsmoking men in the Graz region of the Austrian Special Forces Cobra unit ($n = 46$). All trained aerobically at least 3 d/wk for a minimum of 1 y prior to study participation and had a minimum level of aerobic fitness as assessed with maximal testing ($\text{VO}_{2\text{max}} > 45 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) 4–6 wk prior to baseline. Each volunteer provided written informed consent prior to participating in this investigation. This study was conducted in accordance with the guidelines of the Declaration of Helsinki for Research on Human Subjects and was approved by the Human Ethics Committee at the Medical University of Graz, Austria. Exclusion criteria included use of tobacco products, chronic or excessive alcohol consumption, recent surgery or severe injury or illness, and use of prescription or over-the-counter medications. The Cobra unit physician documented and evaluated any new medications or injuries occurring during the study. One potential participant was excluded prior to randomization due to an exceptionally high $\text{VO}_{2\text{max}} > 65 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ at the initial evaluation. Four randomized participants did not complete the investigation (3 were transferred to the Vienna area Cobra unit and 1 required hospitalization unrelated to the investigation). Therefore, 41 subjects were included in the data analysis.

Dietary records. Dietary data were collected according to guidelines of the Austrian Nutrition Society. The same researcher instructed all

subjects regarding portion sizes and proper recording of foods and beverages consumed. Food records for 7 d prior to each blood collection allowed for nutrient intake assessment and diet duplication. Subjects received copies of their previous 7-d food record and were instructed to replicate that diet prior to their next blood collection and to otherwise maintain their habitual diet throughout the entire study period. All participants had lunch at the Cobra group house and often breakfast and dinner, depending on individual duty assignments. The 7-d food records were analyzed twice (at baseline and after 28 wk) for average number of servings by food group (Supplemental Table 2) and for energy and nutrient content using Opti Diet software (version 3.1.2, GOEmbh), then compared with the recommended dietary allowance established by the German and Austrian Nutrition Societies (16) (Supplemental Table 3).

Body fat determination. Baseline body fat content and distribution were estimated using a computerized optical device (Lipometer, Moeller Messtechnik), which allows rapid, safe, and noninvasive determination of subcutaneous adipose tissue thickness (17).

Screening for $\text{VO}_{2\text{max}}$. Step test $\text{VO}_{2\text{max}}$ and respiratory gas determination was conducted using a bicycle ergometer (Schiller ERG 900S Ergometer). To qualify, 46 potential study participants performed $\text{VO}_{2\text{max}}$ tests with additional blood lactate analysis to determine exercise performance. Respiratory variables were measured using a portable open-air spirometry system (MetaMax I, Cortex).

Medical monitoring. The Cobra unit physician documented all duty days lost due to illness, injury, mission assignments, and other stressors such as circadian imbalance (jet lag). Detailed symptom information was documented for all illnesses.

Physical activity records. The training program, including intensity, duration, extent, and frequency, for each subject was recorded and summarized weekly. This program included both endurance and weight training 2–3 times per week along with additional special training for specific missions. Each subject was instructed to not perform weight training or high intensity/long duration endurance training 4 d prior to blood collections. They were allowed to perform daily cardiovascular exercise at a maximum heart rate of 130 beats/min for up to 30 min during the 4 d prior to blood draw.

Blood collection and sample preparation. Collection of capillary blood (600 μL) used EDTA-coated vials (Sarstedt). After centrifugation at $3000 \times g$ for 10 min plasma was removed and samples were frozen at -70°C for analysis of CP, TNF α , and IL-6. Erythrocytes were washed 3 times with isotonic solution and then lysed with Millipore H_2O . After centrifugation at $3000 \times g$, the supernatant was frozen at -70°C for analysis of hemoglobin (Hb), GPx, and SOD.

Blood chemistry panel. Baseline and final standard blood chemistry used EDTA plasma from peripheral venous blood. Analysis used routine methods and the clinical chemistry analyzer "Eurolyser" (Dia Team, Diagnostica und Arzneimittel Großhandel). Assessment of Hb and iron concentrations used the Advia clinical analyzer (Fa. Bayer).

Analysis of CP, IL-6, and TNF α . Determination of CP required preparation of oxidized bovine serum albumin for standardization, as described elsewhere (18). Measurement of CP used chemiluminescent detection (Lumistar, BMG) after derivatization with dinitrophenylhydrazine. Assessment of plasma protein concentration used the bicinchoninic assay (Pierce). Quantitation of IL-6 and TNF α used an ELISA from Biosource with a spectrophotometric reader (Power Wave, Bio-TEK Instruments). The analytical inter-assay CV were $\leq 3.6\%$ for CP, $\leq 9.6\%$ for IL-6, and $\leq 8.2\%$ for TNF α .

Analysis of SOD and GPx activity. Determination of GPx activity from erythrocyte lysate was performed indirectly by a coupled reaction with glutathione reductase utilizing the ZeptoMetrix assay kit adapted to 96-well plates, with results expressed in units/g Hb. Assessment of SOD activity used erythrocyte lysate with xanthine oxidase in the start

reagent, as previously described (19), expressed in units/mg Hb. Analytical inter-assay CV were $\leq 5.6\%$ for SOD and $\leq 9.2\%$ for GPx.

Statistical analysis. We used SPSS version 12.0 for statistical analysis. Data are presented as means \pm SD. Significance was set at $P < 0.05$. Power analysis and sample size calculation based on previous work with CP (8) indicated 20 subjects per group were adequate for statistical power of $\sim 85\%$ with $\alpha = 0.05$. Comparison of group baseline characteristics and food intake data used a *t* test. Group comparison of nutrient analysis data used 1-way ANOVA and duty days lost due to illness used the binomial test. We analyzed data obtained for CP, TNF α , IL-6, SOD, and GPx using a 5 (time) \times 2 (treatment) repeated measures ANOVA with grouping factors to show group differences over time. When equal variance failed, a comparison was made by the Mann-Whitney rank sum test. To evaluate differences between treatments at specific time points, additional analysis used *t* tests. We used Pearson correlation coefficient and regression analysis to evaluate bivariate relationships.

Results

At baseline, the 2 randomized groups did not differ in age, height, weight, total body fat, lean body mass, VO_{2max}, maximum workload, or clinical chemistry variables (Table 1). Baseline clinical blood chemistry parameters, including glucose, albumin, cholesterol, triglycerides, C-reactive protein, Hb, and iron concentrations and liver enzyme activity, were within normal limits for this population and did not change significantly in any subject throughout the study period (data not shown). The groups had equivalent dietary intake by both food group consumption pattern (Supplemental Table 2) and diet nutrient analysis (Supplemental Table 3). The entire cohort consumed fewer than 4 servings daily of fruit and nonstarchy vegetables.

Assigned duty hours did not differ between the groups at any time point of the study. From baseline to wk 8, both groups' duty hours were ~ 1140 h, increasing to 1480 h from wk 8 to 16 and 1620 h from wk 16 to 28 (data not shown). This increase in duty hours after 8 wk was due to the Austrian chairmanship of the European Union in 2006.

TABLE 1 Baseline characteristics and clinical chemistry data for 41 physically active men¹

Variables	Reference range ²	JPC	Placebo
<i>n</i>		21	20
Age, y		34.3 \pm 5.1	33.8 \pm 5.7
Height, cm		183.2 \pm 11.4	180.8 \pm 8.6
Weight, kg		83.6 \pm 8.1	79.8 \pm 5.4
Total body fat, %		12.9 \pm 3.8	12.4 \pm 2.2
Lean body mass, kg		72.7 \pm 6.6	69.9 \pm 4.7
VO _{2max} , mL·kg ⁻¹ ·min ⁻¹		58.4 \pm 12.5	54.8 \pm 9.3
Maximum workload, W		345 \pm 35	335 \pm 25
Glucose, mmol/L	(3.9–6.1)	4.8 \pm 1.1	4.6 \pm 1.2
Hb, g/L	(136–172)	156 \pm 28	158 \pm 31
Iron, μ mol/L	(14–32)	19 \pm 6	18 \pm 7
Cholesterol, mmol/L	(<5.85)	4.45 \pm 1.15	4.70 \pm 1.00
Triglycerides, mmol/L	(<1.80)	0.86 \pm 0.48	0.94 \pm 0.62
Albumin, g/L	(40–60)	43 \pm 15	44 \pm 19
C-reactive protein, mg/L	(0.5–30)	8 \pm 19	9 \pm 15
Uric acid, μ mol/L	(120–420)	210 \pm 100	290 \pm 80

¹ Values are means \pm SD and did not differ between the groups, $P > 0.05$ (*t* test).

² Reference range for clinical chemistry variables (20).

The group physician documented duty days lost to illnesses (cold/flu symptoms) over the 28-wk observation period, with 134 d lost in the placebo group and 108 d lost in the JPC group ($P = 0.089$). Duty days lost due to illness did not differ between the groups ($P = 0.713$) from baseline to wk 8 (placebo, 73 d; JPC, 67 d). Duty days lost to illness tended to be fewer ($P = 0.068$) in the JPC group (41 d) than in the placebo group (61 d) over the remaining 20 wk (Table 2).

CP concentrations were affected by the time-by-treatment interaction ($P = 0.004$) and by treatment ($P = 0.001$) during the entire study period with a decrease in the JPC group and an increase in the placebo group. At wk 16 ($P = 0.009$) and wk 28 ($P < 0.001$), CP concentration was lower in the JPC group than in the placebo group (Table 3; Supplemental Fig. 1A).

The plasma TNF α concentration changed over time ($P = 0.008$); it increased in both groups from baseline to wk 8, decreased from wk 8 to 16, and was followed by a small increase from wk 16 to wk 28. There tended to be a time \times treatment interaction ($P = 0.092$) during the 28-wk study. The plasma TNF α concentration at wk 16 ($P < 0.001$) and wk 28 ($P = 0.002$) was lower in the JPC group than in the placebo group (Table 3; Supplemental Fig. 1B). Plasma IL-6 concentrations were highly variable and did not change in either group (data not shown).

During the 28-wk study period, there was a positive correlation between SOD and GPx enzyme activity ($P = 0.01$; $r^2 = 0.982$). SOD activity changed over time ($P < 0.001$); it decreased from baseline to wk 8, increased from wk 8 to wk 28, and then returned to baseline values (Table 3). GPx activity, similar to SOD, also showed a time effect ($P = 0.011$), decreasing from baseline to wk 8, then increasing from wk 8 to wk 28, returning to baseline values (Table 3).

Discussion

This was an investigation of a homogenous population of fit men who shared many group meals and often slept in the Cobra group house. The results of this study indicate daily supplementation with JPC for 28 wk protected plasma proteins against free radical-induced oxidative stress compared with a placebo, as demonstrated by decreased CP concentrations. The immune and inflammatory marker, TNF α , was also reduced in the JPC group. The difference was most pronounced from wk 8 to wk 28 (January to June) in conjunction with the additional stress of increased duty hours. The stable activities of antioxidant enzymes GPx and SOD across the study term, combined with reduced concentrations of CP, suggest enhanced antioxidant status in the JPC group as previously reported by others (10,21).

The combination of fruit and nonstarchy vegetable servings averaged 3.2/d in both groups (Supplemental Table 2), below the lowest public health recommendation (1). Dietary intake analysis found folate was low but equivalent in the groups at

TABLE 2 Comparison of illness data in physically active men consuming JPC or placebo for 28 wk

	JPC	Placebo	P-value
<i>n</i>	21	20	
Overall episodes of illness, <i>n</i> /28 wk	17	22	>0.1
Overall duty days lost due to illness, <i>d</i> /28 wk	108	134	0.089
Days lost due to illness baseline–wk 8, <i>d</i> /0–8wk	67	73	>0.1
Days lost due to illness wk 8–wk 28, <i>d</i> /8–28wk	41	61	0.068

TABLE 3 Comparison of plasma CP, TNF α , and RBC SOD and GPx in physically active men consuming JPC or placebo for 28 wk¹

Variable	Group	Wk 0	Wk 4	Wk 8	Wk 16	Wk 28	2-way ANOVA P-values		
							Time	Treatment	Time × treatment
Plasma CP, nmol/mg protein	JPC	0.39 ± 0.05	0.33 ± 0.06	0.32 ± 0.05	0.27* ± 0.03	0.29* ± 0.04	0.112	0.001	0.004
	Placebo	0.38 ± 0.05	0.35 ± 0.04	0.35 ± 0.03	0.38 ± 0.06	0.49 ± 0.05			
Plasma TNF α , ng/L	JPC	1.87 ± 0.64	2.26 ± 0.33	3.50 ± 0.39	0.63* ± 0.53	1.24* ± 0.46	0.008	0.256	0.092
	Placebo	1.29 ± 0.35	2.01 ± 0.36	3.85 ± 0.44	2.01 ± 0.48	2.51 ± 0.72			
RBC SOD, U/mg Hb	JPC	17.7 ± 6.3	16.3 ± 6.2	10.4 ± 5.8	18.0 ± 5.4	17.9 ± 6.3	<0.0001	0.932	0.869
	Placebo	16.2 ± 5.8	14.8 ± 5.8	11.1 ± 6.5	18.0 ± 5.9	16.8 ± 5.5			
RBC GPx, U/g Hb	JPC	212 ± 39	201 ± 26	168 ± 41	202 ± 37	214 ± 32	0.011	0.609	0.919
	Placebo	195 ± 48	191 ± 58	177 ± 29	186 ± 33	205 ± 26			

¹ Values are means ± SD, n = 21 (JPC) or 20 (placebo). *Different from placebo at that time, P < 0.05 (t-test).

60–61% of reference intake amount, whereas dietary intake of antioxidant vitamins C and E was adequate and equivalent in both groups. Intake of β-carotene was also equivalent in both groups, between 80 and 88% of the reference intake amount (Supplemental Table 3).

Peters et al. (22) demonstrated that antioxidant supplementation could attenuate upper-respiratory tract infections in runners. Because running was included in the cohorts' training program twice weekly, we documented illnesses with cold and flu symptoms, such as sore throat and fever. We found a trend for fewer duty days lost due to illness in the JPC group across the entire 28 wk ($P = 0.089$), which was more pronounced during the final 20 wk of the study term ($P = 0.068$), in agreement with previous findings (10). The mean number of duty hours increased from ~1140 to ~1620 in both groups from the baseline to wk 28, representing additional stress for the subjects. It is possible that the additional nutrients provided in the JPC group contributed to modified immune function, resulting in fewer illness days and reduction of circulating cytokines in blood (22).

Leucocytes such as monocytes, macrophages, neutrophils, and natural killer cells produce TNF α in addition to endothelium and adipocytes. TNF α is a central mediator of systemic inflammatory and immune response. There is little influence of exercise alone on this parameter (23,24). The significant increase observed at wk 8 in both groups might be due to the illnesses in the entire cohort before the wk 8 (January) follow-up visit (a total of 140 d; Table 2). After the initial increase of TNF α in both groups, the JPC group had significantly lower concentrations toward the end of the study than the placebo group (Table 3; Supplemental Fig. 1B). As previously mentioned, there were also fewer documented days of illness during this time (wk 8–wk 28) in the JPC group (Table 2).

IL-6 is a cytokine primarily produced by fibroblasts, endothelium cells, and leucocytes like monocytes, macrophages, lymphocytes, and granulocytes. Like TNF α , IL-6 is a mediator of systemic inflammatory and immune reactions, but as opposed to TNF α , IL-6 is released in higher concentrations after exercise (24). IL-6 mediates many aspects of the exercise-induced acute phase response, including upregulation of antioxidant defenses. Synthesis is regulated in part by oxidative stress (25). Sacheck et al. (25) reported no changes from baseline IL-6 after a 12-wk period of supplementation with vitamin E (1000 IU/d) in healthy, trained young and old men. Childs et al. (26) found no significant effect of vitamin C and N-acetyl-cysteine supplementation on IL-6 concentrations. Nantz et al. (10) also did not observe any effect on IL-6 after 11 wk of JPC supplementation in an untrained population. These studies illustrate supplemental antioxidants may not influence IL-6 concentrations.

Protein oxidation can result in loss of enzyme and protein structural functions (27). Protein attack by free radicals, such as reactive oxygen and nitrogen species, free metal ions, and lipid peroxidation end products (including hydroxynonenal or malondialdehyde) can generate CP (28). We observed a significant decrease in CP in the JPC group, whereas the concentrations in the placebo group were constant over the first 16 wk (Table 3; Supplemental Fig. 1A). This reduction of plasma CP concentrations in response to JPC treatment is in agreement with previous results (6,9). The significant differences in CP between the study groups after 16 and 28 wk indicate a different profile of oxidative stress in these groups. Whereas the concentrations in the JPC group decreased further for the remaining study term, the placebo group increased in CP from wk 16 to 28. The etiology of this increased CP in the placebo group is unknown, although the stress profile due to the increased amount of duty hours (in both groups) may have contributed to this observation.

SOD are a family of mineral-containing antioxidant enzymes that scavenge superoxide radicals and are regarded as important enzymes in the antioxidant defense system (29,30). GPx are a family of selenium-containing antioxidant enzymes catalyzing the reduction of reactive oxygen and nitrogen species, such as hydrogen peroxide or lipid peroxides, in the presence of reduced glutathione (31,32). As demonstrated previously (33), GPx activities in erythrocytes decreased when large amounts of β-carotene, vitamin E, vitamin C, and selenium were taken for 15 d. In comparison, the JPC used in this longer study provided modest amounts of these nutrients and the findings did not indicate any effect on SOD or GPx activity. It has been postulated in a review and several studies (30,34–37) that physically active people have adapted antioxidant enzyme systems. Therefore, the stable enzyme activities observed here might be the result of adaptation to chronic aerobic training. However, a time effect occurred across the study term, with significant decreases of both enzyme activities from baseline to wk 8 followed by significant increases in both groups over the remaining time. Both SOD and GPx were correlated over time, suggesting a higher level of oxidative stress over the winter months, with no difference between the study groups.

In conclusion, the groups did not differ in relation to IL-6, SOD, and GPx. However, the JPC group showed positive changes in CP and TNF α concentrations and a trend toward fewer duty days lost due to illness compared with the placebo group. We can postulate that intervention may have been helpful in maintaining resistance against illness in this specific cohort of chronically stressed and exercising men. Applicability of these findings to other populations will require additional study in a larger, more diverse group.

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