

RESEARCH ARTICLE

Systemic inflammatory load in humans is suppressed by consumption of two formulations of dried, encapsulated juice concentrate

Yu Jin¹, Xiangli Cui¹, Udai P. Singh², Alexander A. Chumanevich¹, Brook Harmon³, Philip Cavicchia³, Anne B. Hofseth¹, Venkata Kotakadi¹, Brandy Stroud¹, Suresh R. Volate¹, Thomas G. Hurley³, James R. Hebert³ and Lorne J. Hofseth¹

¹ Department of Pharmaceutical and Biomedical Sciences, South Carolina College of Pharmacy, University of South Carolina, Columbia, SC, USA

² Department of Pathology and Microbiology, School of Medicine, University of South Carolina, Columbia, SC, USA

³ Cancer Prevention and Control Program, University of South Carolina, Columbia, SC, USA

Chronic inflammation contributes to an increased risk for developing chronic conditions such as cardiovascular disease, diabetes, and cancer. A high “inflammatory load” is defined as elevated inflammation markers in blood or other tissues. We evaluated several markers of systemic inflammation from healthy adults and tested the hypothesis that two formulations of encapsulated fruit and vegetable juice powder concentrate with added berry powders (FVB) or without (FV) could impact markers of inflammatory load. Using a double-blind, placebo-controlled approach, 117 subjects were randomly assigned to receive placebo, FV, or FVB capsules. Blood was drawn at baseline and after 60 d of capsule consumption. We measured inflammatory markers (high sensitivity C-Reactive Protein, Monocyte Chemotactic Protein-1, Macrophage Inflammatory Protein 1- β , and Regulated upon Activation, Normal T cell Expressed and Secreted), superoxide dismutase, and micronutrients (β -carotene, vitamin C, and vitamin E). Results showed Monocyte Chemotactic Protein-1, Macrophage Inflammatory Protein 1- β , and RANTES levels were significantly reduced and superoxide dismutase and micronutrient levels were significantly increased in subjects consuming both FV and FVB, relative to placebo. Data suggest a potential health benefit by consuming either formulation of the encapsulated juice concentrates through their anti-inflammatory properties.

Received: November 30, 2009

Revised: February 2, 2010

Accepted: February 7, 2010



Keywords:

Fruit / Human / Inflammation / Juice / Juice Plus

1 Introduction

Chronic systemic inflammation may contribute to reduced human life span [1]. Although there is limited information

on markers of systemic inflammation in healthy adults, there is an association between chronic inflammation and many of the prevalent diseases found in the developed world, including obesity [2], diabetes [3, 4], and cancer [5]. The ability of fruits and vegetables to protect against diseases prevalent in the developed world is well documented [6]. Although the mechanisms of protection are not fully understood, and there are hundreds (if not thousands) of potentially beneficial ingredients in plant foods, it is becoming increasingly clear that their antioxidant and anti-inflammatory properties play a role in this protection [6]. Only a small proportion of US adults meet levels of fruit and vegetable consumption consistent with dietary recommendations [7]. Some of the benefits of increased intake of produce may be achieved by including supplemental juice powder concentrates in the habitual diet. Two formulations have previously shown benefits when compared to placebo

Correspondence: Dr. Lorne J. Hofseth, Department of Pharmaceutical and Biomedical Sciences, South Carolina College of Pharmacy, University of South Carolina, 715 Sumter Street, Room 513D, Columbia, SC 29205, USA

E-mail: hofseth@sccp.sc.edu

Fax: +1-803-777-8356

Abbreviations: **FV**, fruit and vegetable juice powder concentrate; **FVB**, fruit and vegetable juice powder concentrate with added berry powders; **hsCRP**, high sensitivity C-Reactive Protein; **MCP-1**, Monocyte Chemotactic Protein-1; **MIP-1 β** , Macrophage Inflammatory Protein 1- β ; **SOD**, superoxide dismutase; **TNF- α** , tumor necrosis factor- α .

in healthy adults. Specifically, a commercially available encapsulated fruit and vegetable juice powder concentrate (FV) has been shown to improve markers of immune function [8] and a similar fruit and vegetable juice powder concentrate with added berry powder (FVB) was shown to attenuate the formation of carbonyl groups on protein [9]. The objective of this study was to evaluate the impact of these two formulations on several markers of chronic systemic inflammation, when compared to placebo, in healthy adult volunteers. We also examined the ability of these juice concentrate formulations to influence antioxidant function by measuring the concentration of erythrocyte superoxide dismutase (SOD).

The inflammatory markers monitored in this investigation were high sensitivity C-Reactive Protein (hsCRP), Monocyte Chemoattractant Protein-1 (MCP-1), Macrophage Inflammatory Protein 1- β (MIP-1 β), and Regulated upon Activation, Normal T cell Expressed and Secreted (RANTES). The hepatocyte-derived acute-phase reactant hsCRP has been identified clinically as a potential risk marker of primary and secondary adverse cardiovascular events [2, 10]. The pro-inflammatory chemokine-MCP-1 is a member of the cysteine–cysteine subgroup, which is chemotactic for monocytes and T-lymphocytes, and is believed to play a role in the pathogenesis of obesity and diabetes [11]. Additionally, MCP-1 levels are elevated in the epithelium and sub-epithelial tissues of bronchial biopsies from asthmatic subjects [12], and serum MCP-1 levels correlate with the degree of inflammation in patients with hepatitis [13] and with cancer stage [14]. Another cysteine–cysteine member of the chemokine subfamily, MIP-1 β is produced by many white blood cell types, including monocytes, macrophages, and lymphocytes [15]; and is elevated in patients with osteoarthritis [16] and rheumatoid arthritis [17]. Leukocyte migration is induced by RANTES by binding to specific receptors in the seven trans-membrane G-protein-coupled receptor family. It mediates the trafficking of lymphoid cells such as T cells and monocytes, as well as basophils, eosinophils, natural killer cells, dendritic cells, and mast cells. It is produced by CD8+T cells, epithelial cells, fibroblasts and platelets, and is elevated in a wide range of chronic inflammatory conditions, including atherosclerosis, arthritis, atopic dermatitis, asthma, glomerulonephritis, and endometriosis [18] where RANTES is thought to act by promoting leukocyte infiltration to sites of inflammation [19].

2 Materials and methods

2.1 Subjects

A total of 323 subjects between the ages of 22 and 55 years were recruited from the metropolitan Columbia, South Carolina area through flyers and advertisements. All study participants provided written informed consent. Subjects

were ineligible if they were insulin-dependent diabetic patients, pregnant, post-menopausal, not willing to stop taking nutritional supplements, allergic to any of the ingredients in the study capsules, had a diagnosed medical condition, currently taking anti-inflammatory medications, or unable to attend two clinic appointments. A total of 117 eligible subjects were randomized to one of three groups (Fig. 1). Randomization was blocked by gender (male/female), race (white/black/other), and BMI (BMI = weight (kg)/height (m)²; and classified as underweight (< 18.5 kg/m²)/normal weight (≥ 18.5 kg/m² and < 25.0 kg/m²), overweight (≥ 25.0 kg/m² and < 30.0 kg/m²) and obese (≥ 30.0 kg/m²). A total of 117 subjects, 29 males and 88 females, had evaluable data that were used for the definitive statistical analyses. All recruitment, enrollment, and data collection procedures were approved by the University of South Carolina Institutional Review Board (Assurance number: FWA 00000404/IRB Registration number: 00000240).

2.2 Nutritional intervention

This was a double-blind, randomized, placebo-controlled trial. The study required subjects to consume one of two juice concentrate supplements or placebo for a 60-day period. The two treatment groups included an FV blend and an FVB blend of juice powder concentrate (Juice Plus⁺[®], NSA, LLC, Collierville, TN). The placebo capsules were physically identical to the treatment pills. Subjects were assessed at baseline and again after the 60-day intervention

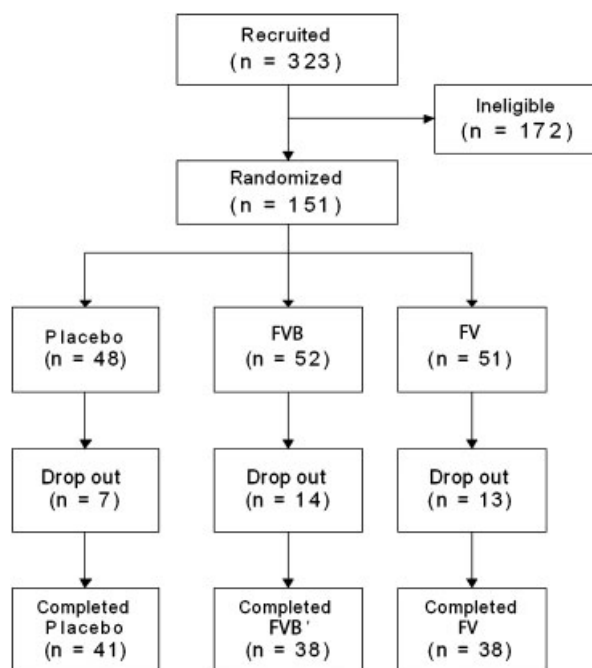


Figure 1. Flow chart of study design.

period. Prior to each clinic appointment subjects were mailed questionnaires to complete and bring with them. They were asked to refrain from strenuous physical activity and excessive alcohol consumption within 72 h prior to each appointment; and asked to refrain from eating or drinking on morning of their appointment.

The questionnaires included a demographics questionnaire (collected at baseline), and diet and physical activity questionnaires (collected at baseline and at 60 d). The demographics questionnaire collected information on age, race, education, employment, and marital status. To assess diet, the National Cancer Institute's Percent Energy from Fat Screener, and Fruit and Vegetables Screeners were used [20]. Physical activity was assessed using the Community Health Activities Model Program for Seniors questionnaire [21]. At each clinic appointment, a fasting blood sample was taken, and information on gender, menstrual status, height, weight, and body composition was collected. Bioelectrical impedance was used to determine percent body fat [22].

At baseline, subjects were provided enough capsules for 60 d. They were instructed to follow their habitual diet and to take their assigned three capsules twice daily with meals (total of six capsules daily for 60 consecutive days). The placebo capsule contents consisted primarily of microcrystalline cellulose, with an equivalent appearance to the active capsules (FV and FVB capsules). The FV capsule contents consisted primarily of a blended 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+® Orchard and Garden Blends, NSA, LLC, Collierville, TN), provided approximately 7.5 mg β -carotene, 234 mg vitamin C, 30 mg vitamin E in the form of RRR- α -tocopherol, 420 μ g folate, 60 mg calcium, and about 42 kJ, as described previously [8]. The FVB capsules consisted of an identical powder concentrate blend of the composition described above with the addition of berry juice powder from: bilberry, blackberry, black currant, blueberry, cranberry, Concord grape, elderberry, raspberry and red currant (Juice Plus+® Orchard, Garden and Vineyard Blends, NSA, LLC, Collierville, TN), which in total provided approximately 7.5 mg β -carotene, 276 mg vitamin C, 72 mg vitamin E in the form of RRR- α -tocopherol, 780 μ g folate, 80 mg calcium, and about 63 kJ, as described previously [9].

2.3 Blood processing

Approximately 30 mL of fasting blood was drawn from each eligible subject on each visit, with 10 mL collected into each of three Vacutainer® blood collection tubes (BD, Franklin Lakes, NJ). A total of 10 mL was processed for vitamin C (ascorbic acid) determination. A total of 10 mL was processed for examination of β -carotene, α -tocopherol, and examination of the inflammatory markers, hsCRP, MCP-1,

MIP-1 β , and RANTES. Briefly, samples were kept vertical in the dark for 45 min at room temperature, and then centrifuged at $1500 \times g$ for 15 min at 4°C for vitamin C determination, serum was collected and added at equal volume to 10% metaphosphoric acid (Sigma, St. Louis, MO) in an amber light-protected, trace-metal-free plastic tube and immediately frozen at -80°C until analysis. Samples were analyzed by an independent commercial diagnostic laboratory (Kronos Science Laboratories, Phoenix, AZ). For β -carotene, α -tocopherol, and hsCRP, serum was collected, and aliquoted into a light-protected amber tube, and shipped frozen to Kronos Science Laboratories for analysis. Briefly, for carotenoids and tocopherols, a reversed-phase HPLC method coupled with multi-wavelength and fluorescence detection was utilized for simultaneous determination in human serum. The method was correlated with NIST Standard Reference Material® 968c, and monitored with NIST Round Robin Proficiency studies twice a year. Total ascorbic acid in serum was measured using HPLC methods coupled with multi-wavelength detection (243 nm). The method was correlated with NIST Standard Reference Material® 970, and monitored with NIST Vitamin C Round Robin Proficiency studies twice a year. The hsCRP was determined using a chemiluminescent immunometric assay performed on the Siemens Immulite 2000 Analyzer (Chicago, IL). Serum for MCP-1, MIP-1 β , and RANTES was added to sterilize Eppendorf tubes, and analyzed using the BioPlex apparatus (BioRad, Hercules, CA). The BioPlex suspension array system uses multiplex suspension array technology for simultaneous quantitative analysis of multiple proteins and peptides from a single sample in a microplate well. We should note here that we also attempted to measure other common markers of inflammation, including of tumor necrosis factor- α (TNF- α), IL-1, and IL-6. However, the assay sensitivity for our particular samples was too low, in that these endpoints were undetectable, or non-reproducible for >70% of the samples.

A 10 mL (Green top; containing clotting inhibitor sodium heparin) tube was processed for examination of whole blood (complete blood count), SOD, and separation of lymphocytes. Briefly, following blood draw, the tubes were kept on an orbital shaker to provide additional assurance against clotting. Sample aliquots were utilized as follows: 1 mL of this blood was separated for complete blood count; and, 4 mL of blood was added to 4 mL of $1 \times$ HBSS, mixed, and layered onto Lymphocyte Separation Medium (MediaTech). Samples were centrifuged at $400 \times g$ for 30 min at room temperature. The lymphocyte layer and 1/2 of the Lymphocyte Separation Media was added to an equal volume of $1 \times$ HBSS. The erythrocyte pellet was collected, and 4 mL of ice-cold ultra-purified water was added, and mixed thoroughly. After 5 min, the aliquot was stored at -80°C for analysis of erythrocyte SOD activity. The erythrocyte SOD activity was measured by the inhibition of the rate of formation of substrate nitroblue tetrazolium, compared to a standard curve. SOD was standardized to Hb

concentration according to standard methods (R&D Systems, Minneapolis, MN). The lymphocyte/HBSS suspension was centrifuged at $200 \times g$ for 10 min at room temperature. Supernatant was again removed, and the pellet was re-suspended in 5 mL $1 \times$ HBSS, and centrifuged at $200 g$ for 10 min at room temperature. Supernatant was removed, and cells were re-suspended in 1 mL of freezing media (20% fetal bovine serum, 10% dimethyl sulfoxide in RPMI-1640 (ATCC, Manassas, VA), counted, aliquoted, and immediately frozen at -80°C .

2.4 Statistical methods

Treatment groups were compared at baseline on demographic and inflammation-related lifestyle variables using Chi Square and one-way ANOVA for categorical and continuous variables, respectively. Pearson product-moment correlations were assessed using Proc Corr in SAS to test the strength of the linear association between variables. Data which did not satisfy the assumption of normality (hsCRP and MCP-1) were log transformed for analysis. For these analyses we calculated change as time 2 values to time 1 values. The intervention effect was assessed with a repeated-measures analysis using Proc Mixed in SAS. Individual contrasts were constructed to test the change in the placebo group against the change in the two treatment arms (*i.e.*, FV and FVB). Outcomes that had a positively skewed distribution were log transformed to meet the assumption of normality, and then back transformed for presentation. All models control for age and gender. Values are presented as the least-squared means and their 95% confidence intervals (95% CI). All analyses were conducted using SAS statistical software (SAS Institute, Cary, NC).

3 Results

3.1 Subject baseline characteristics

Figure 1 outlines the study protocol design and early terminations for each study group. Data are presented on 117 subjects (of 151 (77.5%) eligible and randomized subjects) who have completed the study. A total of 31 subjects failed to complete the study (7 from the placebo group; 13 from the FV group; and 14 from the FVB group). Three subjects reported side effects. Two subjects reported an irregular heartbeat (one in placebo, and one in the FV group), and were asked not to complete the study. One subject reported gastrointestinal discomfort (FV group), and chose not to complete the study. The rest of the subjects who did not complete the study, failed to show for their second appointment for unknown reasons.

For the remaining 117 subjects who completed the study, all the test supplements were well tolerated. At baseline, the

three randomized groups did not differ on age, gender, race, BMI, education, employment, marital, or smoking status (Table 1). Because age, BMI, body fat, physical activity, percent calories from fat, and daily servings of fruits and vegetables can potentially cause changes in inflammatory load, and skew results, we compared these variables between groups at baseline. Table 2 shows that there was no significant difference between the groups on these factors.

3.2 Serum micronutrients

The mean serum micronutrient concentrations for all groups are presented in Table 3. Compared with the placebo group, there were similar and significant increases in β -carotene, ascorbic acid, and α -tocopherol in both the FV and FVB groups. These findings indicate that serum micronutrient levels are boosted by supplementation with FV or FVB capsules compared to placebo capsules, further indicating good compliance by the study subjects. In addition, using returned pill counts (returned $< 20\%$ of pills) we found a high level of compliance; with only two subjects in each of the FV and FVB groups non-compliant.

3.3 Inflammatory load

The key aim of this study was to determine whether a 60-d addition of the FV or FVB capsules to the habitual diet could impact the inflammatory load in healthy people, compared to placebo. To do this, we carried out complete blood counts, measured serum levels of hsCRP, MCP-1, MIP-1 β , and RANTES as pro-inflammatory biomarkers, all of these biomarkers have been implicated clinically as a potential risk marker of primary and secondary adverse cardiovascular events [2, 10]. We also measured erythrocyte SOD levels as an antioxidant biomarker. Results indicate that the complete blood count parameters of hematocrit, hemoglobin, corpuscular hemoglobin, mean cell volume, white blood cell counts, red blood cell counts, and platelet counts did not change in any of the groups (Supporting Information, Table 1), an indication that study subjects were in good health throughout the investigation. There were significant decreases in serum levels of the pro-inflammatory markers, MCP-1, MIP-1 β , and RANTES (Table 4) for both intervention groups compared with placebo. These changes were of similar magnitude for both intervention groups; MCP-1 decreased roughly 35% (32% FV and 38% FVB), MIP-1 β decreased 16% (15% FV and 17% FVB), and RANTES decreased 21% (Table 4). Serum levels of the antioxidant enzyme, SOD were significantly elevated, increasing 96% in FV and 103% in FVB.

Significant negative correlations were observed for SOD *versus* RANTES ($r = -0.56$, $p < 0.001$), SOD *versus* MCP-1 ($r = -0.32$, $p = 0.001$), and SOD *versus* MIP-1 β ($r = -0.28$, $p = 0.004$). SOD was positively correlated with β -carotene

Table 1. Demographics at baseline by treatment group

	Group ^{a)}			p-Value
	Placebo (n = 41)	FVB (n = 38)	FV (n = 38)	
<i>Age</i>				0.24 ^{b)}
21–30 years	23 (56%)	14 (37%)	12 (32%)	
31–40 years	8 (20%)	11 (29%)	12 (32%)	
>41 years	10 (24%)	13 (34%)	14 (36%)	
<i>Gender</i>				0.93 ^{b)}
Male	11 (27%)	9 (24%)	9 (24%)	
Female	30 (73%)	29 (76%)	29 (76%)	
<i>Race</i>				0.88 ^{c)}
African American	6 (15%)	5 (13%)	6 (16%)	
European American	33 (80%)	29 (76%)	28 (74%)	
Other	2 (5%)	4 (11%)	4 (10%)	
<i>BMI</i>				0.95 ^{c)}
Underweight	1 (3%)	1 (3%)	0 (0%)	
Normal weight	20 (49%)	17 (45%)	20 (53%)	
Overweight	10 (24%)	12 (31%)	11 (29%)	
Obese	10 (24%)	8 (21%)	7 (18%)	
<i>Education</i>				0.97 ^{b)}
High school, some college	8 (20%)	7 (18%)	8 (21%)	
College completed	14 (34%)	11 (29%)	13 (34%)	
More than college	19 (46%)	20 (53%)	17 (45%)	
<i>Employment status</i>				0.56 ^{c)}
Full time	27 (66%)	26 (68%)	26 (68%)	
Part time	9 (22%)	10 (26%)	6 (16%)	
Unemployed	5 (12%)	2 (5%)	6 (16%)	
<i>Marital status</i>				0.39 ^{b)}
Married/with partner	25 (61%)	18 (47%)	23 (61%)	
Widowed, divorced, separated, single	16 (39%)	20 (53%)	15 (39%)	
<i>Current smoker</i>				0.39 ^{b)}
No	37 (90%)	37 (97%)	34 (89%)	
Yes	4 (10%)	1 (3%)	4 (11%)	

a) Values are n (%); n = 117.

b) Chi square test.

c) Exact test.

Table 2. Inflammation-related variables at baseline by treatment group

	Group ^{a)}			p-value ^{b)}
	Placebo	FVB	FV	
Age	32.8±9.4	35.6±8.6	36.1±7.8	0.19
BMI	26.4±6.2	27.1±7.2	26.3±5.3	0.86
Percent body fat	30.9±9.1	31.9±8.8	30.5±7.2	0.77
Total physical activity (met-h/wk)	56.1±34.0	49.1±29.2	46.9±35.1	0.43
Percent calories as fat	30.2±3.8	30.9±4.2	29.4±3.2	0.22
Fruit and vegetable (srv/d)	3.1±2.6	2.5±1.7	2.8±1.7	0.53

a) Values are mean ±SD; n = 117.

b) One-way ANOVA.

($r = 0.39$, $p < 0.0001$), and α -tocopherol ($r = 0.29$, $p = 0.002$). Similarly, RANTES was positively correlated with MCP-1 ($r = 0.45$, $p < 0.0001$) and MIP-1 β ($r = 0.57$, $p < 0.0001$).

MCP-1 and MIP-1 β were also positively correlated ($r = 0.50$, $p < 0.0001$). All the three pro-inflammatory biomarkers (RANTES, MCP-1, and MIP-1 β) were negatively correlated

Table 3. Plasma levels of micronutrients baseline and follow-up in placebo, FV and FVB groups

Variable/treatment	Baseline least-squared mean ^{a)} (CI)	Follow-up least-squared mean ^{a)} (CI)	<i>p</i> -value ^{b)}
<i>β</i> -Carotene (μmol/L)			
Placebo	0.42 (0.32, 0.52)	0.45 (0.14, 0.77)	
FV	0.35 (0.24, 0.45)	1.91 (1.59, 2.24)	<0.0001
FVB	0.40 (0.30, 0.50)	1.61 (1.29, 1.93)	<0.0001
<i>Ascorbic acid</i> (μmol/L)			
Placebo	54.5 (47.7, 60.8)	52.2 (46.0, 59.0)	
FV	53.9 (47.1, 60.8)	69.8 (63.0, 76.6)	0.0009
FVB	50.5 (43.7, 56.8)	60.8 (53.9, 67.6)	0.02
<i>α</i> -Tocopherol (μmol/L)			
Placebo	26.7 (24.4, 28.8)	26.7 (24.1, 29.3)	
FV	26.0 (23.7, 28.3)	30.6 (28.1, 33.4)	<0.0001
FVB	27.1 (24.8, 29.3)	32.3 (29.5, 34.8)	<0.0001

a) Least square means adjusting for age and gender.

b) *p*-Value: Change in FV or FVB compared to change in placebo group.

Table 4. Results comparing baseline and follow-up measurements between placebo, FV and FVB groups (positive results: differences between groups are significant at *p*<0.05)

Variable/treatment	Baseline LSMean ^{a)} (CI)	Follow-up LSMean ^{a)} (CI)	<i>p</i> -value ^{b)}
<i>MCP-1</i> (pg/mL) ^{c)}			
Placebo	89.0 (68.1, 116.2)	92.4 (70.4, 121.4)	
FV	82.8 (62.7, 109.2)	57.5 (43.3, 76.4)	0.0006
FVB	94.2 (71.7, 123.8)	58.0 (43.8, 76.7)	<0.0001
<i>MIP-1β</i> (pg/mL)			
Placebo	381.7 (314.8, 448.7)	392.0 (324.8, 459.2)	
FV	358.0 (289.3, 426.7)	305.6 (236.6, 374.6)	0.01
FVB	446.5 (379.5, 513.4)	370.7 (303.5, 437.9)	0.0005
<i>RANTES</i> (pg/mL)			
Placebo	12 722 (9065, 16 380)	13 247 (10 553, 15 941)	
FV	13 819 (10 131, 17 507)	10 876 (8173, 13 579)	0.03
FVB	14 295 (10 656, 17 933)	11 238 (8570, 13 906)	0.02
<i>Superoxide dismutase</i> (U/mg Hb)			
Placebo	7.8 (6.3, 9.3)	10.5 (7.7, 13.4)	
FV	7.7 (6.1, 9.2)	15.1 (12.2, 18.1)	0.01
FVB	7.2 (5.7, 8.7)	14.6 (11.7, 17.4)	0.02

a) Least square means adjusting for age and gender.

b) *p*-Value: Change in FV or FVB compared to change in placebo group.

c) From analysis using log of variable.

with both β -carotene and α -tocopherol (RANTES versus β -carotene, $r = -0.50$, $p < 0.0001$; RANTES versus α -tocopherol, $r = -0.26$, $p = 0.007$; MCP-1 versus β -carotene, $r = -0.44$, $p < 0.0001$; MCP-1 versus α -tocopherol, $r = -0.38$, $p = 0.0001$; MIP-1 β versus β -carotene, $r = -0.36$, $p < 0.0002$; MIP-1 β versus α -tocopherol, $r = -0.28$, $p = 0.004$). Although hsCRP levels did not significantly change in any group, this variable was positively correlated with subject body weight, independent of study group assignment. At baseline the correlation was $r = 0.36$, and for post-intervention it was 0.34 (both $p < 0.05$).

4 Discussion

In this double-blind, randomized, placebo-controlled trial of 117 healthy individuals, we found that a fruit and vegetable juice concentrate powder, either with or without additional berry concentrate, had the capacity to reduce pro-inflammatory biological markers, as well as induce the antioxidant enzyme, SOD. These results are consistent with the hypothesis that these concentrates reduce inflammatory load in healthy people.

A previous study with the FVB showed no change in SOD in a group of already physically fit men undergoing

physically active training during a 28-wk period [9]. Although our results differ from the study by Lamprecht *et al.*, their baseline values were higher than observed here, and they hypothesized that SOD response reflects adaptation to physical activity. Therefore, there was not a detectable additional effect of FVB supplementation in an already highly trained population. In this study, our baseline values were lower, quite possibly because of the more sedentary nature of this otherwise healthy group of volunteers. The effect of FV or FVB on SOD indicates a beneficial effect of the tested interventions in this healthy population. This is consistent with another randomized, placebo-controlled trial, where 1200 IU/d vitamin E was given for 12 wk to patients with tardive dyskinesia, and resulted in elevated SOD levels [23].

Previous studies have shown that fruits and vegetables can reduce inflammatory load, as indicated by markers of inflammation, in human trials. For example, a 4-wk intervention with high intake of carotenoid-rich vegetables and fruit reduced plasma CRP in healthy, non-smoking men [24]. Sanchez-Moreno showed that consumption of a Mediterranean vegetable soup for 14 d increased plasma vitamin C levels, and reduced levels of several pro-inflammatory markers, including MCP-1 [25]. In a cross-sectional study of 3258 elderly men, plasma vitamin C, fruit intake, and dietary vitamin C intake were associated with lower plasma CRP and tissue plasminogen activator [26]. However, in randomized trials, there are conflicting results. For example, Block *et al.* showed vitamin C levels correlated with lower CRP in passive and active smokers [27]. In a double-blind, placebo-controlled trial, Wang *et al.* showed that a high-dose of multivitamin and mineral supplement significantly lowered serum concentrations of CRP compared with that of baseline and the placebo group [28]. Vitamin E (800 U/day) has also been shown to effectively reduce CRP in diabetic patients [29] in a randomized trial. Similar to our results, others also have shown no generalized effect of nutritional components (*e.g.*, vitamins A and C) on CRP, nor TNF- α or IL-6 [30]. In addition, the FVB used in a previous study also had no effect on TNF- α (10) and FV (provided; 7.5 mg β -carotene, 234 mg vitamin C, 45 IU vitamin E, 420 mg folate, 60 mg calcium, and about 42 kJ/capsule, four capsules/day) showed no effect on IL-4, TGF- β , nor IL-6 [8]. There are many possible explanations for these observations, including dosage, length of supplementation period, study population characteristics, inappropriate selection of markers as endpoints, or some combination of these. This highlights the importance of measuring several markers of inflammatory stress in human trials, so erroneous conclusions regarding inflammatory load do not rely on a single marker of systemic inflammation.

Overall, there is relatively consistent evidence, in both humans and animals [31–34], that fruit or vegetable consumption (or their combination or various components) can reduce systemic inflammatory load (clinically potential

risk biomarkers). The specific results depend on many factors, including the particular endpoints and compounds examined. In this study, multiple bioactive compounds, including vitamins, minerals, and fructose, are present in both FV and FVB that have the potential to suppress inflammation. Our results showed that the magnitude of effects on inflammatory markers was similar between FV and FVB for all outcomes. Karlsen *et al.* showed that anthocyanins isolated from bilberries and black currants taken for 3 wk, significantly reduced RANTES in healthy adults, however, there was no significant differences in MCP-1, nor MIP-1 β [35]. The addition of bilberry and black currant juice as part of FVB apparently had no additional effect on RANTES reduction in our data. This inconsistency points out the difficulty in untangling the mechanisms and interactions that are involved in decreasing the inflammatory load. However, the fact that these supplements are derived from a large number of fruits and vegetables, in a sense increases the diversity of the diet, a concept that is associated with healthy diets [36].

MCP-1, MIP-1 β , and RANTES have been shown to be elevated in several inflammatory conditions, including lupus erythematosus, rheumatoid arthritis, multiple sclerosis, and metabolic syndrome [37–40]. However, to our knowledge, there have been no randomized, controlled trials to test the effects of nutrients provided in our FV or FVB on these endpoints. In this study, using a randomized, double-blinded, placebo-controlled design, of 117 healthy individuals, we showed that a fruit and vegetable concentrate resulted in an elevation in micronutrients (as measured by β -carotene, vitamin C, and α -tocopherol) and reduced systemic inflammatory load (as measured by MCP-1, MIP-1 β , and RANTES). We also show that this concentrate can increase the systemic levels of the antioxidant enzyme, SOD. Although the long-term implications of these findings are currently unknown, the close relationship between chronic inflammation and poor human health, suggests such a juice concentrate is a beneficial addition to the habitual diet in support of human health.

Financial supported in part by a research grant from NSA, LLC (Collierville, TN) to the University of South Carolina Research Foundation, in compliance with the guidelines of Funding Food Science and Nutrition Research. We thank all subjects for volunteering for this study. In addition, we would like to thank our team of project co-ordinators, without whom the study could not have been carried out.

The author's responsibilities were as follows – Y. J., X. C., U. P. S., A. B. H., V. K., B. S., S. R. V., and A. A. C. conducted laboratory research; B. H. was the lead project coordinator; P. C., J. R. H., and T. G. H. analyzed data and contributed to interpretation of results. L. J. H. had primary responsibility for the design, implementation, and final content of the manuscript.

The authors have declared no conflict of interest.

5 References

- [1] Finch, C. E., Crimmins EM Inflammatory exposure and historical changes in human life-spans. *Science* 2004, 305, 1736–1739.
- [2] Engstrom, G., Hedblad, B., Stavenow, L., Jonsson, S. *et al.*, Incidence of obesity-associated cardiovascular disease is related to inflammation-sensitive plasma proteins: a population-based cohort study. *Arterioscler. Thromb. Vasc. Biol.* 2004, 24, 1498–1502.
- [3] Barzilay, J., Freedland, E., Inflammation and its association with glucose disorders and cardiovascular disease. *Treat. Endocrinol.* 2003, 2, 85–94.
- [4] Hanley, A. J., Festa, A., D'Agostino Jr, R. B., Wagenknecht, L. E. *et al.*, Metabolic and inflammation variable clusters and prediction of type 2 diabetes: factor analysis using directly measured insulin sensitivity. *Diabetes* 2004, 53, 1773–1781.
- [5] Hofseth, L. J., Ying, L., Identifying and defusing weapons of mass inflammation in carcinogenesis. *Biochim. Biophys. Acta.* 2006, 1765, 74–84.
- [6] Heber, D., Vegetables, fruits and phytoestrogens in the prevention of diseases. *J. Postgrad. Med.* 2004, 50, 145–149.
- [7] Nebeling, L., Yaroch, A. L., Seymour, J. D., Kimmons, J., Still not enough: can we achieve our goals for Americans to eat more fruits and vegetables in the future? *Am. J. Prev. Med.* 2007, 32, 354–355.
- [8] Nantz, M. P., Rowe, C. A., Nieves Jr, C., Percival, S. S., Immunity and antioxidant capacity in humans is enhanced by consumption of a dried, encapsulated fruit and vegetable juice concentrate. *J. Nutr.* 2006, 136, 2606–2610.
- [9] Lamprecht, M., Oettl, K., Schwabeger, G., Hofmann, P. *et al.*, Several indicators of oxidative stress, immunity, and illness improved in trained men consuming an encapsulated juice powder concentrate for 28 weeks. *J. Nutr.* 2007, 137, 2737–2741.
- [10] Devaraj, S., Singh, U., Jialal, I., The evolving role of C-reactive protein in atherothrombosis. *Clin. Chem.* 2009, 55, 229–238.
- [11] Sell, H., Eckel, J., Monocyte chemotactic protein-1 and its role in insulin resistance. *Curr. Opin. Lipidol.* 2007, 18, 258–262.
- [12] Sousa, A. R., Lane, S. J., Nakhosteen, J. A., Yoshimura, T. *et al.*, Increased expression of the monocyte chemoattractant protein-1 in bronchial tissue from asthmatic subjects. *Am. J. Respir. Cell Mol. Biol.* 1994, 10, 142–147.
- [13] Panasiuk, A., Prokopowicz, D., Panasiuk, B., Monocyte chemotactic protein-1 and soluble adhesion molecules as possible prognostic markers of the efficacy of antiviral treatment in chronic hepatitis C. *World J. Gastroenterol.* 2004, 10, 3639–3642.
- [14] Dehqanzada, Z. A., Storrer, C. E., Hueman, M. T., Foley, R. J. *et al.*, Correlations between serum monocyte chemotactic protein-1 levels, clinical prognostic factors, and HER-2/neu vaccine-related immunity in breast cancer patients. *Clin. Cancer Res.* 2006, 12, 478–486.
- [15] Matsukawa, A., Hogaboam, C. M., Lukacs, N. W., Kunkel, S. L., Chemokines and innate immunity. *Rev. Immunogenet.* 2000, 2, 339–358.
- [16] Koch, A. E., Kunkel, S. L., Shah, M. R., Fu, R. *et al.*, Macrophage inflammatory protein-1 beta: a C-C chemokine in osteoarthritis. *Clin. Immunol. Immunopathol.* 1995, 77, 307–314.
- [17] Robinson, E., Keystone, E. C., Schall, T. J., Gillett, N. *et al.*, Chemokine expression in rheumatoid arthritis (RA): evidence of RANTES and macrophage inflammatory protein (MIP)-1 beta production by synovial T cells. *Clin. Exp. Immunol.* 1995, 101, 398–407.
- [18] Appay, V., Rowland-Jones, S. L., RANTES: a versatile and controversial chemokine. *Trends Immunol.* 2001, 22, 83–87.
- [19] Cocchi, F., DeVico, A. L., Garzino-Demo, A., Arya, S. K. *et al.*, Identification of RANTES, MIP-1 alpha, and MIP-1 beta as the major HIV-suppressive factors produced by CD8+T cells. *Science* 1995, 270, 1811–1815.
- [20] Greene, G. W., Resnicow, K., Thompson, F. E., Peterson, K. E. *et al.*, Correspondence of the NCI Fruit and Vegetable Screener to repeat 24-H recalls and serum carotenoids in behavioral intervention trials. *J. Nutr.* 2008, 138, 200S–204S.
- [21] Stewart, A. L., Mills, K. M., King, A. C., Haskell, W. L. *et al.*, Physical activity questionnaire for older adults: outcomes for interventions. *Med. Sci. Sports Exerc.* 2001, 33, 1126–1141.
- [22] Sun, S. S., Chumlea, W. C., Heymsfield, S. B., Lukaski, H. C. *et al.*, Development of bioelectrical impedance analysis prediction equations for body composition with the use of a multicomponent model for use in epidemiologic surveys. *Am. J. Clin. Nutr.* 2003, 77, 331–340.
- [23] Zhang, X. Y., Zhou, D. F., Cao, L. Y., Xu, C. Q. *et al.*, The effect of vitamin E treatment on tardive dyskinesia and blood superoxide dismutase: a double-blind placebo-controlled trial. *J. Clin. Psychopharmacol.* 2004, 24, 83–86.
- [24] Watzl, B., Kulling, S. E., Moseneder, J., Barth, S. W. *et al.*, A 4-wk intervention with high intake of carotenoid-rich vegetables and fruit reduces plasma C-reactive protein in healthy, nonsmoking men. *Am. J. Clin. Nutr.* 2005, 82, 1052–1058.
- [25] Sanchez-Moreno, C., Cano, M. P., de Ancos, B., Plaza, L. *et al.*, Mediterranean vegetable soup consumption increases plasma vitamin C and decreases F2-isoprostanes, prostaglandin E2 and monocyte chemotactic protein-1 in healthy humans. *J. Nutr. Biochem.* 2006, 17, 183–189.
- [26] Wannamethee, S. G., Lowe, G. D., Rumley, A., Bruckdorfer, K. R. *et al.*, Associations of vitamin C status, fruit and vegetable intakes, and markers of inflammation and hemostasis. *Am J Clin. Nutr.* 2006, 83, 567–574.
- [27] Block, G., Jensen, C., Dietrich, M., Norkus, E. P. *et al.*, Plasma C-reactive protein concentrations in active and passive smokers: influence of antioxidant supplementation. *J. Am. Coll. Nutr.* 2004, 23, 141–147.
- [28] Wang, C., Li, Y., Zhu, K., Dong, Y. M. *et al.*, Effects of supplementation with multivitamin and mineral on blood pressure and C-reactive protein in obese Chinese women with increased cardiovascular disease risk. *Asia Pac. J. Clin. Nutr.* 2009, 18, 121–130.

- [29] Upritchard, J. E., Sutherland, W. H., Mann, J. I., Effect of supplementation with tomato juice, vitamin E, and vitamin C on LDL oxidation and products of inflammatory activity in type 2 diabetes. *Diabetes Care*. 2000, 23, 733–738.
- [30] Bruunsgaard, H., Poulsen, H. E., Pedersen, B. K., Nyssönen, K. *et al.*, Long-term combined supplementations with alpha-tocopherol and vitamin C have no detectable anti-inflammatory effects in healthy men. *J. Nutr.* 2003, 133, 1170–1173.
- [31] Mayorga, M., Iborra, A., Estany, S., Martinez, P., Protective effect of vitamin E in an animal model of LPS-induced inflammation. *Am. J. Reprod. Immunol.* 2004, 52, 356–361.
- [32] Guo, L. Y., Hung, T. M., Bae, K. H., Shin, E. M. *et al.*, Anti-inflammatory effects of schisandrin isolated from the fruit of *Schisandra chinensis* Baill. *Eur. J. Pharmacol.* 2008, 591, 293–299.
- [33] Reifen, R., Nur, T., Ghebermeskel, K., Zaiger, G. *et al.*, Vitamin A deficiency exacerbates inflammation in a rat model of colitis through activation of nuclear factor-kappaB and collagen formation. *J. Nutr.* 2002, 132, 2743–2747.
- [34] Mills, J. P., Simon, P. W., Tanumihardjo, S. A., Biofortified carrot intake enhances liver antioxidant capacity and vitamin A status in mongolian gerbils. *J. Nutr.* 2008, 138, 1692–1698.
- [35] Karlsen, A., Retterstol, L., Laake, P., Paur, I. *et al.*, Anthocyanins inhibit nuclear factor-kappaB activation in monocytes and reduce plasma concentrations of pro-inflammatory mediators in healthy adults. *J. Nutr.* 2007, 137, 1951–1954.
- [36] Kant, A. K., Graubard, B. I., A comparison of three dietary pattern indexes for predicting biomarkers of diet and disease. *J. Am. Coll. Nutr.* 2005, 24, 294–303.
- [37] Boiardi, L., Macchioni, P., Meliconi, R., Pulsatelli, L. *et al.*, Relationship between serum RANTES levels and radiological progression in rheumatoid arthritis patients treated with methotrexate. *Clin. Exp. Rheumatol.* 1999, 17, 419–425.
- [38] Vila, L. M., Molina, M. J., Mayor, A. M., Cruz, J. J. *et al.*, Association of serum MIP-1alpha, MIP-1beta, and RANTES with clinical manifestations, disease activity, and damage accrual in systemic lupus erythematosus. *Clin. Rheumatol.* 2007, 26, 718–722.
- [39] Troseid, M., Lappegard, K. T., Claudi, T., Damas, J. K. *et al.*, Exercise reduces plasma levels of the chemokines MCP-1 and IL-8 in subjects with the metabolic syndrome. *Eur. Heart J.* 2004, 25, 349–355.
- [40] Franciotta, D., Martino, G., Zardini, E., Furlan, R. *et al.*, Serum and CSF levels of MCP-1 and IP-10 in multiple sclerosis patients with acute and stable disease and undergoing immunomodulatory therapies. *J. Neuroimmunol.* 2001, 115, 192–198.

**Supporting Information
for Mol. Nutr. Food Res.
DOI 10.1002/mnfr.200900579**

Yu Jin, Xiangli Cui, Udai P. Singh, Alexander A. Chumanevich,
Brook Harmon, Philip Cavicchia, Anne B. Hofseth,
Venkata Kotakadi, Brandy Stroud, Suresh R. Volate,
Thomas G. Hurley, James R. Hebert and Lorne J. Hofseth

**Systemic inflammatory load in humans is suppressed by consumption of two
formulations of dried, encapsulated juice concentrate**