# Effects of a freeze-dried juice blend powder on exercise-induced inflammation, oxidative stress, and immune function in cyclists

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**Abstract:** A freeze-dried fruit and vegetable juice powder (JUICE) was investigated as a countermeasure nutritional strategy to exercise-induced inflammation, oxidative stress, and immune perturbations in trained cyclists. Thirty-four cyclists (25 male, 9 female) were randomized to control (nonJUICE) or JUICE for 17 days. JUICE provided 230 mg·day$^{-1}$ of flavonoids, doubling the typical adult daily intake. During a 3-d period of intensified exercise (days 15–17), subjects cycled at 70%–75% $\dot{V}O_{2max}$ for 2.25 h per day, followed by a 15-min time trial. Blood samples were collected presupplementation, post supplementation (pre-exercise), and immediately and 14-h post exercise on the third day of exercise. Samples were analyzed for inflammation (interleukin (IL)-6, IL-8; tumor necrosis factor alpha (TNF*a*); monocyte chemoattractant protein-1 (MCP-1)), oxidative stress (oxygen radical absorbance capacity (ORAC), ferric reducing ability of plasma (FRAP), reduced and oxidized glutathione, protein carbonyls), and innate immune function (granulocyte (G-PHAG) and monocyte (M-PHAG) phagocytosis and oxidative burst activity). A 2 (group) × 4 (time points) repeated measures ANOVA revealed significant time effects due to 3 days of exercise for IL-6 (396% increase), IL-8 (78% increase), TNF*a* (12% increase), MCP-1 (30% increase), G-PHAG (38% increase), M-PHAG (36% increase), FRAP (12.6% increase), ORAC (11% decrease at 14 h post exercise), and protein carbonyls (82% increase at 14 h post exercise) ($p < 0.01$). No significant interaction effects were found for any of the physiological measures. Although providing 695 gallic acid equivalents of polyphenols per day, JUICE treatment for 17 days did not change exercise-induced alterations in inflammation and oxidative stress or immune function in trained cyclists after a 3-day period of overreaching.

*Key words:* polyphenols, flavonoids, humans, athletes.

**Résumé :** Dans cette étude, on examine un fruit lyophilisé et une poudre de jus de légume (JUICE) comme étant une stratégie nutritionnelle pour contrer l'inflammation induite par l'exercice, le stress oxydatif et les perturbations immunitaires chez des cyclistes entraînés. On répartit aléatoirement 34 cyclistes (25 hommes, 9 femmes) dans deux groupes, l'un de contrôle (non-JUICE) et l'autre expérimental (JUICE) pour une période de 17 jours. Le groupe expérimental reçoit 230 mg·jour$^{-1}$ de flavonoïdes, soit le double de l'apport journalier d'un adulte type. Durant 3 jours d'exercice plus intense (jours 15–17), les sujets pédalent à une intensité sollicitant 70 %–75 % du $\dot{V}O_{2max}$, et ce, 2,25 h par jour, puis effectue une course de 15 minutes contre-la-montre. On prélève des échantillons de sang avant la supplémentation, après la supplémentation (préexercice), puis, au troisième jour, immédiatement après la fin de l'exercice et 14 h plus tard. On analyse l'inflammation du sang (interleukine (IL)-6, IL-8, facteur de nécrose tumorale alpha (TNF*a*), protéine chimiotactique monocytaire-1 (MCP-1)), le stress oxydatif (ORAC, FRAP, glutathion réduit et oxydé, groupements carbonyles dans les protéines) et la fonction immunitaire innée (augmentation de l'activité oxydative et phagocytose des granulocytes (G-PHAG) et des monocytes (M-PHAG)). L'analyse de variance 2 × 4 avec mesures répétées révèle un effet significatif dans le temps causé par les 3 jours d'exercice : IL-6 (augmentation de 396 %), IL-8 (augmentation de 78 %), TNF*a* (augmentation de 12 %), MCP-1 (augmentation de 30 %), G-PHAG (augmentation de 38 %), M-PHAG (augmentation de 36 %), FRAP (augmentation de 12,6 %), ORAC (diminution de 11 % à la 14$^{e}$ h postexercice), groupements carbonyles (augmentation de 82 % à la 14$^{e}$ h postexercice) ($p < 0,01$). L'analyse ne révèle aucune interaction significative en ce qui concerne toutes les mesures physiologiques. Même avec l'apport de 695 équivalents phénoliques d'acide gallique par jour, le traitement JUICE sur une période de 17 jours ne suscite pas de modifications de l'inflammation et du stress oxydatif induits par l'exercice et ne provoque pas d'altération des fonctions immunitaires innées chez des cyclistes entraînés, et ce, après 3 jours de surpassement. [Traduit par la Rédaction]

*Mots-clés :* polyphénols, flavonoïdes, humains, athlètes.

## Introduction

Polyphenols, found in high concentrations in fruits and vegetables, have been linked to beneficial health outcomes in humans (Hooper et al. 2008; Zern and Fernandez 2005). Human studies with large doses of flavonoid aglycones have produced disappointing results, but emerging evidence suggests that chronic ingestion of flavonoid-rich fruit or vegetable extracts attenuates exercise-induced immune dysfunction, inflammation, and oxidative stress (Bloomer et al. 2006; Connolly et al. 2006; Nakazato et al. 2006; Trombold et al. 2011). Likewise, the effectiveness, bioavailability, and bioactivity of flavonoids may be increased when they are consumed in combination from different subclasses (Lila 2007; Silberberg et al. 2006).

Other studies have reported attenuated post-exercise inflammation and oxidative stress with fruit or vegetable extracts, especially when subjects experience significant physiologic stress (Lyall et al. 2009; Nieman et al. 2010; Williamson and Manach 2005). Our lab recently conducted a 10-day study in elite sprint swimmers using a randomized cross-over design with a freshly prepared, flavonoid-rich fruit–vegetable juice blend supplement (505 mg gallic acid equivalents in 473 mL (16 fluid ounces) per day). The swimmers experienced little inflammation, oxidative stress, or immune dysfunction from the interval training sessions, and as a consequence no countermeasure benefits from the juice supplement were measured (Knab et al. 2013).

We reasoned that the flavonoid-rich juice – vegetable juice supplement would prove more efficacious when exercise-induced physiologic stress was high leading to oxidative stress, inflammation, and immune dysfunction. In this study we hypothesized that a freeze-dried powder consisting of fruit–vegetable ingredients (JUICE) high in polyphenols (approx. 37% more than the study with the swimmers (Knab et al. 2013)) would decrease exercise-induced oxidative stress, inflammation, and maintain immune function in highly trained cyclists after 3 days of intense exercise. The JUICE supplement used in this study was similar to that used in the sprint swimmer investigation (Knab et al. 2013), but was changed to a freeze-dried preparation to enhance portability and ease of use by the cyclists.

## Materials and methods

### Subjects

Thirty-four trained endurance cyclists (25 male, 9 female) who regularly competed in road races were recruited into the study and randomized to either control or treatment groups. Subjects avoided the use of large-dose vitamin–mineral supplements, herbs, and medications known to affect inflammation and immune function starting 2 weeks before and throughout the entire study. Subjects trained normally during the study and remained weight stable. All study procedures were reviewed and approved by the Appalachian State University Institutional Review Board, and subjects provided informed consent before the initiation of the study.

### Baseline testing

Two weeks prior to the 3-day period of intensified training (2.5 h per day endurance cycling bouts), subjects reported to the North Carolina Research Campus Human Performance Lab for an appointment between 1430 h and 1730 h for orientation and fitness testing. Subjects were tested for maximal aerobic fitness ($\dot{V}O_{2max}$) on a cycle ergometer (Lode Excaliber Sport, Lode B.V., Groningen, Netherlands) with continuous metabolic measurements using the Cosmed Quark CPET metabolic cart (Rome, Italy). Subjects started cycling at a workload of 150 W and then increased 25 W every 2 min. until they could no longer cycle at >60 RPM. Body composition was measured using the BodPod system (Life Measurement, Concord, Calif., USA). Subjects were randomized to JUICE or nonJUICE groups, and given dates for their participation in the study. Subjects were also given a food recording form with instructions to record all food and beverage intake during the 3-day period prior to the first exercise session. Food record data were analyzed using a computerized dietary assessment program for energy and macronutrient content (Food Processor; ESHA Research, Salem, Ore., USA).

### Study procedures

Supplementation lasted for 17 days (this included the 3-day exercise period) and involved ingesting 3 tablespoons of the freeze-dried juice powder mixed in 177 mL (6 fluid ounces) of pineapple juice (converts to 237 mL (8 fluid ounces) total) in the morning and again at lunch time (for a total of 473 mL (16 fluid ounces) per day). The nonJUICE group was instructed to simply eat their normal diet. Juice powder, tablespoons, and pineapple juice were provided to the subjects. To verify compliance, subjects reported whether they used all of the juice and powder given to them prestudy (31 cans (177 mL (6 ounces)) of pineapple juice and 93 tablespoons of juice powder). The JUICE powder used in this study was a freeze-dried blend of the following ingredients (listed per serving size) and was prepared by Dole Foods (Westlake Village, Calif., USA): 1/3 large red bell pepper, 1/2 small red delicious apple, 1/3 navel orange, 1/2 small carrot, 1/2 cup chopped broccoli, 1/4 1 sprig parsley, 1/2 small tomato, 1/5 cucumber, 1/5 cup blueberries, 1/5 cup pineapples, 1/5 cup blackberries, and 3 medium strawberries. The total phenolic content in milligrams of gallic acid equivalents (mg GAE) in the juice powder preparation was measured to be 696 GAE per 473 mL (16 fluid ounce) serving, as measured using high-performance liquid chromatography. A 473 mL (16 fluid ounce) serving contained 184 calories, 40.3 g total carbs, and 28.7 g sugars (Covance, Princeton, N.J., USA).

After 2 weeks of supplementation, subjects ($N$ = 34) completed three consecutive days of intense bouts of 2.5 h per day exercise at 60% $W_{max}$ with a 15-min time trial at the end. In the morning of the first exercise session, subjects consumed 237 mL (8 fluid ounces) of JUICE. A standardized meal consisting of Boost Plus (Nestle; S.A., Vevey, Switzerland) at 10 kcal·kg$^{-1}$ was ingested at 1200 h. Subjects did not consume JUICE in pineapple juice at lunch time prior to coming to the lab for the exercise trials. Subjects reported to the lab at 1430 h, turned in the 3-day food record, and provided blood samples. Subjects next ingested JUICE (in 177 mL (6 fluid ounces) water) or ingested 237 mL (8 fluid ounces) water (control group) about 15 min prior to exercise. At 1500 h, subjects cycled on their own bicycles on CompuTrainers at 60% $W_{max}$ for 2.25 h followed by a 15-min time trial at the fastest pace possible, with distance measured for a performance outcome. Water was given ad libitum throughout the 2.5-h endurance bouts. Subjects in the treatment group received another dose of JUICE (mixed in water) 1 h into the exercise bout (1600 h). Heart rate and rating of perceived exercise (RPE) were measured every 30 min during the bout, whereas $\dot{V}O_2$ was measured mid-way through the ride for both treatment groups. Subjects repeated this scenario for the next 2 days, but without pre-exercise blood draws. Blood samples were collected again immediately following the third exercise bout on the third day (1730 h). A final blood draw was collected approximately 14 h post exercise the following morning at 0730 h. Subjects completed symptom logs retrospectively at the end of the 2-week supplementation period, and after 3 days of cycling in the lab. The symptom logs included questions on digestive health (e.g., heartburn, bloating, diarrhea, and nausea). Subjects indicated responses using a 12-point Likert scale, with 1 relating to "none at all", 6 "moderate", and 12 "very high".

### Plasma cytokines

Total plasma concentrations of 4 inflammatory cytokines (interleukin (IL)-6, tumor necrosis factor alpha (TNF$\alpha$), IL-8, monocyte chemoattractant protein-1 (MCP-1)) were determined using an electrochemiluminescence-based solid-phase sandwich immunoassay (Meso Scale Discovery, Gaithersburg, Md., USA). All samples and provided standards were analyzed in duplicate, and the intra-assay coefficient of variation (CV) ranged from 4.7% to 9.0% and the inter-assay CV range from 3.5% to 6.6% for all cytokines measured.

The minimum detectable concentration of IL-6 was 0.27 pg·mL$^{-1}$, TNF$\alpha$ 0.50 pg·mL$^{-1}$, MCP 2.4 pg·mL$^{-1}$, IL-8 0.09 pg·mL$^{-1}$). Pre- and post-exercise samples for the cytokines were analyzed on the same assay plate to decrease inter-kit assay variability.

### Oxidative stress and antioxidant capacity

Protein carbonyls were measured according to protocol (Cayman Chemical, 10005020). 220 $\mu$L of sample supernatant was pipetted in duplicate into a micro-well plate and read at 370 nm (Synergy H1 Hybrid Reader, BioTek Instruments Inc., Winooski, Ver., USA). Total protein was determined using the duplicate sample aliquot by adding 20 $\mu$L of sample to the micro-plate in duplicate followed by 180 $\mu$L of guanidine hydrochloride, and 200 $\mu$L of bovine serum albumin standards and read at 280 and 260 nm. Intra-assay CVs for protein carbonyls were 1%.

Total plasma antioxidant power was determined by the ferric reducing ability of plasma (FRAP) assay, a single electron transfer reaction as previously described by Benzie and Strain (1996). Intra- and inter-assay CVs for FRAP were 5% and 7%, respectively.

Red blood cell glutathione was measured using enzymatic recycling method (#703002, Cayman Chemical Company, Ann Arbor, Mich., USA). Briefly, red blood cells were isolated from Heparin treated blood, lysed, and deproteinated. Total glutathione and glutathione (reduced) (GSH) were quantified using glutathione reductase, and concentrations were determined by the Kinetic Method. GSH was calculated by subtracting the measured glutathione disulfide (oxidized) (GSSG) from the total glutathione.

Total oxygen radical absorbance capacity (ORAC) was measured using methods described in other studies (Huang et al. 2002; Ou et al. 2001; Prior et al. 2003). Blanks, trolox standards (25 $\mu$L), and plasma samples from ethylenediaminetetraacetic acid treated blood were loaded into appropriate microtiter plate wells, followed by fluorescein working solution (100 $\mu$L). The plate was then incubated with 2,2'-Azobis(2-amidinopropane)dihydrochloride working solution (25 $\mu$L). ORAC values were calculated by a fluorescence plate reader (Synergy H1 Hybrid Reader, BioTek Instruments Inc., Winooski, Ver., USA) as area under the curve. Intra and inter-assay CVs for ORAC were 4% and 7%, respectively.

### Granulocyte and Monocyte Phagocytosis (G-PHAG, M-PHAG), Oxidative Burst Activity (G-OBA, M-OBA)

G-PHAG, M-PHAG, G-OBA, and M-OBA were assayed as previously described by Konrad et al. (2011). Briefly, phagocytosis was measured through the uptake of Fluorescein isothiocyanate (FITC)-labeled *Staphylococcus aureus* bacteria and oxidative burst was measured through the oxidation of nonfluorescent hydroethidine (HE) to fluorescent ethidium bromide in cells stimulated with unlabeled bacteria. Samples were processed on a Q-Prep™ Workstation (Beckman Coulter, Inc., Brea, Calif., USA) and analysis was performed within 18 hr of blood collection using a Beckman Coulter FC10 500 flow cytometer. After gating on the granulocyte and monocyte populations using forward scatter and side scatter, the mean fluorescence intensity (MFI; x-mean) and percent positive cells for FITC (FL1) and oxidized HE (FL2) were determined.

### Statistical procedures

Data are expressed as mean ± SD and were analyzed using a 2 (group) × 4 (time) repeated-measures ANOVA, between-groups design. When interaction effects were significant ($p \leq 0.05$), changes from presupplementation to each of 3 other time points were compared between groups using student's $t$ tests, with significance set after Bonferroni adjustment at $p \leq 0.017$. Independent student's $t$ tests were used to compare groups for data contained in Table 1 and performance data.

## Results

Subject characteristics are summarized in Table 1 for JUICE and nonJUICE groups. None of the demographic, training, or metabolic measurements differed between groups. No group differences in metabolic measures (oxygen consumption, ventilation, heart rate, power output) were found for the 2.25-h exercise pre-load or the 15-min time trial on each of the 3 exercise days (data not shown). For all 3 days combined, subjects in JUICE and nonJUICE groups during the 2.25-h cycling sessions maintained a 60% $W_{max}$ workload (191 ± 31.0 W and 192 ± 33.3 W, respectively, $p = 0.984$), with no group differences for the average heart rate (142 ± 12.0 bpm and 143 ± 10.8 bpm, respectively, $p = 0.738$), and RPE (12.8 ± 1.7 and 12.2 ± 1.4, respectively, $p = 0.257$). During the 15-min time trials following the 2.25-h exercise pre-load, the average 3-day power output did not differ between JUICE and nonJUICE groups (227 ± 60.1 W and 238 ± 56.7 W, respectively, $p = 0.565$). Macro- and micro-nutrient intake from the 3-day food records (just prior to the 3-day exercise period) revealed no significant group differences (data not shown). Symptom logs showed no group differences for data collected post-supplementation and post–3-days exercise (data not shown).

**Table 1.** Subject characteristics (mean ± SD).

| Variable | JUICE ($N = 16$) | NonJUICE ($N = 17$) | $p$ value |
|---|---|---|---|
| Age (y) | 35.1±8.0 | 35.5±8.2 | 0.904 |
| Gender | M = 11, F = 5 | M = 13, F = 4 | 0.915 |
| Weight (kg) | 75.6±10.4 | 72.2±9.43 | 0.327 |
| % body fat | 19.1±8.0 | 17.5±5.7 | 0.512 |
| $\dot{V}O_{2max}$ (mL·kg$^{-1}$·min$^{-1}$) | 53.7±6.0 | 57.0±7.0 | 0.157 |
| Maximal power output (W) | 319±48 | 319±55 | 0.984 |
| Maximal ventilation (L·min$^{-1}$) | 146±24 | 150±34 | 0.695 |
| Km per week training | 229±94.8 | 285±180 | 0.268 |

**Note:** All variables tested for significance using student's $t$ test except for the use of $x^2$ analysis for gender.

Significant time effects were found for IL-6 ($p < 0.001$), IL-8 ($p < 0.001$), TNF-$\alpha$ ($p = 0.003$), and MCP-1 ($p < 0.001$), but interaction effects were nonsignificant (Table 2). Significant time effects were measured for G-PHAG ($p < 0.001$) and M-PHAG ($p < 0.001$) (Fig. 1A and 1B) and total blood leukocytes (Table 2), but not G-OBA and M-OBA (Table 2); interaction effects for these variables were not significant (G-PHAG $p = 0.194$, and M-PHAG $p = 0.093$).

Significant time effects were measured for ORAC, protein carbonyls, and FRAP, but not GSH–GSSG; none of the interaction effects were significant (Table 3).

## Discussion

Three days of intense cycling induced increases in inflammatory and oxidative stress markers, and significantly decreased G-PHAG and M-PHAG 14-h post exercise in trained competitive cyclists. Contrary to our hypothesis, however, a 2-week supplementation with JUICE, roughly equivalent to 4 servings of fruits and vegetables per day, in trained cyclists did not counter the increase in inflammation, oxidative stress, and immune changes following a 3-day period of prolonged and intensive exercise.

The USDA (USDA Nutrient Data Laboratory 2007) database (http://www.ars.usda.gov/nutrientdata) was used to estimate the flavonoid content of JUICE at 230 mg per 473 mL (16 fluid ounce) serving. The total phenolic content of JUICE was measured at 695 mg GAE per reconstituted serving (approximately 70% of the USDA estimated value). Therefore, on a per weight basis, our fruit and vegetable cocktail (equivalent to 4 servings of fruits and vegetables per day) would represent an approximate doubling of the average American dietary flavonoid intake (Chun et al. 2010). In addition, JUICE was a good source (>10% daily value) of eight vitamins and minerals, and an excellent source (>20% daily value) of 5 other nutrients (i.e., vitamin A, vitamin C, vitamin B6, potassium, and folate).

Previous studies with large doses of single flavonoids such as quercetin had no effect on exercise-induced inflammation (Nieman et al.

**Table 2.** Inflammation and immune function markers.

| | Pre-supplementation | | Post-supplementation | | Post-exercise | | 14-h post-exercise | | |
|---|---|---|---|---|---|---|---|---|---|
| Inflammatory markers | JUICE | NonJUICE | JUICE | NonJUICE | JUICE | NonJUICE | JUICE | NonJUICE | *p* value (time, interaction) |
| IL-6 (pg·mL$^{-1}$) | 0.64±0.37 | 0.60±0.34 | 0.61±0.33 | 0.60±0.45 | 2.87±1.29 | 3.09±2.83 | 0.69±0.32 | 0.66±0.36 | <0.001, 0.958 |
| IL-8 (pg·mL$^{-1}$) | 2.23±0.60 | 2.45±0.92 | 2.25±0.75 | 2.46±1.18 | 4.20±2.35 | 4.22±2.69 | 2.30±0.93 | 2.01±0.85 | 0.001, 0.347 |
| TNF-*α* (pg·mL$^{-1}$) | 4.22±1.43 | 3.93±1.09 | 4.10±1.66 | 3.86±1.20 | 4.70±1.50 | 4.21±1.35 | 4.30±1.22 | 3.94±1.08 | 0.003, 0.797 |
| MCP-1 (pg·mL$^{-1}$) | 204±48.3 | 208±57.5 | 207±47.3 | 206±58.9 | 254±99.4 | 280±85.3 | 222±72.0 | 234±77.7 | <0.001, 0.615 |
| CRP (mg·L$^{-1}$) | 1.04±0.85 | 0.92±0.88 | 1.23±1.10 | 0.90±0.66 | 2.96±2.37 | 2.42±2.32 | 2.48±2.07 | 2.12±2.05 | <0.001, 0.908 |
| **Immune function** | | | | | | | | | |
| G-OBA (MFI) | 11.5±4.52 | 13.7±7.44 | 17.2±11.5 | 17.7±11.8 | 14.0±5.82 | 14.2±6.37 | 12.5±5.28 | 16.6±11.2 | 0.065, 0.695 |
| M-OBA (MFI) | 7.79±2.60 | 8.52±4.28 | 10.1±3.57 | 10.1±4.53 | 9.08±3.04 | 9.90±3.53 | 7.98±2.32 | 11.2±9.43 | 0.384, 0.529 |
| Total Leukocytes (10$^{-9}$·L$^{-1}$) | 6.57±1.39 | 5.95±1.47 | 6.54±1.13 | 5.90±1.37 | 12.6±2.63 | 11.9±4.0 | 5.26±1.08 | 5.27±1.27 | <0.001, 0.825 |

**Note:** Mean ± SD. IL-6, interleukin-6; IL-8, interleukin-8; TNF, tumor necrosis factor; MCP-1, monocyte chemo attractant protein-1; CRP, C-reactive protein; G-OBA, granulocyte oxidative burst activity; M-OBA, monocyte oxidative burst activity; MFI, mean fluorescent intensity.

**Fig. 1.** (A) Granulocyte phagocytosis pre-supplementation (Pre-Supp), post-supplementation (Post-supp) and immediately pre-exercise (Pre-Ex), and 14-h post exercise (14-h post). $p < 0.001$ (time), $p = 0.194$ (interaction). (B) Monocyte phagocytosis pre-supplementation, post-supplementation and immediately pre-exercise, and 14-h post exercise. $p < 0.001$ (time), $p = 0.093$ (interaction).

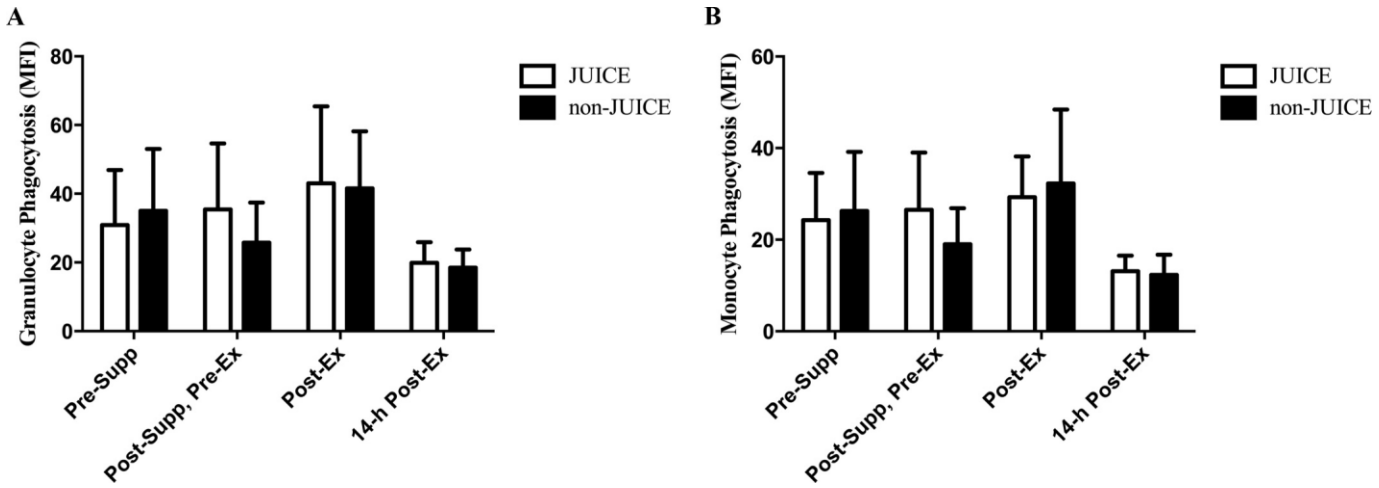


**Table 3.** Oxidative stress and antioxidant capacity markers.

| | Pre-supplementation | | Post-supplementation | | Post-exercise | | 14-h post-exercise | | |
|---|---|---|---|---|---|---|---|---|---|
| Inflammatory markers | JUICE | NonJUICE | JUICE | NonJUICE | JUICE | NonJUICE | JUICE | NonJUICE | *p* value (time, interaction) |
| ORAC (tolox equivalents) | 18090±7093 | 16558±5712 | 20098±4363 | 20166±3764 | 20564±5095 | 20471±5286 | 18646±3901 | 17593±3621 | 0.004, 0.847 |
| Protein Carbonyls (pg·mL$^{-1}$) | 0.80±0.43 | 0.77±0.43 | 1.11±0.73 | 1.21±0.77 | 1.25±0.77 | 1.10±0.41 | 1.49±0.94 | 1.67±1.65 | <0.001, 0.800 |
| FRAP (ascorbic acid equivalents) | 540±106 | 524±88.5 | 533±103 | 517±75.9 | 602±103 | 579±110 | 549±89.2 | 552±96.1 | <0.001, 0.573 |
| GSH–GSSG | 22.6±15.5 | 16.2±6.3 | 25.9±17.0 | 19.7±9.55 | 16.4±9.53 | 19.1±7.13 | 20.70±9.28 | 19.6±10.3 | 0.113, 0.091 |

**Note:** ORAC, oxygen radical absorbance capacity; FRAP, ferric reducing ability of plasma; GSH, glutathione; GSSH, glutathione disulfide (reduced form).

2007), oxidative stress (McAnulty et al. 2008; Shanely et al. 2010), or immune function (Nieman et al. 2007). Combinations of flavonoids may be more bioavailable in the body, and thus, more efficacious in mediating health benefits (Lila 2007). For example, quercetin combined with fish oil and green tea extract had a positive effect on exercise-induced inflammation and immune changes following 3 days of intense exercise in trained cyclists (Nieman et al. 2009).

A wide range of flavonoid and polyphenol doses in fruit and vegetable extracts have been used in exercise-related studies, and much remains to be learned regarding the optimal dosing regimens. Some studies have achieved success in attenuating post- exercise oxidative stress and inflammation using low-to-moderate doses of polyphenols from concentrated extracts of blackcurrents (Lyall et al. 2009), cherry juice blend (Connolly et al. 2006), and other mixed fruit–vegetable concentrates (Bloomer et al. 2006). In our previous study with a fresh fruit–vegetable cocktail supplement in elite swimmers, no effect of the supplement was observed in markers of inflammation, oxidative stress, or immune function post workout (Knab et al. 2013). However, despite the intense nature of the workouts, the rest intervals between sprinting bouts of these athletes led to little or no increases in these measures of physiologic stress, leaving little room for the juice supplement to exert an effect.

In the current study, it was hypothesized that a freeze-dried version of the fresh fruit–vegetable juice providing 37% more polyphenols than that used with the swimmers (Knab et al. 2013), would be more efficacious in reducing inflammation, oxidative stress, and maintaining immune function after 3 days of intense cycling with trained cyclists. Despite the significant effects of ex

ercise in increasing inflammation, oxidative stress, and immune changes, 17 days of JUICE ingestion had no effect on the pattern of change with these biomarkers. The lack of effect from JUICE supplementation may be due to several factors including the dose of polyphenols and the use of highly trained subjects. The dose of polyphenols (696 GAE) in this study is similar to other studies that resulted in positive findings, but with non-athletes (Bloomer et al. 2006; Connolly et al. 2006; Lyall et al. 2009). Highly trained indi- viduals have highly efficient endogenous enzyme systems that result in lower chronic oxidative stress and inflammation, as well as more efficient responses of these systems to an exercise bout (Serrano et al. 2010; Wagner et al. 2010). Thus for most athletes, who may also have sufficient intake of fruits and vegetables in the diet, supplementation with polyphenols and other antioxidants may have little effect on measures of post-exercise oxidative stress and inflammation unless high doses or unique cocktails are ingested for longer periods of time (Margaritis and Rousseau 2008; Nieman et al. 2010). Polyphenols are thought to exert their bene- ficial anti-oxidative and anti-inflammatory effects through their influence in increasing antioxidant capacity and down-regulating genes related to inflammatory processes (Lyall et al. 2009; Williamson and Manach 2005).

In summary, 473 mL (16 fluid ounces) per day of JUICE for 17 days did not counter decreases in phagocytosis or increases in oxidative stress and inflammation after a 3-day period of intensified exercise in trained cyclists. Additional research is needed to determine the optimal polyphenol dosing paradigm for athletes to lessen post-exercise physiologic stress, and whether JUICE may prove more efficacious in healthy but untrained subjects who lack highly developed endogenous physiological defense mechanisms.

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