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Effects of Antioxidant Consumption on Inflammation and Muscle Soreness at Moderate Altitude in Collegiate Football Student-Athletes

Original Research

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Abstract

Introduction: Athletics training and competition is associated with increased oxidative stress and inflammation, especially at moderate altitude. This results from an increased formation of reactive oxygen species due to increased metabolic activity of working cells and tissues as well as decreased oxygen pressure. The oxidative stress can cause inflammation and delayed onset muscle soreness (DOMS), which can be a serious problem for athletes training and competing at a high caliber, as it interferes with optimal sport performance and recovery. A diet intervention was developed to study the relationship between antioxidant-rich food consumption and implications per reduced inflammation and DOMS among athletes.

Methods: During summer 2021, 32 NCAA Division 1 collegiate football student-athletes living and training at moderate altitude were recruited to participate in a seven-week nutrition intervention. Participants were assigned to either an intervention or control group. Participants in the intervention group consumed at least 10,000 Oxygen Radical Absorbance Capacity (ORAC) score units per day through an antioxidant-rich trail mix constructed by the research team. Participants consumed this trail mix in addition to their normal diet. Participants in the control group did not receive trail mix and continued their normal diet. Inflammation was measured pre- and post- intervention through blood biomarkers (high sensitivity C-reactive protein, hs-CRP; Interleukin-6, IL-6) and urine sample analysis (Isoprostane Creatinine ratio, F2/C). DOMS was measured through a pre- and post- survey (Numerical Pain Rating Scale, NPRS).

Results: An independent samples T-test identified the change in mean ORAC scores for the intervention group (M±SD, 149121 units ± 18357 units) was statistically higher than the change in mean ORAC score for the control group (28391 units ± 15359 units): $t(30)=-20.02$, $p<.001$. Paired samples T-tests indicated that hs-CRP and F2/C did not change significantly between time 1 (hsCRP: 1.31 mg/dL ±1.28 mg/dL) (F2/C: 0.41 ± 0.23) and time 2 (hsCRP: 1.50 mg/dL ± 1.92 mg/dL) (F2/C: 0.42 ± 0.11). Separate regression analyses identified pre- F2/C as a significant predictor of post- F2/C for the control group ($B=0.399$, $p<0.001$) and mean ORAC score as a significant predictor of post- F2/C for the intervention group ($B=-3.604E-6$, $p=0.028$). Repeated measures

ANOVA indicated no significant effect of time ($F(1,27)=0.399$, $p=.533$), or group by time ($F(1,27)=0.521$, $p=.477$) on DOMS.
Conclusions: Antioxidant-rich food consumption had minimal impact on inflammation or DOMS induced by physical exercise at moderate altitude among this sample of collegiate football student-athletes. Future research is required to assess the relationship between antioxidant consumption and implications per reduced inflammation and DOMS for student-athletes participating in other collegiate sports.

Key Words: Sports Nutrition, Oxidative Stress, Inflammatory Markers, Delayed Onset Muscle Soreness

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Introduction
Oxidative stress results from high levels of free radicals and low levels of antioxidants in the body. This phenomenon is caused by an imbalance of reactive oxygen species (ROS) and the body's ability to remove these products through antioxidants.[1,4] Although ROS comprise both free radical and non-free radical oxygen intermediates, the terms ROS and free radicals are used interchangeably. Free radicals are molecules with unpaired electrons, which make them extremely unstable. To increase their stability, free radicals scavenge the body to find other electrons to make a pair.[1] This transforms another molecule into a free radical, creating a continual series of unpairing and pairing reactions, which ultimately causes damage to cells and tissues.[1] Antioxidants are molecules that prevent free radicals from sequestering electrons and causing damage. Antioxidants donate electrons to free radicals without becoming destabilized.[1] This ends these cyclical reactions and prevents further damage to cells and tissues. Oxidative stress results when the number of free radicals surpasses the number of antioxidants in the body.[4]

Unregulated oxidative stress can cause acute and chronic inflammation.[5,6] Acute inflammation is the first stage of inflammation mediated through the immune system6 and is usually considered beneficial for the body existing for a short time period.[6] If the inflammation persists, chronic inflammation, the second stage, transpires. Prolonged periods of chronic inflammation can cause cell and tissue damage, often the precursor for many chronic diseases and conditions.[7] Oxidative stress activates numerous transcription factors involved in differential expression of genes responsible for inflammatory processes.4 Additionally, oxidative stress stimulates inflammatory mediators related to chronic disease and condition development and progression.[4,5] Oxidative stress and inflammation associated with the overproduction of free radicals contribute to the development of diabetes mellitus, cardiovascular disease, and neurological disorders.[4,5]

Physical exercise at moderate altitude (~1900-2400 m/6200-7900 ft. above sea level) increases formation of free radicals due to increased metabolic activity of working cells and tissues as well as decreased oxygen pressure.[1,2,8] At moderate altitude, studies have shown a greater increase in oxidative stress and inflammation compared to training at low altitudes. Of note, one study demonstrated elite athletes training at moderate and high altitudes experienced more significant oxidative stress as well as greater cell and tissue damage.[7] There is a need for athletes at moderate altitude to reduce oxidative stress and inflammation to optimize sport performance and recovery. Through the addition of antioxidant-rich foods, oxidative stress and inflammation may be minimized to help maintain training and competing at optimal levels. It may be possible to decrease inflammation and delayed onset muscle soreness (DOMS) through diet alone. Multiple studies have been conducted on the effect of antioxidant supplementation per sport performance and recovery, yet few have explored the effect of antioxidant-rich foods.[1,2] Results are often inconclusive and demonstrate a need for more research in this field.[1,2] Additionally, most studies at moderate altitude are conducted among athletes traveling short-term, not among athletes living, training, and competing at moderate altitude long-term.

One method to increase antioxidant consumption is to incorporate a high Oxygen Radical Absorbance Capacity (ORAC) diet. The ORAC scoring system is a laboratory analysis that provides an overall measure of antioxidant activity for individual foods. The higher the ORAC score, the greater the food antioxidant capacity. Research has shown high ORAC consumption to be safe and effective for health benefits including the potential of reduced oxidative stress and inflammation.[9]

This nutrition intervention examined the effects of antioxidant consumption through antioxidant-rich foods on inflammation and DOMS among athletes living, training, and competing at moderate altitude. The overall objective was to determine whether an increase in the consumption of antioxidant-rich foods could decrease oxidative stress, inflammation and DOMS for athletes.

Scientific Methods

During summer 2021, a seven-week controlled pilot trial was implemented to examine effects of antioxidant consumption on inflammation and DOMS in NCAA Division 1 collegiate football student-athletes at moderate altitude. This study design allowed for prospective research, which is ideal for measuring the effectiveness of a new nutrition intervention.10 A seven-week period was the length of time collegiate football student-athletes were training on campus. Altitude of the study location is ~2106 m/6909 ft. above sea level. All research protocols and procedures were examined and approved by the Institutional Review Board of the university prior to participant recruitment.

Participants

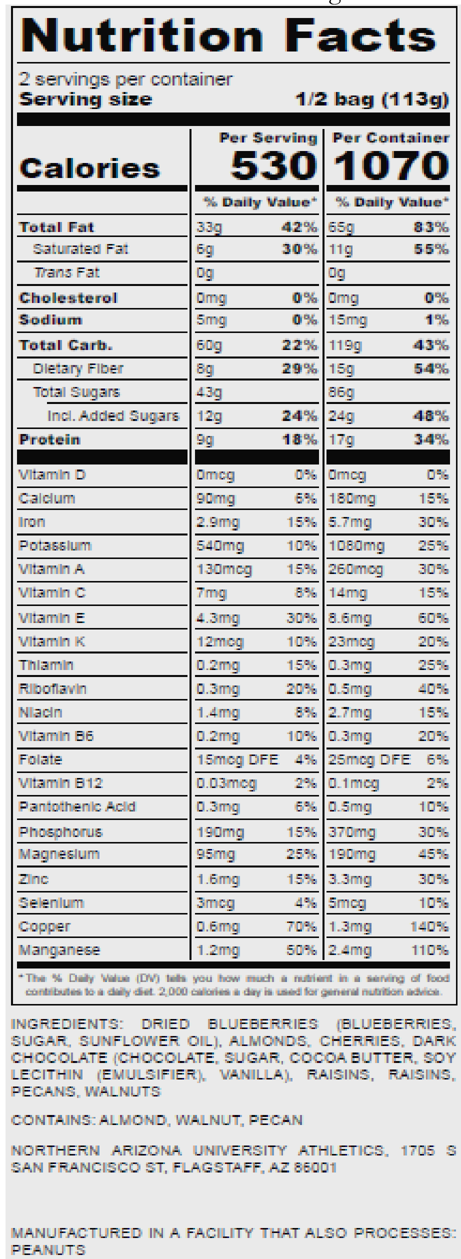
The study population consisted of 32 NCAA Division 1 collegiate football student-athletes living, training, and competing at moderate altitude. Participants were recruited through the football organization and email marketing. Members of the football team received a recruitment email message with information about the diet intervention. Those who were interested in participation were required to fill out a survey explaining their desire to participate and disclose any medical diseases/conditions. The research team, along with members of the football organization, reviewed applicants and made decisions on the final sample of participants. Individuals were selected based on ability to participate in all aspects of the nutrition intervention. All recruited participants completed and submitted an online Qualtrics survey as an initial screening assessment, which included informed consent documentation. Per the initial assessment, all participants identified as 18-25 years old, male sex, enrolled in school fulltime, and football student-athletes. Additionally, participants were free of any injuries or nutrition-related medical diseases/conditions (Celiac Disease, Irritable Bowel Disease, Type 1 Diabetes Mellitus, disordered eating or eating disorder, etc.), as these would limit participation in training and competing or impact results per the nutrition intervention. All participants were followed for a seven-week period, with an additional one-day pre- and post-intervention.

Protocol

Participants were assigned by the research team to either a control or intervention group. All participants recorded daily antioxidant intake through an Oxygen Radical Absorbance Capacity (ORAC) score template. An educational handout containing ORAC score units was also provided to the intervention group to enhance antioxidant-rich food consumption. The ORAC score template allowed athletes to track daily food and beverage consumption so researchers could compute average ORAC score units using validated calculations.9,18 It is estimated the average American consumes 3000-5000 ORAC score units per day, with 10000-12000 ORAC score units exhibiting a significant effect on antioxidant levels and subsequent protection against oxidative stress.18 Thus, it was recommended the intervention group consume at least 10000 ORAC score units per day. To achieve this recommendation, the intervention group was instructed to consume an antioxidant-rich trail mix constructed by the research team everyday (i.e., two servings/the entire bag) throughout the seven-week period. The trail mix consisted of dried fruits (1 oz golden seedless raisins, 1 oz raisins, 1 oz dried blueberries, 1 oz dried cherries), nuts (1 oz raw almonds, 1 oz raw walnuts, 1 oz raw pecans), and 1 oz dark chocolate chips (60-69% cacao) and exceeded 10000 ORAC score units to ensure the intervention group was meeting recommended antioxidant consumption levels (Figure 1). The control group continued consumption of their usual diet. The ORAC score template was distributed weekly to all participants via virtual spreadsheets or in paper format upon request to track antioxidant intake. The intervention group received trail mix distributions weekly at the team training location.

All participants underwent pre- and post-intervention assessment at the Laboratory for Exercise and Nutrition (LEAN) location on campus. Assessments were conducted one-day after a workout pre- and post- intervention. An online Qualtrics survey collected several descriptive statistics, including anthropometric and sociodemographic characteristics. Nutrition information was also collected to assess eating habits, grocery shopping, cooking, and supplementation. These components of the online survey were developed by the research team. High sensitivity-C-reactive protein (hs-CRP) and Interleukin-6 (IL-6) were measured through separate serum samples collected and analyzed at an independent lab. Both measurements have diagnostic accuracy and reliability and are considered two of the best biomarkers used to assess inflammation.11 Unfortunately, IL-6 results were inconclusive due to laboratory error; all measurements pre- and post- study for participants were <2.5 pg/mL, which indicates an undetectable level of inflammation. Researchers also measured F2-Isoprostane Creatinine ratio (F2/C) through urine sample analysis. F2-Isoprostane/Creatinine (F2/C) ratio is considered one of the best biomarkers for measurement of oxidative stress.12,13 Delayed Onset Muscle Soreness (DOMS) was measured through the Numerical Pain Rating Scale (NPRS) survey, which asked participants to rate pain intensity on a scale of zero to ten (zero represents 'no pain at all' and ten represents 'the worst pain ever possible').14 Non-fasting, separate serum samples were collected by licensed phlebotomists, while urine was collected by the research team. The NPRS survey was distributed to participants by researchers in paper format to complete and submit.

Figure 1. Nutrition Facts Label for High ORAC Trail Mix



Nutrition Facts

2 servings per container
Serving size **1/2 bag (113g)**

Calories	**Per Serving 530**		**Per Container 1070**	
		% Daily Value*		**% Daily Value***
Total Fat	33g	**42%**	65g	**83%**
Saturated Fat	6g	**30%**	11g	**55%**
Trans Fat	0g		0g	
Cholesterol	0mg	**0%**	0mg	**0%**
Sodium	5mg	**0%**	15mg	**1%**
Total Carb.	60g	**22%**	119g	**43%**
Dietary Fiber	8g	**29%**	15g	**54%**
Total Sugars	43g		86g	
Incl. Added Sugars	12g	**24%**	24g	**48%**
Protein	9g	**18%**	17g	**34%**
Vitamin D	0mcg	0%	0mcg	0%
Calcium	90mg	6%	180mg	15%
Iron	2.9mg	15%	5.7mg	30%
Potassium	540mg	10%	1080mg	25%
Vitamin A	130mcg	15%	260mcg	30%
Vitamin C	7mg	8%	14mg	15%
Vitamin E	4.3mg	30%	8.6mg	60%
Vitamin K	12mcg	10%	23mcg	20%
Thiamin	0.2mg	15%	0.3mg	25%
Riboflavin	0.3mg	20%	0.5mg	40%
Niacin	1.4mg	8%	2.7mg	15%
Vitamin B6	0.2mg	10%	0.3mg	20%
Folate	15mcg DFE	4%	25mcg DFE	6%
Vitamin B12	0.03mcg	2%	0.1mcg	2%
Pantothenic Acid	0.3mg	6%	0.5mg	10%
Phosphorus	190mg	15%	370mg	30%
Magnesium	95mg	25%	190mg	45%
Zinc	1.6mg	15%	3.3mg	30%
Selenium	3mcg	4%	5mcg	10%
Copper	0.6mg	70%	1.3mg	140%
Manganese	1.2mg	50%	2.4mg	110%

*The % Daily Value (DV) tells you how much a nutrient in a serving of food contributes to a daily diet. 2,000 calories a day is used for general nutrition advice.

INGREDIENTS: DRIED BLUEBERRIES (BLUEBERRIES, SUGAR, SUNFLOWER OIL), ALMONDS, CHERRIES, DARK CHOCOLATE (CHOCOLATE, SUGAR, COCOA BUTTER, SOY LECITHIN (EMULSIFIER), VANILLA), RAISINS, RAISINS, PECANS, WALNUTS

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MANUFACTURED IN A FACILITY THAT ALSO PROCESSES: PEANUTS

Statistical Analysis

Due to the exploratory nature of the nutrition intervention, power analyses to determine required sample size was not conducted. Rather, one of the research goals included the feasibility of conducting a diet intervention among collegiate athletes during summer training. Frequencies were determined for all categorical variables, while means and standard deviations were calculated for all continuous variables. Mean ORAC scores for each participant were calculated using averages from week one through seven. Mean ORAC scores for participants in the intervention group were combined to calculate an overall grand mean score; this was also calculated for the control group. An independent t-tests were used to examine differences in mean ORAC scores between the intervention and control groups. To examine differences in high-sensitivity C-Reactive Protein (hs-CRP) and Isoprostane Creatinine ratio (F2/C) among the entire sample without including the effects of condition, paired samples t-tests were conducted. To determine if the diet intervention impacted changes in hs-CRP and F2/C over time, multiple linear regression models were used to examine hs-CRP and F2/C at seven weeks, using baseline hs-CRP and F2/C as covariates, respectively; mean ORAC score, condition (intervention vs control), and the interaction between mean ORAC score and condition were used as predictors. Differences in DOMS over time and between conditions were examined using repeated measures ANOVA. All analyses were conducted using SPSS version 27. A $p \leq 0.05$ was considered statistically significant for all analyses.

Results
A total of 32 participants completed the study; 17 were assigned to the intervention group, while the other 15 participants were assigned to the control group. Anthropometric and sociodemographic characteristics are outlined in Table 1. Participant nutrition information is summarized in Table 2.

Table 1. Participant Anthropometrics and Sociodemographics (n=32)

	M ± SD	**Percent (%)**
Height (cm)	184.0 ± 6.45	
Weight (kg)	95.51 ± 16.55	
Age (years)	20.75 ± 1.52	
Sex		
Male		100
Female		0

Table 2. Participant Nutrition Information (n=32)

	Participants, Percent (n, %)
Eating Habits	
Excellent	4, 13
Good	19, 59
Average	9, 28
Poor	0, 0
Terrible	0, 0
Independent Grocery Shopping	
Yes	30, 94
No	2, 6
Independent Cooking	
Yes	32, 100
No	0, 0
Supplementation	
Yes	17, 53
No	15, 47

The independent t-test identified the intervention group had a statistically higher mean ORAC score (149121 units ± 18357 units) compared to the control group mean ORAC score (28391 units ± 15359 units) ($p<0.001$).

Paired samples t-tests indicated no statistically significant change in hs-CRP pre- to post- study for either group (intervention: mean difference 2.74, $p=0.494$; control: mean difference 2.13, $p=0.327$) and no change in F2/C over time for either group (intervention: mean difference 0.27, $p=0.406$; control: mean difference 0.22, $p=0.399$). Multiple linear regression results identified no significant predictors of hs-CRP. Multiple linear regression did indicate statistical significance for F2/C at time 2 (pre- to post- intervention) ($R^2=0.381$, $F(4,26)=3.99$, $p=0.012$). Separate regression analyses were conducted for each condition. Results determined the overall model was not statistically significant for F2/C at time 2 for the intervention group ($R^2=0.321$, $F(2,13)=3.07$, $p=0.081$), but mean ORAC score was a significant predictor ($p=0.028$). Results did determine the overall model was statistically significant for F2/C at time 2 for the control group ($R^2=0.716$, $F(2,12)=15.13$, $p<0.001$), with baseline F2/C as the only significant correlate ($p<0.001$, Tables 3 and 4).

Table 3. Mean difference from paired samples t-tests of hsCP and F2/C biomarkers for intervention (n=17) and control (n=15) groups

	Intervention Mean Difference	Intervention p-value	Control Mean Difference	Control p-value
hs-CRP (mg/dL)	2.74	0.494	2.13	0.327
F2/C	0.27	0.406	0.22	0.399

Data are Means ± SD
*Statistical significance at p<0.05

Table 4. Multiple linear regression results of hsCRP and F2/C biomarkers with baseline values, mean ORAC score, study condition, and interaction of ORAC score and condition as predictors

Biomarker	Association	R^2	B	95% CI	p-value*
hs-CRP[a] (mg/dL)	Baseline	0.44	0.38	0.06, 0.70	0.02
	Mean ORAC[c] Score		2.98E-5	0.00, 0.00	0.18
	Condition (Intervention, Control)		-1.12	7.35, 5.11	0.71
	ORAC x Condition		-2.42E-5	0.00, 0.00	0.42
F2/C[b]	Baseline	0.38	0.22	0.07, 0.37	0.01
	Mean ORAC Score		-9.57E-7	0.00, 0.00	0.55
	Condition (Intervention, Control)		0.60	0.18, 1.02	0.01
	ORAC x Condition		-3.15E-6	0.00, 0.00	0.14

[a]High-sensitivity C-Reactive Protein
[b] Isoprostane Creatinine ratio
[c]Oxygen Radical Absorbance Capacity
*Statistical significance at p<0.05

Repeated measures ANOVA evaluated changes in DOMS; results showed no significant effect over time ($F(1,27)=0.399$, $p=0.533$) nor a significant effect by condition ($F(1,27)=0.521$, $p=0.477$).

Discussion

The goal of this controlled pilot trial was to determine if consumption of an antioxidant-rich trail mix decreased oxidative stress, inflammation, and DOMS among NCAA Division 1 collegiate football student-athletes. Although there were no between-group differences seen in hsCRP or DOMS, the mean ORAC score was significantly higher for the intervention group compared to the control group. Among the intervention group, mean ORAC score was a significant predictor of F2/C post-intervention. Over 70% of participants reported their eating habits as good or excellent, and all reported cooking independently. Physically, participants were representative of NCAA Division 1 collegiate football student-athletes; most players have similar anthropometric and sociodemographic characteristics as well as health habits as it relates to nutrition information.[15] Mean ORAC score increased among both the intervention group and control group. Consumption of the antioxidant-rich trail mix caused changes in mean ORAC score among the intervention group to be significantly greater compared to the control group. This result demonstrates changes in diet intake can improve mean ORAC score, reflective of increased antioxidant capacity and nutrient-density, among collegiate football student-athletes. Most studies attempting to improve antioxidant capacity or nutrient-density within football student-athletes or other collegiate or professional sports implement a diet supplement.[1-4,10-11] This is one of the first studies to explore the effects of a nutrition intervention on antioxidant capacity and nutrient-density within the targeted study population.

Although mean ORAC scores increased for both groups and was significantly greater for the intervention group, no changes in hs-CRP were observed over time or between groups. This result implies no differences in acute inflammatory response occur with an increased diet intake of antioxidant-rich food during the short-term, as hs-CRP is an acute inflammatory protein that exhibits elevated expression during inflammatory processes.[16] However, a slight increase in hs-CRP existed among the control group over time (pre- 1.34 mg/dL ± 1.43 mg/dL; post- 1.73 mg/dL ± 1.39 mg/dL), while the intervention group hs-CRP remained relatively consistent pre- (1.28 mg/dL ± 1.20 mg/dL) to post- (1.29 mg/dL ± 2.32 mg/dL) study. Similar studies in non-athletic populations have suggested an inverse relationship between mean ORAC score and hs-CRP.[3,17] A more long-term diet intervention in an athletic population may identify similar results.

There were differences in F2/C outcomes with an increase in mean ORAC scores, specifically among the intervention group. An increase in mean ORAC score to at least 10000 score units per day led to a statistically significant decrease in F2/C pre- to post- study. A statistically significant decrease in this measure indicates the nutrition intervention decreased oxidative stress; studies with antioxidant supplementation have identified similar results.[12-13,18] Although differences in F2/C measures over time between the intervention and control groups did not emerge, participants with highest mean ORAC scores showed the lowest F2/C measures, indicating higher antioxidant consumption may lead to lower oxidative stress. Although participation in the nutrition intervention did not result in a statistically significant change in F2/C over time, there is promise that higher antioxidant consumption in general could cause decreased oxidative stress among athletic populations.

No change in DOMS at 24 hours after exercise occurred within the control group or intervention group, even with statistically significant change in mean ORAC score. This result suggests that increased diet intake of antioxidant-rich food does not impact DOMS among football student-athletes short-term. There is moderate evidence antioxidant consumption through diet supplement decreases objective DOMS at 24, 48, 72, and 96 hours after exercise; however, there is no evidence available on long-term diet intake of antioxidant-rich food or subjective recovery.[19] This indicates longer duration of increased mean ORAC score through diet intake and improved study methods may find decreased DOMS among football student-athletes.

Strengths of this study included an experimental study design, which enabled examination of the cause-and-effect relationship between consumption of antioxidant-rich food, inflammation, and DOMS. All subjective data were collected using various reliable and validated surveys. Objective blood samples were collected by licensed phlebotomists, and urine samples were collected by the research team. Additionally, this study helped identify missing components in research regarding the potential benefits of antioxidant-rich food consumption for athletic populations. Although this was a pilot study, the nutrition intervention was successfully implemented among collegiate football student-athletes which can often be extremely difficult to accomplish.

Limitations of this study included its setting at moderate altitudes as well as sample of collegiate football student-athletes; the specific setting and sample may indicate the findings cannot be extrapolated to other altitudes or athletes.[19] Selection bias may also be present, as participants volunteered to be included in the study. Additionally, the study was not randomized; the research team selected participants to be included in the intervention and control groups. This choice was made to help ensure those participating in the diet intervention adhered to the strict protocols of antioxidant-rich trail mix consumption and mean ORAC score recording. Even so, it was difficult to ensure daily consumption of the antioxidant-rich trail mix and accurate recording of mean ORAC score. Finally, a seven-week period may not provide sufficient time to fully observe results of increased antioxidant consumption. Although a recent study demonstrated variation in oxidative stress after eight weeks, inflammation and DOMS may not be affected in such a short-term period.[20]

Overall, an increase in diet intake of antioxidant-rich food did not improve inflammation or muscle soreness among collegiate football student-athletes over a short-term seven-week period. However, there is some evidence from this study that increased diet intake of antioxidant-rich food may lower oxidative stress and subsequently lead to lower inflammation and DOMS long-term. Other studies suggest an increase in antioxidant consumption through supplementation may decrease inflammation and muscle soreness among collegiate football student-athletes as well as other athletic populations.[3,17,19]

Conclusions

This study is one of few to examine the effects of increased antioxidant consumption through diet intake on inflammation and muscle soreness among athletic populations. Results demonstrated a statistically significant difference in improvement of mean ORAC score between intervention and control groups over a short-term seven-week period. The increase in mean ORAC score was a predictor of decreased oxidative stress. However, these results did not lead to statistically significant changes in inflammation or muscle soreness among collegiate football student-athletes. Some evidence evolved to indicate long-term antioxidant-rich food consumption may decrease inflammation and muscle soreness for athletic populations.

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