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Effect of resistance exercise and carbohydrate ingestion on oxidative stress

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Abstract
Some research studies have produced data indicating that resistance exercise induces oxidative stress, despite minimal increases in VO_2. These studies have primarily relied on oxidative stress markers with low sensitivity and debatable reliability. However, F_2-isoprostanes as measured by gas chromatography mass spectrometry are considered to be a reliable and precise indicator of oxidative stress. Carbohydrate ingestion during exercise is associated with reduced levels of stress hormones, which may influence oxidative stress and plasma antioxidant potential. Therefore, the purpose of this study was to investigate the influence of carbohydrate ingestion during resistance training on F_2-isoprostanes and plasma antioxidant potential. Thirty strength-trained subjects were randomized to carbohydrate (CHO) or placebo (PLA) groups that lifted weights for 2 h. Subjects received 10 ml kg^{-1} h^{-1} CHO (6%) or PLA beverages during the exercise. Blood and vastus lateralis muscle biopsy samples were collected before and after exercise and analyzed for cortisol as a marker of general stress, F_2-isoprostanes as a measure of oxidative stress, and ferric reducing ability of plasma (FRAP) as a measure of antioxidant potential, and for muscle glycogen, respectively. Decreases in muscle glycogen content did not differ between CHO and PLA. Cortisol and FRAP increased significantly in CHO and PLA ($P = 0.008$ and 0.044, respectively), but the pattern of change was not different between groups. F_2-isoprostanes were unaffected by exercise. These results indicate that exhaustive resistance exercise and carbohydrate ingestion have no effect on oxidative stress or plasma antioxidant potential in trained subjects.

Keywords: *Antioxidant potential, F_2-isoprostanes, muscle glycogen, cortisol*

Introduction

Both long-duration and intense acute aerobic exercise can result in increased formation of reactive oxygen species (ROS), resulting in lipid peroxidation and protein alteration [1–5]. The increased oxidative stress is thought to primarily occur as a result of a mass action effect on oxygen consumption (VO_2) [1]. The effect of resistance exercise on oxidative stress has been minimally studied [6–8]. Despite the fact that resistance exercise does not dramatically increase VO_2, Allesio et al. [1] found that isometric, as well as, aerobic exercise increased oxidative stress. Thus, other mechanisms beside the mass action effect of VO_2 may be responsible for increasing oxidative stress during intense muscular contraction. In contrast, Rall et al. [9] found that 12 weeks of progressive resistance exercise did not increase oxidative stress, as measured by urinary 8-hydroxy-2′-deoxyguanosine.

Exhaustive exercise dramatically increases plasma levels of catecholamines which can undergo auto-oxidation to form ROS [10]. Cortisol is typically increased along with catecholamines [11]. Carbohydrate, compared to placebo ingestion has been

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shown to improve maintenance of blood glucose levels, causing a decrease in release of ACTH, cortisol and epinephrine [12]. Thus, carbohydrate supplementation might result in attenuation of oxidative stress by decreasing stress hormone levels. Another method by which carbohydrate might reduce oxidative stress is through increased production of reducing equivalents. Lord-Fontaine et al. [13] reported, for example, that glucose administration protected Chinese hamster ovary cells from heat shock and hydrogen peroxide administration by increasing NADPH from the hexose monophospate shunt.

There is little published data available regarding the effect of carbohydrate on ROS production and antioxidant status, and these studies have used aerobic exercise as the mode [14,15]. We are not aware of any data which has examined oxidative stress with carbohydrate ingestion during resistance exercise. Therefore, we examined whether exhaustive resistance exercise would result in increased oxidative stress and what effect carbohydrate supplementation (1L/h, 6% solution) might have on oxidative stress and plasma antioxidant capacity. We hypothesized that exhaustive resistance training would increase oxidative stress and that carbohydrate ingestion would result in diminished oxidative stress. We hoped to improve upon previous studies by using F_2-isoprostanes as the indicator of oxidative stress. F_2-isoprostanes are regarded as a very sensitive and stable marker of lipid peroxidation [16].

Methods

Subjects

Thirty strength trained athletes were recruited through mass advertising. Male subjects ranging in age from 19 to 27 years were accepted into the study if they had a minimum of six months of total body resistance training experience, were able to back squat to mid-thigh parallel at least 1.25 times their body mass, and were willing to adhere to all aspects of the research design. Subjects agreed to avoid the use of large-dose vitamin/mineral supplements (above 100% of recommended dietary allowances), nutritional supplements, ergogenic aids, herbs and medications for 1 week prior to test sessions. During orientation, a dietitian instructed the subjects to follow a diet moderate in carbohydrate during the 3 days prior to the test session and record intake in a food record. The food records were analyzed using a computerized dietary assessment program (Food Processor, ESHA Research, Salem, Oregon). Informed consent was obtained from each subject, and the experimental procedures were in accordance with the policy statements of the institutional review board of Appalachian State University (ASU).

Research design

Three to six weeks prior to the resistance training session, subjects reported to the ASU Human Performance Lab for orientation and measurement of body mass, body composition and strength. Body composition was assessed from a 3-site skinfold test using a Lange skinfold caliper (Cambridge Scientific Industries, Inc., Cambridge, MD). One-repetition maximum (1-RM) strength was assessed over two testing sessions. Following a two-set warm-up, subjects were allowed three attempts at a given weight to attain 1-RM. Flat bench press, back squat, military press, bent-over row and biceps curl were assessed during the first 1-RM testing session. Incline bench press, front squat, deadlift, upright row and French curl were assessed during the second 1-RM testing session. A standard Olympic barbell was used for all exercises (York Barbell Company, York, PA). Basic demographic and training data were obtained through a questionnaire. After orientation and baseline testing, subjects were randomized to carbohydrate and placebo groups.

Test sessions

A standardized liquid meal (Boost Plus, Mead Johnson Nutritionals, Evansville, IN) was ingested at an energy level of 12 kcal/kg body mass by all subjects prior to 10:00 am on the day of the resistance training session. Subjects next reported to the lab at 3:00 pm, not having ingested energy in any form for at least 5 h, and received carbohydrate (6% or 60 g/l) or placebo beverages (artificially sweetened) 15–30 min pre-exercise (8 ml/kg) and during the 2-h resistance training bout ($10\,ml\,kg^{-1}\,h^{-1}$). The beverages were supplied by the Gatorade Sports Science Institute (Barrington, IL) as in earlier studies [20–22,25]. The carbohydrate and placebo beverages were identical in appearance and taste, sodium ($\sim$19.0 mEq/l) and potassium ($\sim$3.0 mEq/l) concentration and pH ($\sim$3.0). No other beverages or food were ingested during the 2-h resistance training bout or for 1-h post-exercise.

Blood and skeletal muscle biopsy samples were collected $\sim$30 min pre-exercise and immediately post-exercise, with additional blood samples collected 1-h post-exercise. Blood samples were obtained by venipuncture of the antecubital vein and drawn into heparinized vacutainer tubes. The tubes were immediately placed on ice and then spun at 1500*g* for 10 min at 4°C. The plasma was aliquoted into cryotubes, snap frozen in liquid nitrogen and stored at −80°C until analysis. Biopsies were obtained as described below.

The exercise session consisted of 10 different resistance exercises: flat bench press, incline bench press, military press, upright row, bent-over row,

French curl, biceps curl, back squat, front squat and deadlift. Subjects performed 4 sets of 10 repetitions for each resistance exercise, with the first set at 40% of the subject's 1-RM and the subsequent sets at 60% 1-RM. Two minute rest intervals separated sets for the bench press, incline bench press, military press, upright row, bent-over row, French curl and biceps curl. Three minute rest intervals were given following the completion of each exercise and between sets for the back squat, front squat and deadlift.

Skeletal muscle biopsies

Skeletal muscle biopsy samples were acquired before and after exercise following blood sample collection. The exact same procedures were utilized pre- and post-exercise, with incisions made in the same thigh, approximately 3 cm apart. Local anesthesia (1% xylocaine) was injected subcutaneously and into the vastus lateralis. A muscle biopsy sample was then obtained using the percutaneous needle biopsy procedure modified to include suction [17]. Muscle biopsy samples were divided into two pieces and immediately frozen in liquid nitrogen. Samples were stored at -80°C until subsequent muscle glycogen analysis.

Muscle glycogen analysis

Samples were later freeze dried, powdered and dissected free of connective tissue, blood and other non-muscle constituents. A portion of the muscle was extracted with acid, neutralized and glucosyl units were analyzed enzymatically in triplicate using a spectrophotometer [18].

Oxidative stress analysis

Plasma F_2-isoprostanes were determined using gas chromatography mass spectrometry according to the methodology of Morrow [16]. Briefly, free F_2-isoprostanes were extracted from 1 ml of plasma. One to five pmol of deuterated $[^2H_4]PGF_{2\alpha}$ internal standard was added and the mixture vortexed. This mixture was then added to a C_{18} Sep Pak column, followed by silica solid phase extractions. F_2-isoprostanes were converted into pentafluorobenzyl esters and then subjected to thin layer chromatography and then converted to trimethylsilyl ether derivatives. Samples were then analyzed by a negative ion chemical ionization GC-MS using a Nermag R10-10C mass spectrometer interfaced with a DEC-PDP 11/23 Plus computer system.

Plasma antioxidant potential

Total plasma antioxidant potential was determined by the FRAP assay were according to the methodology of Benzie [19]. The basis of this assay is that water soluble reducing agents (antioxidants) in the plasma will reduce ferric ions to ferrous ions, which then react with an added chromogen. Working FRAP solution was prepared daily and consisted of 300 mmol/l acetate buffer with the pH adjusted to 3.6 (3.1 g sodium acetate (Sigma, St Louis, MO) and 16 ml of 1 N acetic acid (Sigma, St Louis, MO) per liter of buffer solution; 10 mmol/l TPTZ (2,4,6-tripyridyl-s-triazine, (Sigma, St Louis, MO) in 40 mmol HCl (Fisher Scientific, Pittsburgh, PA); 20 mmol iron trichloride hexhydrate (Sigma, St Louis, MO) in DI water. Working FRAP reagent was prepared as required by mixing 25 ml acetate buffer, 2.5 ml TPTZ solution and 2.5 ml iron trichloride hexhydrate solution. The working FRAP solution was placed in a water bath and warmed to 37°C.

Then, 100 μl of either standard, sample or blank (deionized water), respectively were added to glass test tubes containing 3.0 ml of warmed FRAP reagent and vortexed. All tubes were then incubated at 37°C for 4 min and read at 593 nm. Samples and standards were analyzed in duplicate, and FRAP values were expressed as vitamin C equivalents as determined by linear regression from a vitamin C standard curve (0–1000 μmol).

Intra-and inter-assay coefficients of variation for the FRAP assay was <10 and 6%, respectively. All samples were corrected for plasma volume shift according to the methodology of Dill et al. [20].

Statistical analysis

Statistical significance was set at the $P < 0.05$ level and values expressed as means ± SEM. Performance measures were compared under carbohydrate and placebo conditions using student *t*-tests. All other data were analyzed using a two (carbohydrate and placebo conditions) × two or three (times of measurement) repeated measures ANOVA. If the condition × time interaction *P*-value was <0.05, the change from pre- to post-exercise values was calculated and compared between conditions using student *t*-tests after Bonferonni correction. Pearson product-moment correlations were used to test the relationship between changes in plasma and muscle measures. SPSS version 11.5 (Chicago, IL) was used for all statistical analysis.

Results

Table I summarizes characteristics by group status for the 30 subjects completing all phases of the study. Subjects in the CHO ($N = 15$) and PLA ($N = 15$) groups did not differ significantly for age, body mass and composition, or years of resistance training. CHO and PLA groups did not differ significantly in 1-RM for 10 different exercises (data not shown). Carbohydrate intake during the 3 days prior to the 2-h resistance

Table 1. Subject characteristics ($N = 30$). Values are means ± SEM.

Variable	Carbohydrate (N=15)	Placebo (N=15)	P-value
Age (years)	21.6 ± 0.5	21.3 ± 0.5	0.627
Stature (m)	1.79 ± 0.02	1.82 ± 0.01	0.117
Body mass (kg)	78.7 ± 2.4	83.1 ± 2.6	0.226
Body composition (% fat)	8.9 ± 1.1	10.5 ± 1.1	0.314
Upper body training (years)	5.2 ± 0.7	5.3 ± 0.5	0.922
Lower body training (years)	4.9 ± 0.8	4.6 ± 0.6	0.761

training test session did not differ significantly between subjects in the carbohydrate and placebo groups (53.9 ± 2.9% and 50.6 ± 2.2% of total energy intake, respectively, $P = 0.371$) (data not shown).

Volume loads for each of the 10 exercises and the total volume load did not differ significantly between CHO and PLA. Total loads for CHO and PLA groups were 19255 + 1022 and 19052 + 846 kg, respectively ($P = 0.879$) (data not shown). Plasma volume decreased less than 1.5% for both groups immediately following the 2-h resistance training bout, and a slight weight gain was measured (0.9 ± 0.1 and 0.6 ± 0.2 kg in the carbohydrate and placebo groups, respectively, $P = 0.149$) (data not shown).

The rate of decrease in muscle glycogen content was significant ($P = 0.000$), but did not differ between CHO and PLA ($P = 0.463$) (Figure 1). Cortisol and FRAP increased significantly over time in CHO and PLA ($P = 0.008$ and 0.044, respectively), but the pattern of change was not different (Figures 2 and 3, respectively). Concentrations of F_2-isoprostanes were unaffected by the exercise ($P = 0.193$) (Figure 4). There were no significant correlations between any markers with the exception of skeletal muscle glycogen content being positively correlated with plasma FRAP ($r = 0.601$, $P = 0.039$) in the PLA group.

Discussion

Intense or long-duration aerobic exercise has been shown to induce oxidative stress [2–5]. However, there are limited data regarding oxidative stress and resistance training [1,6,7,9,21]. Intensive resistance training induces significant depletion of muscle glycogen which could potentially trigger oxidative stress mechanisms [22]. There was a significant decline in muscle glycogen, which was not affected by carbohydrate supplementation. As there was no significant difference in oxidative stress markers, muscle glycogen content did not exert any effects. In support of our findings, Hellsten et al. [23] reported that changes in muscle glycogen level did not affect levels of oxidative stress. Nonetheless, a high, compared to low carbohydrate diet might potentially be important in maintaining hepatic glycogen content and consequently, the ability to maintain blood glucose levels during exercise.

We had hypothesized that a carbohydrate supplement during exercise would diminish oxidative stress through increased blood glucose levels and lowered stress hormone levels [12]. Exhaustive exercise dramatically increases plasma levels of catecholamines which can undergo autooxidation to form reactive oxygen species [10]. Cortisol is a stress hormone which typically parallels increases in catecholamines [11]. Cortisol rose similarly in both CHO and PLA and was not affected by carbohydrate

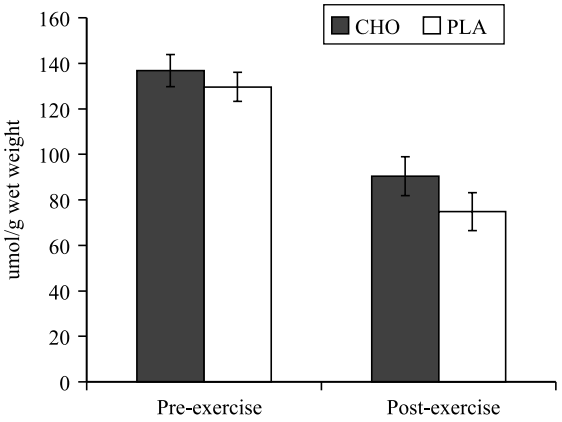

Figure 1. The decrease in skeletal muscle glycogen content did not differ between carbohydrate ($N = 15$) and placebo groups ($N = 15$) after a 2-h resistance training bout (time effect, $P = 0.000$; interaction effect, $P = 0.502$). Values are means ± SEM.

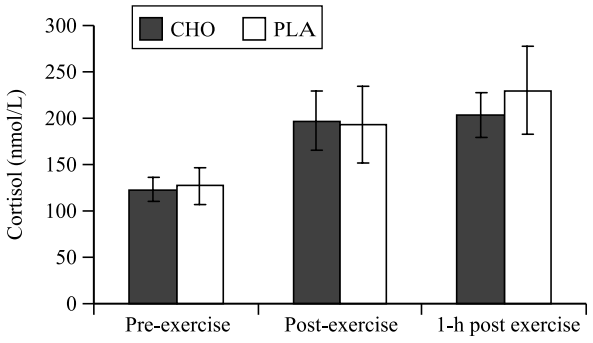

Figure 2. The increase in plasma cortisol did not differ between carbohydrate ($N = 15$) and placebo groups ($N = 15$) after a 2-h resistance training bout (time effect, $P = 0.008$; interaction effect, $P = 0.852$). Values are means ± SEM.

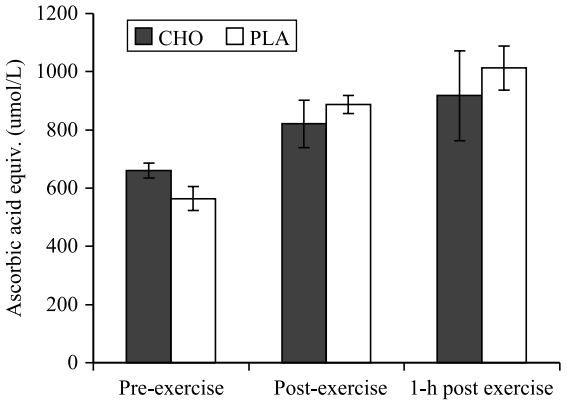

Figure 3. The increase in the ferric reducing ability of plasma (FRAP) did not differ between carbohydrate ($N = 15$) and placebo groups ($N = 15$) after a 2-h resistance training bout (time effect, $P = 0.044$; interaction effect, $P = 0.517$). Values are means ± SEM.

supplementation, nor was cortisol associated with any changes in oxidative stress parameters.

With respect to antioxidant potential, carbohydrate has not been reported to directly increase or contribute to antioxidant status although carbohydrate can be oxidized to form ROS such as reactive carbonyl derivatives [24]. We did not observe any significant effects of carbohydrate administration on FRAP values in CHO vs PLA; however, FRAP values were increased as a result of exercise. Based on findings from previous studies, the increase in plasma antioxidant potential is most likely due to increasing uric acid and vitamin C in the blood during exercise [2,3,23]. Total plasma antioxidant potential has been found to be inversely related to oxidative stress in some disease states [25].

Improving upon previous resistance exercise studies by using F_2-isoprostanes as an indicator of oxidative stress, we found that the resistance exercise did not result in any significant changes from baseline in this sensitive marker. In contrast, we have found F_2-isoprostanes to be significantly elevated after various levels of exhaustive running [2,4,5]. F_2-isoprostanes are of biological interest not only as a specific and sensitive index of lipid peroxidation but also for potential involvement in innate immune system activation. F_2-isoprostanes are known to increase platelet aggregation and smooth muscle constriction and are found in atherosclerotic plaques [16]. The ramification of exercise associated increases in these compounds is not currently known.

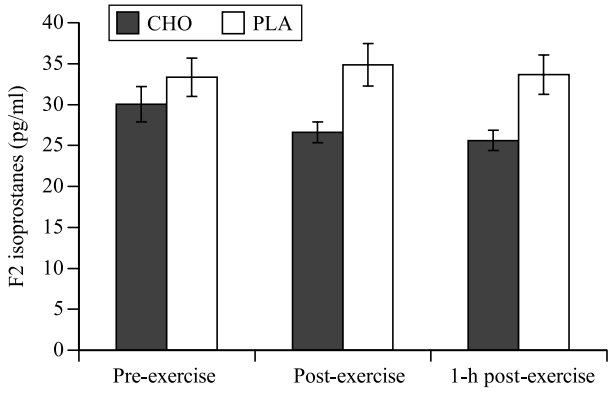

Figure 4. Plasma F_2-isoprostanes were not affected by a 2-h resistance training bout but differed between carbohydrate ($N = 15$) and placebo groups ($N = 15$) (treatment effect, $P = 0.021$; interaction effect, $P = 0.091$). Values are means ± SEM.

Due to the specialized equipment and expense involved in determination of F_2-isoprostanes, most exercise studies typically use MDA or thiobarbituric acid substances (TBARS) as a marker of lipid peroxidation or oxidative stress [1,6,26]. For example, McBride et al. [6] have reported blood MDA concentration to increase after exhaustive resistance exercise, whereas, Alessio et al. [1] found no effect of isometric exercise on MDA as measured by TBARS, but did find an increase in lipid hydroperoxides. However, unless analyzed by HPLC, the MDA-TBAR assay suffers from reliability problems due to non-specificity [27,28]. There was no effect of carbohydrate on F_2-isoprostanes in the current study. We also observed no effect of carbohydrate on F_2-isoprostanes or lipid hydroperoxides in a previous study, although that study involved running [13].

Few studies exist which have examined the effect of macronutrient supplementation on lipid peroxidation and oxidative stress, and these studies have involved aerobic exercise. Karolkiewicz et al. [14] examined the effect of a carbohydrate and protein supplement on reduced glutathione (GSH) and thiobarbituric acid reactive substances (TBARS) in 19 teenage track and field athletes divided into supplement and placebo groups. Carbohydrate and protein supplementation had no effect on plasma GSH or TBARS. Vasankari et al. [15] examined eight athletes who ran 27 km on two separate occasions with random assignment to carbohydrate (105 g of carbohydrate during exercise) or placebo conditions. This study found no effect of carbohydrate supplementation on serum diene conjugation compared to placebo after exercise.

Although aerobic in nature, the above cited studies are in support of our findings of no reduction by carbohydrate on oxidative stress. In agreement, we have not found carbohydrate administration during exhaustive running to affect oxidative stress or plasma antioxidant potential [2]. However, we have recently found that carbohydrate supplementation during exhaustive cycling significantly reduced oxidative stress markers, cortisol and catecholamines and increased plasma antioxidant potential (unpublished data). Therefore, different modes of exercise may produce different responses of oxidative stress and plasma antioxidant potential in response to

carbohydrate feedings. This could be related to differences in mechanical stress.

Conclusions

In summary, the results of this study indicate that exhaustive resistance exercise did not result in increased oxidative stress as measured by F_2-isoprostanes. Furthermore, carbohydrate administration did not affect blood antioxidant capacity or result in differences in F_2-isoprostane levels.

Acknowledgements

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