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Enhanced inflammation with high carbohydrate intake during recovery from eccentric exercise

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Abstract

Inflammation associated with adipose tissue is modulated by macronutrient availability. For example, glucose increases inflammation in obese but not lean individuals. Little is known about how macronutrient intake influences inflammation associated with muscle. The aim of this study was to determine the impact of macro-nutrient intake differences during recovery from eccentric exercise on the inflammatory response. The study was a cross-over design in which young men and women ($n = 12$) completed high and low carbohydrate (CHO) conditions. Both conditions consisted of six sets of ten maximal high-force eccentric contractions of the elbow flexors and extensors followed by a controlled diet for the first 8 h post-exercise. Glucose, insulin, tumor necrosis factor- α , interleukin (IL)-1 β , IL-6, and C-reactive protein were measured from blood samples pre-exercise, 1.5, 4, 8, and 24 h post-exercise. Perceived muscle soreness, strength loss, and serum CK activity were measured through 120 h post-exercise. Perceived soreness was elevated ($P < 0.001$) at all time points post-exercise in both conditions and was higher ($P < 0.05$) in the high compared to the low CHO condition. IL-1 β increased ($P = 0.05$) 24 h post-exercise in the high compared to the low CHO condition. There was a trend ($P = 0.06$) for IL-6 to be elevated in the high compared to the low CHO condition. We conclude that inflammation induced by high-force eccentric exercise in skeletal muscle is greater when a high CHO compared to a low CHO diet is consumed during recovery.

Introduction

Identification of factors influencing inflammation in skeletal muscle may be helpful in understanding inflammatory muscle diseases and inflammation-based functional impairments that involve skeletal muscle, e.g., insulin resistance (Plomgaard et al. 2005). Prolonged, low-level inflammation is associated with risk of some diseases or related conditions such as metabolic syndrome, type 2 diabetes, and cardiovascular diseases (Grundy et al. 2005; Pearson et al. 2003). Additionally, inflammation influences both injury and adaptation processes in skeletal muscle following exercise-induced damage (Tidball 2005). The influence of nutritional factors such as the macronutrient composition of the diet on inflammation linked to skeletal muscle is unclear.

Carbohydrate is a macronutrient of particular interest because inflammation linked to obesity and impaired glucose tolerance has been shown to be augmented by hyperglycemia. Glucose intake (75 g) induces oxidative stress and activates pro-inflammatory factors, e.g., tumor necrosis factor- α (TNF- α) (Dandona et al. 2007). In a study by Gonzalez et al. (2006), acute hyperglycemia in obese, reproductive-aged women caused an increase in TNF- α production. Obese individuals are in a chronic pro-inflammatory state and have elevated levels of inflammatory markers. Thus, the inflammatory process was already initiated in the obese test subjects and hyperglycemia further increased this inflammation as seen by an increase in production of the pro-inflammatory cytokine TNF- α . In another study, Esposito et al. (2002) injected three consecutive boluses of intravenous glucose separated by 2 h each, inducing acute hyperglycemia with each injection. Acute hyperglycemia induced a significant increase in the pro-inflammatory cytokines TNF- α , interleukin (IL)-6, and IL-18 in both control and impaired glucose tolerance subjects. The results of these studies may be an indication that acute hyperglycemia has the ability both to induce an inflammatory response and to increase an inflammatory response that was initiated by another source, such as obesity. However, insulin has anti-inflammatory properties that may offset the influence of glucose ingestion under some circumstances and may differentiate responses

responses between normal individuals and those with impaired glucose tolerance and diabetes (Dandona et al. 2007). It is unclear if rises in blood glucose can enhance acute inflammation in non-obese individuals with normal glucose tolerance.

Some types of exercise naturally cause structural damage to skeletal muscle resulting in an inflammatory response. Eccentric muscle contractions are defined by the muscle being forcibly lengthened as it contracts (Proske and Morgan 2001). Eccentric muscle exercise in untrained skeletal muscle will cause moderate to high levels of muscle damage resulting in decreased strength, increased soreness, and inflammation (Faulkner et al. 1993). Inflammation is the general response of the body to tissue injury, with the overall goal being healing. The inflammatory response is a sequential process involving cytokine and chemokine signals, leukocytes, oxidative stress, and acute phase proteins. All elements of this process have been measured following high-force eccentric exercise (Smith and Miles 2000). The general cytokine cascade in sequential order is TNF- α , IL-1 β , IL-6, IL-1ra, and IL-10 (Petersen and Pedersen 2005). TNF- α and IL-1 β induce lymphocytes, neutrophils, monocytes, and other cells to clear antigens and heal tissue (Ostrowski et al. 1999). TNF- α and IL-1 β also induce the production and release of IL-6. IL-6 promotes some of the proteins in the acute phase response (systemic response), most notably C-reactive protein (CRP).

Exercise recovery nutrition is aimed at consuming some protein and enough carbohydrate (CHO) to generate a rise in insulin that will enhance muscle glycogen replenishment, protein synthesis, and recovery adaptations (Manninen 2006; Ivy et al. 2008). Previously, we had subjects consume an additional 0.25 g of carbohydrate per kilogram body weight per hour for 12 h on top of a baseline diet of 50% carbohydrate on both the day of and the day following exercise. This did not affect IL-6, CRP, or cortisol responses following eccentric exercise (Miles et al. 2007). This was a relatively low CHO load spread out over the course of 48 h. More research is needed to determine whether greater consumption of high CHO foods earlier in recovery from exercise, when high CHO intake is encouraged for recovery, influences the inflammation process.

The purpose of this study was to determine if macro-nutrient intake differences following eccentric exercise influence the inflammation response. We utilized high-force eccentric muscle contractions to induce an acute inflammatory response. The subjects were fed either a high CHO or low CHO diet for the first several hours post-exercise. We hypothesized that the inflammatory response (TNF- α , IL-1 β , IL-6, and CRP) would be higher in the high CHO compared to the low CHO condition. An improved understanding of inflammation in skeletal muscle will help define the role skeletal muscle has in inflammation associated with metabolic diseases, particularly those linked to insulin resistance (Tilg and Moschen 2008).

Methods

Subjects

Fourteen subjects enrolled in the investigation and two did not complete the study due to illness not related to this study. The remaining 12 subjects were 19–36 years of age (male 7, female 5). Both male and female subjects were included in this study. In an effort to reduce the influence of differences in sex, each subject acted as his or her own control. Further, we tested for sex differences in a previous study using a similar exercise (29 men, 22 women) and found that inflammation responses to this exercise were similar between the sexes (Miles et al. 2008). Without including both male and female the *N* would have been reduced and not large enough for significance in many cases. Subjects were non-weight trained and did not do any heavy lifting or lowering with the arms that resulted in soreness of the arms in the 6 months prior to the study. The goal of these criteria was to eliminate the possible effects of the repeated bout effect. This occurs when muscle exposed to eccentric contractions produces a protective adaptation that results in decreased muscle damage with subsequent bouts of eccentric exercise for up to several months (Nosaka et al. 1991). Individuals were excluded if they had a known history or condition of anemia, musculoskeletal limitations, inflammatory conditions, diabetes mellitus, heart disease, known kidney problems (excluding kidney stones), smoking, alcohol use greater than 1 drink per day for women and 2 drinks per day for men, binge drinking (greater than 4 drinks on any one occasion), chronic use of anti-inflammatory medications, use of lipid lowering medications, use of oral contraceptives, regular physical activity that results in bruising or muscle soreness, and pregnancy. To aid in determining exclusion criteria all potential subjects were required to fill out a Health History Questionnaire, a Physical Activity Readiness Questionnaire, and via interview by an investigator, asked several questions regarding the use of their arms during work and recreational activities to determine whether they were likely to have performed heavy lifting or lowering with their arms. Additionally, all subjects were instructed to refrain from using any anti-inflammatory medications throughout the duration of the study. All subjects were informed of the risks and discomforts associated with participation and were required to sign an informed consent document approved by the Montana State University Human Subjects Committee prior to participation.

Research design

The research design was a cross-over protocol in which each subject acted as his or her own control. Each subject completed a high CHO and a low CHO condition. The order of conditions was counterbalanced between subjects and separated by 3–6 weeks within each subject to allow variables to return to baseline between conditions. We have previously used a similar washout period with a similar exercise protocol and observed no differences between conditions for baseline values, suggesting this is a sufficient amount of time for a washout period (Miles et al. 2007). Also, women began each condition within 4 days of the onset of menstrual bleeding to minimize the effect of cyclical hormone variation. On the first morning of each condition, subjects performed high-force eccentric exercise to induce muscle soreness and inflammation. Then, subjects were provided either a high CHO or low CHO diet for the first 8 h post-exercise. Each diet consisted of three meals at 0.5, 3, and 7 h post-exercise. The high CHO diet consisted of corn flakes, 2% milk, apple juice, and Clif bar(s)TM. The macronutrient composition of this diet was 75% carbohydrate, 15% fat, and 10% protein with each subject ingesting 1.45 g carbohydrate per kilogram body weight. The low CHO diet consisted of turkey, cheese, and peanuts. The macronutrient composition of this diet was 6% carbohydrate, 70% fat, and 24% protein with each subject ingesting 0.11 g carbohydrate per kilogram body weight. The diets contained equivalent kilocalories proportional to weight within each subject between conditions. The main goal when designing the diets was to keep them isocaloric, easy to prepare, all while adjusting the CHO load. However, the consequence of this resulted in different fat and protein compositions between the diets as well. This is a limitation of the current study, and any measured changes in inflammation cannot be solely attributed to differing CHO intake. While the subjects could clearly identify differences in the diets, the expected outcomes and influences of the diets were not discussed with the subjects in an effort to not bias the results.

The baseline assessment and blood draw occurred at 7:00 a.m. prior to exercising on the first day of each condition for all subjects for both conditions. Post-exercise strength and soreness assessments were made immediately post-exercise, 1.5, 4, 8, 24, 48, 72, 96, and 120 h post-exercise. Blood samples were collected pre-exercise, 1.5, 4, 8, and 24 h post-exercise for assessment of inflammation. An additional blood sample was collected 120 h post-exercise for analysis of serum CK activity and CRP.

To minimize variability, subjects were required to adhere to several restrictions prior to and during the time of assessment. To obtain optimal baseline assessment of blood, subjects were required to be on an overnight fast and keep physical activity to a minimum the first morning of each condition. Food intake was not controlled or monitored prior to the overnight fast. It was beyond the resources of the study to do so and thus is a limitation of the study as this prior food intake could possibly impact the resulting inflammatory response from the exercise. On subsequent morning blood draws within each condition subjects were required to be fasted at least 8 h and keep physical activity to a minimum. The day prior to and during the assessment period, subjects were required to refrain from strenuous physical activity and physical activity that lasted longer than 60 min in duration. Additionally, from the time of recruitment through completion of the study subjects were instructed not to perform any physical activity they were unaccustomed to or did not do on a regular basis prior to participating in the study. To reduce the influence of illness on inflammatory factors, subjects were only tested if they had been free from known infection for at least 1 week prior to testing.

Anthropometric measurements

Prior to any testing each subject was assessed for anthropometric measurements. Anthropometric measurements consisted of body height and weight, and waist to hip ratio. For the waist to hip ratio a circumference measure was taken at the waist (narrowest point between the ribs and the hips at the end of a normal expiration) and hip (at the level of the greater trochanter) using an anthropometric tape measure. This measure was made over the clothing of the subject (no disrobing occurred).

Eccentric exercise and maximal force production

A computer-controlled isokinetic dynamometer (Kin Com 125E+, Chattecx Corporation, Chattanooga, TN) was used to perform the high force eccentric exercise. The elbow flexor and extensor muscles of one arm were exercised for each condition. For example, if a subject exercised his or her left arm for the high CHO condition then he or she exercised the right arm for the low CHO condition. The exercise protocol was similar to previous exercise protocols used in our lab to allow some comparison between the current study and previous studies (Miles et al. 2007, 2008). The point of rotation in the dynamometer was aligned with the axis of rotation in the elbow. The dynamometer was connected to the arm just below the wrist via a padded support and Velcro strap. Subjects started with their elbow in a fully flexed position. The

dynamometer then moved the elbow to a fully extended position. The subjects were instructed to maximally resist the movement of the elbow being extended by attempting to keep the elbow in a fully flexed position. From the fully extended position, the dynamometer then moved the elbow to a fully flexed position. Subjects also maximally resisted this movement. The movement of the elbow from flexed to extended position and back to flexed position consisted of one repetition. The angular velocity of the dynamometer was set at 0.79 radians (45°) per second. There was a 10-s pause in between each repetition. There were ten repetitions per set with a total of six sets. There was a 5-min rest period between sets. Just prior to the exercise, subjects were given a warm-up and familiarization period to acquaint themselves with the movement of the dynamometer and to ensure their muscles were prepared for maximal force contractions. As the exercise was mechanically very simple, we believe this familiarization period just prior to exercising was sufficient to eliminate any potential differences between the bouts of exercise due to familiarity with the dynamometer and the exercise itself.

Maximal isometric strength was measured pre-exercise, immediately post-exercise, 24, 48, 72, 96, and 120 h post-exercise. The dynamometer was adjusted for each subject the same way as it was adjusted during the high-force eccentric exercise. To test maximal isometric strength the dynamometer was set so the elbow was at an angle of 1.57 rad. (90°). The dynamometer was locked in that position and the subject was instructed to flex or extend their elbow with maximal effort for 3 s. For each measure of maximal isometric strength, the subject performed this procedure three times, and the average of the three trials was used as the maximal isometric strength for that specific time. There was a 30-s rest period in between the three trials for each measure of maximal isometric strength.

Muscle soreness

Perceived muscle soreness was evaluated with the aid of a visual analog scale at pre-exercise, 1.5, 4, 8, 24, 48, 72, 96, and 120 h post-exercise. The scale consisted of a 100-mm line with one end representing no soreness and the other end representing extreme soreness. The subjects were asked to flex and extend their arms several times while holding a 1-kg weight in their hand and putting pressure on both their flexor and extensor muscles with their other hand. Then the subjects placed a vertical line on the scale representing their perceived level of soreness. The subjects were instructed to place a line on the scale in terms of soreness only, not in comparison to other types of pain, e.g., not relative to a broken bone.

Blood analysis

Blood was collected using a standard venipuncture technique at pre-exercise, 1.5, 4, 8, 24, and 120 h post-exercise. Blood was collected from an antecubital vein into chilled evacuated tubes without additive for analysis of glucose, CRP and CK, and containing EDTA for TNF- α , IL-6, IL-1 β , and insulin. The tube without additive was allowed to clot at room temperature and the tube with EDTA was kept chilled on ice until centrifugation within 30 min. Serum and plasma were separated from cells using a refrigerated 21000R Marathon centrifuge (Fisher Scientific, Pittsburgh, PA). Samples were stored at -80°C until analysis.

Serum high-sensitivity CRP (MP Biomedicals, Irvine, CA), plasma high-sensitivity TNF- α (R&D Systems, Minneapolis, MN), plasma high-sensitivity IL-6 (R&D Systems, Minneapolis, MN), plasma high-sensitivity IL-1 β (R&D Systems, Minneapolis, MN), and insulin (Diagnostic Systems Laboratories, Inc., Webster, TX) concentrations were measured using commercially available ELISA kits according to the instructions of the manufacturers. Absorbance of 96-well plates was read at the primary wavelength and corrected for secondary wavelength interference using a μ Quant Universal microplate spectrophotometer (Bio-Tek Instruments, Winooski, VT). All samples were run in duplicate. All samples for a given subject were run on the same assay plate. Average intra-assay coefficients of variation (CV) between duplicate samples were 13.8, 16.2, 9.7, 8.2, and 5.0% for CRP, TNF- α , IL-6, IL-1 β , and insulin, respectively. These are within a twofold range of the intra-assay CV values determined by the manufacturers for 20 or more samples at comparable concentrations.

Serum CK activity was measured using an ultraviolet, kinetic assay utilizing a creatine kinase *n*-acetyl-L-cysteine reagent at 37°C in which CK activity is proportional to the change in NADH production and the increase in absorbance at 340 nm (CK-NAC reagent, Thermo Fisher Scientific, Inc., Waltham, MA). Serum glucose concentration was assayed using a glucose hexokinase reagent set in which glucose is converted to glucose-6-phosphate and then to 6-phosphogluconate with the reduction of NAD⁺ to NADH (Pointe Scientific, Inc. Canton, MI). The absorbance of NADH at 340 nm is directly proportional to the concentration of glucose in the sample and calculated relative to a known standard. These assays were modified for microplate analysis and read using a μ Quant Universal microplate spectrophotometer (Bio-Tek Instruments, Winooski, VT). Samples were run in duplicate. All samples for a given subject were analyzed in the same run of the assay. Intra-assay coefficients of variation were 3.1 and 7.8% for CK and glucose, respectively.

Statistics

Data were analyzed using Statistical Program for Social Sciences (SPSS) for Windows (version 13.0, SPSS Inc., Chicago, IL). Baseline measurements between conditions were analyzed using a paired samples *t* test for continuous variables. To compare conditions over time, a two-way repeated measures analysis of variance (ANOVA) was used. All variables were normally distributed, as determined using the Kolmogorov–Smirnov test. If there were significant main effects or interactions found, paired *t* tests analyses were run to determine the location of the significant differences. Statistical significance was set at an alpha level of 0.05.

Results

Subject characteristics

Six subjects completed the high CHO condition first and six subjects completed the low CHO condition first. Subjects were aged 24.8 ± 6.0 years and weighing 72.9 ± 9.7 kg. Subjects' mean BMI and waist:hip ratio were 23.8 ± 2.9 kg m^{-2} and 0.86 ± 0.08 , respectively. Baseline concentrations of TNF- α , IL-1 β , IL-6, CRP, glucose, and insulin for both conditions are presented in Table 1. Very small, but statistically different baseline concentrations were measured for insulin, where the high CHO condition was slightly greater, and for TNF- α , where the low CHO condition was slightly greater.

Blood glucose and insulin

Blood glucose concentrations were similar between conditions, with no significant changes over time. Insulin concentrations were significantly higher ($P < 0.001$) in the high CHO condition compared to the low CHO condition at all time points except 24 h post-exercise (Fig. 1). Additionally, all post-exercise time points in the high CHO condition were greater than the pre-exercise time point.

Table 1 Baseline values of TNF- α , IL-6, IL-1, CRP, CK, glucose, and insulin for high and low CHO conditions

| Parameters | Mean | SD | <i>P</i> value |
|--------------------------------------|--------|--------|----------------|
| TNF- α (pg ml ⁻¹) | | | |
| High carbohydrate | 1.86 | 0.97 | 0.031 |
| Low carbohydrate | 2.24 | 1.32 | |
| IL-6 (pg ml ⁻¹) | | | |
| High carbohydrate | 1.89 | 1.44 | 0.213 |
| Low carbohydrate | 1.43 | 0.98 | |
| IL-1 β (pg ml ⁻¹) | | | |
| High carbohydrate | 0.19 | 0.14 | 0.464 |
| Low carbohydrate | 0.21 | 0.18 | |
| CRP (mg l ⁻¹) | | | |
| High carbohydrate | 1.81 | 3.14 | 0.853 |
| Low carbohydrate | 1.60 | 2.75 | |
| CK (IU l ⁻¹) | | | |
| High carbohydrate | 279.60 | 241.23 | 0.213 |
| Low carbohydrate | 191.36 | 103.33 | |
| Glucose (mmol) | | | |
| High carbohydrate | 5.82 | 0.50 | 0.286 |
| Low carbohydrate | 5.55 | 0.77 | |
| Insulin ($\mu\text{IU ml}^{-1}$) | | | |
| High carbohydrate | 19.68 | 8.27 | 0.015 |
| Low carbohydrate | 16.03 | 7.38 | |

pg picograms, mg milligrams, kg kilograms, m meter, ml milliliter, dl deciliter, L liter, IU international units, μIU micro-international units

Markers of muscle damage

Perceived muscle soreness was elevated ($P < 0.001$) at all time points post-exercise in both conditions and there was a main condition effect ($P < 0.05$) with higher ratings of perceived soreness in the high CHO condition. Post-exercise isometric strength loss in the biceps was similar between conditions with significant strength loss ($P < 0.001$) immediately post-exercise, 24, and 48 h post-exercise in both conditions. Post-exercise strength loss in

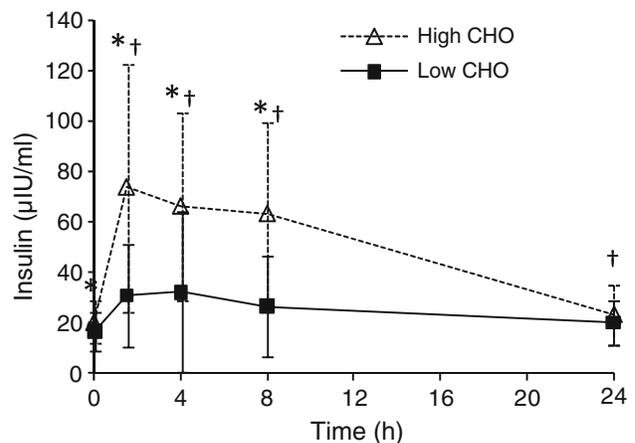


Fig. 1 Insulin concentrations in high and low CHO conditions at pre-exercise, 1.5, 4, 8, and 24 h post-exercise. Values are mean \pm SD, CHO carbohydrate, $n = 10$. * $P < 0.001$ compared to the low CHO condition; † $P < 0.05$ compared to pre-exercise

Table 2 Perceived muscle soreness, percent strength loss, and CK values at all time points measured for both conditions

| Time (h) | Soreness | | % Strength loss biceps | | % Strength loss triceps | | CK | |
|----------|--------------------------|--------------------------|---------------------------|---------------------------|-------------------------|--------------|---------------|---------------|
| | High CHO ^b | Low CHO | High CHO | Low CHO | High CHO | Low CHO | High CHO | Low CHO |
| Pre | 3.2 ± 4.7 | 0.64 ± 1.0 | 0 | 0 | 0 | 0 | 279.6 ± 241.2 | 191.4 ± 103.3 |
| Post | N/A | N/A | -12.0 ± 13.2 ^a | -16.3 ± 11.2 ^a | -3.7 ± 21.8 | -11.0 ± 25.1 | N/A | N/A |
| 1.5 | 17.6 ± 15.0 ^a | 11.7 ± 12.1 ^a | N/A | N/A | N/A | N/A | N/A | N/A |
| 4 | 16.8 ± 16.6 ^a | 7.3 ± 6.5 ^a | N/A | N/A | N/A | N/A | N/A | N/A |
| 8 | 21.7 ± 17.2 ^a | 9.9 ± 6.5 ^a | N/A | N/A | N/A | N/A | N/A | N/A |
| 24 | 48.1 ± 23.6 ^a | 35.2 ± 25.3 ^a | -19.8 ± 18.3 ^a | -7.0 ± 20.5 ^a | -11.8 ± 27.5 | -1.0 ± 29.1 | 241.6 ± 158.0 | 188.6 ± 141.4 |
| 48 | 50.1 ± 29.6 ^a | 46.8 ± 24.9 ^a | -12.5 ± 15.8 ^a | -7.1 ± 19.4 ^a | -0.5 ± 30.6 | 2.2 ± 32.8 | N/A | N/A |
| 72 | 30.1 ± 21.5 ^a | 34.7 ± 17.6 ^a | -2.4 ± 12.2 | -1.0 ± 18.8 | 4.8 ± 32.8 | 8.5 ± 35.1 | N/A | N/A |
| 96 | 16.9 ± 13.5 ^a | 11.2 ± 5.8 ^a | 3.5 ± 10.2 | 2.6 ± 16.5 | 12.4 ± 24.3 | 12.6 ± 37.4 | N/A | N/A |
| 120 | 7.6 ± 7.0 ^a | 2.8 ± 2.8 ^a | 0.0 ± 17.3 | 3.5 ± 19.0 | 15.7 ± 22.4 | 8.1 ± 23.1 | 242.7 ± 204.7 | 179.3 ± 120.3 |

Perceived muscle soreness was evaluated on a 100 mm visual analog scale. Strength loss was measured as percent strength loss compared to baseline measurements made pre-exercise. CK is given as IU/L. Data are mean ± SD

^a $P < 0.001$ compared to baseline

^b $P < 0.05$ compared to low CHO condition (condition effect)

the triceps was similar between conditions with no decreases in strength at any time points post-exercise. CK was parallel between conditions with no significant changes post-exercise. Data for perceived muscle soreness, percent strength loss, and CK are presented in Table 2.

Markers of inflammation

TNF- α was similar between conditions with no significant changes post-exercise (Fig. 2). IL-1 β was higher ($P = 0.05$) in the high CHO condition compared to the low CHO condition 24 h post-exercise (Fig. 3). There was a trend ($P = 0.06$) for a main condition effect for IL-6 concentrations to be higher in the high CHO condition. IL-6 decreased ($P < 0.05$) at 1.5 and 4 h post-exercise, and there was a trend ($P = 0.09$) for an increase 8 h post-exercise in both conditions (Fig. 4). CRP was similar between conditions with no significant changes post-exercise (Fig. 5).

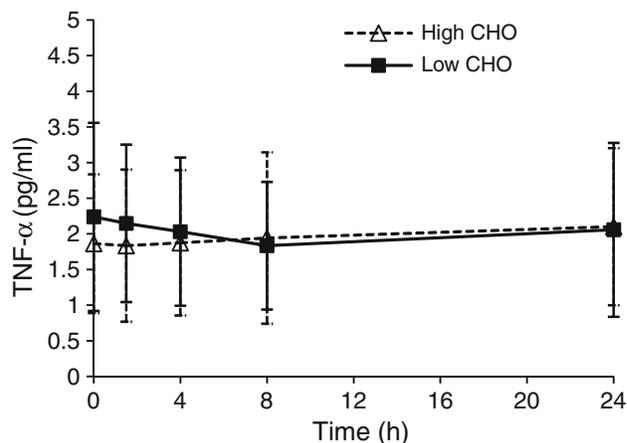


Fig. 2 TNF- α concentrations in high and low CHO conditions at pre-exercise, 1.5, 4, 8, and 24 h post-exercise. Values are mean ± SD, CHO carbohydrate

Discussion

The purpose of this investigation was to determine the influence of macronutrient intake differences following eccentric exercise on the inflammation response. The main finding of the study was that the responses of IL-1 β and soreness were greater in the high compared to the low CHO condition during recovery from eccentric resistance exercise. In an earlier investigation of the influence of carbohydrate supplementation during recovery from eccentric exercise on the inflammatory process, Miles et al. (2007) found that adding 0.25 g kg⁻¹ h⁻¹ CHO to a background diet of 50% CHO during recovery from muscle soreness-inducing exercise did not affect the muscle damage or IL-6 response that occurs several hours post-exercise. In the current study, CHO intake of 4.35 g kg⁻¹ in the high CHO condition across three meals during the first 8 h post-exercise induced a delayed IL-1 β response 24-h post-exercise and greater muscle soreness relative to an isocaloric low CHO condition. While there was a trend for IL-6 to be higher in the high CHO condition, this was not significant and is similar to our finding in our previous study. The subjects in this study had good glycemic control, as evidenced by the lack of increase in blood glucose 1 h after each of the three high CHO meals. In individuals with healthy insulin responses and insulin sensitivity, high CHO foods cause a sharp increase in blood glucose followed by a

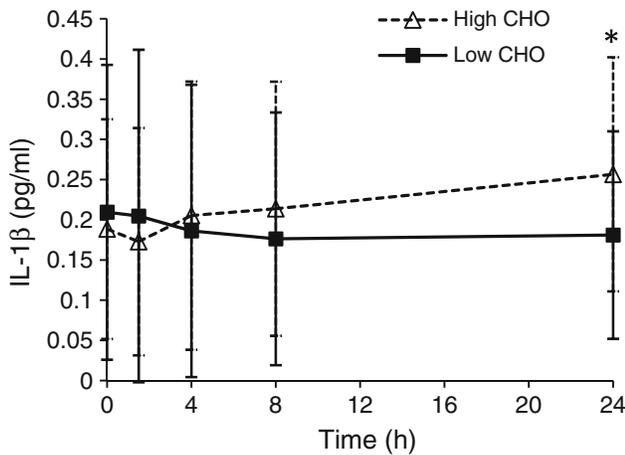


Fig. 3 IL-1 β concentrations in high and low CHO conditions at pre-exercise, 1.5, 4, 8, and 24 h post-exercise. Values are mean \pm SD, CHO carbohydrate. * $P = 0.05$ compared to the low CHO condition at 24 h post-exercise

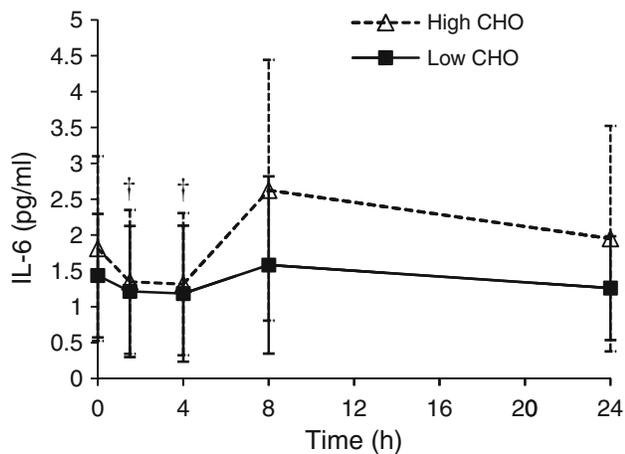


Fig. 4 IL-6 concentrations in high and low CHO conditions at pre-exercise, 1.5, 4, 8, and 24 h post-exercise. There was a time effect where IL-6 was decreased at 1.5 and 4 h post-exercise and a trend for IL-6 to be increased 8 h post-exercise. Values are mean \pm SD, CHO carbohydrate. $\dagger P < 0.05$ compared to pre-exercise

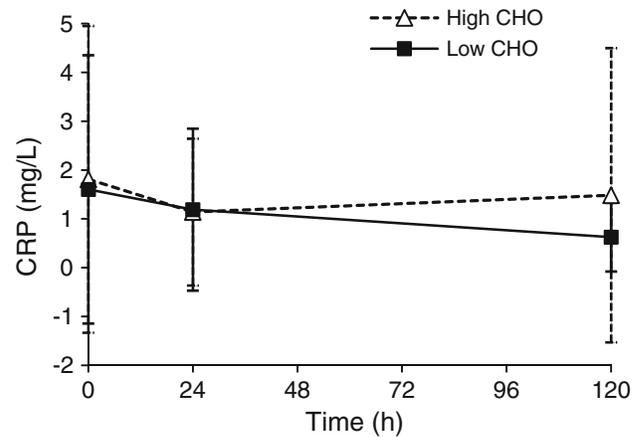


Fig. 5 CRP concentrations in high and low CHO conditions at pre-exercise, 24 and 120 h post-exercise. Values are mean \pm SD, CHO carbohydrate

the skeletal muscle from elevated IL-1 β could result in increased perceived muscle soreness. Secondly, IL-1 β has been shown to enhance production of prostaglandin E₂ (PGE₂) in skeletal muscle (Moldawer et al. 1987). Elevated levels of PGE₂ and muscle soreness after eccentric exercise have been found to coincide suggesting a relationship between IL-1 β , PGE₂, and muscle soreness (Pedersen et al. 1998). The elevated IL-1 β 24 h post-exercise in the high CHO condition could have augmented PGE₂ synthesis in the skeletal muscle, increased sensitivity to pain, and led to increased perceived muscle soreness post-exercise. Thus, higher IL-1 β and soreness may reflect enhanced inflammation in the high CHO compared to low CHO condition. Additionally, we infer from these findings that the inflammation-stimulating effects of the high CHO condition outweighed the potential anti-inflammatory effects of insulin, as reported in previous research (Dandona et al. 2007).

A comparison of the IL-1b increase in the present study to the findings of other investigations provides further evidence that CHO intake has the ability to

sharp decline back to baseline (Foster-Powell et al. 2002). The 1.5, 4, and 8 h post-exercise blood draws were taken 1 h after each meal was consumed. The significant rise in insulin in the high CHO compared to low CHO condition supports the fact that the subjects received a much greater load of CHO during this condition.

Consistent with our hypothesis that high CHO intake would increase inflammation, IL-1 β was higher in the high CHO condition compared to the low CHO condition at 24 h post-exercise. Further, the elevation in IL-1 β is consistent with increased soreness in the high CHO condition via two possible mechanisms. First, IL-1 β has been shown to enhance skeletal muscle protein breakdown (Flores et al. 1989; Nawabi et al. 1990). Elevated protein breakdown in augment the inflammatory cytokine response. Several other studies have used models of eccentric exercise and found no significant changes in IL-1b 24 h post-exercise (Bruunsgaard et al. 1997; Smith et al. 2000; Hirose et al. 2004). Smith et al. (2000) actually observed significant decreases in IL-1b 24 h post-exercise. An increase in the pro-inflammatory cytokines TNF-a and IL-1b is expected at the onset of inflammation. The general cytokine cascade in sequential order is TNF-a, IL-1b, IL-6, IL-1ra, and IL-10 (Petersen and Pedersen 2005). While the increase in IL-1b may have been expected earlier than 24 h post-exercise, recent research by Davis et al. (2007) measured increases in TNF-a, IL-1b, and IL-6 in rat soleus muscle 24–48 h following eccentrically biased downhill running, and Silva et al. (2008) measured increases in TNF-a in plasma 48 h following

eccentric exercise similar to that in the present investigation. Furthermore, Loram et al. (2007) demonstrated that IL-1 β release from skeletal muscle during inflammation is delayed up to 24 h and thus supports the timing of our finding of elevated IL-1 β 24 h post-exercise. Thus, the timing of the increase in IL-1 β is appropriate to the exercise stimulus, and increased IL-1 β in the high CHO but not low CHO condition 24 h post-exercise supports the idea that carbohydrates enhanced the inflammatory response.

The IL-6 response we measured is consistent with either no effect or a small enhancement of inflammation with high CHO intake following eccentric exercise. There was a trend ($P = 0.06$) for IL-6 to be higher in the high CHO condition compared to the low CHO condition with the greatest difference 8 h post-exercise. IL-6 follows a diurnal pattern of being higher at 7:00 a.m. compared to 12:00 or 4:00 p.m. (Miles et al. 2008). As such, there was a trend for an increase at a time when there would typically be a decrease. With respect to our previous work with a lower CHO dose (Miles et al. 2007), a greater influence of CHO toward increasing the IL-6 response was measured in the present study.

An anti-inflammatory effect of the low CHO diet was not likely to have occurred because the IL-6 levels in the low CHO condition in this study were comparable to what was observed in the placebo condition in our previous work (Miles et al. 2007). Even so, with the current study design it cannot be ruled out that the low CHO condition did not attenuate the inflammation and muscle damage to some degree. It has previously been shown that protein, and specifically the branched chain amino acids have the ability to decrease muscle damage and promote repair when ingested post-exercise, and therefore attenuate the resulting inflammation (Negro et al. 2008). The low CHO condition contained 24% of kcals as protein whereas the high CHO condition only had 10% kcals as protein. Thus, this greater protein intake in the low CHO condition could have contributed to the lower inflammation and soreness observed in this condition and is a limitation of the current investigation. It is beyond the scope of this study to determine the exact influence of the differences in protein between conditions. Further research investigating the role of post-exercise protein in combination with CHOs and inflammation is needed.

Normally, endurance exercise causes a significant increase in circulating IL-6 (Petersen and Pedersen 2005). It is accepted that the elevated IL-6 resulting from endurance exercise is released directly from skeletal muscle and promotes liver glycogen breakdown and lipolysis of fatty acids in adipose tissue (Petersen and Pedersen 2005). This response elevates blood glucose and provides additional fuel for the skeletal muscle. It has previously been demonstrated that CHO intake of 0.96 g kg⁻¹ h⁻¹ and lower decreases this IL-6 response associated with endurance exercise (Nehlsen-Cannarella et al. 1997; Nieman et al. 2004; Miles et al. 2006). It is believed the external CHO intake provides sufficient fuel for the skeletal muscles and there is less of an energy requirement on the liver and adipose tissue and thus less IL-6 secreted from skeletal muscle. In the current study, the dose of 1.45 g kg⁻¹ in three post-exercise boluses did not decrease the IL-6 response. This is in agreement with our hypothesis that the observed changes in IL-6 are resulting from an inflammatory response and not due to changes in glucose metabolism as with endurance exercise. Thus, we conclude that the influence of CHO intake on IL-6 differs depending on the type of exercise and when the CHO is consumed. It is clear that endurance exercise induces a large IL-6 response that is attenuated by CHO intake before and during exercise, whereas eccentric exercise induces a smaller IL-6 response that is somewhat augmented by CHO intake consumed during the early recovery period.

We did not measure changes in TNF- α in response to the exercise in either of the conditions. Up-regulation of the pro-inflammatory transcription factor NF- κ B, increases in TNF- α gene expression by monocytes, and increases in TNF- α plasma concentrations in obese but not lean individuals after glucose ingestion have been measured in previous studies (Beutler et al. 1985; Gonzalez et al. 2006). However, many studies fail to measure acute increases in plasma TNF- α from healthy individuals in response to glucose intake or eccentric exercise (Hirose et al. 2004; Gonzalez et al. 2006). Alternatively, given the measurement of increased plasma TNF- α 48 h post-exercise by (Silva et al. 2008), it is possible that changes in TNF- α occurred after our 24 h post-exercise measurement. Additionally, TNF- α has a half life in the plasma of 6 min unless bound by a soluble receptor (Beutler et al. 1985; Aderka et al. 1998). Thus, while we did not measure enhanced TNF- α plasma concentrations in the high CHO compared to low CHO condition, it is possible that potential differences were not captured in plasma concentrations at the times measured.

There were no significant changes in CRP post-exercise in either condition or over time in the current study. These results are similar to previous studies using a similar exercise model (Miles et al. 2007, 2008) and indicate that even given the enhanced inflammation in the high CHO condition, that there was not enough muscle damage to induce a systemic inflammatory response. Studies with greater amounts of exercise stress including 300 maximal eccentric actions (Paulsen et al. 2005) and a 32-km mountain trail race (Miles et al. 2006) induced increases in CRP. It is possible that a similar exercise protocol utilizing a larger muscle group such as the legs could produce a more pronounced inflammatory response and would allow for more significant changes in the measures of inflammation. Given the results of the current

study, it is worthwhile to investigate these dietary impacts on inflammation in the presence of a larger inflammatory stimulus.

While we were successful in differentiating inflammation responses after eccentric exercise in the low compared to high CHO conditions, there are several limitations of the present study. One consideration for the increase in IL-1 β at 24 h post-exercise is that subjects were not in the lab between 8 and 24 h post-exercise. It is possible that other factors (i.e. food intake, physical activity, etc.) could have influenced the increased IL-1 β . However, when considering subjects' individual IL-1 β responses it is likely that the CHO intake was the factor influencing the elevated IL-1 β . There were no obvious outliers causing this increase, and IL-1 β was elevated in the majority of subjects at 24 h post-exercise in the high CHO condition. Additionally, the exercise used to induce inflammation is similar, but not identical, to weight-lifting, but the amount of muscle mass used was less than is typical for resistance exercise. Thus, we have demonstrated the potential for high and low CHO diets to influence inflammation, but further research with greater control during the post-exercise period and exercise stresses that are more applicable to the general population are needed.

In conclusion, there were two significant indications that high CHO intake resulted in a greater inflammatory response following eccentric exercise. IL-1 β and perceived muscle soreness were greater in the high CHO compared to the low CHO condition. Additionally, there was a trend for IL-6 to be greater in the high CHO condition. These results demonstrate that high CHO intake augmented inflammation associated with skeletal muscle; however, lack of change in TNF- α and CRP indicate that the overall inflammation level remained somewhat low. It is beyond the scope of this study to make dietary CHO recommendations with respect to athletic performance and disease prevention. However, these results do support a need for additional investigations on the influence of macronutrient intake that are specifically targeted at both exercise performance and disease measures in specific populations.

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