Carbohydrate Influences Plasma Interleukin-6 But Not C-Reactive Protein or Creatine Kinase Following a 32-Km Mountain Trail Race

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Attenuation of exercise-induced interleukin-6 (IL-6) responses by carbohydrate (CHO) has been demonstrated in studies comparing controlled doses (≥ 0.9 g · kg^{-1} · h^{-1}) to placebo, but not in studies of voluntary intake. This study sought to determine if attenuation of the IL-6 response during a 32.2-km mountain trail race occurs for high compared to low ad libitum CHO intakes. IL-6, C-reactive protein (CRP), and creatine kinase activity (CK) were analyzed from blood samples collected 12 h pre-, 0, 4, and 24 h post-race. Subjects were grouped into low ($n = 14$, 0.4 ± 0.1 g · kg^{-1}· h^{-1}) and high ($n = 18$, 0.8 ± 0.2 g · kg^{-1} · h^{-1}) CHO intake groups. IL-6 0 h post-race ($P < 0.05$) was higher in the low (40.2 ± 22.7 pg · mL^{-1}) compared to the high CHO group (32.7 ± 22.1 pg · mL^{-1}). CRP and CK both increased post-race, but no differences were observed between groups. Attenuation of exercise-induced IL-6 is apparent across a range of CHO intakes.

Key Words: muscle damage, acute phase response, inflammation

Carbohydrate consumption during exercise attenuates a wide range of exercise-induced endocrine and immune responses including plasma epinephrine, cortisol, interleukin-6 (IL-6), IL-10, IL-1 receptor antagonist (IL-1ra), neutrophil, and monocyte concentrations (8, 17, 19). Several of these endocrine and immune components are involved in the inflammatory response to exercise-induced muscle damage (22). The influence of carbohydrate on IL-6 is of particular interest because this cytokine plays an important regulatory role in the inflammatory response to tissue damage (22), but also has important roles in the regulation of blood glucose (4, 6, 24, 28). Thus, IL-6 has two distinctly different roles, one related to inflammation and the other related to energy substrate regulation. The degree to which attenuation of the IL-6 response to exercise influences the response to muscle damage has yet to be determined.

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Strenuous, prolonged endurance exercise and high-force eccentric exercise both are associated with exercise-induced muscle damage and inflammation (7, 11, 13, 15, 22). The efflux of intramuscular enzyme creatine kinase (CK) from muscle to blood is an indicator of skeletal muscle damage (15, 29). C-reactive protein (CRP) is an acute phase protein produced by the liver in response to IL-6 and other cytokines during systemic inflammatory responses (9, 30). Both CK and CRP increase in the circulation in response to exercise-induced muscle damage and there is evidence of a dose response linking the stress and injury of exercise and the levels of these markers (25).

Additionally, the preponderance of research relating to carbohydrate supplementation and exercise-induced endocrine and immune responses utilizes a model of reasonably high carbohydrate intake (> 0.9 g $\cdot$ kg^{-1} $\cdot$ h^{-1}) compared to a zero carbohydrate placebo (16, 17, 18, 19, 23). Neither the influence of ad libitum carbohydrate consumption nor carbohydrate intakes lower than 0.9 g $\cdot$ kg^{-1} $\cdot$ h^{-1} during prolonged endurance events have been investigated.

Based on the clear and consistent attenuation of IL-6 increases during endurance exercise with experimentally controlled CHO consumption compared to placebo, we hypothesize that attenuation of IL-6 increases will occur in individuals who choose to consume more CHO during an endurance event compared to those who choose to consume less CHO. The purpose of this investigation was to determine whether attenuation of the IL-6 response to strenuous endurance exercise associated with exercise-induced muscle damage occurs in higher compared to lower ad libitum intake of carbohydrate. In addition to inducing CRP production by the liver, IL-6 has a negative feedback role in which it acts to down regulate the cytokines stimulating inflammation at the tissue level, tumor necrosis factor-α and IL-1β (22). Thus, we hypothesize that attenuation of the IL-6 response may decrease CRP but increase CK, the marker of muscle tissue injury. A second purpose of this investigation was to determine whether attenuation of the IL-6 response to exercise influences the CK and CRP responses to exercise-induced muscle damage.

Methods

Subjects

Participants in the 2003 and 2004 Ed Anacker Bridger Ridge Runs in Bozeman, Montana volunteered for participation in this investigation after giving written informed consent approved by the Montana State University Institutional Review Board for the Protection of Human Subjects. Thirty-four individuals volunteered. Two subjects did not complete the procedures of the investigation for reasons not related to the investigation. The remaining 32 (18 men, 14 women) completed the race and procedures for this investigation. The level of experience for this type of endurance event ranged from never having completed a comparable event to decades of endurance experience and completion of many comparable events.

Study Design

The baseline blood sample was collected from race participants 12 to 15 h before the 7:00 AM race start. The location of the race starting line precluded collection

of baseline blood samples on the morning of the race. The race consisted of 2073 m of ascent and 2896 m of descent, with peak altitude of the course reaching 2946 m. Thus, the race was performed at moderate altitude and there was an extensive downhill running component. However, the terrain is highly challenging and participants typically have to hike and use their hands to aid their maneuvering in many sections of the course, e.g., very steep ups and downs covered in rocks. Ambient conditions were comparable from 2003 to 2004 with temperature and humidity at 7:00 AM (at starting line), 11:00 AM (at finish line), and 1:00 PM (at finish line) being 10.7 °C and 68%, 27.1 °C and 29%, 28.9 °C and 28% in 2003 and 10.5 °C and 45%, 25.8°C and 25%, 27.0 °C and 23% in 2004. Blood samples were collected within a few minutes of race completion, 4 h later, and approximately 24 h later. Subjects were not given instructions as to what to eat or drink during the race, only to pay attention to their consumption and to save product wrappers so that we could record it post-race. Based on analysis of reported food and drink consumption before and during the race, subjects were grouped according to low CHO intake (< 0.5 g CHO $\cdot$ kg$^{-1} \cdot$ h^{-1}) and high CHO intake (> 0.6 g CHO $\cdot$ kg$^{-1} \cdot$ h^{-1}).

Nutrient Analysis

Nutrient analysis was based on ad libitum intake reported by subjects following the race. Subjects were given some supplements as part of their race packet and there were four aid stations along the course. Items and quantities distributed at the aid stations were known to investigators and the post-race dietary questionnaire addressed the specific items given to subjects and supplied by the race organizers. Additionally, subjects were asked to keep the wrappers of any additional food and drink items that they consumed during the race, i.e., their own food and drink. Nearly all subjects carried bottles with them and volume estimates of water and sports beverages were based on the number of their own containers that they consumed. Thus, we feel a good and reasonable estimate of nutrient intake was obtained, but direct measurements were not made. The remote mountainous locations of the aid stations prevented investigators from going to the aid stations to record this information directly. Information from the dietary intake questionnaire was entered into Nutritionist Pro (version 2.2.16, First DataBank, Inc., San Bruno, CA) for analysis of carbohydrate and other nutrient intake.

Blood Collection and Analysis

Blood was collected from seated subjects using a standard venipuncture technique. A vacuum tube without additive was collected for analysis of CRP, CK, and glucose; and one containing EDTA was collected for analysis of IL-6 and hemoglobin (for correction for shifts in plasma volume). Immediately after collection, EDTA tubes were placed on ice and tubes without additive were allowed to clot before being placed on ice. Pre-race, 0, and 4 h post-race samples were collected on site and transported to our laboratory in shifts for timely centrifugation and storage within 90 min. All samples were stored at –80 °C until analysis. For a given analysis, all samples for an individual were analyzed in the same run or plate of the assay. Interleukin-6, CRP, CK, and glucose levels were corrected for changes in plasma volume using the method of Dill and Costill (3).

Immunoassays of IL-6 and CRP were performed using appropriate dilutions of all samples in duplicate. Plasma IL-6 concentrations were determined using a high-sensitivity EIA kit (R&D Systems, Minneapolis, MN) and serum CRP concentrations were determined using a high sensitivity EIA kit (MP Biomedicals, Irvine, CA). Spectrophotometric measurements were made using a μQuant Universal microplate spectrophotometer (Bio-Tek Instruments, Winooski, VT).

Hemoglobin and glucose concentrations and CK activity were measured in duplicate using assays modified for microplate analysis and read using a μQuant Universal microplate spectrophotometer (Bio-Tek Instruments). Whole blood hemoglobin concentrations were measured using a cyanmethemoglobin hemoglobin procedure (Pointe Scientific, Inc., Lincoln Park, MI). Glucose concentrations were measured using a glucose hexokinase reagent set (Pointe Scientific, Inc.). Serum CK activity was measured using an ultraviolet, enzymatic, kinetic assay (Thermo Electron Corp., Waltham, MA).

During the second year of data collection, muscle and joint soreness assessments were included. Subjects ($n = 20$) rated their perception of muscle and joint soreness using a 100-mm visual analog scale anchored at the low end with "no soreness" and at the high end with "very, very sore."

Statistical Analysis

Data were analyzed using SPSS for Windows, version 13.0 (SPSS, Inc., Chicago, IL). A t-test analysis was used to test for differences between low and high CHO groups for age, BMI, finish time, and muscle and joint soreness 24 h post-race. Repeated measures ANOVAs by sex to determine whether sex differences were present was performed for IL-6 ($P = 0.06$), CRP ($P = 0.13$), and CK ($P = 0.12$). While there was a strong trend for a sex difference in IL-6, both sexes were collapsed for all subsequent analyses. Similarly, no differences were detected between individuals who reported taking non-steroidal anti-inflammatory medications before or during the race compared to those who did not, thus no correction for this variable was made in the statistical analysis. Repeated measures ANOVA by CHO group was performed for glucose, IL-6, CRP, and CK levels over time. Finish time was used as a covariate for IL-6. Age was not a significant covariate. Statistica 6.0 (Statsoft, Inc., Tulsa, OK) was used for Tukey post-hoc analysis when a significant main effect for time or group by time interactions were identified. Pearson product-moment correlations were performed to determine the association between peak responses of IL-6, CRP, and CK. Statistical significance was set at the alpha = 0.05 level.

Results

Group Characteristics and Post-Race Soreness

There were trends for the low CHO group to be older ($P = 0.07$) and have slower finish times ($P = 0.06$) compared to the high CHO group. There also was a trend ($P = 0.06$) for the low CHO group to report greater muscle soreness 24 h following the race. Both groups reported similar levels of joint soreness following the race. Unfortunately, soreness was assessed in 2004 but not 2003, thus the sample size for muscle and joint soreness was smaller ($n = 20$) (Table 1).

Nutrient Intake

The low and high CHO groups differed in their nutrient carbohydrate intake by design. One consequence of this design, based on the ad libitum consumption of the food and drink of the subjects' choice, is that the groups also differed in their intake of energy and other nutrients including branched chain amino acids and electrolytes. The high CHO group also was higher in total energy ($P < 0.05$), sodium ($P < 0.01$), and potassium ($P < 0.05$) intake, but not isoleucine, valine, leucine, or magnesium intake (Table 2).

Table 1   Baseline Characteristics, Race Finish Time, and Soreness 24 h Following the Race for Low and High CHO Intake Groups

	Low CHO (*n* = 14)	High CHO (*n* = 18)	*P*
Age (y)	42.4 ± 15.8	33.6 ± 10.3	0.068
Sex (women/men)	8/6	6/12	
BMI (kg/m²)	22.8 ± 2.3	23.0 ± 2.4	0.839
CHO intake (g·kg^{-1}·h^{-1})	0.4 ± 0.1	0.8 ± 0.2	0.001
Finish time (min)	393 ± 72	345 ± 68	0.061
NSAID use (yes/no)	7/7	6/12	
Muscle soreness (mm)[a]	36.1 ± 17.7	54.8 ± 21.9	0.060
Joint soreness (mm)[a]	29.8 ± 31.7	13.8 ± 19.2	0.175

Note. Values are means ± standard deviation; NSAID, non-steroidal anti-inflammatory medications taken before or during the race; [a]Soreness was assessed in 2004 but not 2003, therefore sample size for the low (*n* = 8) and high (*n* = 12) groups was smaller for these measures.

Table 2   Total Energy and Additional Nutrient Intakes During the Race for Low and High CHO Groups

	Low CHO (*n* = 14)	High CHO (*n* = 18)	*P*
Total energy (kcal)	1082 ± 472	1684 ± 703	0.010
Isoleucine (μg)	522 ± 554	710 ± 838	0.474
Leucine (μg)	903 ± 829	1099 ± 1390	0.644
Valine (μg)	626 ± 592	799 ± 988	0.568
Sodium (mg)	955 ± 493	1661 ± 817	0.008
Potassium (mg)	867 ± 305	1572 ± 1035	0.012
Magnesium (mg)	117 ± 93	188 ± 142	0.120

Note. Values are means ± standard deviation.

Glucose

Plasma glucose concentrations increased ($P < 0.001$) from pre- to post-race (Figure 1). Between group differences were not significant.

Interleukin-6, C-Reactive Protein, and Creatine Kinase

Interleukin-6 differences between groups were present only when race finish time was used as a covariate (Figure 2). Plasma IL-6 concentrations were higher
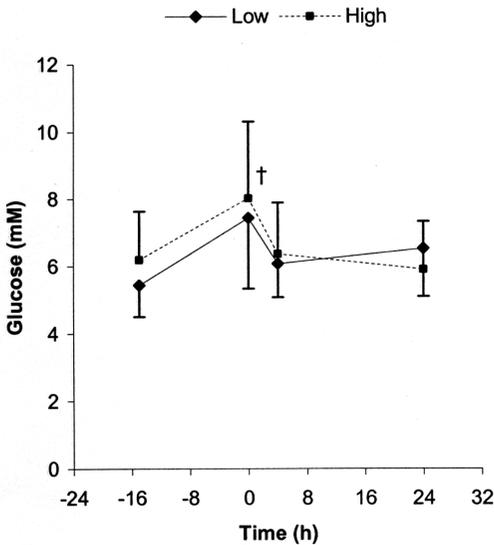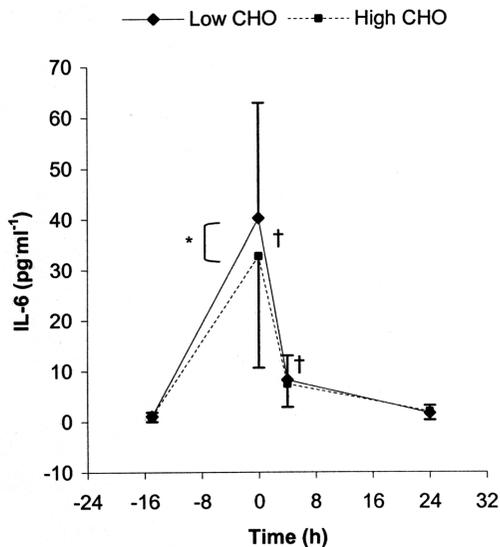

Figure 1—Plasma glucose concentrations for low and high CHO groups pre-race, 0, 4, and 24 h post-race. Values = mean ± standard deviation. †$P < 0.001$ compared to pre-race.

Figure 2—Plasma IL-6 concentrations for the low and high CHO groups pre-race, 0, 4, and 24 h post-race. Values = mean ± standard deviation. *$P < 0.05$ between groups, †$P < 0.001$ compared to pre-race.

($P < 0.001$) 0 and 4-h post-race compared to pre-race and 24 h post-race. With finish time as a covariate, IL-6 was higher in the low compared to the high CHO group at the post-race time point. Serum CRP was higher ($P < 0.001$) 4 and 24 h post-race compared to pre-race (Figure 3). No differences between groups were measured for CRP.

Serum CK activity was higher at all post-race time points compared to pre-race (Figure 4). The interaction between CHO group and repeated measures approached significance ($P = 0.09$), as did the main effect for CHO group ($P = 0.08$).
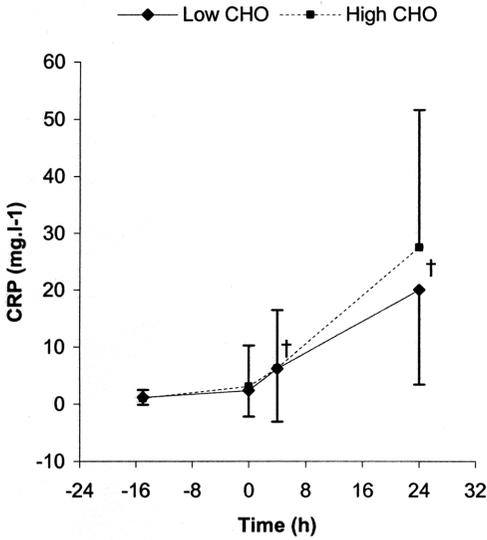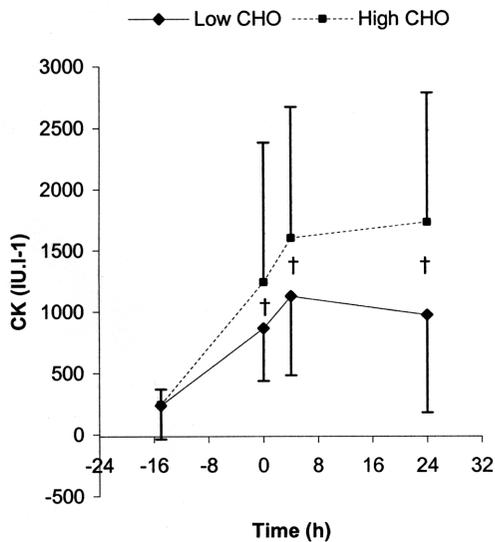

Figure 3—Serum CRP concentrations for the low and high CHO groups pre-race, 0, 4, and 24 h post-race. Values = mean ± standard deviation. †$P < 0.001$ compared to pre-race.

Figure 4—Serum CK activity for the low and high CHO groups pre-race, 0, 4, and 24 h post-race. Values = mean ± standard deviation. †$P < 0.001$ compared to pre-race.

Correlations between IL-6 0 h post-race and CRP ($r = 0.00$) and CK (0.14) at 24 h post-race were very low, indicating that the peak responses of these variables were not associated. There was a significant correlation between CK and CRP at 24 h post-race ($r = 0.40$, $P < 0.05$).

Discussion

The most salient finding of this investigation is that greater ad libitum carbohydrate consumption is associated with lower post-exercise IL-6 levels. There were delayed increases in CRP and CK that did not differ by CHO intake group. Thus, the difference in IL-6 was not associated with differences in the magnitude of the acute phase and muscle damage responses.

This is the first investigation to report lower post-exercise IL-6 concentrations based on higher ad libitum carbohydrate consumption during a prolonged endurance event. Several investigations utilizing well-controlled experimental designs report attenuation of the IL-6 response to endurance exercise with carbohydrate supplementation ($0.96 \text{ g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$) compared to placebo ($0 \text{ g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$) (16, 17, 18, 19, 23). Thus, ad libitum ingestion resulted in carbohydrate intake levels between the carbohydrate supplementation and placebo levels for both the low (about $0.4 \text{ g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$) and high (about $0.8 \text{ g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$) CHO groups. While there are limitations to the research design related to not controlling and directly measuring CHO intake, it is important to relate the findings of the controlled studies to a non-controlled environment in which runners are choosing how much they ingest. Our ability to distinguish IL-6 responses at these intermediate levels, with acknowledged limitations in the experimental design, suggests that there is a dose-response relationship between carbohydrate intake and attenuation of plasma IL-6 concentrations during prolonged endurance exercise.

There was a trend for the high CHO group to have faster race finish times and the difference in the IL-6 response between low and high CHO groups was dependent on correction for race finish time. That is, there was no difference between groups when race finish time was not included as a covariate in the analysis. Exercise intensity is one of several factors known to influence the magnitude of the plasma IL-6 response (10). However, increases in IL-6 release with longer exercise durations also have been reported (23). The low and high CHO groups consumed about 28 and about 56 g of carbohydrate per hour, respectively, and previous research indicates that ingestion of 40 to 75 g of carbohydrate per hour is associated with improved endurance performance (12).

The difference in CHO intake between groups was not reflected in differences in plasma glucose concentrations. This is consistent with some (23) but not all (16, 17, 19) exercise investigations of carbohydrate verses placebo and is a difference between ad libitum intake and controlled intake investigations. There are four possible, not mutually exclusive, explanations for similar plasma glucose concentrations despite the greater intake of carbohydrate in the high CHO group. Administration of recombinant human IL-6 stimulates increases in circulating glucagon, hepatic glucose production, and blood glucose (4, 24, 28). The higher IL-6 levels in the low CHO group could have stimulated greater hepatic glucose output in this group compared to the high CHO group. Further, glucose uptake by active skeletal muscle increases as the intensity of exercise increases (10). Thus,

the greater carbohydrate intake may have been offset by greater muscle uptake of glucose in the high compared to low CHO groups. This is consistent with the glucose homeostasis role of IL-6 during exercise described by Gleeson (6) and Febbraio et al. (4). Third, timing of food and beverage consumption was not controlled, thus subjects may have ingested CHO too early to detect differences in blood concentrations. And finally, because we estimated CHO intake based on recall, albeit a recall in which subjects paid attention to consumption for the purposes of reporting after the race, there may have been error in our estimate.

The substantial downhill running component of the course, the increases in CK and CRP, and the reports of delayed onset muscle soreness suggest that muscle damage occurred during the race and a delayed inflammatory response occurred (7, 11, 13, 15, 22). The efflux of intramuscular CK to the circulation following exercise in which high eccentric contraction forces or prolonged strenuous endurance components is a marker of skeletal muscle damage (15, 29). The time course for muscle tissue damage-induced increases in circulating IL-6 is about 4 to 12 h post-exercise (14, 26, 27). Consequently, we did not measure an association between IL-6 immediately post-exercise and CK. Similarly, Ostrowski et al. (20) also reported a lack of association between IL-6 immediately post-exercise and CK following a marathon. Further, Crosier et al. (2) found no difference in IL-6 responses after two identical bouts of eccentric exercise, one that resulted in muscle damage and one that did not. Collectively, these investigations suggest that the IL-6 measured immediately post-exercise is not associated with markers of muscle damage or the systemic acute phase response.

Toft et al. (26) reported a correlation between IL-6 4 h after eccentric exercise and CK 2 d post-exercise. We measured a similar correlation ($r = 0.428$, $P < 0.05$) between IL-6 and CK at 4 h post-exercise. Thus, we suggest that the IL-6 at this point in time following exercise-induced muscle damage may be more indicative of the tissue damage response than IL-6 measured immediately post-exercise.

C-reactive protein synthesis is induced primarily by IL-6 and predominately occurs in the liver (9, 30). Increases in CRP indicate that a systemic acute phase response is taking place (9, 30). We measured increases in CRP starting at 4 h and peaking at 24 h post-exercise. This time course for post-exercise CRP increases is consistent with previous research (1, 7, 21).

Our finding of similar post-exercise CRP responses in the low and high CHO groups is a further indication that the systemic inflammatory response was not influenced by the difference in IL-6 between groups. Additionally, the correlation coefficient between IL-6 immediately post-race and CRP 24 h post-race was 0.000. It may simply be that the difference between groups was not sufficiently large to influence the systemic inflammatory response. Alternatively, the magnitude of the IL-6 response during exercise does not appear to be associated with the delayed systemic inflammatory response.

We did measure a correlation between CRP and CK 24 h post-exercise. Thus, there is an association between the systemic acute phase response to inflammation and the efflux of CK from damaged muscle tissue. While this is the first investigation to report this association, Strachan et al. (25) reported that both CK and CRP increase as distance of races increased from 15 to 88 km. Fielding et al. (5) reported an association between CK and other components of the inflammatory response including leukocyte infiltration into injured muscle and inflammatory cytokine

production. Similarly, MacIntyre et al. (14) found an association between the delayed increase in IL-6 6 h post-exercise and plasma myosin heavy chain concentrations (indicative of skeletal muscle damage).

In conclusion, while this investigation is based on data collected in an uncontrolled environment and does not utilize a controlled research design, it does provide some clues as to how ad libitum consumption might differ from the controlled CHO consumption at or above 0.90 g · kg^{-1} · h^{-1}. Ad libitum CHO consumption tended to be lower than in controlled intake studies. Attenuation of increases in plasma IL-6 with endurance exercise was observed with ad libitum intake of carbohydrate at higher compared to lower levels. Lower carbohydrate intakes were associated with higher IL-6, but no difference in blood glucose, consistent with the proposed role of IL-6 as a glucoregulatory hormone promoting blood glucose homeostasis. Neither the exercise-induced muscle damage response marked by the increase in CK, nor the acute phase response marked by the increase in CRP were influenced by carbohydrate intake.

Acknowledgments

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