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ORIGINAL RESEARCH

Effects of 4:1 carbohydrate/protein solution versus a carbohydrate-alone solution on IL-6, TNF-α, and cortisol during prolonged cycling in hot environmental conditions

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Purpose: Intense or prolonged exercise and/or heat stress might affect the immune system creating a response similar to trauma or inflammation, resulting in an increase in the susceptibility to viral infections. For example, during prolonged exercise, inflammatory cytokines, such as tumor necrosis factor (TNF)-α, interleukin (IL)-6, and the stress hormone cortisol are produced and released. Although there have been several studies examining the effects of nutritional supplementation on cytokine release in elite athletes, few studies have investigated the effects of different energy drinks during exercise in adverse environmental conditions. Therefore, the purpose of this study was to compare plasma levels of inflammatory cytokines TNF-α and IL-6, and the stress hormone cortisol, during prolonged cycling under hot environmental conditions while ingesting fluid that contains a ratio of 4:1 carbohydrates and protein (4:1 CHO/PRO) versus a carbohydrate-only drink (CHO).

Methods: Six male cyclists (aged 27 ± 8 years; weight 75.5 ± 3.4 kg; VO2max = 66 ± 2.7 mL/kg/min, mean ± standard error) rode on a stationary ergometer on two separate sessions for 2.5 hours at 75% VO2max in an environmental chamber set at 35°C and 60% relative humidity. During the first session the cyclists were given 4 mL/kg body weight of a 6% carbohydrate solution every 15 minutes. During the second session they were given 4 mL/kg body weight of a 4:1 carbohydrate/protein drink every 15 minutes. Subjects were not aware of which drink they were given in each trial. Blood samples were taken pre-, immediately post-, and 12 hours post-exercise. SPSS (IBM Corp, Armonk, NY) was utilized to analyze data through repeated measures analysis of variance.

Results: No significant main effect was observed between treatments in either cortisol ($P = 0.97$), IL-6 ($P = 0.64$), or TNF-α ($P = 0.37$) responses. Total cortisol concentrations were significantly elevated ($P < 0.05$) immediately post-exercise, and from pre- to 12 hours post-exercise with both the 4:1 CHO/PRO and the CHO-alone solutions. TNF-α concentrations were only significantly ($P = 0.045$) elevated post-exercise with the CHO-alone solution. A significant ($P < 0.05$) elevation of IL-6 was seen immediately post-exercise and 12 hours post-exercise with both the CHO-alone and 4:1 CHO/PRO solutions.

Conclusions: Consuming a 4:1 CHO/PRO solution during prolonged cycling under hot environmental conditions has comparable effects on inflammatory cytokines to drinking a CHO-alone solution.

Keywords: carbohydrates, cytokines, heat, performance, protein

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Introduction

It is understood that immune function is affected by physical activity in an adverse environment.[1] While light physical activity even in extreme environmental conditions

promotes a beneficial immune response, highly intense or prolonged exercise and/or heat stress creates a similar response to trauma or inflammation, which can result in an increased susceptibility to viral infections.[1,2] For example, during prolonged exercise, inflammatory cytokines such as TNF-α and IL-6 are produced and released by macrophages and lymphocytes.[3] It has also been demonstrated that heat stress affects physiological responses to exercise, which has been associated with decreased performance in highly trained endurance athletes.[4] Athletes who experience repeated exposure to high heat and humidity during the summer months while in the competitive season can suffer chronic inflammation, decreased muscle glycogen resynthesis, and increased recovery time.[5] During the heat-acclimatization period some of these athletes' performance suffers a decline for periods lasting up to 4 weeks, which in turn can ultimately become a limitation to achieving top level physical performance during competition.[5,6] As a result, there is a need for interventions that can alleviate such symptoms.

In terms of acute nutritional interventions it is well known that improved endurance performance is observed in athletes when consuming carbohydrate beverages during and after exercise, due to the replenishment of glycogen stores.[7,8] Literature in the sports medicine and sports nutrition areas suggests that the ingestion of ≤100 g of protein during or after arduous physical activity can reduce systemic indices of inflammation, reduce perceived muscle soreness or fatigue, and even promote a faster recovery of muscle function when compared to ingesting a placebo.[6,9,10] It is well known that amino acids exert a protective effect on muscle tissue both via direct and indirect mechanisms. Directly, amino acids are known to depress the pathways for Z-line disruption during mechanical trauma. Indirectly, these mechanisms relate to specific amino acid derived metabolites, for example, beta-hydroxy-beta-methylbutyrate, which is a marker of muscle damage used in studies.[11]

Because both the ingestion of carbohydrates and protein alone during and after exercise has resulted in enhanced performance, more recently research has been conducted on the effects of ingesting a mixed carbohydrate and protein drink during or immediately after a high-intensity workout.[12,13] Findings from the above-mentioned studies are still inconclusive since there is substantial variability with respect to outcome measures, protocols, subjects, and products used. Hence, the present study investigated the effects of specific carbohydrate drinks with added protein on cytokines including inflammatory markers in a hot, humid environment.

It is of utmost importance for athletes and the active community in general to find methods that aid heat acclimatization and decrease the level of inflammation observed due to heat stress before and during the competitive period. Therefore, the purpose of this study was to compare plasma differences in inflammatory cytokines TNF-α and IL-6, in addition to the stress hormone cortisol, during prolonged cycling under hot environmental conditions while ingesting fluid that contains a ratio of 4:1 carbohydrates and protein (4:1 CHO/PRO) or a 6% carbohydrate (CHO)-alone drink. It was hypothesized that feedings of 4:1 CHO/PRO compared to CHO-alone would attenuate plasma inflammatory markers immediately after 2.5 hours of intense cycling in the hot and humid environments.

Methods

Subjects

Six highly trained male road cyclists (aged 27 ± 8 years; weight 75.5 ± 4 kg; VO2max = 66 ± 6 mL/kg/min, mean ± standard error [SE]) volunteered to participate in this study. The subjects were highly trained cyclists competing in category 2 road cycling (USA Cycling) events for at least 4 years. Subject anthropometric data is summarized in Table 1. The study was conducted in the winter months to minimize any natural heat acclimatization. Subjects were in the preseason of their training cycle and their first competition was scheduled to take place 1 month after testing. Informed consent was obtained from each subject, and the experimental procedures were in accordance with the policy statements review board of the University of West Florida (UWF).

Experimental procedures

Two to three weeks before the first test session, subjects reported to the UWF Exercise Physiology Laboratory for a study orientation, medical history assessment and anthropometric measures, which included height, weight, and body fat composition via Lange skinfold calipers, and a cardiorespiratory fitness, or VO2max, test. During the orientation session, subjects were educated on avoiding additional supplements

Table 1 Descriptive data for anthropometric measurements of subjects (N = 6)

Anthropometric measure	Mean	SE
Weight (kg)	75.58	1.99
Height (m)	1.75	0.04
BMI (kg/m^2)	23.90	1.12
BF%	12.60	1.22

Abbreviations: SE, standard error; kg, kilogram; m, meter; BMI, body mass index; BF%, body fat percent.

or ergogenic aids, and a list of these aids was supplied to them. Subjects agreed to avoid the use of any ergogenic aids, vitamins or minerals (above 100% of daily recommended allowances), nutritional supplements, herbs, or medications known to affect the immune system for one week before the test sessions. Subjects submitted a weekly nutritional log during the week of the tests. The cyclists brought their own bikes, which were set up at a CompuTrainer™ PRO Model 8001 trainer (RacerMate, Seattle, WA), or cycling computer to determine their VO2max. A graded maximal protocol that started at a resistance of 150 W and increased by 25 W every 2 minutes was used to determine VO2max.[14] Oxygen uptake, ventilation (VE), maximal heart rate (HRmax), maximum exercise workload (Wmax), and respiratory exchange ratio (RER) were measured using a MAX-II System (AEI Technologies Inc, Naperville, IL). The VO2max test was conducted in the laboratory at neutral conditions (21°C, 35% humidity). All of the subjects fulfilled the criteria to achieve maximal effort which included: age predicted maximal heart rate (220 – age), RER equal or higher than 1.15, and plateauing of VO2max.[15]

For this crossover study, each subject came into the laboratory for two randomized, counterbalanced test sessions approximately 1 week apart and they were asked to abstain from any physical activity for 24 hours before each trial. Subjects reported to the laboratory at 9 am for each test session. In order to control caloric intake during test day, and assume consistency among sessions, subjects ingested 16 kcal/kg of a standardized liquid meal that exceeded daily value recommendations for all vitamins and minerals (Boost Plus, Mead Johnson Nutritionals, Evansville, IN).[14] During the sessions, cyclists exercised at 60% Wmax, which equated to 75% of subjects' VO2max in an environmental chamber set at 35°C and 60% relative humidity. The solutions were kept at cool temperatures in blinded plastic water bottles so that the subjects did not know which solution they were given. Only the main investigator had knowledge of the solution given to each subject. Every 15 minutes the cyclists were given either 4 mL/kg body weight of lemon lime carbohydrate-only beverage, Gatorade02 perform, lemon lime flavor, nutrition per serving = 31 g, 120 kcal, carbohydrates 28 g (sucrose), sodium 200 mg, potassium 60 mg (The Gatorade Company, Chicago, IL), or 4 mL/kg body weight of Accelerade, lemon lime flavor, nutrition per serving = 31 g, 120 kcal, carbohydrates 21 g (sucrose), protein 5 g (whey protein isolate), sodium 190 mg, potassium 65 mg, during the other session (PacificHealth Laboratories Inc, Matawan, NJ). Both drinks were isocaloric (31 g = 120 kcal). In order to monitor intensity and workload throughout the trials, power output was recorded every 10 minutes and if subjects fell below the prescribed intensity they were asked to increase their efforts. Blood samples were collected before the test session, immediately post-exercise and 12 hours post-exercise. Sweat loss was calculated from the difference in nude body mass before and after exercise and corrected for fluid intake during the cycling bout. Plasma volume changes due to dehydration were determined according to Dill and Costill (1974).[16]

Plasma cytokine and cortisol measurements

Blood samples (20 mL) were drawn from the antecubital vein into heparinized tubes 10 minutes before the test sessions, immediately after each session, and 12 hours postexercise. The collection tubes were immediately centrifuged and plasma samples frozen at –80°C until analysis. Plasma cortisol was assayed by using an immunosorbent assay kit (Alpco Diagnostics™, Salem, NH). Total plasma concentrations of TNF-α and IL-6 measurement were determined using quantitative sandwich enzyme-linked immunosorbent assay kits provided by Alpco Diagnostics. All samples and provided standards were analyzed in duplicate. An ultrasensitive kit was used to analyze IL-6. Cytokine concentrations were determined by using linear regression from a standard curve which was provided in standards in the kit (ALPCO Diagnostics).

Statistical analysis

Descriptive data are expressed as means ± SE. Differences in body weight pre- and post-exercise were analyzed using a 2 × 2 repeated-measurements analysis of variance (ANOVA). Cytokines and cortisol hormone values were analyzed by using a 2 (4:1 CHO/PRO and CHO groups) × 3 (pre-exercise, immediately postexercise, and 12 hours postexercise measurements) repeated-measurements ANOVA. Newmans-Kuels post hoc tests were used to locate differences when the ANOVA revealed a significant interaction; SPSS version 17 (IBM Inc, Armonk, NY) was utilized to analyze data and statistical significance was set at $P < 0.05$.

Results

All of the subjects maintained the prescribed intensity for heart rate and power output (Table 2). No significant differences ($P > 0.05$) in body weight were found pre- and post-exercise in both conditions (Table 3). A plasma volume decrease of 4.1% ± 3.2% was observed post-exercise in both conditions. Cytokine responses to 4:1 CHO/PRO drink and CHO-alone drink are summarized in Table 4.

Table 2 Mean and SEHR, RER, VE, and power output for subjects during exercise in CHO or 4:1 CHO/PRO conditions

Variable	Condition	
	CHO (mean ± SE)	4:1 CHO/PRO (mean ± SE)
HR	158 ± 3.0	159 ± 2.7
RER	0.86 ± 0.01	0.89 ± 0.00
VE ($L \cdot min^{-1}$)	137.2 ± 2.3	139.3 ± 2.1
Power output (watts)	173 ± 10.1	172 ± 9.8

Abbreviations: SE, standard error; HR, heart rate; RER, respiratory exchange ratio; VE, ventilation; CHO, carbohydrate; PRO, protein.

No significant difference between treatments was observed in cortisol ($P = 0.97$), IL-6 ($P = 0.64$), or TNF-α ($P = 0.37$) responses. A significant difference for time was found with regard to cortisol concentrations ($F(1, 5) = 14.478$, $P = 0.011$). Total cortisol concentrations were significantly elevated ($P < 0.05$) post-exercise and 12 hours post-exercise with both the 4:1 CHO/PRO solution and with the CHO-alone feedings.

A significant difference for time was observed in TNF-α (F (1, 5) = 12.035, $P = 0.002$). TNF-α concentrations were only significantly ($P = 0.045$) elevated post-exercise with the CHO-only solution.

A significant difference for time (F (1, 5) = 33.35, $P = 0.000$) was observed for IL-6. A significant ($P < 0.05$) elevation of IL-6 was seen immediately post-exercise and 12 hours post-exercise with both the CHO-alone solution and the 4:1 CHO/PRO solution.

Discussion

This research study examined whether acute ingestion of a CHO/PRO energy drink, as opposed to a CHO-alone drink, during and after completion of cycling in the heat, would have an impact on plasma inflammatory cytokine markers. In the present study an increase of TNF-α was only observed after exercise with the CHO-alone solution. Although statistically significant, the slight increase in TNF-α observed after exercise with the CHO-alone drink is not likely to be physiologically or metabolically meaningful. Ullum et al[17] demonstrated similar TNF-α responses to our study when they failed to obtain significant increases of plasma TNF-α after 60 minutes of cycling at 75% of maximal oxygen uptake. The researchers argued that concentric exercise, like cycling, which induces less muscle damage, does not influence production of TNF-α.[17] Other studies have shown no differences in TNF-α even after eccentric exercise with post-exercise consumption of a carbohydrate drink or a milk-based carbohydrate-protein drink.[13] Wojcik et al[13] suggest that estimates of protein synthesis should be important in determining potential benefits of different nutrients consumed during and after exercise. In addition, to better understand the effects of a 4:1 CHO/PRO versus a CHO-alone drink on performance in extreme environmental conditions, future studies should include measurements of muscle damage (creatine kinase) and actomyosin protein breakdown (3-methylhistidine) that were not measured in the present study.

Upon completion of the exercise protocol, serum concentrations of IL-6 were significantly higher post-exercise in both conditions. These results concur with several other studies (Pedersen et al,[18]) where IL-6 rises significantly after exercise.[18,19] According to Pedersen et al, IL-6 has not only been shown to increase after exercise, but hyperthermia or heat stress has also shown to trigger increases in IL-6 concentrations.[18] Selkirk, McLellan, Wright, and Rhind observed a temperature-dependent increase in IL-6 in both trained and untrained individuals during a walk to exhaustion at 40°C and 30% humidity.[19] In the present study the similarity of response in IL-6 between treatments suggests that the heat stress mediates the increase of IL-6 rather than an expected attenuation of this by the 4:1 CHO/PRO beverage. Similar results were also observed in one study that measured IL-6 as an inflammatory marker following exercise with acute ingestion of similar supplements.[12] In these studies the level of IL-6 did not alter between placebo, carbohydrate, and protein-carbohydrate beverage ingestion.[12,13]

Plasma cortisol levels in the present study were elevated immediately after exercise and 12 hours post-exercise with both drinks; the 4:1 CHO/PRO drink and the

Table 3 Body weight differences prior to and immediately following exercise in CHO or 4:1 CHO/PRO conditions

	CHO			4:1 CHO/PRO		
	Pre	Post	12-hour post	Pre	Post	12-hour post
TNF-α (pg/mL)	33.3 ± 23.8	35.6 ± 24.3*	34.5 ± 24.0	41.7 ± 21.2	42.68 ± 21.4	41.0 ± 21.2
IL-6 (pg/mL)	0.3 ± 0.1	1.7 ± 0.3*	0.9 ± 0.2*	0.2 ± 0.05	1.2 ± 0.3*	1.2 ± 0.2*
Cortisol (nmol/L)	791.5 ± 121.5	1109.2 ± 69.7*	872.3 ± 60.2*	873.0 ± 60.1	997.34 ± 61.2*	852.1 ± 72.1*

Note: *Statistical significance was set at $P < 0.05$.
Abbreviations: SE, standard error; CHO, carbohydrate; PRO, protein.

Table 4 TNF-α (pg/mL), plasma IL-6 (pg/mL), and cortisol (nmol/L) prior to (pre), immediately following (post), and 12 hours into recovery (12-hour post) from 2.5 hours of biking at 75% VO2max either with CHO or 4:1 CHO/PRO fluid feedings

	CHO	4:1 CHO/PRO
Pre-weight (kg)	76.1 ± 1.9	76.0 ± 2.3
Post-weight (kg)	75.4 ± 2.1	74.3 ± 2.3
Difference (kg)	0.7	1.7
P-value	0.333	0.06

Notes: Values are means ± SE. *Denotes main time effect difference from pre-exercise values ($P < 0.05$).
Abbreviations: CHO, carbohydrate; PRO, protein; TNF, tumor necrosis factor; IL, interleukin.

CHO-alone solution. According to Brenner et al, exercise intensity and exposure to heat are correlated to the release of plasma cortisol levels in athletes.[20] The authors stated that plasma cortisol levels will increase the same manner as with exercise-induced body heating if the heat exposure is of long duration (over 90 minutes) and core temperature rises by at least 1.2°C. The uniform rise of cortisol post-exercise among all conditions noted in the present study agrees with the literature since cyclists in the current study were exposed to extreme environmental conditions for over 120 minutes. Moreover, Brenner et al stated that plasma cortisol release is correlated with the duration and intensity of the exercise.[20] Cortisol values usually increase with aerobic exercise higher than 60% of VO2max, which was the case in the present study. Our results led us to assume that the rise in plasma cortisol observed was a response to the duration and intensity of exercise, and heat exposure, and that the ingestion of a 4:1 CHO/PRO or a CHO-alone drink did not dramatically affect plasma cortisol levels. It is important to mention that plasma cortisol demonstrates diurnal variations and the results of this study might be influenced by these variations.[13]

The present study has several limitations that need to be addressed. The small sample size did not allow us to directly observe differences between conditions. Moreover, the results of this study might not be reflective of a "true" average response because of the small sample size. Most of the cyclists were enrolled in the Marine Corps which imposed considerable time constraints due to duty requirements. Therefore, recruitment and commitment to our study of such high caliber athletes was difficult. Unfortunately, core temperature was not recorded in the current study and we consider the lack of core temperature measurement a limitation in this study. Additionally, the lack of blood glucose measurements during this study is a limitation that should be addressed in future similar studies. Finally, to better understand the effects of a carbohydrate-protein drink on inflammatory markers it is necessary to include a trial comparing the drink with water alone. We consider our "pilot study" results warrant future studies to evaluate the effects of different supplements on inflammatory markers during intense exercise in a hot, humid environment.

Conclusions

This study demonstrated that feedings of a 4:1 CHO/PRO drink during long periods of cycling in hot and humid environments did not greatly attenuate inflammatory responses in cyclists when compared to feedings of a CHO-alone solution. This response needs to be addressed and further investigated since inadequate ergogenic aids might be related to higher inflammation levels or immune deficiencies postcompetition in hot environments.

Disclosure

All authors have participated substantially in the submitted work, have reviewed and approved of the final version of the work, and take responsibility for its content. The submitted manuscript is original and unpublished material, except in abstract form or oral report, and is not under consideration by another journal. All authors report no conflicts of interest in this work.

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