ACUTE HEPATIC RESPONSE TO DIET MODIFICATION AND EXERCISE-INDUCED ENDOTOXEMIA DURING A LABORATORY-BASED DUATHLON

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ABSTRACT: The purpose of the study was to compare the acute hepatic response to diet modification and exercise-induced endotoxemia, and to determine if associations exist between liver damage markers, body core temperature, and IL-6 responses to a laboratory-based duathlon. Eleven moderately-trained healthy males followed a low-carbohydrate (CHO) and a high CHO diet to change their glycogen stores two-days before completing a duathlon. Blood samples were obtained at rest, immediately after and 1- and 2-h following the duathlon for determination of endotoxin-lipopolysaccharide binding protein (LPS-LBP) complex, IL-6, and liver integrity markers AST, ALT, and AST/ALT ratio. Hydration status and body core temperature were assessed at rest, during, and after the duathlon. Athletes were more dehydrated and had higher AST/ALT ratios in the low- compared to the high-CHO diet trial regardless of the measurement time ($p<0.05$). IL-6 increased from resting to immediately after, 1- and 2-h following duathlon regardless of the diet ($p<0.05$). A higher LPS-LBP complex concentration was observed from rest to immediately after the duathlon. No significant correlations were found between LPS-LBP complex levels and body core temperature. In conclusion, athletes on a low-CHO diet showed higher hepatic structural damage and finished more dehydrated compared to athletes on a high-CHO diet. Body core temperature and LPS-LBP complex levels were unrelated beyond the increase in body core temperature explained by exercise. No significant associations were found between body core temperature, IL-6 and LPS-LBP complex concentrations.

KEY WORDS: human, cytokines, lipopolysaccharide, inflammation, exercise

INTRODUCTION

The gastrointestinal system is not only responsible for nutrient absorption, but also for hosting beneficial bacteria. A failure to keep bacteria within the intestines will produce a state called endotoxemia in which bacterial lipopolysaccharides (LPS) or endotoxin translocates to reach the liver. LPS translocation might occur when factors such as a reduction in splanchnic blood flow, ischemia, long stays at altitude, or increased body core temperature affect the integrity of the intestinal wall [23,63]. Indeed, splanchnic blood flow decreases in an inversely proportional manner in relation to the percentage of maximal oxygen consumption (VO_2max) achieved during exercise [20]. LPS elicits a cytokine-mediated pro-inflammatory response that can eventually evolve in sepsis and heat stroke.

The mechanism for cytokine activation involves binding of LPS to serum lipopolysaccharide-binding protein (LBP) to form the LPS-LBP complex [62]. LBP is an acute-phase protein synthesized in hepatocytes considered an opsonin (i.e., can enhance the uptake of bacteria by phagocytic cells) for activation of macrophages (e.g., Kupffer cells) by LPS [38,64]. LPS-LBP complex catalyzes the transfer of LPS to membrane-bound soluble CD14, and is considered a marker of bacterial translocation, transport, and clearance [17].

Once the LPS binds to LBP, the intracellular signal transduction occurs that leads to a proinflammatory response mediated by cytokines such as interleukin-6 (IL-6) released from macrophages [62]. Therefore, a direct association is hypothesized between

LPS-LBP complex and IL-6 [65]. Others have reported subclinical increases in circulating pro-inflammatory IL-6 during strenuous exercise [27,60].

Endurance and resistance training exercise also promote changes in liver damage markers in humans, as previously reported during cycling and running competitions [10,13,35,41,49,52,57]. The chronic effect of exercise in the detoxifying ability of the liver has been studied in animal models where rats who trained for 10-weeks showed lower hepatic damage than their sedentary counterparts [9]. This evidence in an animal model clearly suggests a training adaptation of the liver in its ability to detoxify noxious substances such as LPS. How these findings might be extrapolated to humans is debatable and subject to research.

In spite of the evidence showing that the liver potential to metabolize and clear different drugs is reduced in the presence of LPS [61], the precise mechanisms involved are poorly understood. We suggest a hypothesis that explains that one of such mechanisms might be the initial endogenous energy supply of the liver since LPS clearance is an energy-dependent process carried out by the liver resident macrophages, the Kupffer cells. A similar hypothesis was studied in a rat model where fasting reduced or deactivated temporarily the activity of the Kupffer cells [55]. However, the study was conducted using an animal organ donor model, and not a human exercise-induced hepatotoxicity model.

Therefore, the purpose of this study was to compare the acute hepatic response to initial endogenous hepatic energy levels and exercise-induced endotoxemia, and to determine if associations exist between liver damage markers, body core temperature, and IL-6 responses to a laboratory-based duathlon. Our main hypothesis was that endogenous liver energy stores, as manipulated by diet and endurance exercise, would elicit a differential acute response in markers of liver damage and the LPS-LBP complex-mediated IL-6 response.

MATERIALS AND METHODS

Subjects. Eleven moderately-trained healthy males (age=36.64±4.95 yr; VO_2max=57.36±7.41 ml/kg/min; height= 1.74±0.06 m; weight=74.47±7.66 kg; DEXA body fat=17.22±6.63%; fat-free mass=61.42±5.85 kg) participated in the study. They trained for middle and long distance events such as marathon and triathlon on an average of 11 h•wk^{-1}, including running on average 13 km•wk^{-1} and cycling 24 to 40 km•wk^{-1}. Written informed consent was obtained from each subject prior to participation, and the Institutional Review Board from Auburn University approved the study. Based on the IL-6 response to exercise from previous studies [6,14], and using the nQuery Advisor® statistical sample size software, it was estimated that a sample size of 10 would have a power (β =0.80) to detect an effect size of 0.996 with a 0.05 significance level [43].

Protocol. The protocol initially involved screening with a health history questionnaire for current use of nonsteroidal anti-inflammatory drugs (NSAID). Volunteers were not allowed to participate if they had anemia, any gastrointestinal disorders or other chronic disorders. Participants were also excluded if they had a cardiac pacemaker or other implanted electromedical device, were current cigarette smokers, had an acute or chronic illness or infection, food allergies, or any vaccinations within the previous two-week period.

Liver energy manipulation and exercise intervention. Liver energy stores were modified by diet and exercise as described elsewhere [36]. Briefly, two isoenergetic diets were designed for each participant, a glycogen depletion diet or low carbohydrate (CHO) (low-CHO) and a diet high in CHO (high-CHO). The nutrient content was significantly different for CHO and fat content for both diets. Diets were prepared of commercially available pre-packaged foods and given to each participant 72-h before an exercise trial taking into consideration allotments for breakfast, lunch, dinner, and snacks.

Forty-eight hours before completing a duathlon, participants on the high-CHO diet were instructed to return to the laboratory to complete a 60-min sub-maximal (70% VO_2max) jog on the treadmill, and 24-h before the duathlon, participants were required to rest. During the 48-h in which participants consumed the low-CHO diet, they were required to run on a treadmill for 60-min (70% VO_2max) two-days before the duathlon and 45-min at the same intensity on the day prior to the duathlon. Then, participants were instructed to rest (i.e., no extra exercise) the day before the duathlon.

Duathlon. On the evening before the experimental session, participants were reminded to ingest a silicon-coated pill (HQ Inc., Palmetto, FL, USA). This pill was used as the sensor for determining core temperatures during the experimental duathlon. The sensor was factory-calibrated and was designed to be ingested easily and voided with normal bowel movements within 48-h [31].

On the day of the duathlon participants arrived at the laboratory, returned empty food packages and voided their bladders before body weight was measured. Then, they were instructed to sit quietly for 5-min. Next, a fasting blood sample was obtained. Following the initial blood draw, participants were provided with a standardized breakfast to eat before resting in a comfortable chair. After the 60-min the rest period, participants had 10-min to warm up and then started the duathlon in the following order: a) treadmill run of 5-km (Run-1); b) 30-km stationary cycle (Bike); and c) 10-km treadmill run (Run-2). The subjects ran at 0% grade and were allowed to modify only the treadmill speed. For the cycling part of the race, participants had previously attached their own bicycles to a CompuTrainer™ (Racer Mate, Inc., Seattle, WA, USA). During the duathlon the participants were given the opportunity to drink chilled water ad libitum; solid foods were avoided at all times. Total volume of ingested liquid was measured for further analyses of hydration status. Body temperature was monitored during each stage of the duathlon and VO_2 was also determined seeking to assure an exercise intensity of >70% VO_2max. Once the experimental session was completed, the subjects were provided with rehydration

fluids, fruit, and an appointment for the next visit to the laboratory. Experimental exercise sessions were separated by at least 7 days.

Blood sampling and analytical procedures. Antecubital venous blood samples were obtained at rest, immediately after and again 1- and 2-h after the duathlon. Blood samples were obtained for determination of hematocrit (Hct) and hemoglobin (Hb). Another blood sample was obtained, allowed to clot, centrifugated at 1500g for 15-min to prepare serum aliquots to be stored at -80°C for further analysis of hepatocyte integrity markers aspartate aminotransferase (AST) and alanine aminotransferase (ALT) (Flex®Dimension®, Dade Behring Inc., Deerfield, IL, USA). Enzyme linked-immuno-sorbent assay (ELISA) kits were used for determination of insulin (IN), glucagon (GL) (LINCO, St. Charles, MO, USA), LPS-LBP complex, and a high-sensitivity human IL-6 (HsIL-6) (Cell Sciences, Inc., Canton, MA, USA). Plasma concentration of IL-6 and LPS-LBP were corrected to take into consideration changes in plasma volume due to exercise [11].

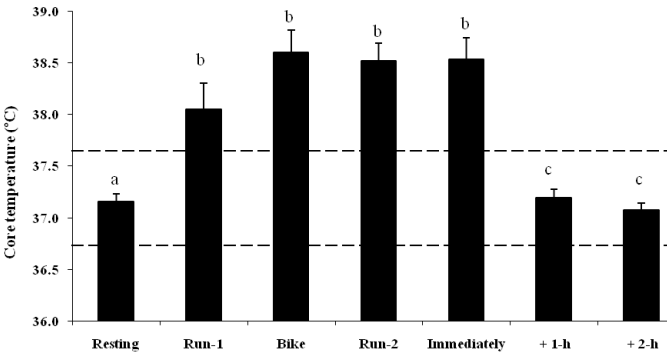
Statistical analysis. Data were analyzed with the Statistical Package for the Social Sciences (SPSS®), version 15.0 for Windows. Descriptive data are presented as means (M) and standard deviation (± SD), and statistical significance was set a priori at $p \leq 0.05$. Paired samples t tests were used to determine significant mean differences between experimental conditions in the dependent variables IN/GL ratio, performance time in the duathlon, diet composition, and hydration status. Factorial 2 x 4 (diets x time points) repeated measures analyses of variance (ANOVA) were computed to analyze AST, ALT, the AST/ALT ratio, LPS-LBP complex, and IL-6. Body core temperature was analyzed by a 2 x 7 (diets x time points) factorial, repeated-measures ANOVA. Percentage VO_2max was analyzed by a 2 x 3 factorial, repeated-measures ANOVA. For all ANOVA tests, appropriate follow-up analyses were computed if significant interactions and/or main effects were found. Finally, a Pearson product-moment correlation was calculated between body composition and IL-6.

RESULTS

Diet and endogenous hepatic energy change. The two diets had similar energy content (~11 MJ); however, the low-CHO diet provided significantly more fat (67%) and less CHO (21%) than the high-CHO diet (25% fat, 63% CHO) ($p<0.001$). The protein content from total energy for both diets was ~11% ($p>0.05$). The mean (SD) fasting IN/GL ratio in the low-CHO Diet (0.27±0.10) was lower ($p \leq 0.05$) than the mean ratio on the high-CHO diet (0.39±0.20), indicating athlete's compliance to the dietary regimen and a change in endogenous hepatic energy status.

Hydration status, body core temperature and duathlon performance. Analysis of hydration status indicated a higher fluid loss (3978.4±1222.4 vs. 3341.8±1235.6 ml) and dehydration (-1.6±1.2 vs. -1.0±1.4%) after the low-CHO diet trial compared to the high-CHO diet trial ($p=0.001$). Body core temperature analyses did not indicate an interaction effect between diet and measurement times ($p=0.626$). In general, body core temperatures increased from resting to the different segments of the race and post-exercise period ($p \leq 0.001$) regardless of the diet consumed (Fig. 1). No differences in mean performance time in the total duathlon were observed during the low-CHO diet (136.38±20.09 min) and the high-CHO (134.88±20.89 min) diet. Regardless of the dietary trial, the subjects performed the duathlon at an intensity of 71.1±2.0% of their individually determined VO_2max.

Liver damage markers. Biochemical and immunological variables measured in this study are presented in table 1. Repeated measures ANOVA indicated no significant interaction between diet and measurement time in the AST, ALT, and/or the AST/ALT ratio ($p>0.05$). For AST, the main effect diet showed significant mean differences in the low- compared to the high-CHO diet (39.23±3.52 vs. 29.40±1.75 U/L; $p=0.007$), whereas the main effect measurement



p<0,05; c +2-h < a< c +1-h < b

FIG. 1. BODY CORE TEMPERATURE DURING A DUATHLON. VALUES ARE COLLAPSED IN SINGLE COLUMNS SINCE NO DIFFERENTIAL EFFECTS BETWEEN DIETARY CONDITIONS WERE OBSERVED. BROKEN LINES REPRESENT UPPER AND LOWER LIMITS FOR NORMAL RESTING BODY TEMPERATURE FOR ADULTS[33]. VALUES ARE MEANS ± SEM.

TABLE I. BIOCHEMICAL AND IMMUNE PLASMA MARKERS FOR THE EXPERIMENTAL CONDITIONS (M ± SD).

	Low-CHO diet				High-CHO diet			
	R	I	+ 1 h	+ 2 h	R	I	+1 h	+2 h
ALT (U/L)[a]	35.68 ± 5.54	37.05 ± 7.28	34.09 ± 6.37	34.05 ± 7.85	32.09 ± 5.36	34.50 ± 7.02	32.77 ± 7.06	33.61 ± 6.27
AST (U/L)[a]	31.82 ± 9.90	40.81 ± 11.56	39.97 ± 11.72	40.54 ± 12.50	24.41 ± 4.90	30.80 ± 6.37	29.97 ± 6.34	31.79 ± 6.33
LPS-LBP (pg/ml)[a]	4.59 ± 1.72	5.91 ± 4.03	4.92 ± 3.14	4.05 ± 1.91	3.84 ± 1.66	4.43 ± 2.10	3.67 ± 1.77	4.07 ± 0.85
IL-6 (pg/ml)[a]	0.82 ± 0.53	9.23 ± 7.38	5.84 ± 4.40	3.99 ± 3.52	0.73 ± 0.56	8.05 ± 5.19	5.77 ± 3.25	3.58 ± 1.78

Legend: R = resting; I = immediately after duathlon; + 1h = 1-h following duathlon; +2 h = 2-h following duathlon.
[a] - $p > 0.05$, for interaction effects (diet condition X measurement time). Significant main effects are presented in the results section.

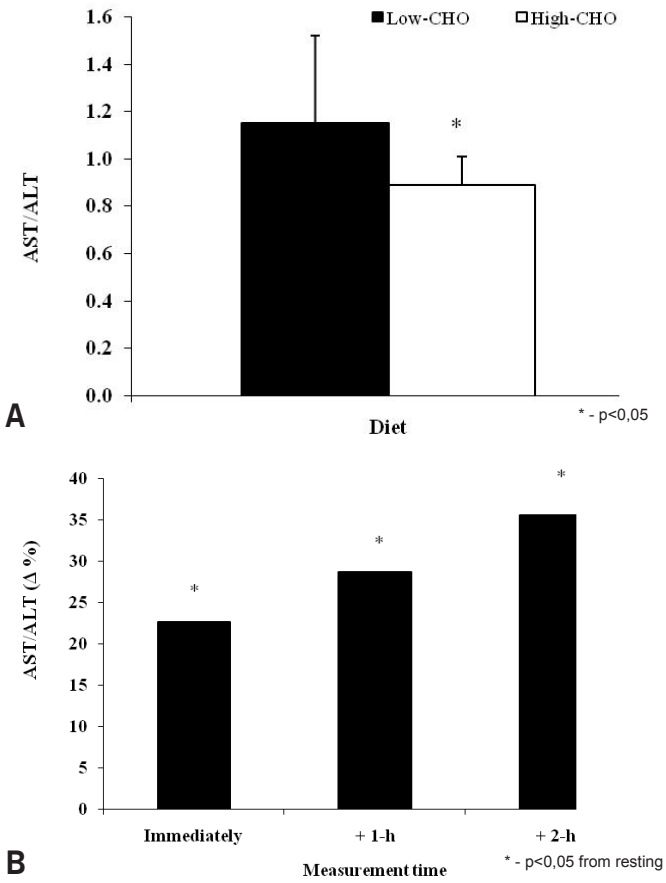


FIG 2A-B. MAIN EFFECT ANALYSES SHOWS:
A) AST/ALT RATIO FOLLOWING A LOW- AND A HIGH CHO DIET, B) THE RELATIVE CHANGE (Δ%) FROM RESTING FOLLOWING A DUATHLON.

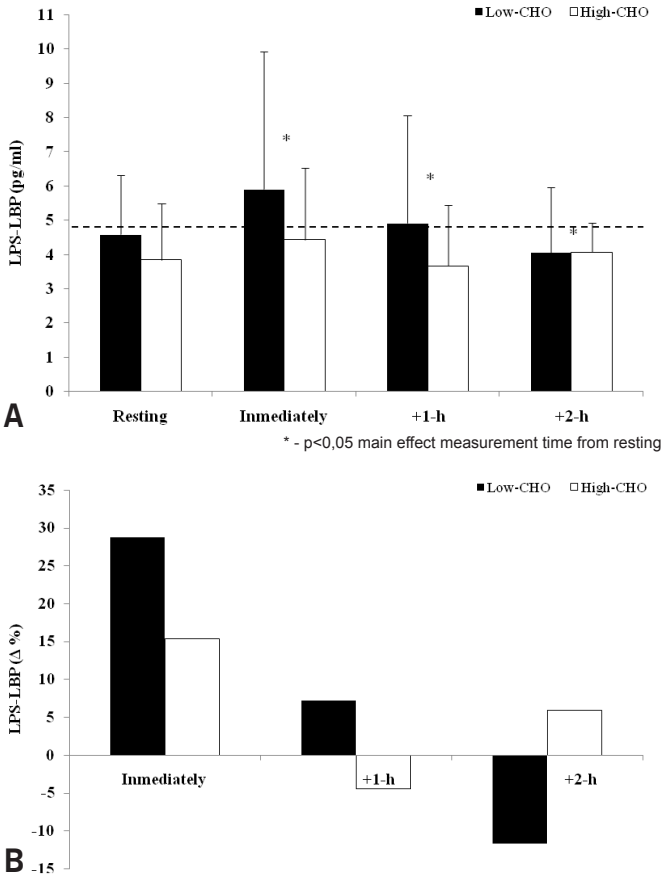


FIG 3A-B. LPS-LBP COMPLEX ACUTE RESPONSE FOLLOWING TWO DIETARY CONDITIONS. PANEL A) SHOWS THE INTERACTION BETWEEN DIETARY CONDITIONS AND MEASUREMENT TIME. MAIN EFFECT MEASUREMENT TIME WAS SIGNIFICANTLY DIFFERENT FROM RESTING ($p<0.05$). BROKEN LINES REPRESENT CUT-OFF POINT FOR MILD ENDOTOXEMIA. PANEL B) SHOWS RELATIVE CHANGE (Δ%) IN LPS-LBP COMPLEX FROM RESTING FOLLOWING A DUATHLON.

time indicated significantly higher mean values immediately, 1- and 2-h following exercise compared to resting values ($p<0.001$). For ALT, a significant measurement time main effect was observed, with baseline values lower than immediately after exercise ($p=0.010$). No significant differences were found between immediately, 1- and 2-h following exercise in ALT values. For AST/ALT ratio, the main effect diet showed a ratio higher in the low- compared to the high-CHO diet (1.15 ± 0.37 vs. 0.89 ± 0.12; $p=0.042$) (Fig. 2a). In addition, the main effect measurement time showed that the AST/ALT ratio increased from resting to immediately following exercise and remained elevated 1- and 2-h following exertion (Fig. 2b).

Endotoxin and IL-6 markers. Regardless of the dietary manipulation, intestinal permeability increased immediately following exercise from resting as determined by the LPS-LBP complex (Fig. 3a, $p\leq0.05$). The mean LPS-LBP complex concentrations increased from resting to immediately after exercise and decreased from resting to 1- and 2-h following exercise (Fig. 3b). Mild-endotoxemia, defined as LPS-LBP complex > 5 pg/ml [51], was found at baseline in three participants (27%) on the low-CHO diet compared to only one (9%) participant on the high-CHO diet. However, endotoxemia was observed in six subjects (55%) immediately following exercise in the low-CHO diet, compared to five subjects (45%) on the high-CHO diet. One-hour after exercise, four (36%) and three (30%) subjects

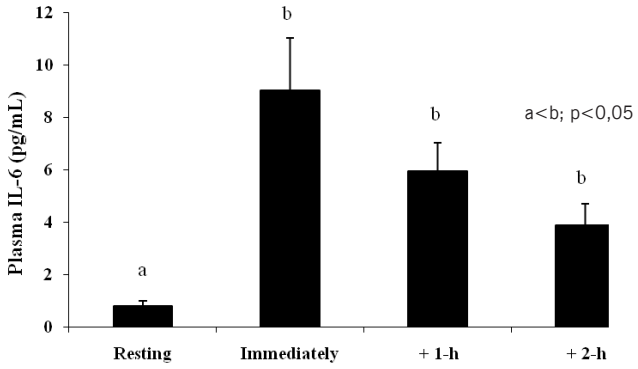


FIG 4. PLASMA IL-6 CONCENTRATIONS FOR MALES PERFORMING A DUATHLON. VALUES ARE COLLAPSED INTO SINGLE COLUMNS SINCE NO DIFFERENTIAL EFFECTS BETWEEN DIETARY CONDITIONS WERE OBSERVED. VALUES ARE MEANS ± SEM.

in the low and the high-CHO diets had endotoxemia. Finally, 2-h following exercise, endotoxemia was found only in one athlete in the low- and the high-CHO diets, respectively. The highest LPS-LBP complex concentration found in a participant was 16.7 pg/ml immediately following exercise in the low-CHO diet condition.

No interaction effect between diet and measurement time on IL-6 was observed ($p>0.05$). Main effect measurement time showed an IL-6 concentration increased from baseline to immediately following duathlon, which remained elevated 1- and 2-h after the exercise sessions (Fig. 4). Pearson-product moment correlations in the low-CHO diet 2-h following exercise indicated an inverse correlation between fat-free-mass and IL-6 ($r=-0.645$; $p=0.032$). Also, in the high-CHO diet 2-h following exercise, an inverse correlation was found between fat-free-mass and IL-6 ($r=-0.653$; $p=0.041$).

DISCUSSION

The primary findings of this study were a higher dehydration level and a mild increase in markers of hepatic damage in the low-CHO diet compared to the high-CHO diet following a duathlon. We found an acute exercise-induced mild endotoxemia in both dietary conditions and did not find changes in physical performance or IL-6 response when initial liver energy status was altered by diet and exercise before a duathlon.

The main energy-requiring processes carried out by the liver are ureagenesis, futile cycling of substrates, gluconeogenesis, protein synthesis, and ketogenesis [37]. We hypothesized that under a low-CHO diet, hepatic glycogen stores and available energy would be reduced and therefore hepatocellular clearance of endotoxins impaired, as suggested by animal models where hepatocyte shrinkage and damage occurred following intense exercise [18,28,30,55]. Thus, in the low-CHO diet trial, we expected to find a higher hepatocellular damage following exercise compared to the high-CHO diet trial. Since LBP is produced by hepatocytes [59], we expected in the low-CHO diet to find an inverse correlation between LPS-LBP and ALT, a serum transaminase more specific to hepatocellular injury [24]. In the low-CHO condition, LPS-LBP complex values were consistently higher than in the high-CHO trial at virtually all sampling times from resting up to the 2-h following exercise; however, no statistical differences or significant associations with ALT were observed.

The AST/ALT ratio is considered a surrogate marker for hepatocyte necrosis and inflammation, with values >1.0 suggestive of hepatocyte damage [1]. In this study, the AST/ALT ratio was > 1.0 immediately following exercise and in the recovery phase (i.e., 1- and 2-h post-exercise), indicating hepatocyte and liver parenchyma structural damage. ALT increased from baseline to immediately following exercise, indicating a mild hepatocyte damage possibly explained by a combination of factors such as exercise intensity, duration, and reperfusion to the liver following exercise [28]. However, even in the presence of a reduced hepatic blood flow induced by intense exercise, the liver is able to maintain its metabolic functions [39]; therefore, using the AST/ALT as a true marker of hepatic damage during exercise may be equivocal [49].

In the present study, the mean exercise intensity (~70% VO_2max) elicited by the subjects during the duathlon in both dietary conditions was high enough to cause intestinal permeability and bacterial translocation from resting as demonstrated by the increased LPS-LBP complex concentration. This exercise intensity caused bacterial translocation immediately following exercise similar to that reported in marathon and ultra-endurance events [3,4,6,7,27,44]. The increased LPS-LBP complex marker indicated a higher transmission of cellular signaling capable of eliciting an increased cytokine response. This finding is similar to previous research in sedentary and athletic populations [20,46,47].

We expected to find a positive correlation between LPS-LBP complex levels and body core temperature since endotoxins are pyrogenic agents capable of eliciting a febrile-like response [48]. No significant correlation coefficients were found between core temperature and endotoxin translocation levels measured immediately, 1- and 2-h following exercise. We suggest that the pyrogenic effect of the increased endotoxemia was not significantly greater than the expected increase in body temperature associated with exercise (body core temperature > 37.8°C). The highest mean core temperature recorded in our participants was 40.69°C in the high-CHO diet condition who completed the run-2 (10 km) in more than 60 min. This figure was slightly below the proposed 41.0°C and 42.0°C shown to impair physiologic functions in humans [32]. LBP is produced in hepatocytes under IL-6 stimulation to capture and present LPS to CD14 and induce the secretion of IL-6 [26]. Therefore, we expected to find a correlation between LPS-LBP complex and IL-6; however, we were unable to demonstrate a significant correlation in either dietary conditions up to 2-h following exercise.

Immune cytokines such as IL-6 have very high metabolic rates [5], and therefore fuel availability before, during and after exercise is of greater concern for athletes. Others [21] have found that compared to high-CHO diets, low-CHO diets significantly impaired immune response (e.g., higher cortisol release, neutrophilia, leucocytosis, neutrophil:lymphocyte ratio, and IL-6; and lower plasma glutamine) following 1-h of cycle ergometer exercise performed at 70% of their individual VO_2max. A very low-CHO diet implies a very high-fat dietary content, which has been shown detrimental to immune function. We expected to find an increase in IL-6 from baseline following exercise in both dietary conditions, as previously reported in measurements taken after light, moderate and hard intensity exercise performed for short- and long-periods of time [2,7,12,22,27,34,40,51]. In our study, IL-6 increased significantly from baseline immediately following the duathlon regardless of the diet, and remained elevated 1- and 2-h following exercise. This finding is consistent to figures shown by marathon runners and athletes running in a treadmill for 2.5-h [40,45].

In the present study, our athletes ran at approximately 70% of their individual VO_2max and were allowed to drink water ad libitum until the last blood sample was drawn 2-h after the duathlon. We reported an 11-fold increase in IL-6 from baseline that also steadily decreased 7- and 5-fold 1-and 2-h following exercise (Fig. 4). Others have found 22-fold increases from baseline immediately and 1-h following 100-min of cycling exercise at 70% VO_2max followed by a time-trial test[8]. A mild dehydration (1.6% body mass)

was found in the low-CHO trial compared to the high-CHO trial that did not influence performance.

In this study we determined body composition by dual energy X-ray absorptiometry (DEXA) method. A direct correlation between FFM and IL-6 has been hypothesized [15,48], based on the contention that skeletal muscle functions as an endocrine organ. Thus, skeletal muscle can account for most of exercise-induced increase in plasma IL-6 [14]. In the present study, since one of the experimental conditions included a glycogen-depletion diet we expected a dramatic increase in IL-6 following exercise in such condition. We found inverse correlations between FFM and IL-6 only 2-h following exercise in both, the low- and high-CHO dietary conditions. Our results suggest that fat tissue accounts for most of the exercise-induced increases in IL-6. We are aware that this finding must be interpreted with caution since IL-6 is not exclusively secreted by muscle tissue but also by several cells and tissues (e.g., fibroblasts, endothelial cells, lymphocytes, macrophages, and adipose tissue during exercise). However, during exercise only a small contribution of IL-6 has been reported for the brain and peritendon [15,16,19,29,42], and therefore, we expected a higher contribution of plasma IL-6 from skeletal muscle following exercise.

The diet content used in this study have previously shown to change both, muscle and hepatic glycogen stores, and therefore, energy stores [36,56]. Previous studies [25,50,58] indicated that low-CHO diets, defined as those having ≥30% fat of total energy, significantly reduced pre-exercise muscle and hepatic glycogen content and might impair exercise performance, yet we did not find differences in duathlon performance times between the experimental trials.

CONCLUSIONS

In conclusion, athletes following a low-CHO diet showed higher acute hepatic structural damage markers and finished more dehydrated compared to athletes on a high-CHO diet. Body core temperature and LPS-LBP complex levels were unrelated beyond an expected increase in body core temperature explained by exercise. No significant associations were found between body core temperature, IL-6 and LPS-LBP complex levels. Further studies are needed that look into the chronic effects of exercise training on liver structure and function.

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