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The Effect of Carbohydrate Ingestion on the Interleukin-6 Response to a 90-minute Run Time Trial

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Fatigue is a predictable outcome of prolonged physical activity; yet its biological cause remains uncertain. During exercise, a polypeptide messenger molecule interleukin-6 (IL-6) is actively produced. Previously, it has been demonstrated that administration of recombinant IL-6 (rhIL-6) impairs 10-km run performance and heightened sensation of fatigue in trained runners. Both high carbohydrate diets and carbohydrate ingestion during prolonged exercise have a blunting effect on IL-6 levels postendurance exercise. We hypothesized that carbohydrate ingestion may improve performance during a prolonged bout of exercise as a consequence of a blunted IL-6 response. Seven recreationally trained fasted runners completed two 90-min time trials under CHO supplemented and placebo conditions in a randomized order. The study was of a double-blinded, placebo-controlled, cross-over study design. Distance covered in 90 min was significantly greater following exogenous carbohydrate ingestion compared with the placebo trial (19.13 ± 1.7 km and 18.29 ± 1.9 km, respectively, p = .0022). While postexercise IL-6 levels were significantly lower in the CHO trial compared with the placebo trial (5.3 ± 1.9 pg·mL^{-1} and 6.6 ± 3.0 pg·mL^{-1}, respectively; p = .0313), this difference was considered physiologically too small to mediate the improvement in time trial performance.

Keywords: cytokines, carbohydrate, prolonged exercise, fatigue, intervention

Exercise fatigue has been described as the failure to continue exercising at a prescribed work rate[1,2] in the presence of an increased subjective perception of effort.[3] Most popular theories consider that fatigue develops as a result of specific biochemical changes in the active muscles[4] although more recently the importance of central neural command is becoming apparent.[5]

It has been suggested that during exercise central command could be altered by changes in a circulating chemical.[6] Indeed elevated plasma levels of the poly-

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peptide messenger molecule interleukin-6 (IL-6) have been associated with increased sensation of fatigue at rest[7] and during exercise, resulting in a significant impairment of 10-km run time trial performance in trained runners.[8] IL-6 is actively produced within contracting skeletal muscles[9] and the central nervous system (CNS)[10] as well as peritendinous tissue[11] and during prolonged exercise plasma IL-6 concentrations consistently increase (for review see Suzuki et al[12]) with levels increasing >100-fold postexercise.[13] Importantly, IL-6 can cross the blood–brain barrier or act at the level of circumventricular organ outside of the blood brain barrier.[14] Furthermore, IL-6 receptors exist at numerous sites in the brain. As previously hypothesized, IL-6 may be taken up by the brain during exercise and the increased release of IL-6 from skeletal muscle during prolonged exercise acts as a feedback mechanism contributing to the development of central fatigue.[6]

It appears that plasma IL-6 response to exercise is attenuated by carbohydrate ingestion[15–17] and by high carbohydrate diets before exercise.[18] This has led to the proposal that IL-6 acts in the manner of an exercise-induced glucose-regulating hormone and is released from either the muscle[19] or the liver[17] to maintain glycemic homeostasis.[17] Studies have investigated the effect of carbohydrate ingestion on IL-6 kinetics during fixed intensity exercise for a fixed duration[15–17] and have reported a blunted IL-6 response and a reduced perception of effort.[15] However, the effect of carbohydrate supplementation on the IL-6 response to exercise during self-paced athletic performance has not yet been evaluated.

Therefore, the purpose of this study was to determine whether carbohydrate ingestion (with the intention of reducing plasma levels of IL-6) during a 90-min self-paced time trial resulted in improved athletic performance.

Methods

Subjects

Seven healthy, recreationally trained male runners (mean ± SD age 24 ± 4 years, mass 74 ± 7 kg, height 178.8 ± 6.5 cm) volunteered for the study. All subjects completed an exercise challenge, which consisted of a 90-min time trial on a motorized treadmill, on two occasions in a randomized order (by laboratory technician who was not involved in the study) and the different treatment conditions each separated by one week. The trials took place in the exercise physiology laboratory at the University of Portsmouth. Approval for this study was obtained from the University of Portsmouth Ethics Committee. Before participation in the trial, all subjects completed informed consent forms and a health questionnaire. Subjects were excluded if they had a history of autoimmune, cardiovascular, endocrine or hematopoietic diseases.

Protocol

Self-Paced Time Trial. All subjects had prior experience of treadmill running and in the week before the trials completed a familiarization 90-min time trial. Trials were conducted at the same time in the morning following an overnight fast. A coefficient of variation of 0.94% for distance covered during 90-min time

trial has been reported for this test (personal communication with M. Barwood, University of Portsmouth). On each trial occasion, subjects reported to the laboratory at precisely the same time of day following an overnight fast. To standardize procedures, subjects were instructed to keep an accurate diary of their exercise training and dietary intake in the week before the first exercise trial, so that they could replicate these factors in the week before the second exercise trial. The study adopted a double-blind, placebo-controlled, cross-over design. Subjects were asked to refrain from heavy exercise in the 72-h period and any exercise in the 24-h period preceding each exercise trial.

Intervention. Following a preexercise blood sample, subjects consumed a fluid bolus (8 mL·kg^{-1} body mass) containing either maltodextrin (8%; CHO) or a placebo (PLA). PLA and CHO beverages were taste-matched by the addition of equal volumes of a lemon flavored sugar-free drink. Within 5 min of consuming the prescribed beverages, subjects began a 90-min self-paced time trial on a motorized treadmill. During each time trial, subjects ingested a beverage of either PLA or CHO (2 mL·kg^{-1} body mass) every 20 min. Neither the true composition of the beverage nor distance completed during each time trial was disclosed to the subjects until completion of both trials. Subjects were provided with elapsed time but not distance feedback. Heart rate, distance and rating of perceived exertion (RPE)[20] were taken every 10 min during each 90-min time trial.

Blood Sampling and Analysis. Venous blood samples were drawn from the antecubital vein before and immediately following each time trial. Blood was collected into appropriate Vacutainer tubes (Becton Dickinson, New Jersey, USA) and centrifuged at 1500 × *g* for 10 min in a refrigerated centrifuge at 4°C. The supernatant was transferred into Eppendorf tubes and immediately frozen at −80°C until later analysis. Plasma IL-6 concentrations were analyzed from tripotassium ethylene diamine tetraacetic acid–treated blood using an enzyme linked immunosorbent assay (IMMULITE, DPC, UK). Plasma glucose and lactate were measured on a BIOSEN C (EKF Diagnostic GmbH, West Germany) analyzer.

Data Analysis

Statistical evaluation of the results that passed the Kolmogorov–Smirnov normality test was carried out using either repeated measures ANOVA or paired Student's *t* tests. When significance was identified with the repeated-measures ANOVA, a Tukey–Kramer multiple comparisons post hoc test was performed to determine the minimum significant difference (MSD). The *q*-value from each pairwise comparison was compared against the MSD to determine significance. Non-Gaussian distributed data were analyzed with a Wilcoxon matched pairs test. All statistics were performed on Graphpad Instat v 3.06. The accepted level of significance was $P < .05$.

Results

Carbohydrate ingestion in a fasted state significantly improved self-paced 90-min time trial running performance compared with the placebo ingestion (19.13 ± 1.7 km and 18.29 ± 1.9 km, respectively, Student's *t* test, $p = .0022$); all seven subjects

completed a greater distance in the CHO time trial than during the PLA time trial (Figure 1).

Preexercise IL-6 values were <2 pg·mL^{-1}, which is below the detection limit of the ELISA. Therefore we examined differences between postexercise IL-6 concentrations using both repeated-measures ANOVA (where preexercise levels were assumed to be 2 pg·mL^{-1}) and a Wilcoxon test comparing only the postexercise plasma IL-6 levels. The ANOVA found no significant differences in postexercise IL-6 between conditions (MSD = 3.997; q-value = 2.366; $p > .05$); however, the Wilcoxon test identified a statistically significant attenuation in the IL-6 response in CHO compared with PLA (5.3 ± 1.9 pg·mL^{-1} vs. 6.6 ± 3.0 pg·mL^{-1}, respectively; $p < .0313$).

Preexercise plasma glucose concentrations were similar between trials (MSD = 3.997; q-value = 0.175; $p > .05$); however, postexercise plasma glucose levels were significantly higher in CHO compared with PLA (Table 1; MSD = 3.997; q-value = 6.866; $p < .001$). Rating of perceived exertion, heart rate (Figure 2) and plasma lactate (Table 1) were not different between trials although they did increase over the duration of the trials ($p < .001$).

On completion of the study three of the seven subjects correctly identified the trial in which they had ingested carbohydrate.

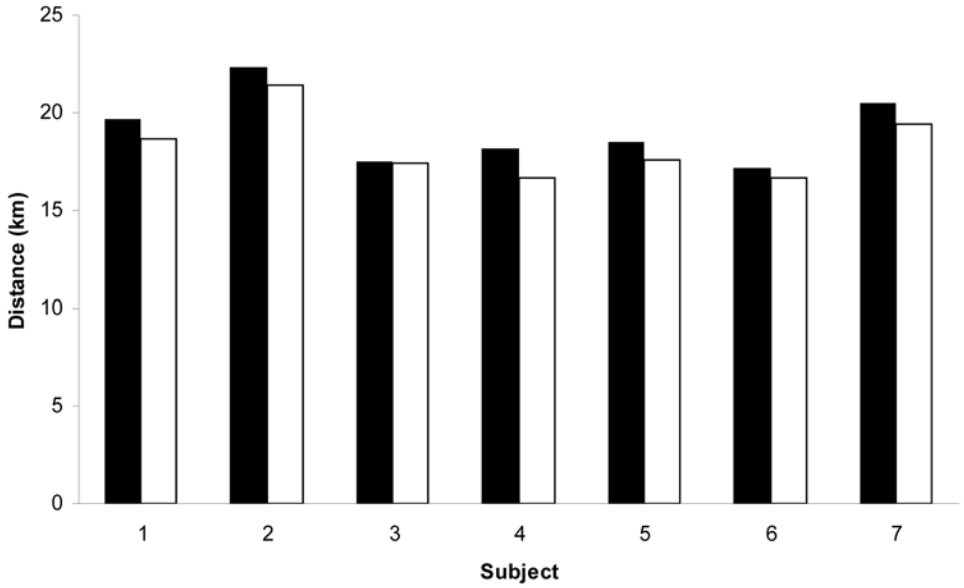

Figure 1 — Effect of CHO (solid bar) and placebo ingestion (clear bar) on total distance run in 90-min time trial (individual subject data, n = 7; $P = .0022$).

Table 1 Effect of 90-min time trial run following either carbohydrate or placebo ingestion on plasma IL-6, glucose and lactate concentrations. Data are mean ± SD, n = 7.

	Preexercise	Postexercise
IL-6 (pg·mL^{-1})†		
CHO	n.d	5.3 ± 1.9**
Placebo	n.d	6.6 ± 3.0
Glucose (mmol·L^{-1})†		
CHO	4.7 ± 0.4	6.8 ± 0.8*
Placebo	4.3 ± 1.4	5.5 ± 0.5
Lactate (mmol·L^{-1})†		
CHO	0.8 ± 0.2	3.7 ± 1.1
Placebo	0.8 ± 0.4	3.3 ± 1.5

n.d. = IL-6 preexercise were below detectable limits of the ELISA (>2 pg·mL^{-1}).

* (ANOVA, $p < .001$).

** (Wilcoxon test, $p = .0313$) CHO trial significantly different to placebo trial.

† ($p < .0001$) main effect of time.

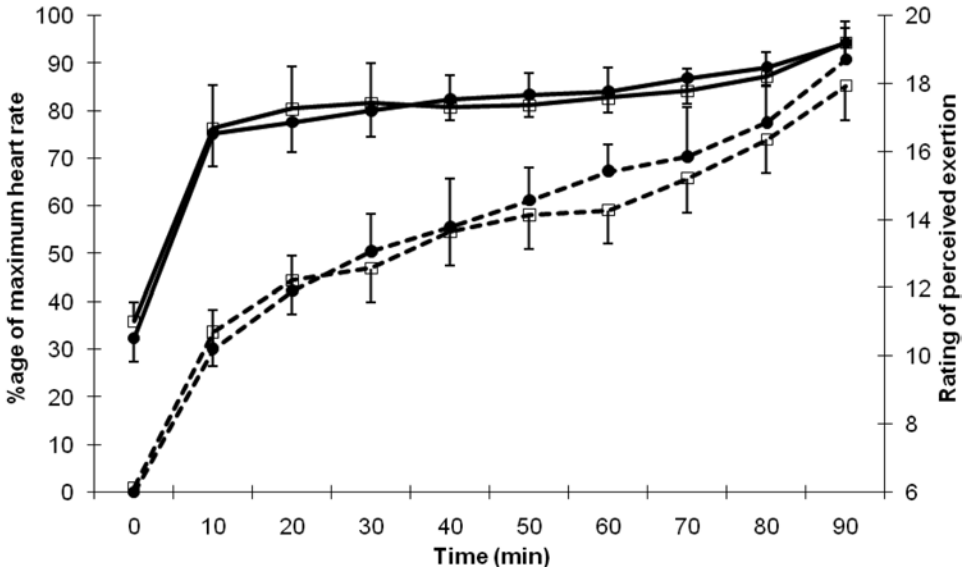

Figure 2 — Effect of CHO (●) and placebo (□) ingestion on heart rate (solid lines) and rating of perceived exertion (broken lines) during 90-min time trial. Main effect of time ($P < .001$). Data are presented as mean ± SD, n = 7.

Discussion

This is the first study to attempt to manipulate the IL-6 response and its effect on self-paced athletic performance. The main finding of this study was that exogenous carbohydrate ingestion significantly improved 90-min time trial performance and this was associated with a lower IL-6 concentration compared with the placebo trial. Our hypothesis that CHO may improve athletic performance by attenuating the IL-6 response to prolonged exercise could be accepted from a statistical standpoint however, it is doubtful that these small differences could mediate the improvement in athletic performance. Previously, it has been demonstrated that moderate IL-6 levels (14 to 21 $pg \cdot mL^{-1}$) can impair 10-km time trial performance[8] but postexercise levels of IL-6 in the current study were substantially lower than those observed by Robson-Ansley et al.[8]

It should be noted that the IL-6 response is augmented with increased exercise intensity.[21] Therefore the true extent of the difference in IL-6 between trials due to CHO ingestion in this study is partially masked by the increased running speed adopted by the subjects during the CHO trial ie, through different mechanisms. It is possible that the IL-6 response in the experimental condition was both attenuated and augmented by the CHO and intensity, respectively, compared with the placebo trial. Unfortunately we were unable to report the actual magnitude of IL-6 response from baseline to postexercise due to the sensitivity of the IL-6 ELISA kit employed for analysis (minimum detection level >2 $pg \cdot mL^{-1}$). Future studies should endeavor to use high sensitivity commercially available ELISA kits with minimum detection limits <0.8 $pg \cdot mL^{-1}$). Nevertheless, our finding that plasma IL-6 concentration was greater in the PLA trial compared with the CHO trial is in agreement with other studies.[15–17]

There are two possible mechanisms that could explain the attenuation in IL-6 release during the CHO trial. Firstly, a fall in intramuscular muscle glycogen stores could stimulate IL-6 release from the working muscle.[22] Due to the fasted state of the subjects before the exercise trials, liver glycogen stores would likely be compromised and therefore only minimally be able to contribute to the maintenance of glucose homeostasis. Hence during the PLA trial intramuscular glycogen would be the main source of substrate during exercise.[23] However, during the CHO trial the ingestion of exogenous glucose, which then enters the circulation, may reduce the use of intramuscular glycogen and attenuate the release of IL-6 from the muscle. Secondly, the increased IL-6 during exercise in the placebo trial was likely to be largely hepatic in origin as proposed by Starkie et al.[17] Hepatic IL-6 appears to have a regulatory function in maintaining blood glucose homeostasis during exercise. Before the release of hepatic IL-6 into the circulation, muscle derived IL-6 can partly mediate the hepatic glucose output by directly acting on hepatocytes to increase hepatic glucose release when blood glucose levels are compromised.[24]

We favor the latter mechanism to explain the results observed in this study based upon the effect that exogenous glucose ingestion has upon intramuscular and hepatic glycogen kinetics. The rate of glycolysis in the muscle is not altered by exogenous glucose supplementation during exercise durations less than 120 min,[25] whereas exogenous CHO ingestion can, at least partially, replace the liver as the source of blood glucose. Therefore, since euglycemia was maintained during both

trials the attenuated plasma IL-6 concentration in the presence of exogenous CHO may be a consequence of the reduced role of hepatic regulation in the maintenance of blood glucose homeostasis. Starkie et al[17] reported that following 60 min of moderate intensity exercise, plasma IL-6 was significantly higher under placebo conditions in comparison with CHO supplemented trial but skeletal muscle IL-6 mRNA was no different between trials suggesting skeletal muscle IL-6 release was unchanged. Hence, CHO ingestion may have blunted hepatic production of IL-6 during exercise resulting in lower IL-6 levels following the CHO trial.

Even though subjects completed a greater distance during the CHO trial their RPE was similar between trials indicating that subjects were able to exercise at a higher intensity for the same perceived exertion. Previously, increased RPE has been associated with an increasing state of hypoglycemia during prolonged exercise.[26] However, Nehlsen-Cannarella et al[15] found RPE was significantly higher when IL-6 concentrations were augmented despite euglycemia being maintained. This role of IL-6 in moderating perception of effort is further supported by research in which administered IL-6 impaired 10-km time trial performance but had no effect on RPE.[8] The authors of that study concluded that elevated IL-6 may have activated serotonergic pathways.

The exact mechanism explaining the improved performance during the CHO exercise trial is not clear but may be linked to an increase in central drive or motivation rather than cytokine or metabolic mediation. It is unlikely that 90-min time trial performance would be limited by endogenous glucose availability although subjects may have compromised liver glycogen as they were in a fasted condition before exercise.[27] Carter et al[28] suggest that CHO receptors in the oral cavity may modulate central pathways associated with motivation and improve athletic performance when metabolic mediation is not a factor.

The IL-6 response to exercise is sensitive to both exercise intensity and duration but the single most important factor determining the magnitude of IL-6 elevation is exercise duration.[29] Due to the study design we were unable to determine whether the magnitude of the attenuated IL-6 response as a consequence of the CHO ingestion was sufficient to explain the improved performance observed in the time trials. We suggest that future studies intending to test the hypothesis that attenuation of the IL-6 response to exercise improves athletic performance should use a preload protocol followed by a time trial (as described elsewhere[30]) to elucidate the separate effects of an intervention such as CHO ingestion on IL-6 kinetics and the effect of IL-6 release on exercise performance. The consequence of attenuating IL-6 production on athletic performance and fatigue during prolonged exercise is unknown and awaits further investigation.

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