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# Influence of Carbohydrate Supplementation on Plasma Cytokine and Neutrophil Degranulation Responses to High Intensity Intermittent Exercise

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Ingesting carbohydrate (CHO) beverages during prolonged, continuous heavy exercise results in smaller changes in the plasma concentrations of several cytokines and attenuates a decline in neutrophil function. In contrast, ingesting CHO during prolonged intermittent exercise appears to have negligible influence on these responses, probably due to the overall moderate intensity of these intermittent exercise protocols. Therefore, we examined the effect of CHO ingestion on plasma interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α) and lipopolysaccharide (LPS)-stimulated neutrophil degranulation responses to high-intensity intermittent running. Six trained male soccer players performed 2 exercise trials, 7 days apart, in a randomized, counterbalanced design. On each occasion, they completed six 15-min periods of intermittent running consisting of maximal sprinting interspersed with less intense periods of running and walking. Subjects consumed either CHO or artificially sweetened placebo (PLA) beverages immediately before and at 15-min intervals during the exercise. At 30 min post-exercise, CHO versus PLA was associated with a higher plasma glucose concentration ($p < .01$), a lower plasma cortisol and IL-6 concentration ($p < .02$), and fewer numbers of circulating neutrophils ($p < .05$). Following the exercise, LPS-stimulated elastase release per neutrophil fell 31% below baseline values on the PLA trial ($p = .06$) compared with 17% on the CHO trial ($p = .30$). Plasma TNF-α concentration increased following the exercise (main effect of time, $p < .001$) but was not affected by CHO. These data indicate that CHO ingestion attenuates changes in plasma IL-6 concentration, neutrophil trafficking, and LPS-stimulated neutrophil degranulation in response to intermittent exercise that involves bouts of very high intensity exercise.

*Key Words:* exercise, immune, nutrition

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## Introduction

Regular carbohydrate (CHO) beverage ingestion during prolonged, continuous strenuous exercise is associated with smaller changes in the plasma concentrations of several cytokines and attenuated neutrophil degranulation responses to a bacterial stimulant (3, 11, 12). In contrast, intermittent exercise of a similar duration elicits only minor changes in cytokine and neutrophil functional responses, and ingesting CHO beverages during the exercise has negligible influence on these measurements (2, 10). Although the exact underlying mechanisms remain to be determined, CHO-beverage ingestion is thought to influence immune cell function during heavy continuous exercise by maintaining plasma glucose levels, thereby reducing hypothalamic-pituitary-adrenal (HPA) axis activation and minimizing the subsequent release of the immunosuppressive hormone cortisol. The previous studies by our group and others who have examined the relationship between CHO supplementation and immune responses to intermittent exercise have found little effect of CHO on plasma cortisol concentration (2, 10), suggesting that the overall intensity of the protocols was not high enough to cause sufficient physiological stress. Therefore, we hypothesize that CHO-beverage ingestion will play a greater role in influencing plasma hormone and immune responses to intermittent exercise that includes exercise bouts of very high intensity.

Much of the research within exercise immunology has concentrated on the effects of prolonged continuous exercise (usually at a constant work rate) on immune function. However, there is considerable participation in multiple-sprint sports, such as soccer, hockey, and rugby, and intermittent training protocols are commonly used by many athletes participating in a wide variety of sports. Prolonged continuous exercise is relatively simple to replicate within a laboratory setting, yet there are great difficulties in trying to map the non-uniform patterns of multiple-sprint sports into discrete bouts of exercise. A protocol has been developed in recent years that has been shown to elicit similar physiological and metabolic responses to intermittent activities such as soccer (Loughborough Intermittent Shuttle Test [LIST]; 13, 14). Importantly, significant elevations in serum cortisol levels have been reported in response to the LIST (21), suggesting that participation in high-intensity intermittent exercise may result in altered immune function.

The acute phase response to tissue injury is comparable to that of inflammation (1). Strenuous exercise is associated with elevated plasma levels of several of the cytokines involved in inflammation, such as tumor necrosis factor-α (TNF-α), interleukin 1-β (IL-1β), IL-6 and IL-10 (4, 6, 11, 16). IL-6 is thought to stimulate the HPA axis as part of the natural negative feedback system that increases the release of cortisol (an anti-inflammatory mediator) from the adrenal cortex and inhibits further release of pro-inflammatory cytokines (1). In addition, IL-6 has been recently suggested to have a role in glucose homeostasis, signaling critically low muscle glycogen levels (19). Thus, it may be that IL-6 influences plasma cortisol levels in two ways: (a) as part of a hormone-like glucoregulatory role, and (b) as part of the inflammatory response. In view of this and the apparent relationship between stress hormone release and immune cell function, it appears that performing intermittent exercise of a high enough intensity to stimulate the release of cortisol may affect both cytokine and neutrophil functional responses. Therefore, the aim of the present study was two-fold: first, to the determine whether high-intensity intermittent exercise elicits changes in plasma TNF-α, IL-6, and lipopolysaccharide (LPS)-stimulated

neutrophil degranulation responses; and second, given the relationship between CHO availability and stress hormone release, to determine whether CHO beverage ingestion can attenuate these changes.

## Methods

Six healthy trained male soccer players (mean ± *SEM:* age, 20 ± 1 years; height 176 ± 2 cm; body mass, 71.7 ± 1.2 kg; $\dot{V}O_{2max}$, 55.6 ± 0.6 ml · kg$^{-1}$ · min$^{-1}$) volunteered to participate in the study. All subjects provided written informed consent prior to the study, which was approved by the Loughborough University Ethics Committee. Subjects did not report any symptoms of infection and were not taking any medication in the 6 weeks before the study.

Each subject's maximal oxygen uptake ($\dot{V}O_{2max}$) was estimated by means of a progressive shuttle run test (18). From this estimate, running speeds corresponding to 55% and 95% $\dot{V}O_{2max}$ were calculated from the tables for predicted $\dot{V}O_{2max}$ values (18). Subjects then performed the LIST for 30 min to familiarize themselves with the required running speeds and experimental procedures.

Subjects performed two exercise trials that were separated by 7 days in a randomized counterbalanced design. During the 2 days before each trial, subjects were asked to refrain from strenuous physical activity and record their food intake in an effort to standardize their nutritional status. They were required to eat the same foods for the 2 days before the second trial. In a further effort to standardize CHO availability during the exercise trials, subjects performed a bout of cycling designed to lower the glycogen content of both type I and II muscle fibers on the evening before each the exercise trial (22). This comprised cycling for 75 min at 70% $\dot{V}O_{2max}$ with 3- × 50-s maximal 'sprints' interspersed after 30 min. Subjects were then provided with a high-energy (60 kJ · kg$^{-1}$ body mass) low CHO (1 g · kg$^{-1}$ body mass) meal and then fasted overnight.

Subjects reported to the laboratory on the morning of each exercise trial and were required to empty their bladder before nude body mass was determined. A cannula (Venflon, 16-18G, Ohmeda, Hatfield, Herts) was inserted into an antecubital vein and kept patent by frequent flushing with sterile saline. Each subject then stood for 15–20 min, after which a pre-exercise blood sample (15 ml) was obtained. A 10-min standardized warm up was then performed and, just prior to the start of the exercise, test subjects consumed 5 ml · kg$^{-1}$ body mass of either a CHO solution (6.4% w/v glucose and maltodextrin [Lucozade Sport, Glaxo Smith Kline, Glos, UK]; CHO trial) or an artificially sweetened placebo solution (PLA trial). Each drink was identical in flavor (lemon) and appearance, and the subjects were unaware of the content of the drinks in each trial. The CHO drink contained sodium (50 mg · 100 ml$^{-1}$) and potassium (8.8 mg · 100 ml$^{-1}$). The osmolality of the drinks was measured using a freezing point osmometer (Advanced Instruments, Norwood, MA) and was 307 and 33 mOsm · kg$^{-1}$ for the CHO and placebo drinks, respectively.

For each exercise, test subjects completed six 15-min periods of intermittent shuttle running consisting of ~10 cycles of maximal sprinting, running, and walking (Figure 1). The subjects were required to run between two lines, 20 m apart at various speeds that related to the previously estimated $\dot{V}O_{2max}$ values. The running and walking speeds during each of the 20 m of the test were dictated by an audio signal from a microcomputer (BBC Master Series) using software developed for this purpose. The 15-min bouts of running were separated by 3-min rest periods.

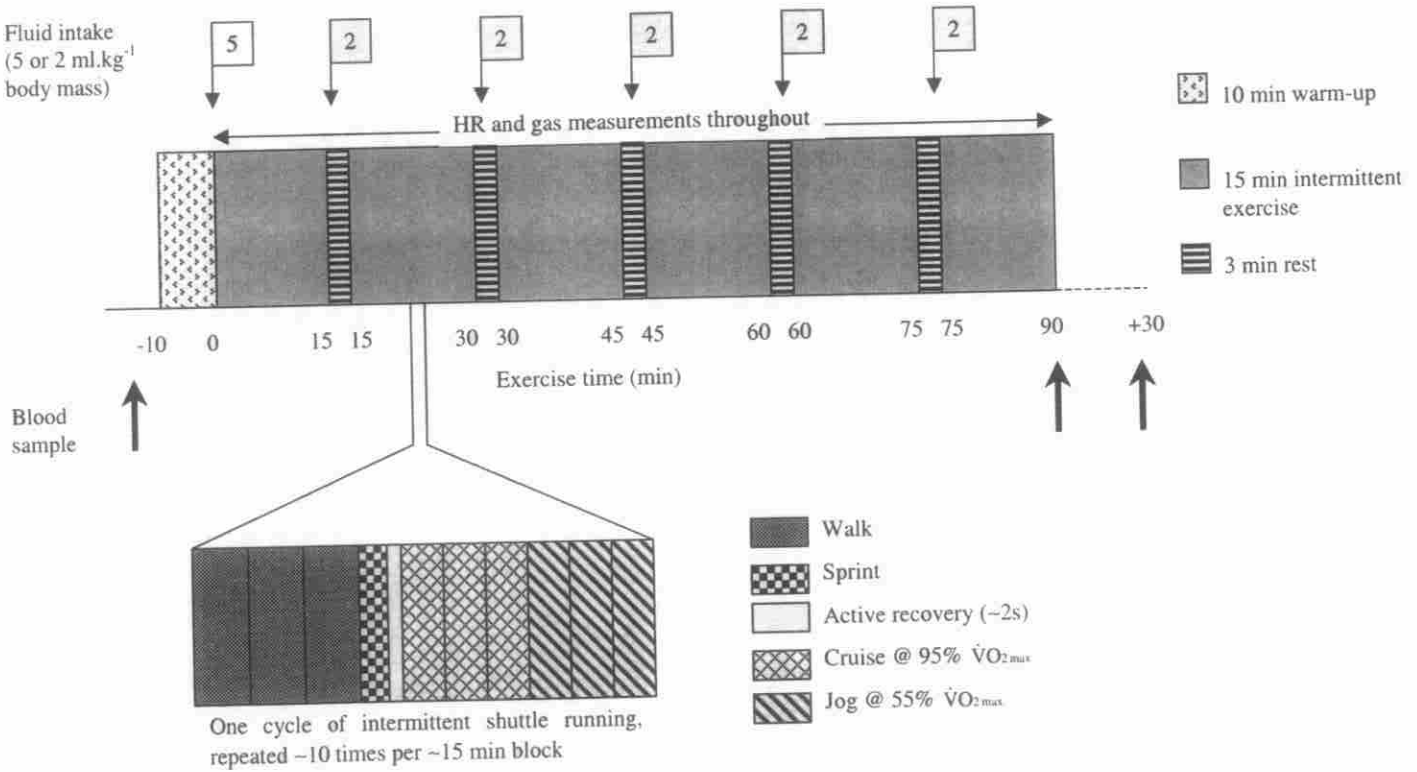


**Figure 1 — Schematic representation of the Loughborough Intermittent Shuttle Running Test (LIST). The mean exercise intensity (excluding the 3-min rest interval) is ~80% $\dot{V}O_{2max}$.**

During these rest periods, subjects consumed a further 2 ml · kg$^{-1}$ body mass of the prescribed solution. Heart rate was measured continuously throughout the exercise using short-range radio telemetry (Sportstester®, Polar Electro, Kempele, Finland). Samples of expired air were collected on two occasions during each 15-min block of the LIST using Douglas bags. A paramagnetic oxygen analyzer (Servomex 1420B, Crowborough, UK) and an infrared carbon dioxide analyzer (Servomex 1415B) were used along with a dry gas meter (Harvard Apparatus, Edenbridge, UK) for determination of $\dot{V}O_2$ and $\dot{V}CO_2$. The mean exercise intensity for the full 90 min of exercise was calculated from these measurements, excluding the 3-min rest periods. Further blood samples (each 15 ml) were taken with the subject standing immediately post-exercise and at 30 min post-exercise. Immediately after the post-exercise blood sample had been obtained, nude body mass was again recorded. No other fluid or food intake was allowed until after the 30-min post-exercise blood sample had been obtained. For all trials, the laboratory temperature was between 16–18 °C and relative humidity was ~56%.

Blood samples were collected into three separate vacutainer tubes (Becton Dickinson, Oxford, UK), two containing $K_3$EDTA and the other containing lithium heparin. Blood taken into one of the $K_3$EDTA vacutainers (4 ml) was used for hematological analysis including hemoglobin, hematocrit, and circulating neutrophil counts using a Technicon H-2 laser system (Bayer Diagnostics, Basingstoke, UK). Plasma volume changes were estimated according to Dill and Costill (8).

Of the 7 ml of blood dispensed into the lithium heparin vacutainer, 1.0 ml of each sample was immediately added to a separate snap-seal microcentrifuge tube (1.5 ml capacity) containing 50 μLof 10 mg/ml bacterial LPS solution (Stimulant, Sigma, Poole, UK). Blood and LPS were mixed by gentle inversion and then incubated for 1 h at 37 °C, being gently mixed again after 30 min. After incubation the mixture was centrifuged for 2 min at 5000 g. The supernatant was immediately stored at –70 °C prior to analysis of elastase concentration.

Elastase concentration in plasma before and after treatment with LPS was determined using a sandwich-type enzyme-linked immunosorbant assay (ELISA) kit specific for polymorphonuclear cell elastase (Merck, Lutterworth, UK), as described by Blannin et al. (5).

The remaining heparinized whole blood and blood taken into the second $K_3$EDTA vacutainer was spun at 1500 g for 10 min in a refrigerated centrifuge (4 °C). This occurred within 15 min of sampling. The plasma obtained was immediately stored at –70 °C. Aliquots of plasma were also analyzed to determine the concentration of glucose (No. 16-50 kit, Sigma, Poole, UK) and cortisol using $^{125}$I radioimmunoassay (ICN Pharmaceuticals, Costa Mesa, CA). The intra-assay coefficient of variation was 2.4%, 1.8%, and 6.3% for glucose, cortisol, and elastase, respectively.

Plasma concentrations of IL-6 and TNF-α were determined in aliquots of $K_3$EDTA plasma with the use of quantitative sandwich-type enzyme-linked immunosorbant assay (ELISA) kits (IL-6: R&D Systems, Abingdon, UK; TNF-α: Diaclone, Besançon, France), which detect both soluble and receptor bound IL-6 and TNF-α. A high-sensitivity kit was used for analysis of IL-6. All assays use monoclonal antibodies specific to the interleukin to be determined as capture antibodies. The detection limit was < 0.1 pg · ml$^{-1}$ for IL-6 and < 3.1 pg · ml$^{-1}$ for TNF-α. The intra-assay coefficient of variation was 4.3% and 2.5% for IL-6 and TNF-α, respectively.

Data in the text, tables, and figures are presented as mean values and the standard errors of the mean (±*SEM*). The data were examined using a two-factor (trial × time of measurement) ANOVA with repeated measures design. Any significant *F* ratios shown were assessed using post hoc Tukey tests and paired *t* tests, where appropriate. If a data set was not normally distributed, statistical analysis was performed on the logarithmic transformation of the data. Assumptions of homogeneity and sphericity in the data were checked and, where appropriate, adjustments in the degrees of freedom for the ANOVA were made. Statistical significance was accepted at $p < .05$.

## Results

Mean daily energy intake and absolute CHO intake were similar on both trials during the 2 days preceding the exercise tests (daily energy intake, CHO: 11.7 ± 1.1 MJ, PLA: 12.9 ± 1.5 MJ; CHO intake, CHO: 5.8 ± 0.6 g · kg$^{-1}$ body mass, PLA: 6.1 ± 0.5 g · kg$^{-1}$ body mass). There were no differences in overall exercise intensity between the CHO and PLA trials; the mean % $\dot{V}O_{2max}$ during the trials was 80.0 ± 0.4%. Heart rate was similar on both trials throughout the LIST (CHO: 164 ± 1 beat · min$^{-1}$, PLA: 162 ± 1 beat · min$^{-1}$; mean of all recordings). Following the exercise, changes in body mass (corrected for fluid intake) were similar on both trials (–1.2 ± 0.1 kg and –1.3 ± 0.0 kg on the CHO and PLA trials, respectively) as was the fall in plasma volume (–2.4 ± 1.6% and –2.3 ± 1.8% on the CHO and PLA trials, respectively).

Plasma glucose concentration was significantly higher on the CHO compared with the PLA trial at 0 and 30 min post-exercise ($p < .01$; Figure 2). At 30 min post-exercise, plasma cortisol levels on the PLA trial were markedly elevated above both pre-exercise values ($p < .01$) and values on the CHO trial ($p < .02$; Figure 3).

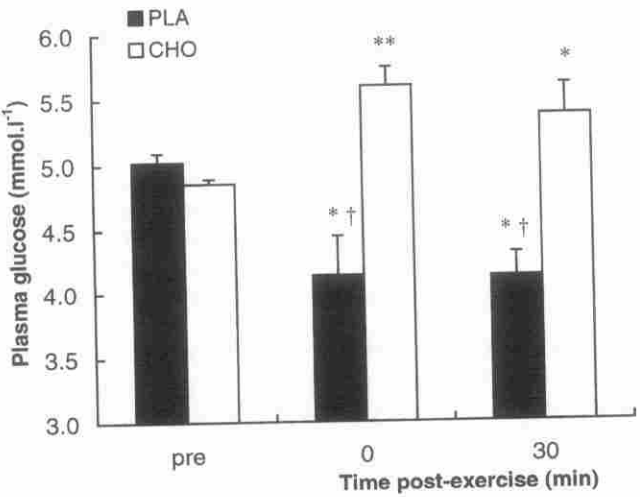


**Figure 2 — Plasma glucose response to the exercise on the carbohydrate (CHO) and placebo (PLA) trials. Values are means ± *SEM*. Significantly different from pre-exercise (within trial): *$p < .05$; **$p < .01$. Significantly lower than CHO: †$p < .01$.**

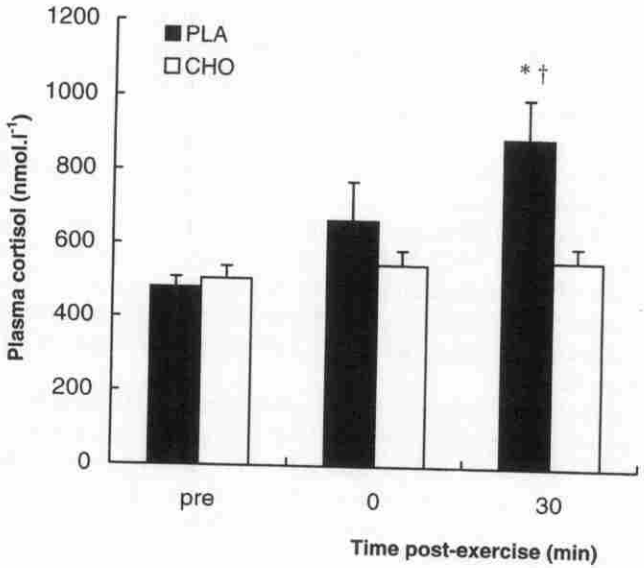


**Figure 3 — Plasma cortisol response to the exercise on the carbohydrate (CHO) and placebo (PLA) trials. Values are means ± *SEM*. Significantly different from pre-exercise (within trial): **p* < .01. Significantly higher than CHO: †*p* < .02.**

**Table 1 Plasma Elastase, Total Lipopolysaccharide (LPS)-Stimulated Elastase Release and Plasma Tumour Necrosis Factor-α (TNF-α) Concentrations During the Carbohydrate (CHO) and Placebo (PLA) Trials**

| Trial | Time post-exercise (min) | | | | | |
|---|---|---|---|---|---|---|
| | Pre-exercise | | 0 | | 30 | |
| Plasma elastase ($\mu g \cdot L^{-1}$) | | | | | | |
| CHO | 136 | (48) | 239 | (28) | 170 | (55) |
| PLA | 87 | (17) | 205 | (40) | 162 | (36) |
| Marginal mean | 112 | (26) | 222 | (24)* | 166 | (31) |
| Total LPS-stimulated elastase release ($\mu g \cdot L^{-1}$) | | | | | | |
| CHO | 791 | (155) | 1531 | (176) | 1838 | (232) |
| PLA | 893 | (100) | 2177 | (185) | 2482 | (163) |
| Marginal mean | 842 | (89) | 1854 | (156)** | 2160 | (166)** |
| Plasma TNF-α ($pg \cdot ml^{-1}$) | | | | | | |
| CHO | 15.3 | (2.9) | 18.7 | (1.9) | 16.1 | (3.5) |
| PLA | 9.4 | (2.5) | 15.8 | (3.6) | 13.4 | (3.0) |
| Marginal mean | 12.4 | (2.0) | 17.2 | (2.0)** | 14.8 | (2.0) |

*Note.* Values expressed as mean ± *SEM*. Marginal means = main effect of time. Significantly different from pre-exercise: **p* < .01; ***p* < .001.

A significant neutrophilia was evident at 0 and 30 min post exercise on both trials (all $p < .01$), the magnitude of which was substantially smaller on the CHO trial ($p < .05$ and $p < .01$ at 0 and 30 min post-exercise, respectively; Figure 4). Plasma elastase concentration was higher immediately after the LIST (main effect of time; $p < .01$) but did not differ between trials (Table 1). Similarly, total LPS-stimulated elastase release was markedly elevated above pre-exercise values immediately and at 30 min post-exercise (main effect of time; both $p < .001$; Table 1). There was also a main effect of trial, with values lower on the CHO trial than on the PLA trial (CHO: $1386 \pm 148\ \mu g \cdot L^{-1}$, PLA: $1851 \pm 187\ \mu g \cdot L^{-1}$; $p < .001$) but no significant Time × Trial interaction effect. Immediately and at 30 min post-exercise, LPS-stimulated elastase release per neutrophil was ~31% lower than pre-exercise values on the PLA trial ($p = .06$); the corresponding fall on the CHO trial was 17% and 9% at these times ($p = .30$; Figure 5).

There was a small yet significant elevation in plasma IL-6 levels on both trials immediately and at 30 min post-exercise ($p < .01$), with values higher on the PLA than on the CHO trial at 30 min post-exercise ($p < .02$; Figure 6). Although there were no significant Time × Trial interaction effects for plasma TNF-α concentration, there was a main effect of time, with values 39% higher post-exercise than pre-exercise ($p < .001$; Table 1).

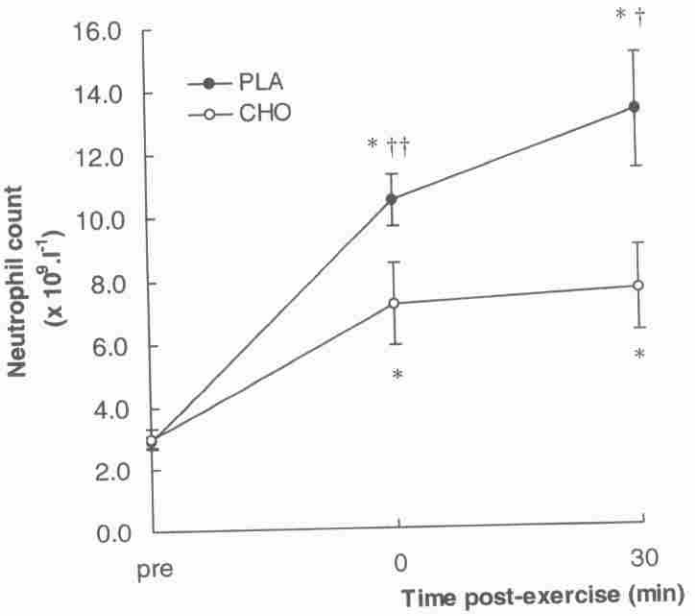


**Figure 4 — Changes in the circulating neutrophil count in response to the exercise on the carbohydrate (CHO) and placebo (PLA) trials. Values are means ± *SEM*. Significantly different from pre-exercise (within trial): *$p < .01$. Significantly higher than CHO: †$p < .05$, ††$p < .01$.**

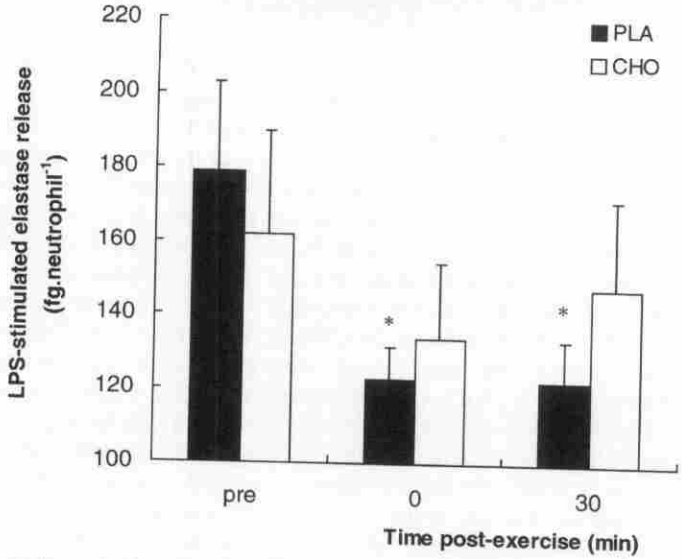


**Figure 5 — Change in lipopolysaccharide (LPS)-stimulated elastase release per neutrophil in response to the exercise on the carbohydrate (CHO) and placebo (PLA) trials. Values are means ± *SEM*. **p* < .06 compared with pre-exercise (within PLA trial).**

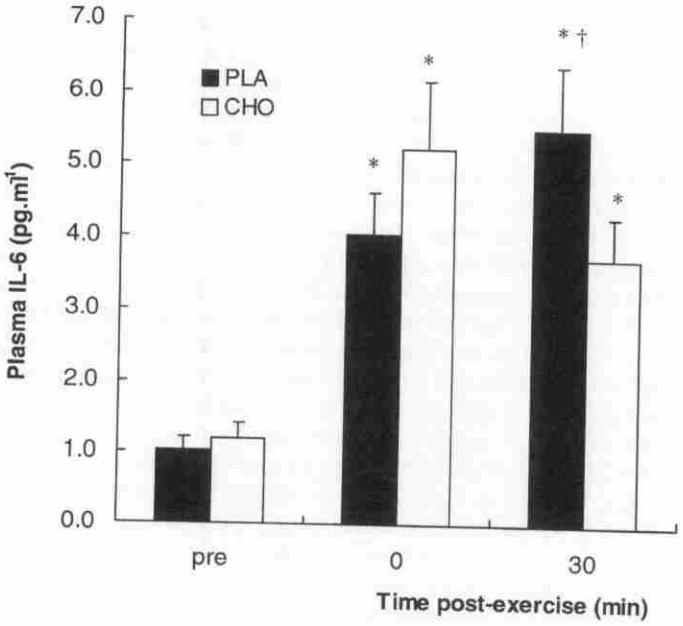


**Figure 6 — Plasma interleukin-6 (IL-6) response to the exercise on the carbohydrate (CHO) and placebo (PLA) trials. Values are means ± *SEM*. Significantly different from pre-exercise (within trial): **p* < .01. Significantly higher than CHO: †*p* < .02.**

## Discussion

These data suggest that performing high-intensity intermittent exercise is associated with a fall in plasma glucose levels, increases in plasma cortisol concentration and total LPS-stimulated neutrophil elastase release, a decrease in LPS-stimulated elastase release per neutrophil, and elevations in plasma levels of IL-6. Ingesting CHO beverages during the exercise attenuates these responses. This is in contrast to previous studies of intermittent exercise (2, 10). However, in these earlier studies the mean intensity of the exercise was <60% $\dot{V}O_{2max}$ compared with ~80% $\dot{V}O_{2max}$ over the 90 min of the exercise in the present study. Performing high-intensity intermittent shuttle running was also associated with elevations in plasma TNF-α concentration that were not affected by CHO beverage ingestion. TNF-α is a pro-inflammatory cytokine, and plasma levels are reported to increase following prolonged, continuous (9, 16) but not intermittent (10) exercise. The authors suggested that the negligible response in the latter study was due to a smaller degree of muscle damage associated with moderate intensity rowing compared with running. Running has a larger eccentric component than rowing exercise, which is predominantly concentric in nature. Therefore, the post-exercise increase in plasma TNF-α observed in the present study may be related to muscle damage sustained during the high-intensity intermittent running exercise. This is supported by the findings of previous work from our laboratory using the same intermittent running protocol, which found that muscle soreness, markers of muscle damage, and lipid peroxidation were elevated following completion of the LIST (21).

During strenuous physical activity, the release of cytokines involved in the inflammatory response follows a sequential pattern: TNF-α and IL-1β are thought to stimulate the local production of IL-6, which in turn induces the acute phase response and the release of the naturally occurring cytokine inhibitor IL-1ra from circulating monocytes. This subsequently inhibits further release of IL-1β, thus helping to down-regulate the inflammatory response (11, 15, 17). Therefore, it may be expected that the pattern of IL-6 release would follow that of TNF-α: The finding that plasma levels of TNF-α peaked immediately post-exercise, whereas plasma IL-6 concentration remained significantly above pre-exercise values at 30 min post-exercise, may lend support to this. However, whereas CHO ingestion did not affect the TNF-α response, it was associated with a marked lowering of the IL-6 response at 30 min post-exercise compared with the PLA trial. This is consistent with previous studies from our group and others that report an association between CHO availability and a lower IL-6 response to strenuous physical activity (4, 11, 12). A recent study has found that there is a net release of IL-6 from active muscle after 1 h of strenuous exercise that appears to be closely related to exercise duration, leading to the suggestion that IL-6 may act in a hormone-like glucoregulatory manner (19). The findings of the present study appear to support this further role for IL-6. Since IL-6 stimulates the HPA axis, ultimately resulting in the release of cortisol, the elevated plasma IL-6 response to the exercise on the PLA trial in the present study could be partly responsible for the overall greater post-exercise cortisol response on that trial. Increased HPA axis activation associated with falls in plasma glucose concentration on the PLA trial would also contribute to the overall cortisol response. Although it is likely that the previous bout of glycogen-lowering exercise exacerbated these responses to the LIST, it should be noted that significant elevations in

both plasma cortisol and IL-6 concentrations have been observed in response to performing the LIST without prior exercise (20).

The finding that CHO ingestion is associated with smaller post-exercise changes in the numbers of circulating neutrophils and neutrophil degranulation responses is consistent with our earlier study of prolonged cycling (3). The higher total LPS-stimulated elastase release on the PLA trial is most likely a reflection of the greater neutrophilia on the PLA compared with the CHO trial (i.e., there were more cells to respond to stimulation). However, compared with pre-exercise values, the amount of elastase being released by each neutrophil in response to LPS fell more following the exercise on the PLA trial than on the CHO trial. Since neutrophils form the first line of defense against invading pathogens, this may suggest that performing this type of exercise without CHO ingestion is associated with compromised innate host-defense, perhaps leaving the individual more susceptible to infection. Furthermore, the effects on neutrophil function may be cumulative, since a recent study of Belgian First Division players found a significant reduction in resting neutrophil phagocytosis and chemotaxis over a season compared with non-playing controls (7). This was associated with a higher incidence of upper respiratory tract infections in the players compared with the controls. Although confounding factors, such as facilitated pathogen exposure through shared drinking bottles and communal changing rooms, should be taken into consideration, the suppression of neutrophil function could partly explain the increased incidence of upper respiratory infection in these individuals.

In conclusion, the results of this study indicate that 90 min of high-intensity intermittent shuttle running elevates plasma cortisol, IL-6, and TNF-α levels, and inhibits bacterially-stimulated neutrophil degranulation responses. With the exception of TNF-α, these responses are largely attenuated by CHO-beverage ingestion. Although the lack of clinically normal reference ranges for immune measures makes it difficult to determine whether the post-exercise fall in neutrophil function predisposes the athlete to infection, these findings do suggest that participants in multiple sprint sports should ensure an adequate CHO intake in order to maintain immune cell function.

## References

1. Bagby, G.J., L.D. Crouch, and R.E. Shephard. Exercise and cytokines: spontaneous and elicited responses. In: *Exercise and Immune Function*, L. Hoffman-Goetz (Ed.). Boca-Raton, FL: CRC, 1996, pp. 55-77.
2. Bishop, N.C., A.K. Blannin, P.J. Robson, N.P. Walsh, and M. Gleeson. The effects of carbohydrate supplementation on immune responses to a soccer-specific exercise protocol. *J. Sports Sci.* 17:787-796, 1999.
3. Bishop, N.C., A.K. Blannin, L. Rand, R. Johnson, and M. Gleeson. The effects of carbohydrate supplementation on neutrophil degranulation responses to prolonged cycling (Abstract). *Int. J. Sports Med.* 21:S73, 2000.
4. Bishop, N.C., N.P. Walsh, D.L. Haines, E.E. Richards, and M. Gleeson. Pre-exercise carbohydrate status and immune responses to prolonged cycling: II. Effect on plasma cytokine concentration. *Int. J. Sport Nutr. Exerc. Metab.* 11:519-528, 2001.
5. Blannin, A.K., M. Gleeson, S. Brooks, and R. Cave. The effects of endurance training in the bacterially stimulated degranulation of human neutrophils in vitro (Abstract). *J. Sports Sci.* 15:28, 1997.

6. Bruunsgaard, H., H. Galbo, J. Halkjaer-Kristensen, T.L. Johansen, D.A. Maclean, and B.K. Pedersen. Exercise-induced increase in serum interleukin-6 in humans is related to muscle damage. *J. Physiol.* 499:833-841, 1997.
7. Bury, T.B, R. Marechal, P. Mahieu, and F. Pirnay. Immunological status of competitive football players during the training season. *Int. J. Sports Med.* 19:364-368, 1988.
8. Dill, D.B., and D.L. Costill. Calculation of percentage changes in volumes of blood, plasma and red cells in dehydration. *J. Appl. Physiol.* 37:247-248, 1974.
9. Dufaux, B., and U. Order. Plasma elastase-alpha 1-antitrypsin, neopterin, tumor necrosis factor, and soluble interleukin-2 receptor after prolonged exercise. *Int. J. Sports Med.* 10:434-438, 1989.
10. Henson D.A., D.C. Nieman, S.L. Nehlsen-Cannarella, O.M. Fagoaga, M. Shannon, M.R. Bolton, J.M. Davis, C.T. Gaffney, W.J. Kelln, M.D. Austin, J.M.E. Hjertman, and B.K. Schilling. Influence of carbohydrate on cytokine and phagocytic responses to 2 h of rowing. *Med. Sci. Sports Exerc.* 32:1384-1389, 2000.
11. Nehlsen-Cannarella, S.L., O.R. Fagoaga, D.C. Nieman, D.A. Henson, D.E. Butterworth, R.L. Schmitt, E.M. Bailey, B.J. Warren, A. Utter, and J.M. Davis. Carbohydrate and the cytokine response to 2.5 h of running. *J. Appl. Physiol.* 82:1662-1667, 1997.
12. Nieman, D.C., S.L. Nehlsen-Cannarella, O.R. Fagoaga, D.A. Henson, A. Utter, J.M. Davis, F. Williams, and D.E. Butterworth. Effects of mode and carbohydrate on the cytokine response to intensive, prolonged exercise. *Med. Sci. Sports Exerc.* 30:671-678, 1998.
13. Nicholas, C.W., C. Williams, H.K.A Lakomy, G. Phillips, and A. Nowitz. Influence of ingesting a carbohydrate-electrolyte solution on endurance running capacity during intermittent, high-intensity shuttle running. *J. Sports Sci.* 13:283-290, 1995.
14. Nicholas, C.W., F.E. Nuttall, and C. Williams. The Loughborough Intermittent Shuttle Test: A field test that stimulates the activity pattern of soccer. *J. Sports Sci.* 18:97-104, 2000.
15. Northoff, H., C. Weinstock, and A. Berg. The cytokine response to strenuous exercise. *Int. J. Sports. Med.* 15:S197-S171, 1994.
16. Ostrowski, K., T. Rohde, S. Asp, P. Scherling, and B.K. Pedersen. Pro- and anti-inflammatory cytokine balance in strenuous exercise in humans. *J. Physiol.* 515(Pt. 1):287-291, 1999.
17. Pedersen, B.K., K, Ostrowski, T. Rohde, and H. Bruunsgaard, H. The cytokine response to strenuous exercise. *Can. J. Physiol. Pharmacol.* 76:505-511, 1998.
18. Ramsbottom, R., J. Brewer, and C. Williams. A progressive shuttle run test to estimate maximal oxygen uptake. *Br. J. Sports Med.* 22:14-144, 1988.
19. Steensberg, A., G. van Hall, T. Osada, M. Sacchetti, B. Saltin, and B.K. Pedersen. Production of interleukin-6 in contracting human skeletal muscles can account for the exercise-induced increase in plasma interleukin-6. *J. Physiol.* 529:237-242, 2000.
20. Thompson, D. *Muscle Damage and Soreness Following Prolonged Intermittent Shuttle Running and the Effect of Vitamin C Supplementation.* Dissertation, Loughborough University, 2000.
21. Thompson, D., C. Williams, M. Kingsley, C.W. Nicholas, H.K.A. Lakomy, F. McArdle, and M.J. Jackson. Muscle soreness and damage parameters after prolonged intermittent shuttle-running following acute vitamin C supplementation. *Int. J. Sports Nutr.* 22:68-75, 2001.
22. Vollestad, N., K.I. Tabata, and J.L. Medbo. Glycogen breakdown in different human muscle fibre types during exercise of short duration. *Acta Physiologica Scand.* 144:135-141, 1992.