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Jonathan Peake
University of Queensland

Jeremiah J. Peiffer
Edith Cowan University

Christopher R. Abbiss
Edith Cowan University

Kazunori Nosaka
Edith Cowan University

Paul B. Laursen
Edith Cowan University

See next page for additional authors

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ORIGINAL RESEARCH

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Carbohydrate Gel Ingestion and Immunoendocrine Responses to Cycling in Temperate and Hot Conditions

Jonathan Peake, Jeremiah J. Peiffer, Chris R. Abbiss, Kazunori Nosaka, Paul B. Laursen, and Katsuhiko Suzuki

Purpose: Heat stress might attenuate the effects of carbohydrate on immunoendocrine responses to exercise by increasing endogenous glucose production and reducing the rate of exogenous carbohydrate oxidation. The authors compared the efficacy of carbohydrate consumption on immune responses to exercise in temperate vs. hot conditions. ***Methods:*** Ten male cyclists exercised on 2 separate occasions in temperate (18.1 ± 0.4 °C, 58% ± 8% relative humidity) and on another 2 occasions in hot conditions (32.2 ± 0.7 °C, 55% ± 2% relative humidity). On each occasion, the cyclists exercised in a fed state for 90 min at ~60% VO_{2max} and then completed a 16.1-km time trial. Every 15 min during the first 90 min of exercise, they consumed 0.24 g/kg body mass of a carbohydrate or placebo gel. ***Results:*** Neutrophil counts increased during exercise in all trials ($p < .05$) and were significantly lower (40%, $p = .006$) after the carbohydrate than after the placebo trial in 32 °C. The concentrations of serum interleukin (IL)-6, IL-8, and IL-10 and plasma granulocyte-colony-stimulating factor, myeloperoxidase, and calprotectin also increased during exercise in all trials but did not differ significantly between the carbohydrate and placebo trials. Plasma norepinephrine concentration increased during exercise in all trials and was significantly higher (50%, $p = .01$) after the carbohydrate vs. the placebo trial in 32 °C. ***Conclusion:*** Carbohydrate ingestion attenuated neutrophil counts during exercise in hot conditions, whereas it had no effect on any other immune variables in either temperate or hot conditions.

Keywords: exercise, leukocytes, cytokines, metabolism

Carbohydrate metabolism is a key factor influencing immune changes during exercise. Changes in blood glucose availability during exercise mediate the systemic production and release of interleukin (IL)-6, IL-8, IL-1 receptor antagonist (IL-1ra), and IL-10. Carbohydrate consumption during exercise attenuates changes in these cytokines after exercise by enhancing blood glucose availability (Bishop, Gleeson, Nicholas, & Ali, 2002; Febbraio et al., 2003; Nieman, Davis, et al., 2005; Nieman

Peake is with the School of Human Movement Studies, University of Queensland, Brisbane, QLD 4072 Australia. Peiffer, Abbiss, Nosaka, and Laursen are with the School of Exercise, Biomedical and Health Sciences, Edith Cowan University, Joondalup, Western Australia, Australia. Suzuki is with the Faculty of Human Sciences, Waseda University, Tokorozawa, Japan.

et al., 2003; Nieman, Henson, Davis, et al., 2006; Starkie, Arkinstall, Koukoulas, Hawley, & Febbraio, 2001). Changes in circulating leukocyte counts after exercise are also diminished in response to carbohydrate ingestion (Bishop et al., 2002; Nieman et al., 2003, 2001, 1998a). This effect likely occurs via a reduction in the systemic release of cytokines and stress hormones that are responsive to changes in blood glucose availability (Bishop et al., 2002; Nieman et al., 2001, 1998a). Depletion of muscle glycogen during exercise also enhances gene expression of cytokines such as IL-6 and IL-8 in skeletal muscle during exercise (Chan, Carey, Watt, & Febbraio, 2004; Febbraio et al.; MacDonald, Wojtaszewski, Pedersen, Kiens, & Richter, 2003; Nieman, Davis, et al., 2005; Nieman et al., 2003). In addition to carbohydrate metabolism, muscle damage influences cytokine responses to exercise (Nieman, Dumke, et al., 2005).

Exercise in the heat might alter the effects of carbohydrate consumption on changes in blood leukocyte counts and systemic cytokine concentrations. Exercise in hot conditions (>30 °C) enhances blood glucose availability by increasing total carbohydrate oxidation and hepatic glucose production (Hargreaves, Angus, Howlett, Conus, & Febbraio, 1996; Jentjens, Wagenmakers, & Jeukendrup, 2002). Furthermore, exercise in the heat accelerates muscle glycogen depletion and reduces the oxidation rate of exogenous carbohydrate (Jentjens, Wagenmakers, & Jeukendrup, 2002). The reduced rate of exogenous carbohydrate oxidation during exercise in the heat might result from impaired gastric emptying and intestinal absorption. The excess carbohydrate that is not absorbed (or oxidized) might accumulate with fluid in the gastrointestinal tract, leading to gastrointestinal discomfort (Jentjens et al., 2006).

One study (Lim, Byrne, Chew, & Mackinnon, 2005) examined the effects of carbohydrate on alterations in leukocyte subsets and cortisol in soldiers marching on a treadmill for 3 hr in hot conditions (35 °C, 55% relative humidity). Another study (Mitchell, Dugas, McFarlin, & Nelson, 2002) investigated the effects of hydration status on changes in leukocyte mobilization, lymphocyte proliferation, and natural killer cell activity after 75 min of cycling at 55% VO_{2peak} in warm conditions (22 °C, 30% relative humidity) and hot conditions (38 °C, 45% relative humidity). No studies have specifically addressed whether the effects of carbohydrate on immune responses differ during exercise in cool or temperate conditions (i.e., ≤18 °C) compared with hot conditions (i.e., >30 °C). Because exercise in the heat increases endogenous glucose production (Hargreaves et al., 1996) and reduces the oxidation of exogenous carbohydrate (Jentjens et al., 2002), carbohydrate supplementation might have less effect on immunoendocrine responses during exercise in hot versus temperate conditions.

We aimed to examine whether the efficacy of carbohydrate supplementation on changes in circulating leukocyte subsets, cytokines (IL-1ra, IL-6, IL-8, IL-10, TNF-α, and granulocyte-colony-stimulating factor), markers of neutrophil activation (myeloperoxidase and calprotectin), and stress hormones (epinephrine, norepinephrine and cortisol) is reduced after strenuous exercise in hot compared with temperate conditions. Our rationale for conducting this study was that heat stress increases endogenous glucose production and reduces the oxidation rate of exogenous carbohydrate during exercise. Consequently, heat stress might attenuate the effectiveness of carbohydrate as a countermeasure against immunosuppression after exercise. Most other studies in this area have examined the effects of carbohydrate during exercise in a fasted state. We examined the

effects of carbohydrate during exercise in a fed state because endurance athletes are more likely to consume a preexercise meal before competition than to compete in a fasted state.

Methods

Experimental Design and Approach to the Problem

We designed a randomized, counterbalanced, placebo-controlled experimental protocol. A group of cyclists was recruited to take part in four exercise trials: (a) exercise with carbohydrate ingestion in temperate conditions (*M* ± *SD* 18.1 ± 0.4 °C, 58% ± 8% relative humidity), (b) exercise with placebo ingestion in temperate conditions, (c) exercise with carbohydrate ingestion in hot conditions (32.2 ± 0.7 °C, 55% ± 2% relative humidity), and (d) exercise with placebo ingestion in hot conditions. Blood was sampled before, during, and after exercise; blood was analyzed for circulating leukocytes, cytokines, markers of neutrophil activation, and stress hormones. Data were compared between carbohydrate and placebo trials within temperate and hot conditions.

Participants

Ten endurance-trained male cyclists with a minimum of 2 years competitive cycling experience volunteered to participate in the study. Their mean (*SD*) age was 27 (6.7) years, body mass was 77.9 (6.6) kg, height was 1.81 (0.06) m, sum of seven skinfolds was 66 (12) mm, VO_{2max} was 4.8 (0.3) L/min, and peak power output was 343 (25) W. The cyclists were riding 250–300 km/week at the time of the study. All participants completed a medical questionnaire and gave written informed consent before the study. The experimental procedure was approved by the Central Human Research Ethics Committee at Edith Cowan University.

Exercise Testing

Exercise testing was performed using a Velotron cycle ergometer (RacerMate, Seattle, WA, USA) and the Velotron coaching software (Version 1.5). The cycle ergometer was adjusted to the dimensions of each cyclist's own bicycle, equipped with aerodynamic handlebars, and fitted with the cyclist's own pedals, thereby allowing each cyclist to use his own shoe and cleat system.

On their first visit to the exercise laboratory, the cyclists performed a VO_{2max} test. Gas exchange was measured throughout the entire test using a ParvoMedics TrueOne 2400 diagnostic system (Sandy, UT, USA). Heart rate was recorded with the use of the ParvoMedics system and compatible chest electrode (Polar Electro Oy, HQ, Kempele, Finland). From the VO_{2max} test, peak power output was calculated, and the power output corresponding to 80% of the individual second ventilatory threshold (Lucia, Hoyos, Perez, & Chicharro, 2000), or 60% VO_{2max}, was established. After the VO_{2max} test the cyclists completed a familiarization 16.1-km performance time trial.

After this initial testing, the cyclists returned to the exercise laboratory on four separate occasions, as described previously. All exercise trials were separated by at least 1 week. The order of these trials was randomized and counterbalanced.

On each occasion, the cyclists were required to ride on the cycle ergometer for 90 min at ~60% VO_{2max}. Gas analysis was performed every 15 min during exercise, and workload was adjusted accordingly to maintain this intensity. After this steady-state exercise, the cyclists completed a 16.1-km performance time trial. All exercise (steady state and time trial) was performed in a climate chamber (2.9 × 6.8 × 2.7 m).

The mean (*SD*) durations of the four time trials were as follows: 25 min 26 s (1 min 40 s) for the placebo trial in 18 °C, 25 min 25 s (1 min 45 s) for the carbohydrate trial in 18 °C, 27 min 32 s (1 min 53 s) for the placebo trial in 32 °C, and 26 min 35 s (1 min 26 s) for the carbohydrate trial in 32 °C. The duration of the time trial was significantly shorter in response to carbohydrate than placebo ingestion in 32 °C (mean difference 57 s, 95% confidence interval 3 s to 1 min 12 s, $p = .04$) but not in 18 °C ($p = .89$). The duration of the time trial was significantly longer for the placebo trial in 32 °C than for the placebo trial in 18 °C (mean difference 1 min 26 s, 95% confidence interval 36 s to 3 min 36 s, $p = .01$). The duration of the time trial for the carbohydrate trial in 32 °C also tended to be longer than that in 18 °C (mean difference 1 min 11 s; 95% confidence interval −1 s to 1 min 24 s; $p = .05$).

All trials were conducted between 9:00 and 11:00 a.m. During exercise, a fan was placed 1.5 m in front of the cyclists. The speed of the fan was set at 30 m/s to simulate the environmental conditions experienced when cycling outdoors (Saunders, Dugas, Tucker, Lambert, & Noakes, 2005). The cyclists wore Lycra shirts and shorts during exercise. Exercise testing was performed between the months of October and December, when daily ambient temperatures ranged from 12.1 ± 3.3 °C to 23.1 ± 3.9 °C.

Diet and Carbohydrate Supplementation

The cyclists were instructed to avoid training and to consume meals consisting of 6 g carbohydrate per kg body mass on the day before the exercise trials. The cyclists were provided with details of the carbohydrate content of common foods. They also recorded their food intake in the 24-hr period before their first exercise trial and were instructed to record and eat the same type and amount of food at the same time before each trial. The cyclists were allowed to eat a meal of their choice 2 hr before the exercise trials. We adopted this approach because otherwise the cyclists would have fasted for more than 12 hr since their last meal. In a practical setting, it is unlikely that cyclists would compete in a fasted state. We elected not to standardize the carbohydrate content of the preexercise meal because athletes vary with regard to the amount of food they prefer to consume before exercise.

In a double-blind fashion and randomized and counterbalanced order, the cyclists consumed either a 25% weight/volume sucrose (carbohydrate) gel or placebo gel (Cottee's, Ringwood, VIC, Australia) before and during the 90-min steady-state cycling. They consumed 0.48 g of carbohydrate (or placebo) per kg of body mass immediately before the warm-up and then 0.24 g of carbohydrate (or placebo) per kg of body mass every 15 min during the 90 min of exercise (i.e., at 15, 30, 45, 60, and 75 min). This feeding regimen is similar to those in previous studies that have used 6–8% carbohydrate beverages rather than gels (Jentjens et al., 2002; Nieman, Davis, et al., 2005). The gels did not contain any electrolytes.

The cyclists were not able to distinguish between the tastes of the carbohydrate and placebo gels. The average rate of carbohydrate consumption during the 90 min of exercise was 1 ± 0.06 g/min, and the average amount of carbohydrate consumed during the 90 min was 131 ± 19 g. Cyclists drank plain water ad libitum throughout the 90 min of exercise. The mean (*SD*) volume of fluid consumed during exercise was similar between the carbohydrate and placebo trials in 18 °C (1.0 ± 0.4 L) and in 32 °C (2.0 ± 0.4 L). We chose this regimen of carbohydrate supplementation and fluid replacement to simulate feeding strategies cyclists use when racing. The cyclists did not consume any gels during the time trial but were allowed to continue drinking water ad libitum.

Core Temperature

To determine core body temperature, a sterile disposable rectal thermistor (Monatherm Thermistor, 400 Series, Mallinckrodt Medical, St. Louis, MO, USA) was self-inserted at a 0.12-m depth past the anal sphincter. Core temperature was recorded every second during exercise using a data logger (Grant Instruments, Shepreth Cambridgshire, UK).

Blood Sampling and Processing

Venous blood samples were collected from a forearm vein before the warm-up, immediately after the 90 min of steady-state exercise (90 min), and immediately after the time trial. Blood was collected into sterile Vacutainers containing either K_2-EDTA for blood cell counts and the separation of plasma or serum-separation tubes (Becton Dickinson, Franklin Lakes, NJ, USA). Before the K_2-EDTA tubes were centrifuged, 1 ml of whole blood was removed to obtain complete blood cell counts. The tubes were then centrifuged at 2500 rpm for 10 min at 4 °C. After blood collection, the serum-separation tubes were left at room temperature to clot before they were centrifuged. The K_2-EDTA plasma was divided into 1-ml aliquots, and the serum was divided into 0.7-ml aliquots. All plasma and serum samples were stored at –80 °C until the day of analysis.

Blood Analysis

Plasma glucose concentration was measured spectrophotometrically on an automated analyzer (Hitachi Model 7170, Japan) using an enzymatic reaction involving hexokinase (GLU-HK [M], Shinotest Co., Tokyo, Japan). Complete blood cell counts were measured using a Beckman Coulter-Counter Gen-S (France SA, Villepinte, France). Plasma epinephrine and norepinephrine concentrations were measured by enzyme-linked immunosorbent assay (ELISA; Labor Diagnostika Nord, Nordhorn, Germany). Plasma granulocyte-colony-stimulating factor (G-CSF) and serum cortisol concentrations were measured using ELISA kits from IBL (Gunma, Japan; Hamburg, Germany). The serum concentrations of IL-6, IL-1ra, and tumor-necrosis factor (TNF)-α were measured using Quantikine high-sensitivity ELISA kits (R&D Systems, Minneapolis, MN, USA). Serum IL-8 and IL-10 concentrations were measured using OptEIA kits (Becton Dickinson, San Diego, CA, USA). Plasma myeloperoxidase and calprotectin concentrations were measured

using ELISA kits from HyCult Biotechnology (Uden, The Netherlands). Stress-hormone and serum cytokine concentrations were calculated by comparison with a standard curve established in the same set of measurements. These measurements were taken using a microplate reader (VERSAmax, Molecular Devices, Sunnyvale, CA, USA). The intra-assay coefficients of variation and sensitivity of all assays are presented in Table 1. Leukocyte counts were adjusted for percentage changes in blood volume, whereas plasma and serum variables were adjusted according to percentage changes in plasma and blood volume, as calculated from hemoglobin and hematocrit (Dill & Costill, 1974).

Statistical Analysis

The data were checked for normal distribution using the Kolmolgorov–Smirnov statistic. The data for all physiological variables, G-CSF, and cortisol were normally distributed and are presented as $M \pm SD$. The data for neutrophil, lymphocyte, and monocyte counts and IL-1ra, IL-8, TNF-α, myeloperoxidase, epinephrine, and glucose concentrations were normally distributed after log transformation and are presented as geometric means ± 95% confidence intervals. The data for IL-6, IL-10, calprotectin, and norepinephrine concentrations were not normally distributed and are presented as medians ± interquartile range.

The normally distributed data were analyzed using a 2- (trials; carbohydrate vs. placebo) × 3- (time points; pre-warm-up, 90 min, post–time trial) factor repeated-measures ANOVA to determine time effects and Time × Trial interactions within each condition (i.e., temperate and hot conditions). Student's paired *t* tests were used to compare differences between trials and individual time points. The nonnormally distributed data were analyzed using the nonparametric Friedman's ANOVA on ranks to determine time and trial effects. Wilcoxon's signed rank tests were then used to assess differences between specific time points and trials. The false-discovery-rate procedure (Curran-Everett, 2000) was used for multiple comparisons between time points and carbohydrate versus placebo trials. Statistical significance was set at $p < .05$. Statistical analysis was carried out using SPSS Version 15.0 (SPSS Inc., Chicago, IL, USA).

Table 1 Coefficient of Variation and Sensitivity of Enzyme-Linked Immunosorbent Assays

Parameter	Intra-assay coefficient of variation	Sensitivity
Epinephrine	4.3%	11 pg/ml
Norepinephrine	4.0%	44 pg/ml
G-CSF	2.0%	1.2 pg/ml
Cortisol	4.4%	2.5 ng/ml
IL-6	6.6%	0.039 pg/ml
IL-1ra	5.4%	22 pg/ml
TNF-α	6.9%	0.12 pg/ml
IL-8	3.3%	0.8 pg/ml
IL-10	4.0%	2 pg/ml
Myeloperoxidase	4.1%	0.4 ng/ml
Calprotectin	5.0%	1.6 ng/ml

Results

Physiological Variables

The percentage of VO_{2max} and maximum heart rate maintained during steady-state exercise was similar between the carbohydrate and placebo trials in 18 °C (61% ± 3% VO_{2max} and 77% ± 3% HR_{max}) and in 32 °C (61% ± 3% VO_{2max} and 84% ± 2% HR_{max}). Heart rate during the time trials was similar between the carbohydrate and placebo trials in 18 °C (90% ± 5% HR_{max}). In contrast, during the time trials in 32 °C heart rate tended to be higher ($p = .07$) after carbohydrate ingestion (93% ± 5% HR_{max}) than after placebo ingestion (90% ± 6% HR_{max}). Fluid consumption was similar between the carbohydrate and placebo trials in 18 °C (1.0 ± 0.4 L) and in 32 °C (2.0 ± 0.4 L). Dehydration (% change in body mass) was similar between the carbohydrate and placebo trials in 18 °C (−0.8% ± 0.7%) and in 32 °C (−1.0% ± 1.1%). Plasma volume decreased to a similar extent during the carbohydrate and placebo trials in 18 °C (−6.4% ± 5.6%) and in 32 °C (−7.2% ± 5.9%). Core temperature increased during exercise (time effect $p < .0001$). Core temperature was significantly higher after the carbohydrate trial than after the placebo trial in 32 °C (Time × Trial interaction $p = .002$) but not 18 °C (Figure 1).

Plasma Glucose Concentration

Plasma glucose concentration increased during all trials (time effect $p < .05$). The pattern of changes in plasma glucose concentration did not differ significantly between the carbohydrate and placebo trials in either 18 °C or 32 °C (Figure 2). A significant trial effect ($p = .003$, power = 0.98) was evident for the trial in 32 °C, however; plasma glucose concentration was higher (20%, $p = .008$) at the end of exercise in the carbohydrate trial than in the placebo trial.

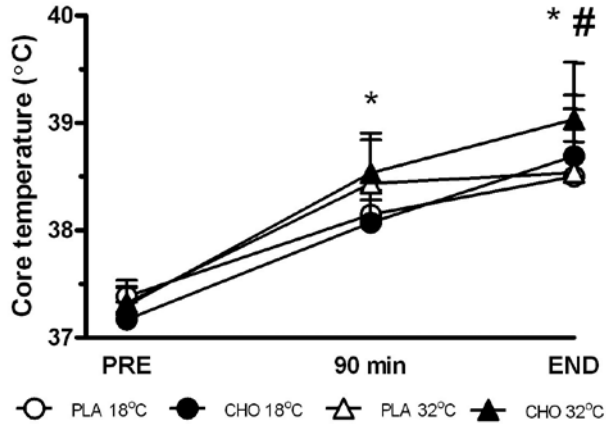

Figure 1 — Core temperature, *M* ± *SD*. PLA = placebo trial; CHO = carbohydrate trial. *Significantly different from preexercise values, $p < .05$. #Significantly different between carbohydrate and placebo conditions in 32 °C, $p < .05$.

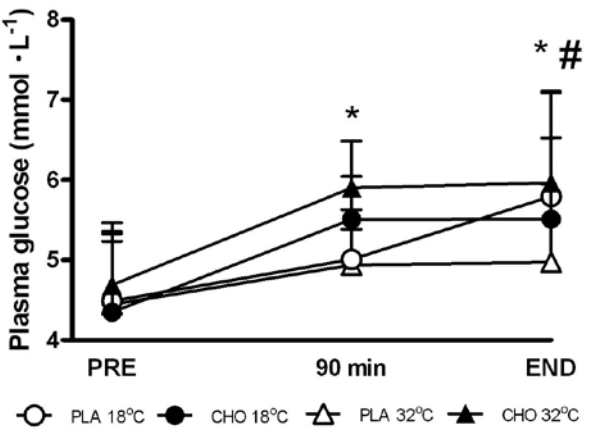

Figure 2 — Plasma glucose concentration, geometric means ± 95% confidence interval. PLA = placebo trial; CHO = carbohydrate trial. *Significantly different from preexercise values, $p < .05$. #Significantly different between carbohydrate and placebo conditions in 32 °C, $p < .05$.

Leukocytes

Neutrophil counts were lower (40%) after the carbohydrate trial than after the placebo trial in 32 °C (interaction $p = .0001$, power = .99; Figure 3). A similar difference in neutrophil counts was evident between the trials in 18 °C, but this difference did not reach statistical significance (interaction $p = .085$, power = .52). Lymphocyte and monocyte counts also increased significantly during all trials but did not differ significantly between the carbohydrate and placebo trials in either 18 or 32 °C (Table 2).

Cytokines

The concentrations of serum IL-6, IL-8, and IL-10 and plasma G-CSF increased significantly during all trials (time effect $p < .05$; Figure 4 and Table 3). Serum IL-1ra and TNF-α concentrations increased during exercise in 32 °C (time effect $p < .05$) but not in 18 °C (Table 3). The pattern of changes in IL-6, IL-8, TNF-α, and G-CSF was similar between the carbohydrate and placebo trials in both 18 and 32 °C. IL-1ra was 25% lower after exercise in the carbohydrate trial than in the placebo trial in 32 °C, but this difference did not reach statistical significance (interaction $p = .06$, power = .44). IL-10 was 10–20% lower after exercise in the carbohydrate trial than in the placebo trial in both conditions, but these differences were not statistically significant (Wilcoxon's rank test 18 °C, $p = .19$; 32 °C, $p = .30$).

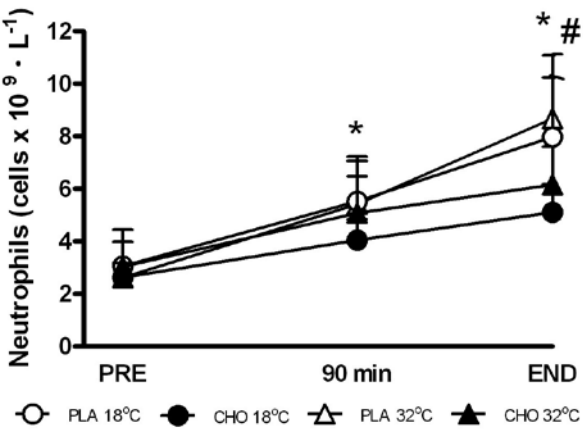

Figure 3 — Neutrophil counts, geometric means ± 95% confidence interval. PLA = placebo trial; CHO = carbohydrate trial. *Significantly different from preexercise values, $p < .05$. #Significantly different between carbohydrate and placebo conditions in 32 °C, $p < .05$.

Table 2 Lymphocyte and Monocyte Counts

	Pre-warm-up	90 min*	Post–time trial *
Lymphocytes (cells × 10^9/L)			
placebo trial in 18 °C	1.7 (0.3)	2.5 (0.5)	3.6 (0.7)
carbohydrate trial in 18 °C	1.8 (0.2)	2.7 (0.4)	4.1 (0.7)
placebo trial in 32 °C	1.8 (0.3)	3.0 (0.7)	4.0 (0.7)
carbohydrate trial in 32 °C	1.8 (0.3)	2.7 (0.6)	3.8 (0.7)
Monocytes (cells × 10^9/L)			
placebo trial in 18 °C	0.4 (0.1)	0.7 (0.2)	0.8 (0.2)
carbohydrate trial in 18 °C	0.4 (0.1)	0.6 (0.2)	0.7 (0.2)
placebo trial in 32 °C	0.4 (0.1)	0.6 (0.1)	0.9 (0.1)
carbohydrate trial in 32 °C	0.4 (0.1)	0.6 (0.1)	0.9 (0.1)

Note. Data are presented as geometric means (95% confidence intervals).

*Significantly different from preexercise values, $p < .05$.

Myeloperoxidase and Calprotectin

The plasma concentrations of myeloperoxidase and calprotectin increased during both trials (time effect $p < .01$) but did not differ significantly between the carbohydrate and placebo trials in either 18 or 32 °C (Table 4).

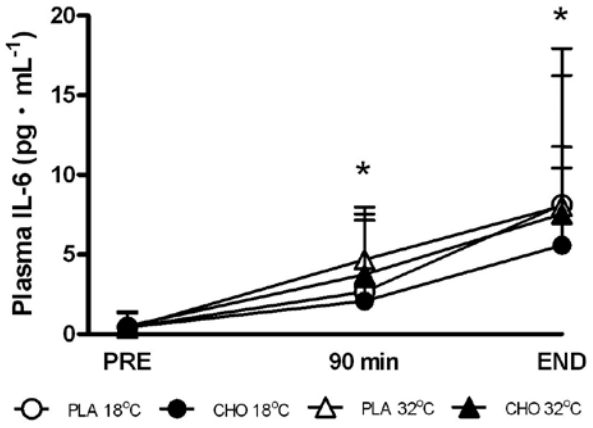

Figure 4 — Serum IL-6 concentration, medians ± interquartile range. PLA = placebo trial; CHO = carbohydrate trial. *Significantly different from preexercise values, $p < .05$.

Table 3 Cytokine Concentrations

	Pre-warm-up	90 min	Post–time trial
IL-1ra (pg/ml)			
placebo trial in 18 °C	270 (87)	284 (110)	328 (298)
carbohydrate trial in 18 °C	240 (75)	212 (55)	230 (51)
placebo trial in 32 °C	233 (46)	366 (114)*	539 (615)
carbohydrate trial in 32 °C	253 (91)	371 (217)	401 (151)*
IL-8 (pg/ml)			
placebo trial in 18 °C	10 (1)	12 (2)	13 (3)
carbohydrate trial in 18 °C	10 (1)	13 (4)	16 (4)*
placebo trial in 32 °C	12 (1)	17 (3)*	21 (3)*
carbohydrate trial in 32 °C	12 (2)	17 (2)*	20 (3)*
IL-10 (pg/ml)			
placebo trial in 18 °C	2.9 (4.3)	4.9 (5.5)*	5.1 (4.8)*
carbohydrate trial in 18 °C	2.8 (3.4)	4.1 (5.4)	4.9 (4.9)*
placebo trial in 32 °C	3.1 (3.7)	6.7 (7.4)*	12.3 (11.1)*
carbohydrate trial in 32 °C	3.9 (3.4)	7.3 (10.4)*	9.5 (18.3)*
TNF-α (pg/ml)			
placebo trial in 18 °C	1.1 (0.1)	1.1 (0.2)	1.2 (0.1)
carbohydrate trial in 18 °C	1.2 (0.2)	1.2 (0.1)	1.3 (0.1)
placebo trial in 32 °C	1.3 (0.2)	1.5 (0.2)*	1.4 (0.2)
carbohydrate trial in 32 °C	1.3 (0.3)	1.6 (0.2)*	1.6 (0.3)
G-CSF (pg/ml)			
placebo trial in 18 °C	3.7 (1.3)	5.0 (2.2)	4.6 (2.4)
carbohydrate trial in 18 °C	3.4 (0.8)	5.4 (1.6)*	5.6 (1.5)*
placebo trial in 32 °C	3.6 (0.6)	6.1 (1.3)*	6.1 (1.1)*
carbohydrate trial in 32 °C	4.4 (1.7)	7.2 (2.3)*	7.8 (1.9)*

Note. Data for G-CSF are presented as means (SD). Data for IL-1ra, IL-8, and TNF-α are presented as geometric means (95% confidence interval). Data for IL-10 are presented as medians (interquartile range).

*Significantly different from preexercise values, $p < .05$.

Table 4 Myeloperoxidase and Calprotectin Concentrations

	Pre-warm-up	90 min	Post–time trial
Myeloperoxidase (ng/ml)			
placebo trial in 18 °C	73 (35)	101 (21)	169 (49)*
carbohydrate trial in 18 °C	57 (12)	78 (20)*	139 (29)*
placebo trial in 32 °C	54 (14)	126 (32)*	214 (53)*
carbohydrate trial in 32 °C	59 (15)	116 (34)*	179 (36)*
Calprotectin (ng/ml)			
placebo trial in 18 °C	870 (1,069)	1,677 (1,313)*	2,265 (1,706)*
carbohydrate trial in 18 °C	647 (755)	1,580 (1,520)*	2,299 (1,708)*
placebo trial in 32 °C	590 (513)	1,917 (495)*	2,259 (1,388)*
carbohydrate trial in 32 °C	818 (1,466)	1,882 (1,591)*	1,658 (1,588)*

Note. Data for myeloperoxidase are presented as geometric means (95% confidence interval). Data for calprotectin are presented as medians (interquartile range).

*Significantly different from preexercise values, $p < .05$.

Stress Hormones

The plasma concentrations of epinephrine and norepinephrine increased during all trials (time effect $p < .0001$). Plasma norepinephrine concentration was higher (50%) after the carbohydrate trial than after the placebo trial in 32 °C (Wilcoxon's rank test $p = .01$; Figure 5). Norepinephrine was also higher (80%) after the carbohydrate trial than after the placebo trial in 18 °C, but this difference was not statistically significant (Wilcoxon's rank test $p = .15$). Plasma epinephrine concentration did not differ significantly between the carbohydrate and placebo trials in either 18 or 32 °C (Table 5). Serum cortisol concentration decreased during exercise (time effect $p < .0001$) and did not differ significantly between the carbohydrate and placebo trials in either 18 or 32 °C (Table 5).

Discussion

The aim of this study was to compare the efficacy of carbohydrate on changes in circulating leukocyte subsets, cytokines, and markers of neutrophil activation after strenuous exercise in temperate (18 °C) and hot conditions (32 °C). Carbohydrate attenuated total leukocyte and neutrophil counts after exercise in 32 °C but not in 18 °C. Contrary to our expectations, carbohydrate had no significant effect on lymphocyte and monocyte counts, cytokines, and markers of neutrophil activation after exercise in either 18 or 32 °C. Therefore, it remains unknown whether ambient temperature during exercise alters the efficacy of carbohydrate on other immune variables.

Blood glucose availability is a key factor affecting immunoendocrine responses to exercise (Bishop et al., 2002; Febbraio et al., 2003; Nieman, Davis, et al., 2005; Nieman et al., 2003; Nieman, Henson, Davis, et al., 2006; Starkie et al., 2001). In contrast to other studies (Hargreaves et al., 1996; Jentjens et al., 2002), we found no difference in plasma glucose concentration after exercise in 18 °C compared with 32 °C. In the current study, the temperature differential between temperate and hot conditions was 14 °C, whereas in other studies it was 19–20 °C (Hargreaves et al.;

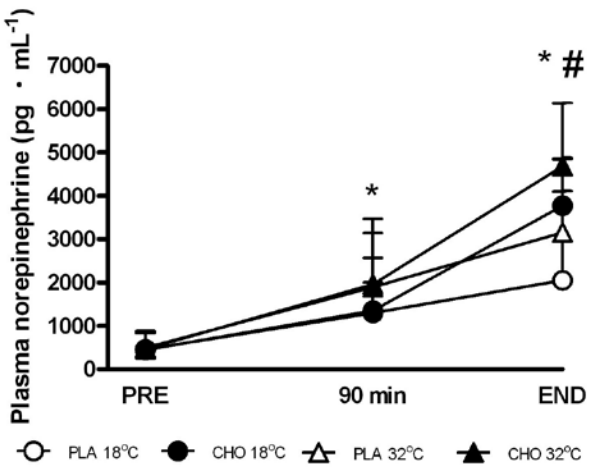

Figure 5 — Plasma norepinephrine concentration, medians ± interquartile range. PLA = placebo trial; CHO = carbohydrate trial. *Significantly different from preexercise values, $p < .05$. #Significantly different between carbohydrate and placebo conditions in 32 °C, $p < .05$.

Table 5 Epinephrine and Cortisol Concentrations

	Pre-warm-up	90 min	Post–time trial
Epinephrine (pg×mL^{1})			
placebo trial in 18 °C	32 (16)	129 (90)*	348 (418)*
carbohydrate trial in 18 °C	23 (21)	56 (45)*	551 (361)*
placebo trial in 32 °C	23 (18)	190 (175)*	616 (708)*
carbohydrate trial in 32 °C	28 (21)	111 (77)*	676 (340)*
Cortisol (ng×mL^{1})			
placebo trial in 18 °C	98 (14)	78 (12)*	47 (11)*
carbohydrate trial in 18 °C	97 (10)	93 (27)	51 (12)*
placebo trial in 32 °C	98 (13)	69 (19)	44 (16)*
carbohydrate trial in 32 °C	102 (12)	69 (14)*	42 (8)*

Note. Data for cortisol are presented as *M* (*SD*). Data for epinephrine are presented as geometric means (95% confidence interval).

*Significantly different from preexercise values, $p < .05$.

Jentjens et al., 2002). Differences in glucose kinetics during exercise in temperate versus hot conditions might therefore depend on the magnitude of the temperature differential. Plasma glucose concentration was higher after the carbohydrate trial than after the placebo trial in 32 °C, but the difference (20%) was smaller than that reported in other carbohydrate-supplementation trials (Bishop et al., 2002; Nieman, Davis, et al., 2005; Nieman et al., 2003, 1998b; Starkie et al., 2001). The possible

reasons for these minor differences in plasma glucose concentrations are discussed in more detail later in the article.

Contrary to our hypothesis, we found that carbohydrate significantly attenuated neutrophil counts after exercise in 32 °C but not in response to exercise in 18 °C. This effect was not likely a result of the effects of carbohydrate on glucose-sensitive stress hormones, because carbohydrate did not attenuate changes in serum cortisol and plasma catecholamine concentrations. These findings contrast with other research (Bishop et al., 2002; Nieman et al., 2003, 2001, 1998a). In the current study, carbohydrate might simply have attenuated neutrophil counts during exercise in 32 °C (but not in 18 °C), because neutrophil counts tended to be 10–20% higher after exercise in 32 °C versus 18 °C (data published elsewhere; Peake et al., 2008). Heat stress during exercise promotes the demargination of neutrophils from endothelial surfaces into the bloodstream, possibly through increased sheer stress.

The lack of any significant effect of carbohydrate on exercise-induced changes in the serum concentration of IL-6 also contrasts with most research findings (Bishop et al., 2002; Febbraio et al., 2003; Nieman, Davis, et al., 2005; Nieman et al., 2003; Nieman, Henson, Davis, et al., 2006; Starkie et al., 2001). Two other studies have reported no significant effect of carbohydrate on alterations in plasma IL-6 concentration after exercise (Nieman et al., 2001; Starkie, Angus, Rolland, Hargreaves, & Febbraio, 2000). Carbohydrate ingestion consistently attenuates IL-6 during exercise that challenges blood glucose availability (Bishop et al., 2002; Nieman, Davis, et al., 2005; Nieman et al., 2003, 1998b; Starkie et al., 2001). We found that plasma glucose concentration increased slightly (~10%) during the placebo trials. Therefore, this endogenous glucose production might have reduced the effect of supplemental carbohydrate on changes in serum IL-6 concentration. We also allowed the cyclists in our study to consume a preexercise meal to simulate preparation for a competitive event. Athletes are more likely to consume a preexercise meal before competition than to compete in a fasted state. This meal might have helped maintain blood glucose availability during exercise (Sherman, Peden, & Wright, 1991), resulting in a smaller effect of carbohydrate ingestion.

One study (Bishop, Blannin, Walsh, & Gleeson, 2001) reported that the effects of carbohydrate on immunoendocrine responses are diminished during cycling to fatigue. This effect is a result of the fact that carbohydrate prolongs time to fatigue, resulting in greater immune changes in response to the longer exercise duration. In the current study, carbohydrate ingestion enabled the cyclists to maintain a higher intensity during the time trial in 32 °C—as indicated by the improved performance and the trend ($p = .07$) toward a higher heart rate during the time trial. We also noted, however, that carbohydrate did not significantly influence exercise intensity or immunoendocrine responses after the time trial in 18 °C. Therefore, whether exercise intensity influences the efficacy of carbohydrate ingestion on immunoendocrine responses remains uncertain.

Carbohydrate might have less effect on changes in IL-6 during cycling than during running, possibly because muscle damage during running might contribute to the release of IL-6 from skeletal muscle (Nieman et al., 1998b; Starkie et al., 2000). Furthermore, all other studies investigating the efficacy of carbohydrate ingestion on immunoendocrine responses to exercise have used carbohydrate beverages rather than gels. Glucose transport across the intestinal wall might differ between carbohydrate beverages containing electrolytes such as sodium and gels without

sodium. We used gels rather than carbohydrate drinks because this allowed the cyclists to adopt their own hydration strategies during exercise. In other studies, participants have been required to drink a fixed volume of carbohydrate beverage at fixed intervals, a regimen that might not reflect hydration strategies used during competitive cycling.

Other studies have reported that carbohydrate ingestion attenuates changes in the systemic concentrations of IL-1ra and IL-10 (Nieman, Davis, et al., 2005; Nieman et al., 2003; Nieman, Henson, Davis, et al., 2006). This effect might be linked to a decrease in the release of IL-6 from skeletal muscle, because infusion of recombinant human IL-6 stimulates a rise in the plasma concentrations of IL-1ra and IL-10 (Steensberg, Fischer, Keller, Moller, & Pedersen, 2003). We found no effect of carbohydrate on IL-6; this might explain why carbohydrate also had no significant effect on serum IL-1ra and IL-10 concentrations in the current study. Interindividual variation might also account for the lack of any significant effect of carbohydrate ingestion on serum IL-1ra concentration after exercise in 32 °C. Muscle glycogen depletion stimulates IL-8 gene expression in skeletal muscle (Chan et al., 2004), but our findings and those of others (Nieman, Henson, Davis, et al., 2006) indicate that carbohydrate ingestion does not influence the systemic concentration of IL-8 after exercise. Plasma G-CSF concentration increases after brief maximal exercise (Yamada et al., 2002) and prolonged exercise (Nieman, Henson, Davis, et al., 2006; Suzuki et al., 2003, 2006). Our findings suggest that metabolic stress is not a stimulus for the production and release of G-CSF into the circulation.

Several studies have reported that exercise increases neutrophil degranulation, as indicated by elevated plasma concentrations of elastase and myeloperoxidase (Peake, 2002; Peake et al., 2004; Suzuki et al., 2003). More recent studies have also observed that plasma calprotectin concentration is elevated after exercise (Fagerhol, Nielsen, Vetlesen, Sandvik, & Lyberg, 2005; Mooren et al., 2006). Calprotectin (otherwise known as S100A8/A9) is secreted from monocytes and neutrophils by activation of protein kinase C, in response to a variety of inflammatory conditions. It is involved in regulating leukocyte chemotaxis, adhesion, and arachidonic-acid metabolism (Nacken, Roth, Sorg, & Kerkhoff, 2003). No studies have examined the effect of carbohydrate on changes in these markers of neutrophil activation after exercise. Carbohydrate attenuates neutrophil oxidative-burst activity after exercise (Nieman et al., 1998a). It also prevents the exercise-induced decline in elastase released from neutrophils stimulated with lipopolysaccharide, possibly by attenuating plasma cortisol concentration (Bishop et al., 2002; Bishop, Walsh, & Scanlon, 2003). We found no effect of carbohydrate on changes in plasma myeloperoxidase and calprotectin concentrations. Therefore, carbohydrate might have specific effects on different neutrophil functions during exercise.

Our findings differ from those of other studies indicating that carbohydrate attenuates plasma epinephrine concentration after exercise (Nieman, Henson, Davis, et al., 2006; Starkie et al., 2000). This disparity could be the result of the relatively minor differences in plasma glucose concentration between the carbohydrate and placebo trials in the current study. Carbohydrate ingestion might attenuate epinephrine responses to a greater extent during exercise in which plasma glucose concentration remains stable or decreases (in the absence of supplemental carbohydrate). Unexpectedly, we observed that plasma norepinephrine concentration

was higher after the carbohydrate trial than the placebo trial in 32 °C. As discussed previously, plasma glucose concentration was higher after the carbohydrate trial than the placebo trial in 32 °C. This additional glucose availability might have enabled the cyclists to maintain a higher intensity and heart rate during the time trial. This greater intensity and sympathetic drive are likely reflected by the higher plasma norepinephrine concentration in response to carbohydrate ingestion after the time trial in 32 °C. As an α-agonist, norepinephrine also plays a greater role in restricting blood flow to the splanchnic region during high-intensity exercise than does epinephrine. These factors could partially account for the different effects of carbohydrate on epinephrine and norepinephrine responses. We could not interpret the effects of carbohydrate on the cortisol response to exercise, because serum cortisol concentration decreased during exercise. This decrease was likely caused by circadian variation (Nieman, 1996).

The higher core temperature during exercise in 32 °C than in 18 °C likely reflects a reduced capacity to dissipate body heat in hot ambient conditions. The higher core temperature might also account for the higher heart rates during steady-state exercise in 32 °C, because extra stress is placed on the cardiovascular and thermoregulatory systems to minimize heat storage in the body. The greater volume of fluid consumed during exercise in 32 °C likely helped compensate for the greater cardiovascular stress. Heart rates were similar during the time trials in 18 °C and 32 °C, yet time-trial performance was significantly slower in 32 °C. These data suggest that the control of skin blood flow—and therefore core temperature—was more important than maintenance of muscle blood flow and power output during high-intensity exercise in hot ambient conditions.

In summary, carbohydrate ingestion attenuated total leukocyte and neutrophil counts during exercise in 32 °C, whereas it had no effect on other immune variables in all other trials. Further studies are warranted to determine whether ambient temperature alters the efficacy of carbohydrate on exercise-induced immune changes. The current study highlights several important factors to consider when designing studies to examine the efficacy of carbohydrate on immunoendocrine responses to exercise. These factors include preexercise meal consumption; the intensity, duration, and mode of exercise; and the form of carbohydrate supplement (i.e., drink vs. gel).

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