Influence of carbohydrate on cytokine and phagocytic responses to 2 h of rowing

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ABSTRACT

HENSON, D. A., D. C. NIEMAN, S. L. NEHLSEN-CANNARELLA, O. R. 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.*, Vol. 32, No. 8, pp. 1384–1389, 2000. **Purpose:** This study examined the influence of carbohydrate (C) versus placebo (P) beverage ingestion on the phagocytic and cytokine responses to normal rowing training by 15 elite female rowers. **Methods:** Athletes received C or P before, during and after, two, 2-h bouts of rowing performed on consecutive days. Blood was collected before and 5–10 min and 1.5 h after rowing. Metabolic measures indicated that training was performed at moderate intensities, with some high-intensity intervals interspersed throughout the sessions. **Results:** Concentrations of blood neutrophils and monocytes, phagocytic activity, and plasma IL-1ra were significantly lower postexercise after C versus P ingestion. No differences were observed for oxidative burst activity, IL-6, IL-8, or TNFα. Glucose was significantly higher after 2 h of rowing with C ingestion; however, cortisol, growth hormone, epinephrine, norepinephrine, and CRP were not affected by carbohydrate. **Conclusions:** These data indicate that carbohydrate compared with placebo ingestion attenuated the moderate rise in blood neutrophils, monocytes, phagocytosis, and plasma IL-1ra concentrations that followed 2-h bouts of training in elite female rowers. No changes in blood hormone concentrations were found. **Key Words:** INFLAMMATORY RESPONSE, IMMUNE RESPONSE, IL-1ra, OXIDATIVE BURST, NEUTROPHILS

Tissue injury that results from prolonged strenuous exercise has been shown to elicit an acute phase response that is analogous to inflammation (1). This response is characterized by the release of pro-inflammatory cytokines such interleukin (IL)-1β, IL-6, and TNFα, which work synergistically but are limited or reversed through several pathways, including the production of anti-inflammatory cytokines IL-1-receptor antagonist (IL-1ra), IL-4, and IL-10 (1,2,6,12,16,18,20,30). In addition, pro-inflammatory cytokines released in response to exercise-induced muscle injury are believed to stimulate the hypothalamic-pituitary-adrenal (HPA) axis, providing a natural negative-feedback mechanism that increases the adrenal release of cortisol and limits further the release of IL-1β and IL-6. Low concentrations of blood glucose have also been linked to HPA activation and subsequent cortisol production. In addition to its effects on the cytokine response, intense exercise results in an increase in the number and phagocytic activity of blood neutrophils and monocytes (14,17,19,26). Less clear, however, is the impact of heavy exertion on oxidative burst activity, with some studies reporting an increase (17,22,25,29) and others a decrease (14,21). Various nutritional and chemical intervention has been attempted to alter the inflammatory changes after heavy exertion, with the most effective results observed through carbohydrate supplementation (4,7,9,12,14–17,24,25). Two previous studies from our laboratory involving 30 marathon runners and 10 triathletes suggest that carbohydrate compared to placebo ingestion is associated with a diminished pro- and anti-inflammatory cytokine response, lower granulocyte and monocyte phagocytosis and oxidative burst activity, fewer perturbations in blood immune cell counts, higher plasma glucose concentrations, and an attenuated cortisol and growth hormone response (12,14,16,17). It has

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been suggested that the overall hormonal and inflammatory responses observed with carbohydrate compared with placebo supplementation are related to a reduced level of physiologic stress as reflected by the reduced plasma cortisol levels. Although the underlying mechanisms remain to be determined, this effect may be due to the maintenance of plasma glucose concentrations and the reduced activation of the HPA axis. Furthermore, its clinical significance is unclear (7,10,11).

The physiology of rowing has recently been reviewed and indicates that elite rowers are capable of extremely high workloads (8,23,27,28). In high-load training phases before World Championships, training volume approaches 190 $min \cdot d^{-1}$, of which between 55–65% is rowing, and the rest is composed of nonspecific exercises, such as weight lifting, stretching, and calisthenics (28). The specific rowing component is primarily performed as an endurance-type training, with only 4–10% of total rowed time spent at high intensities (28). Actual race events often involve about 70% of the whole body muscle mass, with an average power of 450–550 W for a 5.5- to 8-min race (28). Several investigators have recently reported that the stress hormone, cortisol, is significantly elevated after rowing training, a response that is primarily attributed to the large amount muscle mass that is utilized (23,27). Because cortisol is known to influence the pro- and anti-inflammatory cytokine and immune responses, it is likely that elite rowers may experience cyclic and stressful changes in inflammation and immune (3,13).

This counterbalanced, double-blind, placebo-controlled study investigated the influence of carbohydrate versus placebo beverage consumption on both pro-inflammatory (IL-6, IL-8) and anti-inflammatory (IL-1ra) cytokine responses, phagocytic and oxidative burst activities, and hormonal responses to 2 h of rowing in 15 elite female rowers. The purpose was to determine whether carbohydrate versus placebo ingestion would attenuate inflammatory changes that occur in response to a normal bout of training in female rowers preparing for the year 2000 Olympic games in Sydney, Australia.

METHODS

Subjects

Fifteen elite female rowers located at the ARCO Olympic Training Center in Chula Vista, CA, were used in the study. Blood samples were collected from the athletes in late May, approximately 3 months before the 1998 World Championships. At this time, the female rowers were averaging 12–13, 90- to 120-min training sessions per week and were estimated by the coach to be about 5% above projected race times. Informed consent was obtained from each subject, and the experimental procedures were in accordance with the policy statements of the institutional review board of Appalachian State University.

Research Design

Blood samples were collected from the athletes during a 3-d period at the Olympic Training Center. During the first day, blood samples were collected at 0600–0700 h after the athletes had fasted for at least 9 h, had avoided exercise for 12 h, and had rested in the seated position for at least 15 min. These samples were analyzed for various immune and hormonal parameters, and represented the preexercise samples for the following 2 d. Subjects reported in writing that they were free of infectious disease symptoms and had avoided vitamin-mineral supplements for 4 d before blood sampling.

On days 2 and 3, the athletes received either 6% carbohydrate (60% sucrose, 40% glucose; 0.20 $kcal \cdot mL^{-1}$) or placebo beverages before, during, and after 2 h of rowing in a randomized, counterbalanced design, with each athlete serving as their own control. The beverages were supplied by the Gatorade Sports Science Institute (Barrington, IL). Treatments were double blinded, and carbohydrate and placebo beverages were identical in appearance and taste. Except for carbohydrate concentration, the two fluids were identical in sodium ($\sim$19.0 $mEq \cdot L^{-1}$) and potassium ($\sim$3.0 $mEq \cdot L^{-1}$) concentration, and pH ($\sim$3.0).

Subjects reported to the training site (Lake Otay, Olympic Training Center) on both days at 0545 h in a 9-h fasted state. The 15 athletes drank 12 $mL \cdot kg^{-1}$ body mass of a carbohydrate or placebo beverage and then rowed from 6:00–8:00 a.m. The athletes used their normal equipment on the lake (eight and four double-oared shells, double and single sculls) engaging in a normal 2-h training bout, with 3-min rest periods taken every 15 min. To determine workload, all athletes wore heart rate monitors (Polar Electro Inc., Woodbury, NY), with heart rates recorded every 15 min. A Cosmed K4 β-2 metabolic unit (Cosmed, Rome, Italy) was passed from athlete to athlete in the eight oared-shell to measure oxygen consumption and ventilation. During rowing, subjects drank 4 $mL \cdot kg^{-1}$ body mass of carbohydrate or placebo every 15 min. Blood samples were collected within 10 min after exercise (0800), and then again 1.5 h later (0930). During the postexercise period, all 15 athletes rested quietly while drinking carbohydrate or placebo beverages at a rate of 8 $mL \cdot kg^{-1} \cdot h^{-1}$ (no food or other beverage was ingested).

Cardiorespiratory Fitness Assessment

Maximal oxygen consumption, ventilation, and respiratory rate were measured in the rowers using a SensorMedics VMax29 metabolic cart (Yorba Linda, CA) during a 1500-m standard time trial row on the Concept II-B ergometer (Norrisville, VT) (27). Maximal heart rate was measured during this test using Polar Vantage X1 chest-strap transmitters (Polar Electro Inc.).

Blood Samples

All blood samples were collected from an antecubital vein with subjects in the seated position. The blood was collected into tubes containing sodium heparin as the anti-

TABLE 1. Subject characteristics ($N = 15$).

Age (yr)	22.4 ± 0.5
Height (cm)	181 ± 1
Body mass (kg)	76.1 ± 1.8
Body mass index ($kg \cdot m^{-2}$)	23.1 ± 0.4
Body fat (%)	17.9 ± 0.6
$\dot{V}O_{2max}$ ($L \cdot min^{-1}$)	4.07 ± 0.08
HR_{max} ($beats \cdot min^{-1}$)	186 ± 3
$\dot{V}E_{max}$ ($L \cdot min^{-1}$)	150 ± 4
RR_{max} ($breaths \cdot min^{-1}$)	59.8 ± 1.4
Hemoglobin ($g \cdot L^{-1}$)	13.4 ± 0.3
Hematocrit (L)	0.392 ± 0.007

$\dot{V}O_{2max}$, maximal oxygen uptake; HR_{max}, maximal heart rate; $\dot{V}E_{max}$, maximal minute ventilation; RR_{max}, maximal respiratory rate.

coagulant. Routine complete blood counts (CBC) were performed by a clinical hematology laboratory (Lab Corp, Burlington, NC), and provided leukocyte subset counts, hemoglobin, and hematocrit.

Leukocyte phagocytosis/oxidative burst assays. The phagocytosis assay was carried out within 4 h of blood collection. During the interim, the blood was stored at room temperature in the original blood collection tubes. The assay utilized a FITC-labeled bacteria (*Staphylococcus aureus*; Molecular Probes, Eugene, OR) to quantify the degree of phagocytosis by granulocytes and monocytes. Monocyte and granulocyte percentages were determined using four-color flow cytometric phenotyping (CD3-FITC, CD8-PE, CD45-PerCP, and CD4-APC). To determine the extent of oxidative burst exhibited by granulocytes and monocytes, we employed 2′,7′-dichlorodihydrofluorescein diacetate (DCF-DA; Molecular Probes), a nonfluorescent molecule that is oxidized to green fluorescent dichlorofluorescein (DCF) when oxidants are generated in the oxidative burst in response to a challenge with *S. aureus*. Bioparticle reagents of unlabeled and labeled *S. aureus* were suspended into phosphate-buffered saline (PBS) at a working concentration of 1.6×10^6 bioparticlesμL^{-1}. After determining the number of phagocytic cells in 100 μL of whole blood and adding 117 FITC-labeled bacteria per cell, the mean channel fluorescence (FITC) was analyzed to determine the degree of engulfed bacteria. To determine the oxidative burst activities, either DCF-DA (final concentration, 100 μM) (basal activity level), or DCF-DA and 117 unlabeled bioparticles per cell (stimulated activity level) were added to 100-μL whole blood. After incubating the samples for 60 min (37°C) in the dark, lysing the RBC with 1% NH_4Cl, centrifuging, and resuspending the pellets, the samples were acquired on the flow cytometer. For each sample, 10,000 phagocytes (monocytes and granulocytes) were acquired. Oxidative burst activity was determined by subtracting basal levels from the stimulated levels.

Cytokine measurements. Interleukin-6 (IL-6) and tumor necrosis factor alpha (TNFα) were measured with Enzyme Immunoassay Kits (Immunotech, Westbrook, ME). A standard curve was constructed for each run for each cytokine using standards provided in the kits. Concentrations for both controls and serum samples were generated from standard curves using linear regression analysis. These assays were a two step "sandwich" enzyme immunoassay in which samples and standards were incubated in a 96-well microtiter plate coated with a cytokine capturing antibody. After appropriate incubation times, the wells were washed and a second detection antibody conjugated to acetylcholinesterase (IL-6) or alkaline phosphatase (TNFα) was added. The plates were incubated and washed, and the amount of bound enzyme labeled detection antibody was measured by adding a chromogenic substrate. The plates were then read at the appropriate wavelength (405 nm). The minimum detectable concentration was 0.10 $pg \cdot mL^{-1}$ for IL-6 and 0.11 $pg \cdot mL^{-1}$ for TNFα. Total plasma interleukin-1 receptor antagonist (IL-1ra) and interleukin-8 (IL-8) was measured with a quantitative sandwich ELISA technique, using monoclonal antibodies specific for IL-1ra or IL-8 as capture antibodies (R&D Systems, Inc., Minneapolis, MN). A standard curve was constructed from standards run with the samples. The minimum detectable level of plasma was 14 $pg \cdot mL^{-1}$ for IL-1ra and 1.0 $pg \cdot mL^{-1}$ for IL-8.

Hormones, glucose, lactate, and plasma volume. Plasma cortisol and growth hormone was assayed using competitive solid-phase ^{125}I radioimmunoassay (RIA) techniques according to manufacturer's instructions (Diagnostic Products Corporation, Los Angeles, CA). For plasma epinephrine and norepinephrine, blood samples were drawn into chilled tubes containing EGTA and glutathione (Amersham, no. RPN532 Vacutainer tubes, Arlington Heights, IL), centrifuged, and the plasma stored at −80°C until analysis. Plasma concentrations of epinephrine were determined by high pressure liquid chromatography (HPLC) with electrochemical detection as described previously (17). Total plasma C-reactive protein (CRP) was measured with a quantitative sandwich ELISA technique, using monoclonal antibodies specific for CRP as capture antibodies and employing the manufacturer's suggested protocol (Hemagen Diagnostics, Inc., Walthman, MA). A standard curve was

TABLE 2. Blood leukocyte concentrations in response to 2 h of rowing under carbohydrate and placebo conditions ($N = 15$).

10^9 cells$\cdot L^{-1}$	Preexercise (0600)	Postexercise (0800)	1.5-h Post (0930)	*P*-Value (Condition × Time)
Total leukocytes				
Carbohydrate	5.51 ± 0.27	5.79 ± 0.44**	5.59 ± 0.34**	<0.001
Placebo	5.51 ± 0.27	7.69 ± 0.66	7.28 ± 0.50	
Neutrophils				
Carbohydrate	2.85 ± 0.23	3.73 ± 0.39**	3.44 ± 0.30**	<0.001
Placebo	2.85 ± 0.23	5.39 ± 0.57	5.08 ± 0.4	
Monocytes				
Carbohydrate	0.43 ± 0.03	0.37 ± 0.03**	0.33 ± 0.02**	<0.001
Placebo	0.43 ± 0.03	0.47 ± 0.04	0.40 ± 0.02	

** $P < 0.01$, change from baseline significantly different between placebo and carbohydrate conditions.

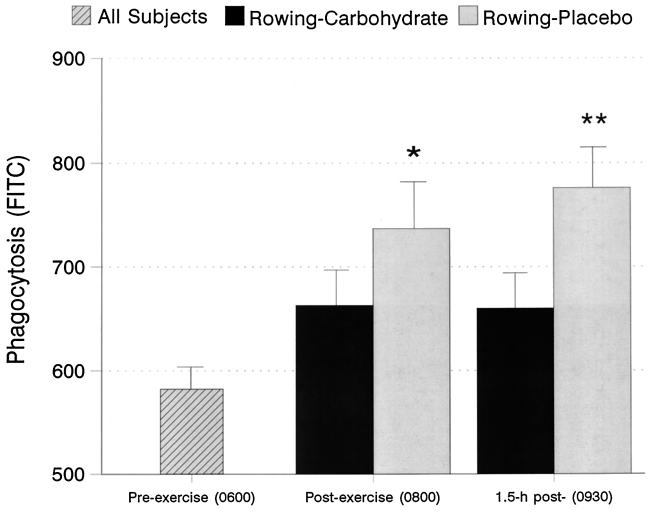

Figure 1—Effects of carbohydrate on phagocytic activity in response to 2 h of rowing ($N = 15$). Carbohydrate versus placebo ingestion produced a significant difference in the pattern of change over time ($P = 0.005$). * $P < 0.05$, ** $P < 0.01$, change from baseline significantly different between placebo and carbohydrate conditions.

constructed from standards run with the samples. The minimum detectable level of plasma CRP was 0.25 $\mu g \cdot mL^{-1}$. Plasma glucose was analyzed spectrophotometrically (prerun, immediate- and 1.5-h postrun samples) as described previously (17). Blood samples were analyzed for lactate according to manufacturer guidelines (Diagnostic Products Corporation). Plasma volume changes were estimated using the method of Dill and Costill (5).

Statistical Analysis

Statistical significance was set at the $P < 0.05$ level, and values are expressed as mean ± SE. Leukocyte and lymphocyte subsets, hormone values, and all immune function measures were analyzed using 2 (carbohydrate and placebo groups) × 3 (times of measurement) repeated measures ANOVA. Athletes served as their own controls; thus, change variables computed from baseline for the immediate- and 1.5-h postexercise values were compared between conditions using paired *t*-tests. A Bonferroni adjustment was made for the multiple comparisons and statistical significance was set at $P < 0.025$.

RESULTS

The average physical characteristics of the 15 elite female rowers are summarized in Table 1. No significant change in plasma volume or body mass occurred after the carbohydrate and placebo rowing sessions, indicating that fluid intake matched sweat losses (data not shown). Heart rate, measured eight times (at the end of each 15 min) during the 2-h rowing sessions, averaged 151 ± 2 beats·min^{-1} (82.4 ± 1.3% of maximum heart rate) and 152 ± 2 beats·min^{-1} (82.2 ± 1.5%) for the carbohydrate and placebo sessions, respectively. Ratings of perceived exertion at the end of the rowing sessions averaged 14.6 ± 0.4 (carbohydrate) and 14.7 ± 0.4 (placebo), indicating a "somewhat hard" to "hard" workload using the 6–20 scale. Steady state samples taken for approximately 10 min in a subset of eight rowers indicated a mean oxygen uptake of 2,307 ± 169 mL·min^{-1} (57% of $\dot{V}O_{2max}$), ventilation of 76 ± 12 L·min^{-1}, respiratory rate of 43 ± 2 breaths·min^{-1}, and respiratory exchange ratio of 1.00 ± 0.05. Although lactate levels were slightly higher after carbohydrate ingestion, the levels did not average greater than 2 mmol·L^{-1} after either session (data not shown). All rowers ingested the placebo and carbohydrate beverages according to the prescribed schedule. The air temperature at lake level averaged 15.5°C with a relative humidity of 70%.

The patterns of change over time between carbohydrate and placebo rowing sessions were significantly different for total leukocytes, neutrophils, and monocytes (Table 2). These differences were highlighted by a less pronounced leukocytosis and neutrophilia, and a decreased monocyte concentration in the carbohydrate session at both postexercise time points.

The increase in phagocytic activity was significantly lower after the carbohydrate versus placebo rowing session (Fig. 1); however, differences were not observed for oxidative burst activity (Table 3). Significant interaction effects were observed for IL-1ra with postexercise elevations less pronounced in the carbohydrate session (Fig. 2). No differences were observed for IL-6, IL-8, or TNFα (Table 3).

Glucose was significantly higher after two hours of rowing with carbohydrate ingestion compared to placebo (Table 4). However, the patterns of change in cortisol, growth

TABLE 3. Phagocyte oxidative burst activity and serum cytokine concentrations in response to 2 h of rowing under carbohydrate and placebo conditions ($N = 15$).

	Preexercise (0600)	Postexercise (0800)	1.5-h Post (0930)	*P*-Value (Condition × Time)
Oxidative burst (mean fluorescence channel, FITC)				
Carbohydrate	79.9 ± 7.9	76.9 ± 6.4	96.6 ± 7.9	0.376
Placebo	79.9 ± 7.9	82.3 ± 8.3	107.0 ± 6.9	
IL-6 (pg·mL^{-1})				
Carbohydrate	1.40 ± 0.65	1.60 ± 0.45	1.27 ± 0.57	0.858
Placebo	1.40 ± 0.65	1.93 ± 0.46	1.47 ± 0.42	
IL-8 (pg·ml^{-1})				
Carbohydrate	3.33 ± 1.56	4.20 ± 2.13	3.27 ± 1.76	0.128
Placebo	3.33 ± 1.56	2.87 ± 1.58	3.67 ± 2.22	
TNFα (pg·mL^{-1})				
Carbohydrate	23.7 ± 4.6	25.7 ± 6.2	27.1 ± 5.9	0.485
Placebo	23.7 ± 4.6	23.9 ± 4.7	23.5 ± 4.1	

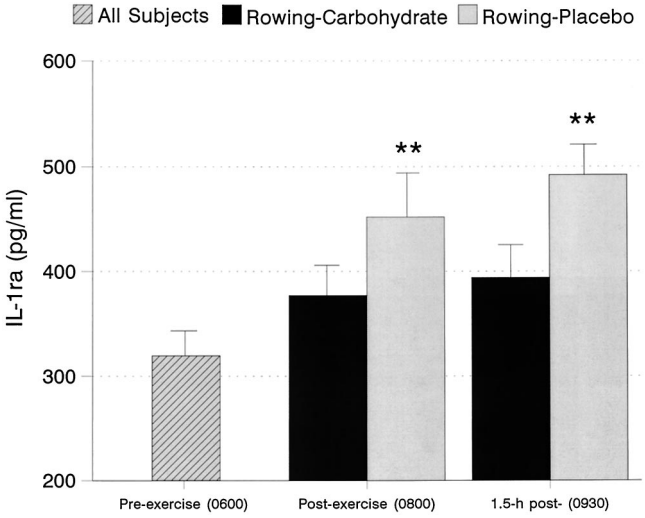

Figure 2—Effects of carbohydrate on plasma IL-1ra concentration in response to 2 hours of rowing ($N = 15$). Carbohydrate versus placebo ingestion produced a significant difference in the pattern of change over time ($P = 0.001$). ** $P < 0.01$, change from baseline significantly different between placebo and carbohydrate conditions.

hormone, CRP (Table 4), and epinephrine and norepinephrine (data not shown) did not differ between sessions.

DISCUSSION

The data suggest that carbohydrate versus placebo ingestion attenuates postexercise elevations in neutrophil and monocyte numbers, phagocytic activity, and plasma IL-1ra concentrations. However, the elevations that were observed were smaller in magnitude than that reported with long-duration, high-intensity bouts of running and cycling, and are consistent with a more moderate training intensity. As described for other elite rowers (28), the present training sessions were performed at moderate intensities, although some high-intensity intervals were interspersed throughout the 2-h sessions. The observations that plasma lactate concentrations did not exceed 2 mmol·L^{-1}, that hormone perturbations were minimal, and that the rowers performed at 57% of their $\dot{V}O_{2max}$ are consistent with this training level interpretation. Although the mechanisms of these carbohydrate effects are not yet understood, they may be related to several factors including energy balance, glycogen stores, blood glucose flux, and/or muscle inflammation (12,14,16,17).

Prolonged and intense aerobic exercise has been shown to induce a sequential release of TNFα, IL-1β, IL-6, and IL-1ra (6,12,16,18,20,30). It has been proposed that exercise-induced injury of skeletal muscle fibers triggers the local production of IL-6, possibly through stimulation by TNFα and IL-1β (20). IL-6, in turn, induces the acute phase response and the production of IL-1ra from blood monocytes, which inhibits further production of IL-1β, thus providing a negative feedback system that prevents an exaggerated inflammatory response. Carbohydrate ingestion has been shown to attenuate the IL-6 and IL-1ra responses to 2.5 h of high-intensity running and cycling (12,16). In the present study, the pattern of change in IL-1ra, but not IL-6, was affected by carbohydrate ingestion, with carbohydrate resulting in a 20% increase in IL-1ra at the 1.5-h postexercise time point, compared with a 50% increase with placebo. Because IL-1ra release typically follows IL-6 release, it is unknown why only the anti-inflammatory phase was affected in this study. However, consistent with the moderate intensity training regimen, the IL-1ra increase observed in the placebo condition was significantly less than the 230–500% increases reported to follow prolonged, high-intensity cycling and running (6,12,16), suggesting that the inflammatory response to rowing training and the antiinflammatory significance of IL-1ra may be minor. Although significant elevations in serum concentrations of IL-6 have consistently been reported after strenuous exercise (6,12,16,18,20), the absence of an IL-6 response in the present study may indicate a lower degree of muscle damage associated with moderate intensity rowing and/or may reflect the predominant concentric nature of muscular contraction characteristic of rowing exercise. Indeed, Bruunsgaard et al. (2) have shown that exercise involving concentric muscle activity (normal bicycle exercise) is associated with a much lower IL-6 response than eccentric exercise (e.g., braked cycling during reversed revolutions).

The observation that oxidative burst activity was unaffected by carbohydrate ingestion is inconsistent with our previous study of 10 triathletes, in which carbohydrate attenuated the modest, postexercise increases in monocyte and neutrophil oxidative burst activity after 2.5 h of running and

TABLE 4. Plasma glucose and hormone concentrations in response to 2 h of rowing under carbohydrate and placebo conditions ($N = 15$).

	Preexercise (0600)	Postexercise (0800)	1.5-h Post (0930)	*P*-Value (Condition × Time)
Glucose (mmol·L^{-1})				
Carbohydrate	4.93 ± 0.13	4.69 ± 0.27*	4.03 ± 0.15	0.015
Placebo	4.93 ± 0.13	4.01 ± 0.15	4.03 ± 0.22	
Cortisol (nmol·L^{-1})				
Carbohydrate	955 ± 94	518 ± 69	416 ± 64	0.071
Placebo	955 ± 94	600 ± 85	372 ± 54	
Growth Hormone (ng·mL^{-1})				
Carbohydrate	6.81 ± 2.59	3.81 ± 1.83	1.13 ± 0.23	0.932
Placebo	6.81 ± 2.59	3.19 ± 0.55	0.88 ± 0.05	
CRP (μg·mL^{-1})				
Carbohydrate	1.03 ± 0.52	1.05 ± 0.56	0.93 ± 0.51	0.544
Placebo	1.03 ± 0.52	1.65 ± 0.79	1.22 ± 0.79	

* $P < 0.05$, change from baseline significantly different between placebo and carbohydrate conditions.

cycling under placebo conditions (17). Carbohydrate versus placebo ingestion did result in less pronounced elevations in blood monocyte and neutrophil concentrations, and phagocytic function. However, the magnitude of these rowing-induced elevations were less profound than observed with running and cycling bouts of longer duration and higher intensity (14,17). We suggest that the modest neutrophil/monocyte trafficking and function changes observed in the present study primarily reflect a reduced level of inflammation associated with a low degree of muscle damage that may follow prolonged, moderate-intensity rowing. This suggestion is consistent with the minimal pro-inflammatory cytokine response observed in the study.

In summary, 2-h bouts of moderate-intensity rowing by elite female rowers resulted in minimal changes in the number and function of neutrophils and monocytes and plasma concentrations of IL-1ra. Although carbohydrate attenuated elevations in IL-1ra and phagocytic activity, the significance of these effects is unclear in light of the moderate degree of perturbations that occurred. These data suggest that carbohydrate supplementation has a minor influence on immune and inflammatory changes when exercise intensity is not sufficient to induce a significant stress hormone response.

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REFERENCES

1. BAGBY, G. J., L. D. CROUCH, and R. E. SHEPHERD. Exercise and cytokines: spontaneous and elicited responses. In: *Exercise and Immune Function,* L. Hoffman-Goetz (Ed.). Boca Raton, FL: CRC Press, 1996, pp. 55–77.
2. 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.
3. BUDGETT, R. G., and G. N. FULLER. Illness and injury in international oarsmen. *Clin. Sports Med.* 1:57–61, 1989.
4. CASTELL, L. M., J. R. POORTMANS, and E. A. NEWSHOLME. Does glutamine have a role in reducing infections in athletes? *Eur. J. Appl. Physiol.* 73:488–451, 1996.
5. 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.
6. DRENTH, J. P., S. H. M. VAN UUM, M. VAN DEUREN, G. J. PESMAN, J. VAN DER VEN-JONGEKRIJG, and J. W. M. VAN DER MEER. Endurance run increases circulating IL-6 and IL-1ra but downregulates ex vivo TNFα and IL-1β production. *J. Appl. Physiol.* 79:1497–1503, 1995.
7. GLEESON, M., A. K. BLANNIN, N. P. WALSH, N. C. BISHOP, and A. M. CLARK. Effect of low- and high-carbohydrate diets on the plasma glutamine and circulating leukocyte responses to exercise. *Int. J. Sport Nutr.* 8:49–59, 1998.
8. HAGERMAN, F. C. Applied physiology of rowing. *Sports Med.* 1:303–326, 1984.
9. MACKINNON, L. T., and S. L. HOOPER. Plasma glutamine and upper respiratory tract infection during intensified training in swimmers. *Med. Sci. Sports Exerc.* 28:285–290, 1996.
10. MITCHELL, J. B., D. L. COSTILL, J. A. HOUMARD, M. G. FLYNN, W. J. FINK, and J. D. BELTZ. Influence of carbohydrate ingestion on counterregulatory hormones during prolonged exercise. *Int. J. Sports Med.* 11:33–36, 1990.
11. MURRAY, R., G. L. PAUL, J. G. SEIFENT, and D. E. EDDY. Responses to varying rates of carbohydrate ingestion during exercise. *Med. Sci. Sports Exerc.* 23:713–718, 1991.
12. NEHLSEN-CANNARELLA, S. L., O. R. FAGOAGA, D. C. NIEMAN, et al. Carbohydrate and the cytokine response to 2.5 hours of running. *J. Appl. Physiol.* 82:1662–1667, 1997.
13. NIELSEN, H. B., N. H. SECHER, N. J. CHRISTENSEN, and B. K. PEDERSEN. Lymphocytes and NK cell activity during repeated bouts of maximal exercise. *Am. J. Physiol.* 271:R222–R227, 1996.
14. NIEMAN, D. C., O. R. FAGOAGA, D. E. BUTTERWORTH, et al. Carbohydrate supplementation affects blood granulocyte and monocyte trafficking but not function after 2.5 h of running. *Am. J. Clin. Nutr.* 66:153–159, 1997.
15. NIEMAN, D. C., D. A. HENSON, D. E. BUTTERWORTH, et al. Vitamin C supplementation does not alter the immune response to 2.5 hours of running. *Int. J. Sports Nutr.* 7:174–184, 1997.
16. NIEMAN, D. C., S. L. NEHLSEN-CANNARELLA, O. R. FAGOAGA, et al. Influence of mode and carbohydrate on the cytokine response to heavy exertion. *Med. Sci. Sports Exerc.* 30:671–678, 1998.
17. NIEMAN, D. C., S. L. NEHLSEN-CANNARELLA, O. R. FAGOAGA, et al. Influence of mode and carbohydrate on the granulocyte and monocyte response to intensive, prolonged exercise. *J. Appl. Physiol.* 84:1252–1259, 1998.
18. NORTHOFF, H., C. WEINSTOCK, and A. BERG. The cytokine response to strenuous exercise. *Int. J. Sports Med.* 15:S197–S171, 1994.
19. ORTEGA, E. Physiology and biochemistry: influence of exercise on phagocytosis. *Int. J. Sports Med.* 15:S172–S178, 1994.
20. OSTROWSKI, K., T. ROHDE, M. ZACHO, S. ASP, and B. K. PEDERSEN. Evidence that interleukin-6 is produced in human skeletal muscle during prolonged running. *J. Physiol. (Lond.)* 508:949–953, 1998.
21. PYNE, D. B., M. S. BAKER, J. A. SMITH, R. D. TELFORD, and M. J. WEIDEMANN. Exercise and the neutrophil oxidative burst: biological and experimental variability. *Eur. J. Appl. Physiol.* 74:564–571, 1996.
22. SATO, H., T. ABE, T. KIKUCHI, et al. Changes in the production of reactive oxygen species from neutrophils following a 100-km marathon. *Nippon Eiseigaku Zasshi* 51:612–616, 1996.
23. SECHER, N. H. Physiological and biomechanical aspects of rowing: implications for training. *Sports Med.* 15:24–42, 1993.
24. SHEPHARD, R. J., and P. N. SHEK. Heavy exercise, nutrition and immune function: is there a connection? *Int. J. Sports Med.* 16: 491–497, 1995.
25. SINGH, A., M. L FAILLA, and P. A. DEUSTER. Exercise-induced changes in immune function: effects of zinc supplementation. *J. Appl. Physiol.* 76:2298–2303, 1994.
26. SMITH, J. A., and D. B. PYNE. Exercise, training, and neutrophil function. *Exerc. Immunol. Rev.* 3:96–116, 1997.
27. SNEGOVSKAYA, V., and A. VIRU. Elevation in cortisol and growth hormone levels in the course of further improvement of performance capacity in trained rowers. *Int. J. Sports Med.* 14:202–206, 1993.
28. STEINACKER, J. M., W. LORMES, M. LEHMAN, and D. ALTENBURG. Training of rowers before world championships. *Med. Sci. Sports Exerc.* 30:1158–1163, 1998.
29. SUZUKI, K., S. NAGANUMA, M. TOTSUKA, et al. Effects of exhaustive endurance exercise and its one-week daily repetition on neutrophil count and functional status in untrained men. *Int. J. Sports Med.* 17:205–212, 1996.
30. WEINSTOCK, C., D. KÖNIG, R. HARNISCHMACHER, J. KEUL, A. BERG, and H. NORTHOFF. Effect of exhaustive exercise stress on the cytokine response. *Med. Sci. Sports Exerc.* 29:345–354, 1997.