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Blood Leukocyte mRNA Expression for IL-10, IL-1Ra, and IL-8, but Not IL-6, Increases After Exercise

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ABSTRACT
The primary purpose of this project was to study exercise-induced leukocyte cytokine mRNA expression. Changes in plasma cytokine levels and blood leukocyte mRNA expression for interleukin-6 (IL-6), IL-8, IL-10, and IL-1 receptor antagonist (IL-1Ra) were measured in 12 athletes following 2 h of intensive cycling (~64% Wattsmax) while ingesting a carbohydrate or placebo beverage (randomized and double blinded). Blood samples were collected 30 min preexercise and immediately and 1 h postexercise. Carbohydrate compared with placebo ingestion attenuated exercise-induced changes in plasma cortisol (8.8% vs. 62%, respectively), epinephrine (~9.2% vs. 138%), IL-6 (10-fold vs. 40-fold), IL-10 (8.9-fold vs. 26-fold, and IL-1Ra (2.1-fold vs. 5.6-fold). Significant time effects were measured for blood leukocyte IL-8 (2.4-fold increase 1 h postexercise), IL-10 (2.7-fold increase), IL-1Ra (2.2-fold increase), and IL-6 (0.8-fold decrease) mRNA content, with no significant differences between Cho and Pla test conditions. In summary, gene expression for IL-8, IL-10, and IL-1Ra, but not IL-6, is increased in blood leukocytes taken from athletes following 2 h of intensive cycling and is not influenced by carbohydrate compared with placebo ingestion. mRNA expression was high enough to indicate a substantial contribution of blood leukocytes to plasma levels of IL-8, IL-10, and IL-1Ra during prolonged exercise.

INTRODUCTION
A marked increase in the plasma concentrations of several cytokines, including interleukin-6 (IL-6), IL-10, IL-8, IL-1 receptor antagonist (IL-1Ra), granulocyte colony-stimulating factor (G-CSF), monocyte chemoattractant protein-1 (MCP-1), and macrophage inflammatory protein-1 β (MIP-1β), occurs in response to prolonged and intensive exercise. Sources of production of these cytokines are still being explored.

After heavy exercise workloads, the mRNA content for tumor necrosis factor-α (TNF-α), IL-8, IL-1β, and IL-6 within muscle biopsy samples is increased, with the greatest fold increases measured for IL-6 and IL-8 mRNA (20–30-fold change from rest when rest=1).2,3,8-12 Although muscle cells produce most of the IL-6 during exercise,11-13 production of other cytokines within muscle biopsy samples may come from several types of cells. Blood mononuclear cells do not appear to contribute IL-1β, TNF-α, or IL-6 during sustained exercise.10 In a previous study, we noted that IL-8 mRNA expression was highest in postexercise muscle biopsy samples that visually appeared to have the most blood.2 IL-8 is produced by a variety of cells throughout the body, including endothelial cells and blood mononuclear cells,13 but the primary source during exercise is unknown. Recently, Akerstrom et al.16 showed that IL-8 protein expression occurred within the cytoplasm of muscle fibers following 3 h of bicycle exercise but could not rule out contributions from other cell types, including endothelial cells and leukocytes. The anti-inflammatory cytokines IL-10 and IL-1Ra are derived from a variety of cell sources, including blood monocytes, but sources of production during exercise have not yet been determined. Ostrowski et al.10 proposed that exercise triggers IL-6 production within muscle cells, leading to a stimulation of IL-1Ra release from circulating blood mononuclear cells. Steensberg et al.17 showed that infusion of recombinant human IL-6 (rHuIL-6) increased plasma IL-1Ra and IL-10 levels compared with saline infusion but did not identify the sources of production.
EXERCISE AND BLOOD CYTOKINE mRNA

In multiple studies, we and others have shown that carbohydrate compared with placebo ingestion attenuates exercise-induced increases in blood neutrophils, monocyte, T, and natural killer (NK) cell counts, plasma cortisol, epinephrine, IL-6, IL-10, and IL-1Ra, but not IL-8, IL-3, IL-4, IL-12, interferon-γ (IFN-γ), and IL-1β.1–6,18–20 Carbohydrate may exert these effects through multiple mechanisms, including elevation in blood glucose and tissue glucose uptake, decreases in cytokine mRNA expression, reductions in proinflammatory signals, and an attenuation of IL-6 release from the working muscle tissue.1,3,6,17,19 Carbohydrate ingestion during prolonged exercise permits investigation of the relative importance of these stimuli under randomized, placebo-controlled conditions.

The purpose of this project was to study the effect of carbohydrate compared with placebo ingestion on plasma hormone and cytokine levels and leukocyte cytokine mRNA expression following 2 h of intensive cycling. We hypothesized that carbohydrate compared with placebo ingestion would attenuate plasma hormone and cytokine levels after exercise. Based on limited information, we also hypothesized that blood leukocyte mRNA for IL-10, IL-8, IL-1Ra, but not IL-6, would increase during exercise, but not to the same magnitude during the carbohydrate trials. The cytokines measured in this study are based on prior studies showing that of plasma cytokines measured to date by exercise immunologists, IL-6, IL-8, IL-10, and IL-1Ra are among those that increase most during exercise.1–7 We and other investigators have shown little or no exercise-induced increases for IL-2, IL-3, IL-4, IL-12, interferon-γ (IFN-γ), TNF-α, and IL-1β.5,7

MATERIALS AND METHODS

Subjects

Twelve trained male cyclists were recruited as experimental subjects through local and collegiate cycling clubs. Written informed consent was obtained from each subject, and the experimental procedures were approved by the university institutional review board.

Research design

Two to three weeks prior to the first test session, subjects reported to the ASU Human Performance Laboratory for orientation and measurement of body composition and cardiorespiratory fitness. Body composition was assessed by hydrostatic weighing using an electronic load cell system (Exertech, Dresbach, MN), estimated residual volume, and the Siri equation.27 VO2max was determined using a graded maximal protocol (25 W increase every 2 min starting at 150 W) with the subjects using their own bicycles on CompuTrainer Pro Model 8001 trainers (RaceMate, Seattle, WA). Oxygen uptake and ventilation were measured using the MedGraphics CPX metabolic system (MedGraphics Corporation, St. Paul, MN). Heart rate was measured using a chest heart rate monitor (Polar Electro Inc., Woodbury, NY). Basic demographic and training data were obtained through a questionnaire.

Subjects agreed to avoid the use of large-dose vitamin/mineral supplements (>100% of recommended dietary allowances), nutritional supplements, ergogenic aids, herbs, and medications known to affect immune function for 1 week prior to test sessions. During orientation, a dietician instructed the subjects to follow a diet moderate in carbohydrate during the 3 days prior to the test session and record intake in a food record. The food records were analyzed using a computerized dietary assessment program (Food Processor, ESHA Research, Salem, OR).

On test session dates, a standardized liquid meal (Boost Plus, Mead Johnson Nutritional, Evansville, IN) was ingested at an energy level of 67 kJ·kg⁻¹ (16 kcal·kg⁻¹) body mass by subjects prior to 10:00 AM. Boost Plus is a nutritionally complete, high-energy oral supplement with an energy density of 6.4 kJ·mL⁻¹ (1.52 kcal·mL⁻¹) and contains 16% of energy as protein, 34% as fat, and 50% as carbohydrate. In quantities of 1000 mL, Boost Plus exceeds daily value recommendations for all major vitamins and minerals.

Subjects came to the laboratory for two 2-h test sessions and ingested carbohydrate or placebo beverages in a randomized, counterbalanced design. The test sessions were 1–2 weeks apart. Subjects cycled for 2.0 h at ~60%–65% Wattsmax. On test session dates, subjects reported to the laboratory at 3:00 PM, not having ingested energy in any form after 10:00 AM. Blood samples were collected ~30 min preexercise, immediately postexercise, and 1 h postexercise. Blood samples were drawn from an antecubital vein with subjects in the seated position. Experimental subjects ingested carbohydrate (6% or 60 g·1⁻¹) or placebo beverages 15–30 min preexercise (12 ml·kg⁻¹) and during the 2-h cycling bout (4 ml·kg⁻¹·15 min⁻¹). The beverages were supplied by the Gatorade Sports Science Institute (Barrington, IL). The carbohydrate and placebo beverages were identical in appearance and taste and in sodium (~19.0 mEq·1⁻¹) and potassium (~3.0 mEq·1⁻¹) concentration and pH (~3.0). No other beverages or food were ingested during the test sessions.

During the test sessions, subjects cycled using their own bicycles on trainers (CompuTrainer Pro Model 8001) with the exercise load set at ~60%–65% maximal Watts and ~75% VO2max. Metabolic measurements were made every 30 min of cycling using the MedGraphics CPX metabolic system to verify workload.

Leukocyte mRNA extraction and cDNA synthesis

The QIAampRNA Blood Mini Kit Protocol (Qiagen, Valencia CA) was used to extract mRNA. From each subject, two 1.5-mL aliquots of whole blood collected in EDTA were puri-

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>21.0 ± 1.0a</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.78 ± 0.01</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>71.6 ± 1.9</td>
</tr>
<tr>
<td>Body composition (% fat)</td>
<td>11.9 ± 1.0</td>
</tr>
<tr>
<td>Heart rate, maximal (beats·min⁻¹)</td>
<td>191 ± 2</td>
</tr>
<tr>
<td>Powermax (W)</td>
<td>335 ± 8</td>
</tr>
<tr>
<td>VO2max (ml·kg⁻¹·min⁻¹)</td>
<td>56.9 ± 1.3</td>
</tr>
<tr>
<td>Minute ventilation, maximal (l·min⁻¹)</td>
<td>166 ± 6</td>
</tr>
</tbody>
</table>

*aMean ± SE.
fied for RNA. Briefly, erythrocytes were selectively lysed, and leukocytes were recovered by centrifugation. Samples were briefly centrifuged through a QIAshredder spin column, ethanol was added to adjust binding conditions, and the sample was applied to a Qiagen spin column. RNA was bound to the silica gel membrane during a brief centrifugation step. Contaminants were washed away, and total RNA was eluted in 30 μL RNase-free water.

The extracted RNA (7.5 μL of sample) was dissolved in diethylpyrocarbonate-treated water and quantified spectrophotometrically at 260-nm wavelength. RNA was reverse transcribed into cDNA in a 50-μL reaction volume containing 19.25 μL RT buffer, 10 μL 10× RT buffer, 11 μL 25 mM MgCl2, 10 μL deoxyNTPs mixture, 2.5 μL random hexamers, 1 μL RNase inhibitor, and 1.25 μL multiscribe reverse transcriptase (50 U/μL). Reverse transcription was performed at 25°C for 10 min, 37°C for 60 min, and 95°C for 5 min, followed by quick chilling on ice, and stored at –20°C until subsequent amplification.

**Quantitative real-time PCR analysis**

Quantitative real-time polymerase chain reaction (PCR) analysis was done as per the manufacturer’s instructions (Applied Biosystems, Foster City, CA) using TaqMan Gene Expression Assays. DNA amplification was carried out in 12.5 μL TaqMan Universal PCR Master Mix (AmpliTaq Gold DNA polymerase, passive reference 1, buffer, dNTPs, AmpErase UNG), 1 μL cDNA, 9 μL RNase-free water, and 1.25 μL 18S primer (VIC) and 1.25 μL primer (FAM) (for endogenous reference and target cytokine) in a final volume of 25 μL well. Human control RNA (calibrator RNA) was also used and served

### Table 2. Plasma Glucose, Insulin, Cortisol, and Epinephrine in 12 Cyclists Under Carbohydrate (C) and Placebo (P) Conditions Before and After 2 h of Intensive Cycling

<table>
<thead>
<tr>
<th>Variable</th>
<th>Preexercise</th>
<th>Postexercise</th>
<th>1 h post-exercise</th>
<th>p value: interactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mmol·L⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>5.52 ± 0.13</td>
<td>6.46 ± 0.36*</td>
<td>6.15 ± 0.43</td>
<td>0.004; 0.510</td>
</tr>
<tr>
<td>P</td>
<td>5.38 ± 0.15</td>
<td>5.05 ± 0.21</td>
<td>4.90 ± 0.11</td>
<td></td>
</tr>
<tr>
<td>Insulin (pmol·L⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>122 ± 40.4</td>
<td>226 ± 51.9</td>
<td>249 ± 51.5*</td>
<td>0.050; 0.097</td>
</tr>
<tr>
<td>P</td>
<td>76.8 ± 17.5</td>
<td>97.5 ± 21.4</td>
<td>27.7 ± 6.28</td>
<td></td>
</tr>
<tr>
<td>Cortisol (mmol·L⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>319 ± 31</td>
<td>347 ± 52*</td>
<td>263 ± 42*</td>
<td>&lt;0.001; 0.042</td>
</tr>
<tr>
<td>P</td>
<td>303 ± 30</td>
<td>490 ± 45</td>
<td>434 ± 51</td>
<td></td>
</tr>
<tr>
<td>Epinephrine (pmol·L⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>808 ± 140</td>
<td>734 ± 120</td>
<td>638 ± 130</td>
<td>0.049; 0.022</td>
</tr>
<tr>
<td>P</td>
<td>732 ± 90</td>
<td>1740 ± 580</td>
<td>771 ± 110</td>
<td></td>
</tr>
</tbody>
</table>

*aInteraction: The first p value represents the condition (carbohydrate vs. placebo) × time (3 time points) interaction; the second p value represents the overall time effect.

*bSignificant difference in change from preexercise in carbohydrate compared with placebo trials, p < 0.05.

### Table 3. Plasma Cytokine Levels in 12 Cyclists Under Carbohydrate (C) and Placebo (P) Conditions Before and After 2 h of Intensive Cycling

<table>
<thead>
<tr>
<th>Variable</th>
<th>Preexercise</th>
<th>Postexercise</th>
<th>1 h post-exercise</th>
<th>p value: interactions*</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6 (pg·mL⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>1.28 ± 0.68</td>
<td>14.6 ± 2.44*</td>
<td>8.77 ± 1.37*</td>
<td>0.007; &lt;0.001</td>
</tr>
<tr>
<td>P</td>
<td>0.56 ± 0.23</td>
<td>23.0 ± 4.70</td>
<td>12.6 ± 2.66</td>
<td></td>
</tr>
<tr>
<td>IL-8 (pg·mL⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>5.53 ± 0.73</td>
<td>9.62 ± 1.08</td>
<td>10.3 ± 0.73</td>
<td>0.453; &lt;0.001</td>
</tr>
<tr>
<td>P</td>
<td>4.93 ± 0.41</td>
<td>10.1 ± 1.12</td>
<td>10.2 ± 0.86</td>
<td></td>
</tr>
<tr>
<td>IL-1Ra (pg·mL⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>202 ± 56</td>
<td>323 ± 63</td>
<td>624 ± 275*</td>
<td>0.010; 0.061</td>
</tr>
<tr>
<td>P</td>
<td>184 ± 22</td>
<td>374 ± 54</td>
<td>1215 ± 503</td>
<td></td>
</tr>
<tr>
<td>IL-10 (pg·mL⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>1.67 ± 0.14</td>
<td>16.2 ± 2.75*</td>
<td>16.5 ± 4.06*</td>
<td>0.003; &lt;0.001</td>
</tr>
<tr>
<td>P</td>
<td>1.83 ± 0.11</td>
<td>25.3 ± 4.32</td>
<td>48.9 ± 13.8</td>
<td></td>
</tr>
</tbody>
</table>

*aInteractions: The first p value represents the condition (carbohydrate vs. placebo) × time (3 time points) interaction; the second p value represents the overall time effect.

*bSignificant difference in change from preexercise in carbohydrate compared with placebo trials, p < 0.05.
as a calibrator for each plate. Samples were loaded in a MicroAmp 96-well reaction plate. Plates were run using the ABI Sequence Detection System. After 2 min at 50°C and 10 min at 95°C, plates were amplified by 50 repeated cycles, of which 1 cycle consisted of a 15-sec denaturing step at 95°C and a 1-min annealing/extending step at 60°C. Data were analyzed by ABI software using the cycle threshold (CT), which is the value calculated and based on the time (measured by PCR cycle number) at which the reporter fluorescent emission increases beyond a threshold level (based on the background fluorescence of the system), and it reflects the cycle number at which the cDNA amplification is first detected. We have previously reported detailed methodology concerning the dual amplification technique. Samples were run in duplicate, and the intraassay and interassay CVs were determined to be 1.69% and 1.65% for the ΔCTs, respectively.

Calculations for relative quantification

Quantification of cytokine gene expression for IL-8, IL-10, IL-1Ra, and IL-6 was calculated using the ΔΔCT method as described by Livak and Schmittgen. This method uses a single sample, the calibrator sample, for comparison of every unknown sample’s gene expression. This method of analysis and quantification has been shown to give similar results to the standard curve method. Briefly, ΔCT (CT(FAM) − CT(VIC)) was calculated for each sample and calibrator. ΔΔCT (ΔCT(calibrator) − ΔCT(sample)) was then calculated for each sample, and relative quantification was calculated as 2ΔΔCT. Initial exclusion criteria consisted of FAM CT ≥40 and VIC CT ≥23.

Hormones, glucose

Plasma concentrations of cortisol were determined using the competitive solid-phase 125I radioimmunoassay (RIA) technique (Diagnostic Products Corporation, Los Angeles, CA) with cortisol-specific antibody (Ab)-coated tubes (Coat-A-Count tubes). Intraassay (CVintra) and interassay (CVinter) coefficients of variation were 4.5% and 5%, respectively. Assay sensitivity was 5.5 nmol/L (0.2 pg/dL). RIA kits were also used to determine plasma concentrations of insulin in duplicate according to the manufacturer’s instructions (Diagnostic Products Corporation). CVintra and CVinter were 5% and 7%, respectively. Assay sensitivity was 1.2 µg/dL. For plasma epinephrine, blood samples were drawn into chilled tubes containing EGTA and glutathione and centrifuged, and the plasma was stored at −80°C until analysis. Plasma concentrations of epinephrine were determined by competitive EIA (Labor Diagnostika Nord, Nordhorn, Germany) using the microtiter plate format as described previously. The CVintra and CVinter variation were 11% and 14%, respectively. The sensitivity of this assay for plasma samples is 11 pg/mL. Plasma glucose was analyzed spectrophotometrically using a glucose oxidase reagent (Randox Laboratories Ltd., Belfast, Northern Ireland) in a microtiter plate-based assay.

Plasma cytokine measurements

Total plasma concentrations of IL-1Ra, IL-6, IL-8, and IL-10 were determined using quantitative sandwich ELISA kits provided by R&D Systems, Inc. (Minneapolis, MN). All samples and provided standards were analyzed in duplicate. The minimum detectable concentrations were IL-1Ra <22 pg · mL⁻¹, IL-6 <0.70 pg · mL⁻¹, IL-6 (high sensitivity) <0.094 pg · mL⁻¹, IL-8 <10 pg · mL⁻¹, and IL-10 <3.9 pg · mL⁻¹. A high sensitivity kit was used to analyze IL-6 in the prerace plasma samples. Because of the lack of high-sensitivity kits for IL-8 and IL-10, we extrapolated data below the minimum detectable level using a software program suited to this task (SOFTmax, Molecular Devices, Sunnyvale, CA). Preexercise and postexercise samples for IL-8, IL-10, and IL-1Ra were analyzed on the same assay plate to decrease interkit assay variability.

Statistical analysis

Values were expressed as mean ± SE. Performance measures across the two exercise trials were compared using paired
RESULTS

Subject characteristics and results from the maximal cardiopulmonary tests for the 12 male cyclists are listed in Table 1. Three-day food records indicated no significant differences in macronutrient intakes between test sessions, with a mean energy intake of 13.1 ± 0.9 MJ · day⁻¹ (3119 ± 213 kcal · day⁻¹) and a percent of energy intake of 60.2% ± 2.6% carbohydrate, 27.5% ± 1.9% fat, and 14.4% ± 1.0% protein. Power output and heart rate did not differ between the two test sessions, and averaged 214 ± 5 W (63.9 ± 0.7% Wmax), 159 ± 3 heart beats/min (83.3 ± 1.1% HRmax), and 73.6 ± 1.5% VO2max.

Plasma glucose, insulin, cortisol, and epinephrine data are summarized in Table 2. Significant condition (carbohydrate vs. placebo) × time interaction effects were measured for each of these variables. Postexercise levels of glucose were higher in the carbohydrate than in the placebo trials (17% vs. −6.1%, respectively), and levels of cortisol (8.8% vs. 62%, respectively) and epinephrine (-9.2% vs. 138%, respectively) were lower.

Plasma cytokine data are summarized in Table 3. Significant condition × time interaction effects were measured for IL-6, IL-1Ra, and IL-10 but not IL-8. In the carbohydrate compared with placebo trials, lower plasma levels were measured for IL-6 (10-fold vs. 40-fold, respectively, immediately postexercise), IL-10 (8.9-fold vs. 26-fold, respectively, 1 h postexercise), and IL-1Ra (2.1-fold vs. 5.6-fold, respectively, 1 h postexercise).

We determined that leukocyte mRNA expression for IL-8, IL-10, and IL-1Ra, but not for IL-6, increased postexercise to a similar degree during the carbohydrate and placebo cycling trials. Our data also indicate that exercise induced increases in plasma cortisol, epinephrine, IL-6, IL-1Ra, and IL-10 were attenuated when cyclists ingested carbohydrate compared with placebo beverages, confirming previously published findings from our laboratory and others.1–3,5,9,19,25,26

This is the first study to show that heavy exercise workloads increase mRNA content in blood leukocytes for IL-10, IL-1Ra, and IL-8, but not IL-6, under both carbohydrate and placebo conditions. The mechanisms involved in this disparity between the IL-6 response and other cytokines remain unclear at this time. Two other studies have shown that exercise does not induce IL-6 mRNA expression in blood mononuclear cells.10,14

The absolute abundance of IL-8, IL-1Ra, and IL-10 mRNA from blood leukocytes in our subjects was high (i.e., low mean ΔCT values), indicating a substantial potential contribution to plasma cytokine levels. For the same amount of total mRNA, IL-8 and IL-10 mRNA abundance in blood leukocytes was well above levels we have previously reported from muscle biopsy.

The absolute abundance of IL-8, IL-1Ra, and IL-10 mRNA from blood leukocytes in our subjects was high (i.e., low mean ΔCT values), indicating a substantial potential contribution to plasma cytokine levels. For the same amount of total mRNA, IL-8 and IL-10 mRNA abundance in blood leukocytes was well above levels we have previously reported from muscle biopsy.

FIG. 1. IL-8 mRNA expression in blood leukocytes from cyclists during 2 h of cycling at ~64% Wmax under carbohydrate and placebo conditions. Values are expressed as a fold change from rest, with rest equal to 1. Time effect, p = 0.003; carbohydrate/placebo condition × time effect, p = 0.397.

FIG. 2. IL-10 mRNA expression in blood leukocytes from cyclists during 2 h of cycling at ~64% Wmax under carbohydrate and placebo conditions. Values are expressed as a fold change from rest, with rest equal to 1. Time effect, p = 0.003; carbohydrate/placebo condition × time effect, p = 0.397.

FIG. 3. IL-6 mRNA expression in blood leukocytes from cyclists during 2 h of cycling at ~64% Wmax under carbohydrate and placebo conditions. Values are expressed as a fold change from rest, with rest equal to 1. Time effect, p = 0.040; carbohydrate/placebo condition × time effect, p = 0.817.

FIG. 4. IL-8 mRNA expression in blood leukocytes from cyclists during 2 h of cycling at ~64% Wmax under carbohydrate and placebo conditions. Values are expressed as a fold change from rest, with rest equal to 1. Time effect, p = 0.003; carbohydrate/placebo condition × time effect, p = 0.397.
samples.\textsuperscript{2,9} IL-8 was approximately 1000-fold greater and IL-10 was 128-fold greater in the leukocyte pellets compared with the muscle biopsy samples.\textsuperscript{2} Despite the low numbers of leukocytes expected in postexercise muscle tissue, we propose that part of the IL-8 mRNA expression in this tissue is from the leukocytes.

The pattern of change in plasma IL-8 paralleled increases in blood leukocyte IL-8 mRNA during both exercise trials, suggesting that gene expression and production and release of IL-8 are regulated by factors related to exercise workload and not hormonal influences altered by carbohydrate or placebo ingestion. Previous research has indicated that IL-8, a chemotactic cytokine, is produced by multiple cell types, including endothelial cells, fat cells, mononuclear cells in blood, macrophages, and cells in bone, skin, and muscle.\textsuperscript{15} IL-8 is an important mediator of inflammatory disorders and stimulates polymorphonuclear cell adherence, degranulation, and respiratory burst activity.\textsuperscript{15} IL-8 is rapidly induced by many stimuli including, TNF-\(\alpha\), IL-1, and bacterial agents, but whether these are important during exercise conditions is unknown.

Postexercise plasma levels of IL-1Ra, IL-10, and IL-6, however, did not parallel increases in blood leukocyte cytokine mRNA expression during both exercise trials. These data indicate that postexercise plasma IL-1Ra, IL-10, and IL-6 levels under carbohydrate/placebo conditions represent the cumulative effects of multiple factors that transpire following gene expression in several cell sources. Febbraio et al.,\textsuperscript{19} for example, have shown that IL-6 release from the contracting muscle is decreased when subjects ingest carbohydrate compared with placebo beverages despite similar increases in muscle IL-6 mRNA, and they reasoned that IL-6 release is regulated by substrate availability. Most studies have shown that cycling exercise induces short-lived but significant increases in muscle mRNA for IL-6, IL-8, IL-1\(\beta\), and TNF-\(\alpha\), and that increases are similar under carbohydrate compared with placebo conditions despite lower plasma IL-6 levels.\textsuperscript{2,8,19,22} Thus, cytokine mRNA expression in blood leukocyte or muscle biopsy samples or both does not necessarily correspond with cytokine release or plasma cytokine levels.

In summary, our most important finding is that gene expression for IL-8, IL-10, and IL-1Ra, but not IL-6, is increased in blood leukocytes taken from athletes following 2 h of intensive cycling and is not influenced by carbohydrate compared with placebo ingestion. mRNA expression was high enough to indicate a substantial contribution of blood leukocytes to plasma levels of IL-8, IL-10, and IL-1Ra during prolonged exercise. Except for IL-8, however, postexercise plasma levels of these cytokines did not parallel blood leukocyte mRNA expression during each of the carbohydrate/placebo trials, indicating that cytokine production and release are influenced by posttranscriptional events or that cytokines are being produced and released from other cell types.

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