Carbohydrate and the cytokine response to 2.5 h of running


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Nehlsen-Cannarella, S. L., O. R. Fagoaga, D. C. Nieman, D. A. Henson, D. E. Butterworth, R. L. Schmitt, E. M. Bailey, B. J. Warren, A. Utter, and J. M. Davis. Carbohydrate and the cytokine response to 2.5 h of running. J. Appl. Physiol. 82(5):1662–1667, 1997.—This randomized, double-blind, placebo-controlled study was designed to determine the influence of 6% carbohydrate (C) vs. placebo (P) beverage ingestion on cytokine responses (5 total samples over 9 h) to 2.5 h of high-intensity running (76.7 ± 0.4% maximal O2 uptake) by 30 experienced marathon runners. For interleukin-6 (IL-6), a difference in the pattern of change between groups was found, highlighted by a greater increase in P vs. C immediately pre- and postrun (753 vs. 421%) and 1.5 h postrun (193 vs. 86%) [F(4,112) = 3.77, P = 0.006]. For interleukin-1-receptor antagonist (IL-1ra), a difference in the pattern of change between groups was found, highlighted by a greater increase in P vs. C 1.5 h postrun (231 vs. 72%) [F(2,50) = 6.38, P = 0.003]. No significant interaction effects were seen for bioactive IL-6 or IL-1. The immediate postrun plasma glucose concentrations correlated negatively with those of plasma cortisol (r = −0.67, P < 0.001); postrun plasma cortisol (r = 0.70, P < 0.001) and IL-6 levels (r = −0.54, P = 0.003) correlated positively with levels of IL-1ra. Taken together, the data indicate that carbohydrate ingestion attenuates cytokine levels in the inflammatory cascade in response to heavy exertion.

interleukin-1-receptor antagonist

STRENUOUS PHYSICAL EXERCISE such as long-distance running typically results in muscle soreness and injury. The response to tissue injury after exercise is analogous to the acute-phase response to inflammation resulting from infection (2). Intense exercise elicits some of the cytokines involved in inflammation, such as tumor necrosis factor-α (TNF-α), interleukin (IL)-1α, IL-1β, IL-6, and IL-1ra to investigate effects on the inflammatory process and the release of cytokines, we designed a randomized, double-blind, placebo-controlled study to investigate the influence of carbohydrate ingestion on the inflammatory cytokine response to 2.5 h of running. We studied IL-1β, IL-6, and IL-1ra to investigate effects on the early and late phases of inflammation and on the anti-inflammatory resolution phase, respectively. We hypothesized that carbohydrate vs. placebo supplementation would keep plasma glucose levels at a higher level, attenuating the rise in epinephrine and cortisol and both pro- and anti-inflammatory cytokines.

METHODS

Subjects

Thirty experienced marathon runners (24 men and 6 women) were recruited who met the following subject-selection criteria: 25–59 yr of age; marathon race time of <4 h within the previous year; average training distance of ≥30 km/wk during the previous year; completion of at least two...
Experimental Design

Runners were unevenly randomized into carbohydrate (n = 17) or placebo (n = 13) supplement groups. Runners from each group recorded food intake for 3 days before the simulated marathon race, choosing foods from a list that ensured a carbohydrate intake of ~60% of total calories. Nutrient intake was assessed by using the computerized dietary-analysis system Food Processor Plus, version 6.0 (ESHA Research, Salem, OR).

This study was conducted during the months of May and June. During the subjects’ first appointment, their height, weight, body composition, and maximal cardiorespiratory fitness were measured. Body composition was assessed from hydrostatic weighing, and VO2max was determined by utilizing a graded maximal treadmill protocol (5, 23). O2 uptake and ventilation were measured by using the MedGraphics CPX metabolic system (MedGraphics St. Paul, MN). Maximal heart rate was measured by using the Quinton Q4000 Stress Test System (Quinton Instrument, Seattle, WA). Training history and demographic factors were assessed through use of a questionnaire.

Within 2 wk of their first appointment, subjects reported to the Human Performance Laboratory in a 12-h fasted and rested condition at 0700. Subjects indicated that they had avoided intensive exercise the day before testing and all exercise for at least 12–15 h and that they were free of symptoms associated with respiratory infections. After the subjects rested for 10–15 min, a blood sample was taken from each. Next, the runners consumed 0.75 liter of a 6% carbohydrate (Gatorade, Quaker Oats, Baxtoning, IL) or placebo beverage before the run. The beverages were prepared by the Gatorade Sports Science Institute. Treatments were double blind, and carbohydrate and placebo beverages were identical in appearance and taste. Except for carbohydrate concentration, the two fluids were identical in sodium (3.0 eq/l) and pH (7.5–8.0) concentration and in pH (3.0).

The marathoners ran on treadmills from 0730 to 1000 at a pace adjusted to elicit a workload ~75–80% of VO2max. Metabolic and heart rate measurements were made every 20 min during the run to ensure that subjects were maintaining the appropriate workload. Runners ingested 0.250 liter of carbohydrate or placebo fluid every 15 min during the run. Immediately after the 2.5-h run, at 1000, blood samples were obtained from the runners, followed by samples taken at 1130, 1300, and 1600. Subjects drank 500 ml/h of carbohydrate or placebo fluid during the first 1.5 h of recovery and then 250 ml/h during the last 4.5 h of recovery. After the 1130 blood sampling, subjects ate a meal ad libitum, choosing foods from the same food list they had adhered to during the 3 days before the study.

Cytokine Measurements

Five blood samples per subject were drawn from an antecubital vein into heparinized tubes with subjects in the seated position (after 10–15 min of rest, except for the immediate postrun sample). The collection tubes were immediately chilled and centrifuged, with plasma samples frozen at –80°C until analysis (cytokine, hormone, and glucose levels). IL-6 (both total plasma levels and bioactive) and cortisol were measured from all five blood samples. Several parameters (IL-1β, IL-1α, glucose, and catecholamines) that were anticipated to be near baseline levels by 1.5 h postexercise were not measured at the 3- and 6-h postrun time points.

Total plasma IL-1β and IL-6 measurements. IL-1α and IL-6 were measured with MEDIGENIX solid-phase enzyme-amplified sensitivity immunoassay (INCELL, Stillwater, MN) enzyme-linked immunoabsorbent assay kits. These assays are based on an allogenic system, in which a blend of monoclonal antibodies directed against distinct epitopes of each interleukin molecule is used to capture antibodies. The minimum detectable concentration of plasma IL-1β and IL-6 is 2 pg/ml.

Plasma bioactive IL-6 assay. A standard proliferative bioassay (9) was used to measure the activity of human IL-6. Briefly, multiple dilutions of plasma samples (plasma inhibitors of IL-6 were inactivated by heating) and a standard (human recombinant IL-6; Collaborative Biomedical Products/Becton-Dickinson Labware, Bedford, MA) were tested in culture for their ability to stimulate proliferation of IL-6-responsive 7TD1 mouse hybridoma cells (American Type Culture Collection, Rockville, MD). The assays were done in triplicate. The amount of proliferation was assessed by the level of [3H]thymidine uptake. Interassay variability was monitored by using an internal control in each assay. The final 5–10 mean control concentration was 4.5 ± 0.65 U/ml, giving us confidence in the sensitivity and reproducibility of this assay. The IL-6 bioassay has a detection limit of ~1 pg/ml of active cytokine.

Total plasma IL-1α. Total plasma IL-1α was measured with the use of a quantitative sandwich enzyme-linked immunoabsorbent assay technique by using monoclonal antibodies specific for IL-1α as capture antibodies (R&D Systems, Minneapolis, MN). The minimum detectable level of plasma IL-1α is <14 pg/ml.

Hormones, Glucose, and Plasma Volume

Plasma cortisol was assayed by using a competitive solid-phase 125I radioimmunoassay technique (Diagnostic Products, Los Angeles, CA). For plasma epinephrine and norepinephrine, blood samples were drawn into chilled tubes containing EGTA and glutathione (no. RPN532 Vacutainer tubes, Amersham) and centrifuged, and the plasma was stored at ~80°C until analysis. Plasma concentrations of epinephrine were determined by high-pressure liquid chromatography with electrochemical detection (19). Plasma was analyzed spectrophotometrically for glucose (prerun, immediately postrun, and 1.5-h postrun samples) (17). Plasma volume changes were estimated by using the method of Dill and Costill (11).

Statistical Analysis

Data are expressed as means ± SE. Leukocyte subsets, hormone values, and all immune function measurements were analyzed by using 2 (carbohydrate and placebo groups) × 3 or 5 (times of measurement) repeated-measures analysis of variance. The change from baseline for the immediate postexercise and 1.5-, 3-, and 6-h recovery values was compared between groups by using Student’s t-test. For these four multiple comparisons, a Bonferroni adjustment was made, with statistical significance set at P < 0.013, and values between this and 0.05 were treated as trends. Pearson product-moment correlations for glucose, cortisol, epinephrine, and various cytokine measurements were calculated within the group of marathon runners to test the strength of these associations.

RESULTS

Table 1 summarizes subject characteristics for the runners in the carbohydrate and placebo groups. Groups
Table 1. Subject characteristics

<table>
<thead>
<tr>
<th></th>
<th>Carbohydrate</th>
<th>Placebo</th>
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<tbody>
<tr>
<td>Age, yr</td>
<td>40.5 ± 1.8</td>
<td>42.9 ± 2.3</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.73 ± 0.02</td>
<td>1.76 ± 0.02</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>68.4 ± 2.2</td>
<td>68.8 ± 2.1</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>13.9 ± 1.1</td>
<td>12.9 ± 0.9</td>
</tr>
<tr>
<td>(\dot{V}O_2)max, ml·kg(^{-1})·min(^{-1})</td>
<td>53.4 ± 1.3</td>
<td>53.4 ± 1.6</td>
</tr>
<tr>
<td>HR(_{max}), beats/min</td>
<td>177 ± 3</td>
<td>177 ± 3</td>
</tr>
<tr>
<td>(V_e)(_{max}), l/min</td>
<td>141 ± 7</td>
<td>144 ± 9</td>
</tr>
<tr>
<td>(RER_{max})</td>
<td>1.31 ± 0.04</td>
<td>1.22 ± 0.02</td>
</tr>
<tr>
<td>Running distance, km/wk</td>
<td>67.5 ± 5.0</td>
<td>60.1 ± 5.0</td>
</tr>
<tr>
<td>Running experience, yr</td>
<td>12.7 ± 1.7</td>
<td>16.7 ± 2.5</td>
</tr>
<tr>
<td>Marathon personal best, min</td>
<td>200.6 ± 5.5</td>
<td>201.1 ± 5.5</td>
</tr>
<tr>
<td>Total marathons completed</td>
<td>15.9 ± 3.5</td>
<td>23.4 ± 6.4</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 17 carbohydrate and 13 placebo subjects. \(\dot{V}O_2\)max, maximal \(O_2\) uptake; HR\(_{max}\), maximal heart rate; \(V_e\)\(_{max}\), maximal minute ventilation; \(RER_{max}\), maximal respiratory exchange ratio.

did not differ significantly in any of the training and fitness parameters measured. The 30 runners can be characterized as nonelite but highly experienced and committed to marathon running.

Nutrient analysis of the 3-day food records revealed no significant differences between groups. Energy intake for all subjects combined was 2,368 ± 114 calories/day, with the proportion of carbohydrate at 62.6 ± 1.5%, fat at 22.3 ± 1.2%, and protein at 15.7 ± 0.4%.

The carbohydrate and placebo groups did not differ significantly in any of the performance measurements taken during the 2.5-h run, except for the average respiratory exchange ratio (0.93 ± 0.01 and 0.89 ± 0.01, respectively, \(P = 0.009\)) and the ending rating of perceived exertion by using the 6–20 scale (14.8 ± 0.3 and 16.3 ± 0.4, respectively, \(P = 0.004\)). As a group, the 30 marathon runners averaged 11.9 ± 0.2 km/h during the 2.5-h run, with a heart rate of 151 ± 2 beats/min or 0.35% of \(V\dot{O}_{2max}\). The laboratory temperature averaged 23.8 ± 0.2°C, with a relative humidity of 51.9 ± 0.6%.

All runners consumed fluids according to the research design, including 2.5 liters during the 2.5-h run. The average runner lost 0.35 ± 0.14 kg of body weight (0.4 ± 0.2%). Plasma volume changes were minimal, and the pattern of change over all time points did not differ significantly between the two groups \([F(3,26) = 0.90; P = 0.453]\); for the pre- to immediate postexercise period, plasma volume change for the carbohydrate and placebo groups was only −1.5 ± 0.4 and −1.0 ± 0.4%, respectively.

The cytokine data are summarized in Table 2 and Figs. 1 and 2. The pattern of change over time between groups was not significantly different for bioactive IL-6 or total plasma IL-1β but was significant for total plasma IL-6 \([F(4,112) = 3.77, P = 0.006]\) and IL-1ra \([F(2,50) = 5.5, P = 0.003]\). For total plasma IL-6, a greater increase from prerun levels was measured for the placebo vs. carbohydrate groups at the immediate postrun (753 and 421%, respectively, \(P = 0.028\)) and 1.5-h postrun (193 vs. 86%, respectively, \(P = 0.018\)) time points. By 1.5 h postrun, plasma IL-1ra had risen 231 vs. 72% in the placebo and carbohydrate groups, respectively (\(P = 0.013\)). Significant time effects were found for IL-6 (bioactive) but not IL-1β (total). For all subjects combined, IL-6 (bioactive) rose 49% immediately after the run, returning close to prerun levels by 1.5 h postrun.

Figure 3 and Table 3 summarize the plasma cortisol, catecholamine, and glucose data. The patterns of change over time between groups for cortisol \([F(4,25) = 3.46, P = 0.022]\) and glucose \([F(2,27) = 10.0, P = 0.001]\), but not epinephrine \([F(2,26) = 1.94, P = 0.164]\), were significantly different, with cortisol higher and glucose lower for the carbohydrate group compared with the placebo group. *Significantly different from prerun between groups, \(P < 0.05\).

Table 2. Bioactive interleukin-6 and total plasma interleukin-1β concentrations in carbohydrate and placebo groups before and after 2.5 h of treadmill running at 76.7 ± 0.4\% \(\dot{V}O_2\)max

<table>
<thead>
<tr>
<th></th>
<th>Prerun (0715)</th>
<th>Postrun (1000)</th>
<th>1.5 h Postrun (1130)</th>
<th>3 h Postrun (1300)</th>
<th>6 h Postrun (1600)</th>
<th>Effect, Group × Time</th>
<th>Effect, Time</th>
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<tbody>
<tr>
<td>Interleukin-6</td>
<td></td>
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<tr>
<td>C (n = 12)</td>
<td>15.4 ± 2.0</td>
<td>24.9 ± 4.3</td>
<td>13.4 ± 2.0</td>
<td>15.0 ± 2.8</td>
<td>11.2 ± 1.7</td>
<td>0.710; &lt;0.001</td>
<td></td>
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<tr>
<td>P (n = 6)</td>
<td>12.9 ± 2.5</td>
<td>15.6 ± 1.8</td>
<td>9.61 ± 1.44</td>
<td>9.75 ± 2.3</td>
<td>8.53 ± 1.27</td>
<td></td>
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<tr>
<td>Interleukin-1β</td>
<td></td>
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<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>C (n = 15)</td>
<td>16.7 ± 3.6</td>
<td>17.4 ± 3.9</td>
<td>14.6 ± 2.3</td>
<td></td>
<td></td>
<td>0.606; 0.113</td>
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<tr>
<td>P (n = 13)</td>
<td>15.8 ± 1.8</td>
<td>13.4 ± 1.5</td>
<td>11.8 ± 1.0</td>
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</table>

Values are means ± SE, expressed as pg/ml; n = no. of subjects. C, carbohydrate group; P, placebo group; nos. in parentheses, time on 24-h system.
concentrations lower in the placebo group after the 2.5-h run. The immediate postrun glucose concentration correlated negatively with cortisol ($r = 0.67$, $P = 0.001$) and epinephrine ($r = 0.54$, $P = 0.002$). The 1.5-h postrun total IL-1ra correlated positively with the averages of immediate postrun and 1.5-h postrun cortisol levels ($r = 0.70$, $P < 0.001$) (Fig. 4) and total plasma IL-6 ($r = 0.54$, $P = 0.003$). No significant correlation between postrun cortisol and total plasma IL-6 was found.

**DISCUSSION**

In agreement with Drenth et al. (13), we showed that intensive, prolonged exercise is associated with a strong increase in plasma concentrations of both IL-6 and IL-1ra but not IL-1β. We have extended this information, showing that carbohydrate vs. placebo ingestion was associated with higher plasma glucose and lower plasma cortisol, IL-6, and IL-1ra concentrations. These data suggest that carbohydrate ingestion attenuates both the pro- and anti-inflammatory responses to heavy exertion, a conclusion strengthened by the significant correlations between plasma glucose and cortisol, cortisol and IL-1ra, and IL-1ra and IL-6.

As a part of the body's natural negative-feedback system, proinflammatory cytokines activate the HPA axis and the sympathoadrenergic system, exerting strong anti-inflammatory actions (2). Epinephrine and cortisol both inhibit the release of IL-1 and IL-6 from isolated monocytes (1). We sought to alter the stress hormonal response to heavy exertion through carbohydrate ingestion and then study whether the plasma inflammatory cytokine response was affected. Release of adrenocorticotropic hormone and cortisol during exercise has been linked, in part, to decreases in blood glucose concentrations (10, 18, 20, 21), with a variable

| Table 3. Catecholamine and glucose response in C and P groups after 2.5 h of treadmill running |
|---------------------------------|--------------------------|--------------------------|--------------------------|
| Prerun (0715) | Postrun (1000) | 1.5 h Postrun (1130) | Group × Time Effect, Time |
| Epinephrine, nmol/l | | | |
| C | 0.81 ± 0.14 | 1.03 ± 0.19 | 0.76 ± 0.10 | 0.164; 0.034 |
| P | 0.73 ± 0.11 | 1.37 ± 0.12 | 1.16 ± 0.21 | |
| Norepinephrine, nmol/l | | | |
| C | 1.80 ± 0.29 | 6.57 ± 0.42 | 2.74 ± 0.41 | 0.117; < 0.001 |
| P | 2.43 ± 0.29 | 8.34 ± 0.64 | 3.16 ± 0.40 | |
| Glucose, mmol/l | | | |
| C | 4.80 ± 0.13 | 6.65 ± 0.17 | 4.97 ± 0.22* | 0.001; < 0.001 |
| P | 4.87 ± 0.21 | 5.26 ± 0.39 | 4.44 ± 0.16 | |

Values are means ± SE for 17 carbohydrate and 13 placebo subjects. Significantly different from prerun between groups: *$P < 0.05$; †$P < 0.0125$.

Fig. 2. Pattern of change in total plasma interleukin-1-receptor antagonist (IL-1ra) over time between groups was significantly different [F(2,50) = 6.38, $P = 0.003$], with plasma concentrations rising higher 1.5 h postrun in placebo group compared with Carb group. *Significantly different from prerun between groups, $P < 0.05$.

Fig. 3. Pattern of change in cortisol over time between groups was significantly different [F(4,25) = 3.46, $P = 0.022$]. Difference in change from prerun between groups tended to be higher in placebo vs. Carb group at both immediate postrun ($P = 0.073$) and 1.5-h postrun ($P = 0.054$) timepoints.

Fig. 4. Plasma levels of cortisol (average of immediate postrun and 1.5 h postrun) correlated significantly with 1.5-h postrun levels of total plasma IL-1ra ($r = 0.70$, $P < 0.001$). ▲, Carb group; star, placebo group.
effect on epinephrine (20, 21). Although decreases in blood glucose are not typical during and after intensive long-distance running, the data from the present study do suggest that carbohydrate ingestion is associated with higher plasma glucose and lower plasma cortisol levels after 2.5 h of intensive running. For all subjects, there was a strong negative correlation between postrun plasma glucose and cortisol levels, with a moderate negative effect on epinephrine.

The onset of inflammation is brought about by tissue macrophages, smooth muscle cells, and fibroblasts responding to muscle cell injury and producing cytokines of the IL-1 and TNF families; these are called early or “alarm” cytokines (3, 14). IL-1 and TNF act on fibroblasts and endothelial cells to produce a secondary front of cytokines, which includes IL-6 and IL-8 (3). The main mediator of the acute-phase response is IL-6, which, in turn, is regulated by IL-1 (15). The elevation of IL-6 helps induce synthesis of acute-phase proteins (inflammatory); it also upregulates IL-1ra production and activates the HPA axis, both anti-inflammatory. These parameters correlated in our study. As reviewed by Bagby et al. (2), few studies have examined the tissue site of cytokine production in response to exercise. Ullum et al. (28) were unable to detect exercise-induced changes in blood mononuclear cell mRNA for various cytokines and surmised that a tissue other than blood produces IL-6 and IL-1. Probable sources are the active muscle, other metabolically active tissues in which proinflammatory events are occurring (2, 4, 7), and the brain (30).

The data from the present study suggest that carbohydrate must be having some effect starting in the metabolically active tissue areas because of its effect in lowering IL-6, an important inducer of the inflammatory cascade. In the carbohydrate group, total plasma IL-6 was lower than in the placebo group, which was then linked to lower total IL-1ra and cortisol. Although plasma glucose was higher in the carbohydrate group, and correlated strongly and negatively to cortisol, it is likely that the first step in the chain of events occurred within the metabolically active tissues. Alternatively, the energy demands of the brain may not have been satisfied throughout the 2.5-h run in the placebo group, and this deficiency could have generated a stress signal. Both of these pathways may have simultaneously contributed to the stress response.

Carbohydrate ingestion had a significant effect on the pattern of response of total plasma IL-6 but not bioactive IL-6 after 2.5 h of running. For all subjects combined, total plasma IL-6 rose 551% immediately after exercise compared with 49% for bioactive IL-6. We used a standard proliferative bioassay, in which plasma samples were tested in culture for their ability to stimulate proliferation of IL-6-responsive 7TD1 mouse hybridoma cells (9). We expected to find that the proliferative response was higher in the placebo vs. the carbohydrate group because of its higher total plasma IL-6. These are the first bioactive IL-6 data to be presented in the exercise immunology literature, and further research is warranted to establish the usefulness of this assay under exercise conditions.

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