Influence of Carbohydrate Ingestion on Cytokine Responses Following Acute Resistance Exercise

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The effect of carbohydrate supplementation (CHO) on interleukin 2 (IL-2) and interleukin 5 (IL-5) secretion following acute resistance exercise was examined in 9 resistance-trained males. Subjects completed a randomized, double-blind protocol with exercise separated by 14 days. The exercise consisted of a high intensity, short rest interval squat workout. Subjects consumed 1.0 g · kg body mass–1 CHO or an equal volume of placebo (PLC) 10 min prior to and 10 min following exercise. Blood was collected at rest (REST), immediately post exercise (POST), and at 1.5 h of recovery (1.5 h POST). Isolated peripheral blood mononuclear cells were stimulated with PHA and assayed for IL-2 and IL-5 secretion. IL-2 secretion was significantly decreased at POST for both the PLC and CHO groups. However, the degree of decrease was less in the CHO group (16%) than in the PLC group (48%), and this difference was statistically significant. These responses were transient, and the values returned to normal by 1.5 h POST. A mild and transient but significant decrease in IL-5 secretion by the PLC group was observed at POST (26%) compared to REST. No significant decrease was observed in IL-5 secretion for CHO from REST to POST (12%). These data support a possible effect of carbohydrate supplementation on IL-2 and IL-5 secretion following high-intensity resistance exercise.

Key Words: carbohydrates, exercise, immune function, cortisol, interleukins

Introduction

The human immune system often responds to stress with a transient immunosuppression. Exercise is one stressor that can influence function of the immune system. The immune system’s response to exercise varies greatly with the intensity of effort relative to the individual’s maximal performance and to the individual’s training.
status (19, 25). Data from our laboratory support a protective effect of training on immune system function following a high intensity resistance exercise bout (23). In contrast, bouts of high intensity aerobic exercise, such as marathon running, are thought to induce transient immunosuppression (1, 11, 15, 26).

Many of the immune system alterations that occur following exercise have been attributed to the immunomodulatory effects of hormones (4, 27). For example, cortisol has been reported to inhibit mitogen-induced lymphocyte proliferation via a suppression of monocyte function and a decrease in interleukin-2 release (4). Cortisol may also lead to decreases in circulating lymphocyte numbers by inhibiting lymphocyte entry into the blood compartment and stimulating lymphocyte emigration from the blood into the peripheral lymphoid tissues (4, 27).

A recent series of experiments has tested the hypothesis that carbohydrate ingestion, by suppressing cortisol release, can attenuate post-exercise immunosuppression (6, 14, 16, 17, 21, 22). The results of these investigations revealed that, compared to placebo, carbohydrate ingestion during exercise results in an attenuated cortisol response and fewer perturbations in immune cell counts. Additionally, carbohydrate ingestion has been shown to maintain phytohemagglutinin (PHA) -induced lymphocyte proliferation post-exercise (7). We have recently studied the effects of carbohydrate supplementation on immune system function following acute resistance training exercise (9). PHA-induced lymphocyte proliferation following a high intensity short rest interval squat workout was not significantly different between carbohydrate and placebo groups.

Cytokines are low-molecular weight proteins secreted by several cell types that control interactions among cells involved in the immune, inflammatory, and several other cellular responses. With respect to T cell responses, a subset of cells known as T helper-1 cells (Th1) secrete cytokines that modulate cell-mediated immunity. These include interleukin 2 (IL-2) and interferon gamma. A second T cell subset, known as T helper 2 cells (Th2), secrete a different subset of cytokines that modulate humoral immunity and antibody production. These include interleukin 4 (IL-4) and 5 (IL-5). Thus, measurement of the relative secretion of Th1 cytokines as compared with Th2 cytokines offers an index of the type of immune response that is active at a given time.

Nieman et al. (20) recently demonstrated a beneficial effect of carbohydrate supplementation on the cytokine IL-10 and on the IL-1 receptor antagonist IL-1ra responses following a marathon race. The effect was linked to a higher plasma glucose concentration and a lower cortisol concentration in the carbohydrate compared to the placebo group. To our knowledge, no studies have examined the effect of carbohydrate supplementation on the cytokine response following high intensity resistance training. The purpose of the present study was to determine whether carbohydrate ingestion would alter IL-2 or IL-5 secretion during the short-term recovery from a high intensity resistance exercise session. Additionally, this investigation attempted to quantify the relationships among blood glucose, cortisol, and the IL-2 and IL-5 response to resistance exercise.

Methodology

Some data reported for this paper are from a subset of subjects who participated in a previously published investigation (9). All cytokine data are original and have not been previously reported.
Subjects

Nine healthy, resistance-trained males participated in this investigation. All subjects had been performing resistance training for ≥ 3 months prior to the study and could back squat at least 150% of their body mass. Due to the relationship between muscle damage and an inflammatory response, all subjects were required to be performing back squats during training. Descriptive data for the subjects are presented in Table 1. All subjects read and signed an informed consent, and completed a health history questionnaire in accordance with guidelines set forth by the Advisory Committee for Human Experimentation at the University of Kansas. Subjects refrained from caffeine and alcohol consumption for 12 h and exercise for 24 h prior to all testing sessions.

Research Design

The subjects were tested on three occasions. The first testing session was used to collect anthropometric data and to determine each subject’s one-repetition maximum (1RM) in the back squat. Subjects reported to the laboratory for the second test session between 3 and 7 days following initial testing. The second and third sessions were treatment conditions in which the subjects consumed either a carbohydrate (CHO) or placebo (PLC) beverage, followed by a resistance exercise session. A second dose of the treatment beverage was consumed 10 min following the training session. The treatment beverages were administered in a double-blind, randomized fashion. The second and third test sessions were separated by 14 days.

Initial Testing

Height and body mass were measured using a wall-mounted stadiometer and an electronic scale, respectively. Body composition was determined via 7-site skinfold (8). The subject’s 1RM back squat was determined using previously described methods (23).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>25.0 ± 2.9</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>174.4 ± 8.2</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>80.1 ± 9.8</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>10.1 ± 3.7</td>
</tr>
<tr>
<td>1RM squat (kg)</td>
<td>153.2 ± 20.9</td>
</tr>
<tr>
<td>Training experience (y)</td>
<td>8.3 ± 5.6</td>
</tr>
</tbody>
</table>

Table 1 Subject Characteristics (N = 9)
**Pre-testing Dietary Controls**

Subjects recorded their food intake in a dietary journal for the 3 days prior to each treatment session. Subjects were given detailed instructions on how to complete the journal. The goal of the dietary control was to ensure that subjects consumed a consistent diet, both in terms of energy content and macronutrient intake. Dietary analyses were performed using commercially available software (Food Processor, v. 7.01, ESHA Research, Salem, OR, USA).

**Treatment Conditions**

Subjects reported for the treatment conditions in an 8-h fasted state. Upon arrival, subjects consumed a low-carbohydrate meal consisting of commercially available food bars (Atkins Nutritionals, Inc.) and water. The meal contained 1764 kJ, 24 g fat, 5.2 g carbohydrate, and 19 g protein. The subjects then rested for 1.5 h in the laboratory before the first blood sample was collected. Following this initial blood sample collection, subjects ingested the first dose of the treatment beverage. The subjects began the resistance exercise session 10 min after ingestion of the treatment beverage. The 10-min window between ingestion and exercise was employed in an attempt to increase blood glucose levels while avoiding the possibility of reactive hypoglycemia (29). Figure 1 presents a timeline of the test-day procedures.

![Figure 1 — Individual treatment session timeline.](image-url)
The resistance training protocol involved a high volume of large muscle mass exercises with short rest intervals, and was chosen to maximize the cortisol response to the exercise bout (10). The training session began with two warm-up sets of 10 repetitions of back squats at 40% and 50% of 1RM. This was followed by 5 sets of 10 repetitions of the back squat at 65% 1RM and then 3 sets of 10 repetitions of half squats at 85% 1RM. Subjects rested for 60 s between each set. If subjects were unable to complete the prescribed number of repetitions for a given set, the resistance was lowered by 2.5 kg for the following set. The total time to complete the training protocol was 18–20 min. Total exercise volume was calculated as the number of sets × repetitions per set × resistance. Subjects consumed the second dose of their assigned treatment beverage 10 min after completion of exercise and then remained in the laboratory for 1.5 h.

**Supplementation**

During each testing session, the subjects consumed either a CHO supplement (Gatorlode®, 20% maltodextrin and dextrose solution, Quaker Oats, Inc.) or a PLC beverage (aspartame and citrus flavoring, Quaker Oats, Inc.). The treatment beverages consisted of a volume of fluid that provided 1.0 g · kg body mass⁻¹ of carbohydrate or an equal volume of placebo. Two doses of the assigned treatment beverage were consumed each test day, the first dose at 10 min prior to initiating exercise, the second dose 10 min following the post-exercise blood draw.

**Blood Collection**

Blood was collected from an antecubital vein at baseline (REST), immediately post exercise (POST), and at 1.5 h post exercise (1.5 h POST). For each of the three time points, a total volume of 28 ml of blood was collected into four vacutainers: a standard serum tube for the determination of serum cortisol, a heparinized vacutainer for IL-2 and IL-5 analysis, and two vacutainers containing EDTA for plasma glucose and complete blood counts, respectively.

**Cortisol, Glucose, and Complete Blood Counts**

Blood samples for glucose and cortisol were analyzed using standard biochemical techniques. A clinical hematology laboratory performed the complete blood count analysis. A description of those techniques has been reported previously (9). Plasma volume shift was calculated from the hemoglobin and hematocrit values (5). All blood variables were corrected for plasma volume changes.

**Cytokine Assays**

Mononuclear cells were isolated from heparinized peripheral blood using Ficoll-Hypaque (Pharmacia, Piscataway, NJ, USA) density gradient centrifugation as previously described (3). Cells were seeded in triplicate wells in 48-well tissue culture plates (Corning, Corning, NY, USA) at a concentration of 1 × 10⁶ cells/ml and either non-treated or treated with 10 μg · ml⁻¹ phytohaemagglutinin (PHA; Gibco BRL, Gaithersburg, MD, USA). Cells were incubated at 37 °C in a humidified CO₂ incubator for 24 h. Supernatant fluid was removed from each well, and the
amounts of interleukin 2 and interleukin 5 secreted were assessed using Quantikine™ kits (R&D Systems, Minneapolis, MN, USA).

**Data Analysis**

Data are expressed as means ± standard deviation. Concentrations of IL-2 and IL-5, plasma glucose, and plasma cortisol were compared using a 2 × 3 (treatment by time) repeated measures analysis of variance (ANOVA). Post hoc analyses of significant effects were completed using paired t tests, and Pearson product-moment correlations were used to determine the relationships among glucose, cortisol, and IL-2 and IL-5 concentrations. Paired t tests were used to compare dietary intake and work completed between treatments. The level of significance was set at p ≤ .05.

**Results**

**Subject Characteristics**

Table 1 summarizes the various physical and training characteristics of the 9 subjects. The subjects were experienced, non-competitive weight trainers. The average ratio of 1RM squat relative to body mass was 1.91 ± 0.16, indicating a high level of strength in the subjects.

**Dietary Records**

Nutrient analysis of 3-day food diaries prior to each test session revealed a consistent dietary intake. The average energy intake for the subjects was 13,637 ± 3,870 kJ · d⁻¹ prior to the CHO treatment and 12,171 ± 4,244 kJ · d⁻¹ prior to PLC. The macronutrient composition of the dietary intake was also similar between treatments: CHO: carbohydrate (p = .796) = 370 ± 141 g · d⁻¹; fat (p = .087) = 115 ± 41 g · d⁻¹; protein (p = .287) = 205 ± 144 g · d⁻¹; PLC: carbohydrate = 386 ± 140 g · d⁻¹; fat = 90 ± 27 g · d⁻¹; protein = 153 ± 47 g · d⁻¹.

**Resistance Exercise**

No difference in total exercise volume completed during the exercise bout was observed between treatment conditions. The volume load lifted during CHO treatment was 9,986.0 ± 1,229.0 kg, while volume load lifted during PLC was 9,740.0 ± 1,331.1 kg.

**Interleukin Secretion**

Kolmogrov-Smirnov tests indicated that the interleukin secretion data were normally distributed (for IL-2 Z = .876, p = .426; for IL-5, Z = .568, p = .904), supporting the use of parametric statistics to analyze these data. PHA-stimulated IL-2 and IL-5 secretion are shown in Figures 2 and 3, respectively. In the PLC treatment, IL-2 secretion decreased by 48% at POST compared to REST whereas, in the CHO treatment, IL-2 secretion decreased by only 16% (Figure 2). This resulted in a significant difference between the treatment conditions at POST (p = .008). By 1.5 h POST, the PHA-induced secretion levels for CHO and PLC returned to those observed at REST, indicating that the decrease in IL-2 secretion at POST was transient.
A similar trend was observed also for IL-5 (Figure 3). In the PLC treatment, a 26% decrease in IL-5 secretion was seen at POST compared to REST ($p = .03$), whereas in the CHO treatment, IL-5 secretion had decreased by only 12% (nonsignificant). IL-5 secretion had begun to return to pre-exercise levels at 1.5 h POST. There were no significant differences in IL-5 between treatments. The decrease in IL-5 from REST to POST during PLC was statistically significant ($p = .03$), while there was no significant change during CHO.
Glucose, Cortisol, and Blood Leukocytes

Exercise induced significant alterations in glucose, cortisol, and leukocytes (Table 2), and these data have been reported previously (9). No significant correlations were observed among the dependent variables of glucose, cortisol, IL-2, and IL-5 at any of the measurement times.

Table 2 Plasma Glucose (mmol/L), Serum Cortisol (nmol/L), and Blood Leukocyte Subset Responses (10⁹ · L⁻¹) Responses to Resistance Exercise With Carbohydrate or Placebo (N = 9)

<table>
<thead>
<tr>
<th>Variable</th>
<th>REST</th>
<th>POST</th>
<th>1.5 h</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M ± SD</td>
<td>M ± SD</td>
<td>M ± SD</td>
<td>Treatment × Time Effects</td>
</tr>
<tr>
<td>Glucose</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHO</td>
<td>5.42 ± 0.74</td>
<td>9.51 ± 2.86*</td>
<td>7.52 ± 2.34</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>PLC</td>
<td>5.91 ± 0.73</td>
<td>7.57 ± 1.86*</td>
<td>6.54 ± 1.03</td>
<td>.022</td>
</tr>
<tr>
<td>Cortisol</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHO</td>
<td>500 ± 129</td>
<td>618 ± 139*</td>
<td>588 ± 173</td>
<td>.007</td>
</tr>
<tr>
<td>PLC</td>
<td>444 ± 113</td>
<td>602 ± 97*</td>
<td>600 ± 201</td>
<td>.817</td>
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<tr>
<td>Lymphocytes</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>CHO</td>
<td>1.50 ± 0.56</td>
<td>3.12 ± 1.26*</td>
<td>1.02 ± 0.31*</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>PLC</td>
<td>1.61 ± 0.48</td>
<td>3.14 ± 1.12*</td>
<td>1.16 ± 0.37*</td>
<td>.974</td>
</tr>
<tr>
<td>Monocytes</td>
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<tr>
<td>CHO</td>
<td>0.44 ± 0.11</td>
<td>0.74 ± 0.28*</td>
<td>0.39 ± 0.11</td>
<td>&lt;.001</td>
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<tr>
<td>PLC</td>
<td>0.47 ± 0.10</td>
<td>0.78 ± 0.24*</td>
<td>0.39 ± 0.09</td>
<td>.770</td>
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<tr>
<td>Neutrophils</td>
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<tr>
<td>CHO</td>
<td>3.05 ± 0.80</td>
<td>4.05 ± 1.09*</td>
<td>4.33 ± 2.12*</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>PLC</td>
<td>2.88 ± 0.63</td>
<td>3.73 ± 0.78*</td>
<td>4.31 ± 1.40*</td>
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<tr>
<td>Basophils</td>
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<td></td>
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<tr>
<td>CHO</td>
<td>0.04 ± 0.05</td>
<td>0.08 ± 0.04</td>
<td>0.05 ± 0.05</td>
<td>.136</td>
</tr>
<tr>
<td>PLC</td>
<td>0.07 ± 0.05</td>
<td>0.07 ± 0.03</td>
<td>0.07 ± 0.04</td>
<td>.348</td>
</tr>
<tr>
<td>Eosinophils</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHO</td>
<td>0.23 ± 0.13</td>
<td>0.37 ± 0.28*</td>
<td>0.17 ± 0.09*</td>
<td>.001</td>
</tr>
<tr>
<td>PLC</td>
<td>0.27 ± 0.16</td>
<td>0.37 ± 0.23*</td>
<td>0.18 ± 0.10*</td>
<td>.642</td>
</tr>
</tbody>
</table>

Note. *Denotes significant difference from REST within treatments (p < .025). #Denotes significant difference from REST between treatments (p < .025). From ref. 9.
Immune system function has been shown to be suppressed following both brief, high intensity exercise (2, 9, 10, 18, 19, 23, 24) and prolonged, vigorous exercise (1, 11, 15, 26). It has been suggested that carbohydrate supplementation may minimize the immune system changes that occur following exercise (6, 14, 16, 17, 21, 22), and our data support this theory. In the current study, carbohydrate supplementation had a significant effect on the pattern of change in IL-2 secretion and a slight effect on IL-5 secretion following a resistance exercise bout. The 16% decrease in IL-2 secretion in the CHO condition at POST compared to the 48% decrease in IL-2 secretion in the PLC condition suggested that with CHO, cytokine secretion was maintained at higher levels with PLC. With CHO, T cells were still able to respond to PHA stimulation by secreting IL-2. Since we observed a similar effect on IL-5 secretion, this may suggest that both subsets of T cells are affected.

The CHO treatment was effective for increasing blood glucose concentrations above that observed with placebo. Central to the hypothesis of carbohydrate ingestion displaying an interactive effect on the immune response to exercise is an increase in blood glucose levels. Blood glucose concentration was increased during both exercise conditions and others have also observed increases in plasma glucose in response to exercise with placebo or water (6, 7, 12, 13). The increased blood glucose concentrations during the PLC treatment is likely due to an increase in liver glycogenolysis and a decrease in glucose uptake by working skeletal muscle. The significantly larger increase in glucose levels from REST to POST observed with CHO indicates that the CHO treatment effectively elevated blood glucose levels above that observed with PLC.

Nieman et al. (20) have recently demonstrated a beneficial effect of carbohydrate supplementation on IL-10 and IL-1ra concentrations following a marathon race. A specific link between carbohydrate ingestion, higher plasma glucose and insulin, and lower plasma cortisol and anti-inflammatory cytokine levels was demonstrated in that study (20) as well as previous investigations using similar research designs (14, 21). Alterations in serum cortisol are thought to be responsible for many of the changes in immune status commonly observed following vigorous exercise.

Alterations in cell trafficking following exercise should be considered regarding the findings of the current study. The lymphocytosis observed immediately following heavy exercise typically consists of elevations of all lymphocyte subtypes, but also of a proportionally greater increase in circulating NK cells (6, 7, 27). This alteration in lymphocyte subset composition would lead to a proportional decrease in T-helper cells in the isolated PBMC from which cytokines were assayed and thus may be largely responsible for the decrease in cytokine secretion observed in both conditions at this time point. Carbohydrate ingestion has been shown to alter circulating lymphocyte subpopulations following endurance exercise, specifically by decreasing the number of T-cells immediately post exercise (6, 7). The lack of flow cytometry data measuring alterations in T cell numbers during the recovery period is a potential limitation to the current study. However, the findings of Henson et al. (6, 7) were also characterized by significant differences between CHO and PLC conditions in the total numbers of circulating lymphocytes at post exercise and recovery time points. The present data, while limited to a comparison of total lymphocyte numbers (reported previously), provide no indication of any effect of CHO ingestion on immune cell trafficking.
The hormone epinephrine has also been implicated in the immune response to exercise and may have affected the present results. Steensberg et al. (28) have recently reported a negative correlation between plasma epinephrine and circulating IL-2 producing CD8+ T cells following long endurance exercise. Therefore, it is possible that plasma epinephrine levels may have been significantly increased at POST in PLC above levels seen in CHO, resulting in fewer circulating Th1 cells and an attenuated IL-2 production as compared to CHO. It is unfortunate that epinephrine levels were not measured in the present data. However, previous studies of carbohydrate’s effect on the immune response to exercise, using an endurance exercise model, revealed no difference in epinephrine levels between CHO and PLC conditions (6, 14). Additionally, Nieman et al. have characterized the response of epinephrine following acute exhaustive resistance exercise as a moderate increase when compared to prolonged, high intensity endurance exercise (19). In light of these reports, the potential to ascribe the between-treatment difference observed in IL-2 secretion immediately following this resistance exercise bout solely to changes in epinephrine is diminished.

The resistance exercise protocol employed in the present study elicited a significant increase in serum cortisol from REST to POST, but no differences were found between treatments. We expected to find a significantly attenuated cortisol response in the CHO treatment but this did not occur (data reported previously). It is possible that the exercise protocol was of insufficient duration or volume to elicit differences in plasma cortisol between treatments. Previous studies that have observed an attenuated cortisol response with carbohydrate ingestion during exercise have all employed endurance exercise protocols of 60 min or longer in duration (6, 7, 12, 13). In contrast, the protocol employed in the current study was only ~15 min in duration. Given this, it seems likely the cortisol responses in the present study were not due to changes in glucose concentration, but were primarily a response to the stress of the high intensity training session. The present work suggests that cortisol is not the only modulator of immune responses. We observed that CHO and not PLC attenuated exercise-induced changes in IL-2 and IL-5 secretion under conditions where cortisol levels were similar. These effects seemed to involve both Th1 (cellular) and Th2 (humoral) immune responses.

In summary, CHO versus PLC supplementation before and following heavy resistance exercise minimized the decrease in IL-2 and IL-5 secretion after training. No association could be established, however, between serum cortisol levels and cytokine secretion, suggesting additional, non-cortisol-mediated transient suppression of the immune response. Future tests of the interaction between carbohydrate ingestion and immune function should compare the effects of longer-term dietary interventions of low- and high-carbohydrate diets, and longer duration, higher volume exercise sessions to improve the strength of the intervention.

References


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