Influence of carbohydrate status on immune responses before and after endurance exercise

J. B. MITCHELL, F. X. PIZZA, A. PAQUET, B. J. DAVIS, M. B. FORREST, AND W. A. BRAUN
Exercise Physiology Laboratory, Texas Christian University, Fort Worth, Texas 76129

Mitchell, J. B., F. X. Pizza, A. Paquet, B. J. Davis, M. B. Forrest, and W. A. Braun. Influence of carbohydrate status on immune responses before and after endurance exercise. J. Appl. Physiol. 84(6): 1917–1925, 1998.—To determine the effect of carbohydrate (CHO) status on immune responses after long-duration exercise, on two occasions, 10 men completed a glycogen-depleting bout of cycle ergometry followed by 48 h of either a high-CHO diet (HiCHO; 8.0 g CHO/kg) or a low-CHO diet (LoCHO; 0.5 g CHO/kg). After the 48 h, subjects completed a 60-min ride at 75% maximal O2 uptake (EX). Blood samples were taken predi depletion, pre-EX, post-EX, and 2 and 24 h post-EX and were assayed for leukocyte number and function, glucose, glutamine, and cortisol. The glucose responses were significantly higher in the HiCHO (4.62 ± 0.26 mM) vs. the LoCHO (3.19 ± 0.15 mM) condition post-EX, and glutamine was significantly higher in the HiCHO (0.472 ± 0.036 mM) vs. the LoCHO (0.410 ± 0.025 mM) condition throughout. Cortisol levels were significantly greater in the LoCHO (587 ± 50 nM) vs. the HiCHO (515 ± 62 nM) condition throughout the trial. Lymphocyte proliferation (phytohemagglutinin) was significantly depressed after exercise. However, there was no difference between conditions, and the depression was not correlated with elevations in cortisol. Circulating numbers of leukocytes, neutrophils, lymphocytes, and lymphocyte subsets were significantly greater in the LoCHO vs. the HiCHO condition at the post-EX and 2 h post-EX time points. These data indicate that the exercise and diet manipulation altered the number of circulating leukocytes but did not affect the decrease in lymphocyte proliferation that occurred after exercise.

blood glucose; glutamine; leukocytes; lymphocyte proliferation

ACUTE BOUTS of strenuous exercise have been shown to bring about alterations in the immune system, in the form of both depressed functional responses (9, 10, 29) and changes in the number of circulating leukocytes (9, 10, 29, 37). The altered immune responses resulting from exercise are short lived, and the clinical relevance is uncertain (14, 38). There may be a connection, however, between altered immune responses and the increase in the occurrence of infection that has been reported in some athletes during heavy training and after competition (2, 22, 25, 27, 32, 36).

The precise cause of the alterations in immune function has not been clearly identified. It is known, however, that heavy training and long-duration competitive events place considerable demands on the body’s energy reserves. Depending on the dietary and exercise regimens of an individual, carbohydrate (CHO) stores can be acutely and/or chronically reduced (4, 19). Furthermore, plasma glutamine levels have been shown to undergo a reduction in response to heavy training (30), and they may be a metabolic marker for the overtraining syndrome (34). Because the cells of the immune system are highly dependent on blood glucose and glutamine as energy substrates (26, 31, 36), it is possible that the exercise-induced alterations in immune function are partially related to the levels of available substrates, such as glucose and glutamine, which can undergo significant decreases in response to specific dietary and/or exercise regimens (13, 19).

Furthermore, when the energy demands are high (for instance, in the case of prolonged exercise), the stress response is more pronounced, as evidenced by a concomitant elevation in counterregulatory hormones. Decreases in substrate availability produce elevations in cortisol, epinephrine, and growth hormone. Exercise alone also produces increases in these hormones; however, the combination of exercise and a pronounced counterregulatory response may have a more exaggerated effect on immune function.

Therefore, the purpose of this investigation was to determine the effect of preexercise carbohydrate status on immune cell numbers and lymphocyte proliferation before and after exercise. Specifically, the effects of high- and low-CHO diets (HiCHO and LoCHO, respectively) on lymphocyte proliferation and leukocyte counts were examined before and up to 24 h after 60 min of continuous submaximal exercise that was preceded by glycogen-depleting exercise and 48 h of dietary control (either 8.0 or 0.5 g CHO/kg body mass).

METHODS

Subjects. Ten moderately trained men capable of completing 60 min of submaximal cycle ergometry were selected as subjects. Each subject was initially tested for maximal O2 uptake (V˙O2max) on an ergometer by using a graded protocol. Results from this test were used to establish the work loads for subsequent submaximal exercise tests. All subjects signed an institutionally approved informed consent form, and they completed a medical history questionnaire to screen for major contraindications to exercise testing. Subject characteristics are as follows (means ± SE): age, 24.7 ± 4.8 yr; height, 179.0 ± 6.4 cm; body mass, 79.32 ± 7.45 kg; %fat, 10.17 ± 2.18; and V˙O2max, 3.93 ± 0.70 l/min.

Experimental design and protocol. After the initial test for V˙O2max, each subject participated in two experimental trials administered in a randomized, counterbalanced design with at least 2 wk between trials. During the 24-h period before the experimental trial, subjects consumed a standard mixed diet and did not engage in strenuous exercise. Each experimental trial spanned 3 days, with testing performed at the same time of day for each subject.

The two trials were a HiCHO and a LoCHO condition. In each condition, subjects reported to the laboratory between 0600 and 0900 and completed a bout of exercise designed to deplete muscle glycogen (5). During the next 48 h, the subjects remained inactive and consumed a diet designed either to replenish CHO stores (target CHO intake of 8.0 g CHO/kg body mass) or to maintain a CHO-depleted state.
CARBOHYDRATE STATUS AND IMMUNE RESPONSES

period of dietary control, subjects completed a 60-min ride at a target intensity of 75% of \( V_{O2\max} \). During the final 24 h, subjects remained inactive and consumed a self-selected, normal mixed diet, which was duplicated for the two conditions.

Experimental testing. The first blood sample was taken before the depletion ride and after 15 min of supine rest. The subjects were weighed, and then they completed the 60-min depletion ride at 70% of \( V_{O2\max} \), followed by six 1-min sprints, with 1 min of rest between each sprint. Respiratory exchange measurements were taken by Douglas bag and analyzed on a metabolic cart (Vista; Vacumed, Ventura, CA) during the 60-min ride to verify exercise intensity. After the depletion ride, in the HiCHO condition, they were given a prescribed diet of readily available foods. The recommended food items were fruit, fruit juice, bagels, bread, cola, and pasta with tomato sauce. In the LoCHO condition, because of the difficulty in self-selecting a LoCHO diet, they were given the actual food to be consumed for the next 48 h. This consisted of cheese, eggs, bacon, tuna, and hamburger. Based on computer tables of caloric equivalency and percent contribution of fat and CHO. These diets corresponded to 7.62 and 0.43 g CHO/kg body mass for the HiCHO and LoCHO conditions, respectively.

On returning to the laboratory for the experimental exercise, the subjects again rested in a supine position before a second blood sample was taken. They were again weighed and then completed a 60-min ride on a cycle ergometer at a target intensity of 75% \( V_{O2\max} \). In the HiCHO condition, the subjects consumed a beverage that delivered 50 g CHO, and in the LoCHO condition an equivalent amount of water was consumed. This was done to further accentuate the differences in CHO status between the two conditions. Respiratory exchange measurements were taken by Douglas bag to verify exercise intensity and to determine the rates of fat and CHO oxidation. CHO oxidation was calculated by using the respiratory exchange ratio, liters of \( O_2 \) consumed, and standard tables of caloric equivalency and percent contribution of fat and CHO. Immediately after exercise, and at 2 and 24 h after the ride, blood samples were taken after 15 min of supine rest. Subjects were allowed to consume only water from the end of exercise until the blood sample at 2 h.

Blood analysis. At each time point, blood was drawn from an antecubital vein into three different evacuated collection tubes (Vacutainer; Becton Dickinson, Mountain View, CA). The first contained heparin and was used for the lymphocyte proliferation measurements. The second contained EDTA and was used for the determination of leukocyte counts and lymphocyte subpopulations via flow cytometry. The third was a plain vacutainer to obtain serum for measurement of cortisol and blood glucose. Aliquots of whole blood were immediately removed from the latter tube and deproteinized in 8% perchloric acid to obtain an extract for the determination of lactate and glutamine.

Lymphocyte proliferation was conducted by using a whole blood method with the mitogen phytohemagglutinin (PHA; Difco Labs, Detroit, MI) (6). Briefly, after sampling, the blood was diluted 1:10 with glutamine-deficient RPMI 1640 (Sigma Chemical, St. Louis, MO) supplemented with either 0.0, 0.20, 0.50, or 2.0 mM glutamine. Two sets of triplicates of 100 µl of the diluted blood were then plated into microtiter plate wells for each level of glutamine (24 wells for each sample). PHA (100 µl) at a concentration of 100 mM was added to one set of the triplicate wells. The PHA also contained glutamine at concentrations of 0.0, 0.2, 0.5, and 2.0 mM to keep the final concentration of glutamine in the wells at the desired level. The second set of triplicate wells was used as a control and did not receive PHA. All samples were then incubated for 72 h in a humidified atmosphere of 5% \( CO_2 \) at 37°C (model 8241; National Appliance, Portland, OR). After they were incubated, the cells were pulsed with 0.5 µCi tritiated thymidine (ICN Radiochemicals, Irvine, CA); 8 h later, the cells were harvested onto glass fiber filters with the use of a semiautomated cell harvester (model M12V; Brandel, Rockville, MD). Radiolabeled thymidine uptake was determined by using liquid scintillation counting (model LS 5801; Beckman Instruments, Irvine, CA).

The flow cytometry procedure involved preparation of the cells by using a Coulter Immunoprep Leukocyte Preparation System (Q-Prep; Coulter Electronics, Hialeah, FL). A 100-µl aliquot of the cell preparation from the Q-Prep was washed with PBS and incubated at room temperature for 10 min with 5 µl of a monoclonal antibody for lymphocyte subsets and interleukin-2 (IL-2) receptor expression (Becton Dickinson). The monoclonal antibodies were conjugated with FITC, phycoerythrin (PE), and peridinin-chlorophyll-a-protein (PERCP) (Becton Dickinson). The conjugated monoclonal antibodies were specific for T lymphocytes [CD3+(PERCP)], helper/inducer lymphocytes [CD3+(PERCP)]CD4+(FITC)], cytotoxic/suppressor lymphocytes [CD3+(PERCP)]CD8+(PE)/CD4-(FITC)], B lymphocytes [CD20+(PERCP)], natural killer cells [CD16+(FITC)]CD56+PE), and IL-2 receptor expression [CD25+(FITC)] on CD3+ cells. The antibody-labeled cells were analyzed by using a Cytoron Absolute flow cytometer (Ortho Diagnostics, Raritan, NJ). Complete and differential blood counts were also determined from the blood drawn into EDTA-treated tubes by using an automated analyzer (Sysmex K-1000; TOA Electronics, Kobe, Japan).

From the serum samples, blood glucose was determined by using a colorimetric method (procedure 510; Sigma Chemical) on a spectrophotometer (Spectronic 601; Spectronic Instruments, Rochester, NY). Cortisol was determined by using an RIA method (procedure 2000-SP; Diagnostic Systems Laboratories, Webster, TX) followed by detection of counts per minute (cpm) on a Logic 1118 gamma counter (Abbott Laboratories, North Chicago, IL). Blood lactate was determined by perchloric extracts by using the spectrophotometric enzymatic method described by Lowry and Passonneau (20). Perchloric acid extracts were also assayed for blood glutamine by using the spectrophotometric enzymatic method described by Lund (21).

Statistical analysis. All data were analyzed using a two-factor ANOVA for repeated measures. The first factor (condition) had two levels, HiCHO and LoCHO. The second factor (time) had various levels, depending on the frequency of sampling (five levels for all of the variables based on blood samples). The dependent variables were body mass and CHO oxidation; blood glucose, lactate, and glutamine; leukocyte counts and lymphocyte subpopulations; and proliferation responses expressed as total cpm and cpm per lymphocyte subset. Differences detected by the ANOVAs were located with a Newman-Keuls post hoc test. Pearson correlations were run between various blood variables at specific time points. Significance was accepted at the \( P < 0.05 \) level.

RESULTS

Metabolic data. The resting body mass in the HiCHO conditions did not change during the 48 h of dietary
control (79.30 ± 2.51 vs. 79.39 ± 2.43 kg for predepletion and preexercise, respectively). However, during this period in the LoCHO condition, the subjects lost body mass (79.79 ± 2.30 vs. 78.94 ± 2.40 kg for predepletion and preexercise, respectively). The change in body mass for the HiCHO condition (0.08 ± 0.16 kg) was significantly less than in the LoCHO condition (−0.85 ± 0.28 kg).

There were no differences in the submaximal O₂ uptake (V˙O₂) responses (2.79 ± 0.12 and 2.85 ± 0.14 l/min for the HiCHO and LoCHO conditions, respectively; Table 1). The average exercise intensities relative to VO₂max were 71.17 ± 1.28 and 72.70 ± 0.77% for the HiCHO and LoCHO conditions, respectively. The rates of CHO oxidation were significantly greater in the HiCHO condition throughout the 60 min of exercise (Table 1).

After exercise, blood glucose levels in the HiCHO condition were significantly greater than in the LoCHO condition (Fig. 1). At all other time points, the levels were not different between conditions. Blood lactate followed a similar pattern, with the HiCHO condition producing significantly higher levels at both the pre- and the postexercise time points (Fig. 2). The analysis of blood glutamine levels showed a significant main effect for condition, with the levels in the HiCHO condition being higher than in the LoCHO condition (Fig. 3). Cortisol responses also showed a main effect for condition and time (Fig. 4). The post hoc analysis of time effects revealed that the postexercise values were higher than those observed at 2 and 24 h postexercise in both the HiCHO and LoCHO conditions.

Proliferation data. The proliferation responses from the cells incubated at 0.2, 0.5, and 2.0 mM glutamine were significantly higher than those in 0.0 mM glutamine. However, there were no differences in the responses among the three levels of glutamine (data for the predepletion time point shown in Table 2). Because of the similarity in the responses from the three levels of glutamine, the responses from the cells incubated at 0.5 mM were used for the statistical analyses. This level was chosen because it most closely approximates physiological levels as determined by enzymatic methods (39). The proliferation responses were expressed in three ways: 1) as total cpm of the stimulated cells – cpm of the control cells, 2) as cpm (− control) per lymphocyte, and 3) as cpm (− control) per CD3+ cell.

Table 1. Exercise respiratory-exchange data

<table>
<thead>
<tr>
<th></th>
<th>15 min</th>
<th>30 min</th>
<th>45 min</th>
<th>60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>V˙O₂, l/min</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HiCHO</td>
<td>2.72 ± 0.14</td>
<td>2.79 ± 0.12</td>
<td>2.83 ± 0.10</td>
<td>2.81 ± 0.12</td>
</tr>
<tr>
<td>LoCHO</td>
<td>2.80 ± 0.15</td>
<td>2.87 ± 0.13</td>
<td>2.85 ± 0.14</td>
<td>2.86 ± 0.15</td>
</tr>
<tr>
<td>CHO oxidation, g/min</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HiCHO</td>
<td>3.21 ± 0.18</td>
<td>3.26 ± 0.15</td>
<td>3.25 ± 0.15</td>
<td>3.10 ± 0.17</td>
</tr>
<tr>
<td>LoCHO</td>
<td>2.56 ± 0.26*</td>
<td>2.45 ± 0.25*</td>
<td>2.37 ± 0.26*</td>
<td>2.51 ± 0.24*</td>
</tr>
</tbody>
</table>

Values are means ± SE. V˙O₂, O₂ uptake; CHO, carbohydrate; HiCHO, high-carbohydrate diet; LoCHO, low-carbohydrate diet. *Significant difference from HiCHO condition, P < 0.05.
With the use of total cpm control (Fig. 5), there were no differences between conditions. However, for both the HiCHO and LoCHO conditions, the response at 2 h postexercise was significantly lower than that at the predepletion time point. With the use of either the cpm per lymphocyte (Fig. 6) or the cpm per CD3+ cell (Fig. 7), there was again no difference between conditions. The postexercise responses for cpm per lymphocyte were lower than at all other time points, and the postexercise values for cpm per CD3+ cell were lower than at all other time points except the preexercise value. Correlations between cortisol and proliferation responses expressed as cpm per lymphocyte and per CD3+ cells were not significant (HiCHO, \( r = 0.05 \) and 0.23; LoCHO, \( r = 0.55 \) and 0.55 for cortisol vs. cpm per lymphocytes and per CD3+ cells, respectively). Similarly, correlations between proliferation and blood glutamine levels were not significant.

Cytometric data. The number of leukocytes was significantly greater at the postexercise and 2-h postexercise time points in the LoCHO compared with the HiCHO condition (Table 3). A similar difference was observed in neutrophils, but it was only significant at the 2-h postexercise time point. The exercise-induced elevation produced a neutrophil percentage of 82% (vs. 54% predepletion) in the LoCHO condition compared with 68% (vs. 56% predepletion) in the HiCHO condition at the 2-h postexercise time point. Lymphocytes were different between conditions only at the postexercise time point. The elevation in neutrophil percentage at the 2-h postexercise time point corresponded to a reduction in lymphocyte percentage to 13% (vs. 36% predepletion) in the LoCHO condition and 23% (vs. 33% predepletion) in the HiCHO condition. Significant differences between specific time points within conditions for leukocyte, neutrophil, and lymphocyte numbers are noted in Table 3. In the LoCHO condition, the correlation (\( n = 10 \)) between cortisol and leukocyte number at the postexercise time point approached significance (\( r = 0.627, P = 0.052 \)), whereas that between cortisol and neutrophil number at the same time point reached significance (\( r = 0.762, P = 0.010 \)). The corresponding correlations in the HiCHO condition were not significant (\( r = 0.417, P = 0.23 \); and \( r = 0.39, P = 0.265 \), respectively)

The numbers of CD3+, CD4+, CD8+, and CD20+ cells were significantly greater in the LoCHO compared with the HiCHO condition at the postexercise time point. However, at the 2-h postexercise time point, the trend was reversed (Table 4). The CD4+/CD8+ ratio was significantly lower in the LoCHO condition postexercise, but it was greater at the 2 h time point compared with the HiCHO condition. The number of CD56+ cells was greater in the LoCHO condition only at the postexercise time point. Significant differences between specific time points within conditions for lymphocyte subsets are noted in Table 4. There was a significant main effect for condition and time for the IL-2 receptor expression (CD25+) on T lymphocytes (Fig. 8), with the HiCHO responses greater than those in the LoCHO condition. In both conditions, the preexercise responses were lower than the predepletion responses, and the 2-h postexercise responses were lower than predepletion and postexercise responses.
DISCUSSION

The primary findings of this study were that the preexercise diet and exercise regimen, which was designed to produce a reduction in substrate availability in the LoCHO condition, did not bring about differences in exercise-induced decreases in lymphocyte proliferation compared with the HiCHO condition. There were, however, significant differences between conditions in the number of circulating leukocytes, neutrophils, lymphocytes, and lymphocyte subsets which may have been mediated by differences in hormonal responses that were present as a result of the dietary manipulation.

Metabolic responses. Preexercise CHO status can be assessed indirectly via such markers as changes in body mass and in blood glucose and lactate levels. The significant loss of mass during the 48-h period of dietary control in the LoCHO condition probably represents the loss of water that had been stored with liver and muscle glycogen. The significantly lower resting lactate levels and the tendency for a reduced resting blood glucose in the LoCHO condition are also suggestive of a reduced CHO availability.

During exercise, the significantly lower rates of CHO oxidation in the LoCHO condition and the lower postexercise blood glucose and lactate levels would be expected when CHO availability is reduced (4). The mean blood glucose of 3.25 mM at the end of exercise in the LoCHO condition was comparable to that typically associated with fatigue encountered at the end of endurance exercise (4). Collectively, the metabolic responses indicate that there was a lowered CHO status in the LoCHO condition.

Proliferation responses. The lymphocyte proliferation responses followed a pattern of postexercise decreases that has been identified by previous researchers (8, 9). It was hypothesized that, after the 60 min of exercise in a CHO-depleted state, lymphocyte function would be impaired to a greater extent because of a reduced availability of substrate, i.e., glucose and/or glutamine. Although there were significant exercise-induced decreases in proliferation responses, there was no effect of the dietary manipulation, regardless of the method of determining the response.

The question of the degree of decline in blood glucose and/or glutamine that would elicit a depressed prolifera-

<table>
<thead>
<tr>
<th>Glutamine Concentration</th>
<th>HiCHO, counts/min</th>
<th>LoCHO, counts/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0 mM</td>
<td>2,597 ± 869*</td>
<td>2,375 ± 485*</td>
</tr>
<tr>
<td>0.2 mM</td>
<td>24,655 ± 3,330</td>
<td>26,599 ± 2,238</td>
</tr>
<tr>
<td>0.5 mM</td>
<td>27,658 ± 3,571</td>
<td>25,798 ± 2,555</td>
</tr>
<tr>
<td>2.0 mM</td>
<td>23,479 ± 3,130</td>
<td>21,505 ± 3,239</td>
</tr>
</tbody>
</table>

Values are means ± SE. Proliferation responses are shown for predepletion time point. * Significant main effect for concentration, with 0.0 mM different from all others, P < 0.05.
ative response has not been clearly identified. A method-
ological issue that should be kept in mind when analyz-
ing these and other results is the tendency for lower
plasma glutamine levels obtained with enzymatic meth-
ods (0.5 mM) as opposed to those reported in studies
using high-performance liquid chromatography and
other methods (1.0 mM) (23). Despite these method-
ological discrepancies, what is important in the present
study is the relative differences between conditions.

The influence of CHO status on the availability of
 glutamine and its concomitant effects on immune re-
sponses can be addressed from the standpoint of the
influence of both diet and exercise. Greenhaff et al. (13)
observed a significant decrease in resting plasma gluta-
mine as a result of 4 days on a low-CHO (3% of total
kcal) diet, whereas Blomstrand et al. (1) reported a
significantly lower plasma glutamine with placebo com-
pared with CHO ingestion, 5 min after 80 min of ex-
ercise. Others have reported significant decreases in
 glutamine in response to demanding exercise bouts,
such as a marathon (18, 30), or after heavy training
which elicited symptoms of the overtraining syndrome
(23, 30). Although it is recognized that cells of the
immune system use glutamine at relatively high rates
(26), a diet or exercise-induced decrease in blood gluta-
mine that leads to impaired cellular function has not
been identified. It is difficult to conclude, therefore, that
the decline of ~17% to mean values below 0.4 mM in
the LoCHO condition represents a decrease that would
impair lymphocyte proliferation. A comparable percent
decline in plasma glutamine was, however, observed
after a marathon (31) and in overtrained swimmers.
However, in neither case was the decline directly linked
to impaired immune function (23).

Some investigators have shown that in vitro proli-
feration remains optimal with glutamine levels ranging
from 0.3 to 1.0 mM (30, 31, 33). On the other hand,
others report that the proliferative response is en-
hanced with increasing concentrations ranging from
0.1 to 1.0 mM, and that 0.5 mM is necessary for a
complete transition through the cell cycle (17, 39). The
similarity in the in vitro proliferation responses from
cells incubated with 0.2, 0.5, and 2.0 mM glutamine in
the current study supports the idea that glutamine
levels below the normal physiological range may be
adequate to support lymphocyte proliferation. Con-
versely, Moriguchi et al. (24) have demonstrated that
glutamine supplementation in rats prevented exercise-
duced decreases in both plasma glutamine levels and
lymphocyte proliferation. These authors concluded that
glutamine supplementation may have exerted its ef-

ts by directly affecting the responsiveness of lympho-
cytes to mitogens via an enhanced fuel availability.

It has been suggested that exercise-induced changes
in proliferative responses may be caused by the redistri-
bution of cells rather than by actual alterations in the
functional capacity of lymphocytes (10, 14). Frisina et
al. (8) reported a decrease in mitogen-induced re-
sponses that was correlated (r = 0.78) with changes in
T-cell number. Hinton et al. (14) reported similar
relationships and also found that cultures of pure

Table 3. Leukocyte, neutrophil, and lymphocyte counts

<table>
<thead>
<tr>
<th></th>
<th>Pre-Dep</th>
<th>Pre-Ex</th>
<th>Post-Ex</th>
<th>2 h Post-Ex</th>
<th>24 h Post-Ex</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Leukocytes, ×10^9/l</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HiCHO</td>
<td>5.80 ± 0.49</td>
<td>5.54 ± 0.38</td>
<td>8.17 ± 0.51&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>6.60 ± 0.40</td>
<td>5.62 ± 0.46</td>
</tr>
<tr>
<td>LoCHO</td>
<td>5.71 ± 0.45</td>
<td>5.77 ± 0.39</td>
<td>9.79 ± 0.59&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>11.46 ± 1.09&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>5.09 ± 0.37</td>
</tr>
<tr>
<td><strong>Neutrophils, ×10^9/l</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HiCHO</td>
<td>3.22 ± 0.30</td>
<td>3.12 ± 0.31</td>
<td>4.76 ± 0.49&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>4.56 ± 0.40&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>3.44 ± 0.42</td>
</tr>
<tr>
<td>LoCHO</td>
<td>3.08 ± 0.28</td>
<td>3.16 ± 0.28</td>
<td>5.20 ± 0.50&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>9.59 ± 1.11&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>2.95 ± 0.28</td>
</tr>
<tr>
<td><strong>Lymphocytes, ×10^9/l</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HiCHO</td>
<td>1.92 ± 0.22</td>
<td>1.82 ± 0.16</td>
<td>2.66 ± 0.23&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>1.51 ± 0.12&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>1.63 ± 0.15</td>
</tr>
<tr>
<td>LoCHO</td>
<td>2.05 ± 0.20</td>
<td>1.96 ± 0.16</td>
<td>3.62 ± 0.24&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>1.30 ± 0.12&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>1.63 ± 0.14&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are means ± SE. Pre-Dep, prederepletion; Pre-Ex, preexercise; Post-Ex, postexercise. *Significant difference from HiCHO condition at
specified time point. Letters indicate significant time effects, P < 0.05. <sup>a</sup>Different from prederepletion; <sup>b</sup>different from preexercise.
T lymphocytes did not show postexercise decreases in proliferation. When Nieman et al. (29) adjusted for the number of CD3+ cells in concanavalin A-stimulated whole blood samples taken before and after high- and moderate-intensity exercise, there was less depression of the mitotic response compared with the nonadjusted responses, particularly with moderate-intensity exercise. In the current investigation, the most noticeable effect of using cpm divided by either lymphocyte number or CD3+ cells was that the depression of proliferation moved from 2 h postexercise to immediately after exercise. This shift can be attributed to the redistribution of circulating cells at the various time points. Thus, in the face of exercise-induced lymphocytosis, the proliferative response of the lymphocytes and, in particular, the T lymphocytes was reduced on a per-cell basis. These responses were obtained regardless of condition; thus these responses were not caused by dietary manipulation.

The monoclonal antibody (CD25+) for the IL-2 receptor has been suggested as a more accurate means of assessing in vivo activation of lymphocytes than in vitro mitogen-stimulated proliferation (9). Previous investigators have reported mixed results regarding the effect of acute exercise on the number of CD25+ cells (9, 11, 35). The current results are difficult to interpret because of the lack of a significant interaction and the variable pattern of significant time effects. However, the significant main effect for condition suggests a tendency for a lower response cause by the LoCHO diet.

Immunosuppressive effects of cortisol, particularly relative to lymphocyte proliferation, have been reported (40, 42). However, on the basis of previous findings from exercise studies, the relationship between elevated cortisol and decreased lymphocyte proliferation is not consistent (28, 29). In the present data, the temporal relationship between cortisol elevations and depressed lymphocyte proliferation (expressed per lymphocyte and CD3+ cell) suggests that a cortisol-

### Table 4. Lymphocyte subsets

<table>
<thead>
<tr>
<th></th>
<th>Pre-Dep</th>
<th>Pre-Ex</th>
<th>Post-Ex</th>
<th>2 h Post-Ex</th>
<th>24 h Post-Ex</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CD3+, ×10^9/l</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HiCHO</td>
<td>1.47 ± 0.17</td>
<td>1.36 ± 0.13</td>
<td>1.70 ± 0.15&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>1.13 ± 0.10&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>1.19 ± 0.10&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>LoCHO</td>
<td>1.58 ± 0.17</td>
<td>1.45 ± 0.13</td>
<td>2.15 ± 0.10&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>0.91 ± 0.09&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>1.23 ± 0.10&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>CD4+, ×10^9/l</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HiCHO</td>
<td>0.86 ± 0.11</td>
<td>0.79 ± 0.09</td>
<td>0.83 ± 0.08</td>
<td>0.65 ± 0.07&lt;sup&gt;a&lt;/sup&gt;&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.68 ± 0.07&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>LoCHO</td>
<td>0.92 ± 0.11</td>
<td>0.82 ± 0.09</td>
<td>0.98 ± 0.08&lt;sup&gt;a&lt;/sup&gt;&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.56 ± 0.06&lt;sup&gt;a&lt;/sup&gt;&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.71 ± 0.07&lt;sup&gt;a&lt;/sup&gt;&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>CD8+, ×10^9/l</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HiCHO</td>
<td>0.51 ± 0.06</td>
<td>0.48 ± 0.05</td>
<td>0.64 ± 0.06&lt;sup&gt;a&lt;/sup&gt;&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.41 ± 0.03</td>
<td>0.42 ± 0.03</td>
</tr>
<tr>
<td>LoCHO</td>
<td>0.55 ± 0.06</td>
<td>0.52 ± 0.05</td>
<td>0.88 ± 0.05&lt;sup&gt;a&lt;/sup&gt;&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.31 ± 0.04&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>0.42 ± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>CD4+/CD8+</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HiCHO</td>
<td>1.69 ± 0.06</td>
<td>1.62 ± 0.05</td>
<td>1.34 ± 0.06&lt;sup&gt;a&lt;/sup&gt;&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.60 ± 0.03</td>
<td>1.59 ± 0.03</td>
</tr>
<tr>
<td>LoCHO</td>
<td>1.69 ± 0.06</td>
<td>1.58 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.13 ± 0.05&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>1.79 ± 0.04&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>1.69 ± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>CD20+, ×10^9/l</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HiCHO</td>
<td>0.22 ± 0.03</td>
<td>0.25 ± 0.03</td>
<td>0.29 ± 0.02&lt;sup&gt;a&lt;/sup&gt;&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.23 ± 0.02</td>
<td>0.22 ± 0.02</td>
</tr>
<tr>
<td>LoCHO</td>
<td>0.27 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.27 ± 0.03</td>
<td>0.34 ± 0.02&lt;sup&gt;a&lt;/sup&gt;&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.19 ± 0.02&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>0.22 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>CD56+, ×10^9/l</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HiCHO</td>
<td>0.26 ± 0.03</td>
<td>0.24 ± 0.04</td>
<td>0.70 ± 0.10&lt;sup&gt;a&lt;/sup&gt;&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.17 ± 0.21</td>
<td>0.23 ± 0.04</td>
</tr>
<tr>
<td>LoCHO</td>
<td>0.24 ± 0.03</td>
<td>0.27 ± 0.05</td>
<td>1.18 ± 0.17&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>0.16 ± 0.03</td>
<td>0.21 ± 0.03</td>
</tr>
</tbody>
</table>

Values are means ± SE. *Significant difference from HiCHO condition. Letters indicate significant time effects, P < 0.05. <sup>a</sup>Different from predepletion; <sup>b</sup>different from preexercise.

---

**Fig. 8. CD25+ cells.** □, High-carbohydrate condition (HiCHO); ▶, low-carbohydrate condition (LoCHO). *Main effect for condition. <sup>a</sup>Significant main effect for time, with preexercise and 2-h postexercise values different from all others.
induced suppression may have occurred; however, correlations between these two variables were not significant. Therefore, despite the significant condition main effect for cortisol, it is not possible to draw definitive conclusions regarding the effects of the glucose counter-regulatory hormone responses on functional immune responses.

An additional issue that should be considered is the potential influence of dietary fat on immune responses. In the LoCHO condition, >200 g of fat were consumed, which amounted to ~60% of the caloric consumption. A high-fat diet can have inhibitory effects on the immune system, as shown by impaired lymphocyte proliferation and other functional responses (3, 25, 36). Although the majority of these effects have been produced with chronic consumption of a high-fat diet, potential effects of the 48 h of high-fat intake in the present study should not be overlooked.

Cell number responses. The pattern of change in total leukocytes, neutrophils, lymphocytes, and lymphocyte subsets in both the HiCHO and LoCHO conditions is similar to that reported in previous investigations (11, 12, 16, 28, 29). Exercise has been shown to produce significant leukocytosis, neutrophilia, lymphocytosis, and elevations in lymphocyte subsets. The leukocytosis and neutrophilia may be sustained for a few hours of recovery; however, the lymphocytosis and elevations in lymphocyte subsets are typically followed by a significant depression during recovery (11, 29). It has been suggested that the changes in cell numbers are caused by the exercise-induced elevation in stress hormones and/or hemodynamic factors, both of which are intensity-dependent (7, 11, 15, 29, 41). In the present investigation, the two bouts of exercise were identical in intensity. Thus, the greater cell number shifts in the LoCHO condition may have been caused by the hormonally induced indirect influence of differences in energy substrate availability. The presence of moderate correlations between cortisol and leukocyte and neutrophil numbers at the postexercise time point supports this conclusion. The clinical significance of the alterations in cell number in general, and the more exaggerated responses in the LoCHO condition, in particular, is uncertain.

In conclusion, although the diet and exercise regimen altered indicators of carbohydrate status during the 60 min of exercise, lymphocyte proliferation responses in the LoCHO condition were not different from those in the HiCHO condition. Thus, moderate differences in substrate availability did not explain exercise-induced changes in lymphocyte function. The significant differences between conditions in the number of circulating leukocytes, neutrophils, lymphocytes, and lymphocyte subsets which were observed may be explained by greater responses of stress hormones that were the result of the dietary manipulation. The clinical implications of the more pronounced numerical shifts in the LoCHO condition are not known. However, if possible, from a practical perspective, dietary CHO intake should be such that the more exaggerated responses are avoided.

This study was supported by the TCU Research and Creative Activities Fund.

Address for reprint requests: J. B. Mitchell, PO Box 297730, Texas Christian Univ., Fort Worth, TX 76129 (E-mail: J.Mitchell@TCU.EDU).

Received 16 June 1997; accepted in final form 9 March 1998.

REFERENCES