Indomethacin does not influence natural cell-mediated cytotoxic response to endurance exercise

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Indomethacin does not influence natural cell-mediated cytotoxic response to endurance exercise

W. A. BRAUN,1 M. G. FLYNN,2 D. E. JACKS,3 T. MCLoughlin,3 J. Sowash,3 C. P. LAMBERT,3 AND E. MYLona3

1Human Performance Laboratory, California State Polytechnic University, Pomona, California 91768; 2Wasti Human Performance Laboratory, Purdue University, West Lafayette, Indiana 47907; and 3Exercise Physiology Laboratory, University of Toledo, Toledo, Ohio 43606

Braun, W.A., M. G. Flynn, D. E. Jacks, T. McLoughlin, J. Sowash, C. P. Lambert, and E. Mylona. Indomethacin does not influence natural cell-mediated cytotoxic response to endurance exercise. J. Appl. Physiol. 87(6): 2237–2243, 1999.—Natural cell-mediated cytotoxicity (NCMC) has been shown to be attenuated during recovery from high-intensity or prolonged exercise. Two theories have been proposed to explain the transient suppression of NCMC: prostaglandin-induced inhibition of natural killer (NK) cell activity or a numerical redistribution of NK cells. This study was designed to examine the effects of oral indomethacin (a prostaglandin inhibitor) on NCMC before and after 1 h of high-intensity running (85% maximal oxygen uptake). A secondary purpose was to compare whole blood and isolated peripheral blood mononuclear cell assay procedures for assessing NCMC. Ten male distance runners completed two trials that were preceded by either 48 h of indomethacin (Indo; 150 mg/day) or no treatment (control). NK (CD3+/CD16+/CD56−) cell concentrations were significantly elevated postexercise but were not affected by Indo. NCMC was significantly suppressed at 1.5 h of recovery relative to preexercise only with the whole blood assay procedure. Indo was not found to influence NCMC, leukocyte, or lymphocyte subset concentrations. Mean cytotoxic response was significantly greater with the whole blood method.

EXERCISE HAS THE POTENTIAL to influence host defense by altering the ability of the immune system to defend against infectious agents. A single bout of intense or prolonged exercise has been shown to result in suppression of natural cell-mediated cytotoxicity (NCMC) during recovery from the exercise (3, 24, 25, 30). Considerable attention has been given to the postexercise suppression of NCMC because it may play a role in the increased incidence of infections reported by athletes during periods of hard training (11, 18, 21, 23, 27). Although the postexercise suppression of NCMC has been well documented, identification of the mechanisms responsible has been elusive. Proposed mechanisms that have received the most attention are prostaglandin-induced inhibition of natural killer (NK) cell function (6, 26, 30) or the numerical redistribution of NK cells during recovery from exercise (15, 29).

Pedersen et al. (26) and Tvede et al. (30) have suggested that suppression of NCMC may be related to an increase in prostaglandin synthesis by neutrophils and monocytes. It has been proposed that prostaglandins downregulate the activity of NK cells. Furthermore, in vivo and in vitro administration of indomethacin, a prostaglandin inhibitor, have been associated with a maintenance of NCMC during recovery from exercise (12, 24–26, 30). In contrast, Nieman et al. (15) reported that preincubation of isolated mononuclear cells with indomethacin had no effect on the suppression of NCMC during recovery from prolonged endurance exercise.

A reduction in the venous concentration of NK cells has been consistently observed during recovery from high-intensity and prolonged exercise (12, 15, 17, 20, 26, 29). As a result, it has been suggested that the suppression of NCMC may be due to the reduced number of NK cells in the circulation during recovery from exercise (15, 29). Furthermore, when NCMC has been expressed per NK cell, suppression during recovery from exercise has not been observed (15, 17, 19). Therefore, postexercise NCMC suppression may reflect a change in NK cell concentration rather than impaired function of individual NK cells.

There are some important methodological differences in the studies conducted to date. In addition, investigations in which isolated mononuclear cell assays have been used to examine the influence of oral or in vitro indomethacin may have some important limitations. In the isolated, peripheral blood mononuclear cell (PBMC) assay, cells are washed and, as a result, are not incubated with other immune cells and with endocrine and other factors that were present when the blood was obtained. During the isolation procedure, a selective loss of CD8+ cells has been reported (8, 14, 29). Furthermore, use of such an assay method may not allow for an effective assessment of an oral dose of indomethacin because of the repeated washing of the cells. Therefore, a whole blood assay may provide a better means of assessing the influence of oral indomethacin on the NCMC response to exercise.

The whole blood method is being used more frequently for the assessment of NCMC responses to exercise. It is possible that the whole blood assay more closely represents the in vivo environment than does the isolation method and that the two methods may result in differences in cytotoxic response. Therefore, the purpose of the present investigation was to study the effects of oral indomethacin on the NCMC...
responses to 1 h of high-intensity running by using both an isolated mononuclear cell assay and a whole blood assay. A secondary purpose of the investigation was to determine whether cytotoxic responses would differ between assay methods. Through this design we sought to better elucidate mechanisms involved in the postexercise suppression of NCMC.

METHODS

Subjects

Ten well-trained male distance runners volunteered to participate in this study. Written consent was obtained by using a university-approved form, and each subject completed a medical history questionnaire. Subjects had an average of 14 yr of running experience and reported an average training volume of 74 km/wk.

Preliminary Testing

Before experimental trials, all subjects performed a maximal oxygen uptake ($\dot{V}O_{2\text{max}}$) test with 12-lead electrocardiogram by using a graded treadmill protocol. Initial treadmill velocity was set at a pace 53.3 m/min slower than the subject’s self-reported 10-km race pace. Treadmill velocity was increased by 26.6 m/min every 3 min until the velocity was 26.6 m/min faster than the self-reported 10-km race pace. From this point, treadmill grade was increased by 2% every 2 min until the subject reached the point of volitional fatigue.

Expired gases were sampled continuously from a mixing chamber (0.67 l/min). Concentrations of expired oxygen and carbon dioxide were determined by using calibrated Applied Electrochemistry SA-3 (Applied Electrochemistry, Sunnyvale, CA) and CD-3A (Ametek, Thermomax Instruments Division, Pittsburgh, PA) analyzers, respectively. The volume of inspired air was measured by using a Rayfield airflow meter (Rayfield Equipment, Waitsfield, VT). An automated gas-analysis system (Rayfield Equipment) was used for determining $\dot{V}O_{2\text{max}}$, ventilation, and respiratory exchange ratio.

Experimental Testing

Subjects completed two 1-h treadmill runs at velocities calculated to elicit 80% of $\dot{V}O_{2\text{max}}$. One trial was preceded by 48 h of oral indomethacin dosing (50 mg, 3 x daily; 150 mg/day). This dosage was selected to match dosages reported by earlier investigators (24). Trials were separated by 2 wk with order of treatment [indomethacin or no treatment (control)] randomized and counterbalanced. For the indomethacin condition, the final dose was taken 1 h before the start of the experimental trial.

Subjects reported to the laboratory between 0600 and 0730 h, with trial start time held constant within subjects. Each 1-h run was preceded by a 5-min warm-up with incremental velocity increases until the desired pace was achieved. Expired gases were collected into Douglas bags at 4, 20, 40, and 59 min of each run. Gases were analyzed as described in Preliminary Testing, and gas volume was determined using a Tissot spiro meter (Warren E. Collins, Braintree, MA). Rating of perceived exertion (6-20 scale) (4) and heart rate by radio telemetry (Polar Favor, Polar Electro, Port Washington, NY) were obtained and recorded at the same time points. Subjects were permitted to drink water ad libitum during the course of each run. The mean environmental conditions in the laboratory were 22°C temperature and 63% relative humidity.

Blood Collection and Analysis

All blood samples were drawn from an antecubital vein with the subject in a seated position. Samples were obtained after 15 min of rest before the run (Pre), immediately after completion of the run (Post), and after 1.5 h of recovery (1.5 Post). Blood samples (30 ml) were collected into evacuated tubes for determination of NNMC (PBMC isolation and whole blood assays: lithium heparin tubes), complete blood count (K-EDTA tubes), and mononuclear cell populations (sodium citrate-citric acid tubes).

Mononuclear Cell Population

Mononuclear cell populations were determined by direct immunofluorescence [fluorescence-activated cell sorter (FACS), Becton-Dickinson, San Jose, CA] by using the whole blood lysis technique. Whole blood aliquots were incubated for 15 min at room temperature with monoclonal antibodies conjugated with phycoerythrin and fluorescein isothiocyanate (Becton-Dickinson). FACS lysing solution was added, and the solution incubated for 15 min before centrifugation (30 s at 400 g). Supernatant was removed, and the cells were washed before being fixed with 2% paraformaldehyde. Mononuclear cell populations were not determined for isolated PBMCs.

NCMC

Isolation assay. PBMCs were isolated from 15 ml of heparinized whole blood by density gradient centrifugation with Histopaque (Sigma Chemical, St. Louis, MO). Isolated cells were incubated for 5 min with ammonium chloride to remove erythrocytes and then washed with RPMI before resuspension in cell medium at a concentration of $5 \times 10^6$ cells/ml. Cell culture medium (RPMI 1640) was supplemented with 15% fetal calf serum (Sigma Diagnostics, St. Louis, MO) and contained 2 ml penicillin-streptomycin, 0.5 ml amphotericin B, 1 ml 100 mM sodium pyruvate, and 1 ml 100 mM nonessential amino acid per 100 ml of medium. Isolated cell suspension (100 µl) was added to each of 12 microplate wells and 100 µl of $^{51}$Cr-labeled K562 human erythroleukemic cells (American Type Culture, Rockville, MD) were added to wells in triplicate at concentrations of 2 x 10^5, 1 x 10^5, 0.5 x 10^5, and 0.25 x 10^5 cells/ml to yield effector-to-target ratios of 2.5:1, 5:1, 10:1, and 20:1. Spontaneous and total lysis were determined at the four target concentrations by substituting 100 µl of RPMI and 100 µl of 1% Triton X-100 for effector cells, respectively. Microplates were then incubated for 4 hr at 37°C and 5% CO_2. After incubation, 100 µl of chilled medium were added to each well and the plates were centrifuged (10 min at 400 g). Supernatants (100 µl) were transferred to glass test tubes and counted for 5 min on a gamma radiation counter (GammaTrac 1191, TM Analytic). NCMC was calculated as

$$%\text{LYSIS} = \frac{[(\text{cpm}_{\text{test}} - \text{cpm}_{\text{spontaneous}})/(\text{cpm}_{\text{total}} - \text{cpm}_{\text{spontaneous}})] \times 100}$$

where $\text{cpm}_{\text{total}}$, $\text{cpm}_{\text{spontaneous}}$, and $\text{cpm}_{\text{test}}$ are counts per minute of test, spontaneous, and total lysis, respectively.

Whole blood assay. Whole blood aliquots (150 µl) were added to each of 12 microplate wells, and 50 µl of $^{51}$Cr-labeled K562 cells (4 concentrations) were added to the whole blood aliquots in triplicate as described above. Spontaneous and total lysis were determined at the four target concentrations by substituting 150 µl of RPMI and 150 µl of 1% Triton X-100 for effector cells, respectively. Before incubation, effector-
target suspensions were centrifuged (10 min at 120 g). Cell suspensions were incubated for 4 h at 37°C and 5% CO₂, after incubation, 100 µl of chilled medium were added to each well and the plates were centrifuged (10 min @ 400 g). Cell-free supernatants (100 µl) were removed and counted as described above. NCMC was calculated as percent cytotoxicity as described by Fletcher et al. (9).

\[
\text{NMCW} = \left( \frac{\text{Vtot} - \left( \frac{\text{Vb} \times \text{Hct}}{\text{Vtot}} \right)}{\text{TR}} \right) \times 100
\]

where ER is mean cpm of experimental release, SR is mean cpm for spontaneous release, TR is mean cpm for total release, Vtot is total volume/well, Vb is volume of blood/well, Hct is sample hematocrit, and B is background cpm for the gamma counter.

NCMC was also determined as percent cytotoxicity per NK cell (CD3⁻ /CD16⁻/CD56⁻) as described by Baron et al. (1). Expression of cytotoxicity per NK cell enabled the determination of a 1:1 (effector-to-target) ratio through kinetic analysis.

Statistical Analyses

Data were analyzed by using three-way (treatment × method × time) and two-way (method × time) repeated-measures analysis of variance. Independent variables included treatment (indomethacin or control), assay method (whole blood or isolation assay) and time (pre-, post-, or 1.5 postexercise). Dependent variables included percentage and number of lymphocytes, neutrophils, T cells, B cells, NK cells, T cell subsets, and NCMC (%lysis and 1:1 effector-to-target ratio). Cytotoxicity (%lysis) was analyzed by using a three-way analysis of variance (treatment × method × time). Two-way analysis of variance (treatment × time) was employed to analyze all other dependent variables. One-way analysis of variance was used to compare mean values for heart rate, percentage of VO₂max, and rating of perceived exertion between treatment conditions (control and indomethacin trials). When significant differences were detected (P < 0.05), a Tukey post hoc analysis was employed to locate the specific mean differences.

RESULTS

Subject characteristics of the 10 well-trained male distance runners are summarized in Table 1. Within the previous year, the average subject had completed a 10-km race in 37.2 min and was training 74.5 km/wk. Exercise trials (60 min) were run at an average intensity of 85.5% of VO₂max and 13.4 km/h. There were no differences between exercise trials based on treatment

Table 1. Subject characteristics and descriptive data

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Means ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>36.0 ± 7.50</td>
</tr>
<tr>
<td>Height, cm</td>
<td>68.6 ± 6.36</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>69.9 ± 8.87</td>
</tr>
<tr>
<td>VO₂max, ml·kg⁻¹·min⁻¹</td>
<td>57.4 ± 6.58</td>
</tr>
<tr>
<td>HRmax, beats/min</td>
<td>182.0 ± 6.43</td>
</tr>
<tr>
<td>Running distance, km/wk</td>
<td>74.5 ± 12.53</td>
</tr>
<tr>
<td>Running experience, yr</td>
<td>14.3 ± 2.40</td>
</tr>
<tr>
<td>10-km personal best, min</td>
<td>37.21 ± 1.64</td>
</tr>
</tbody>
</table>

Values are for 10 subjects. VO₂max: maximal O₂ uptake; HRmax: maximal heart rate.

Table 2. Exercisetrial results (control vs. indomethacin)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>Indomethacin</th>
</tr>
</thead>
<tbody>
<tr>
<td>VO₂max, %VO₂max</td>
<td>85.7 ± 1.70</td>
<td>85.3 ± 1.10</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>161.8 ± 2.62</td>
<td>163.3 ± 2.38</td>
</tr>
<tr>
<td>RPE</td>
<td>13.24 ± 0.46</td>
<td>13.31 ± 0.30</td>
</tr>
</tbody>
</table>

Values are means ± SE. Values reflect mean data for measurements made after 4, 20, 40 and 59 min of running at a workload calculated to elicit 80% VO₂max. VO₂, O₂ consumption; RPE, rating of perceived exertion (control and indomethacin) for running intensity (% VO₂max), heart rate, or rating of perceived exertion by using the Borg 6–20 scale (4) (Table 2).

Leukocyte number and leukocyte subsets were not different between treatments at any time points. However, significant grand mean time effects were present for leukocyte, neutrophil, monocyte, and lymphocyte numbers (Table 3). B-cell concentration was unaffected by time and treatment. T-lymphocyte concentration was significantly elevated at Post relative to Pre and 1.5 Post measurements, but there were no differences on the basis of treatment (Table 3). NK cell number was significantly elevated at Post relative to Pre and 1.5 Post measurements but was not different on the basis of treatment (Table 3).

Significant time effects were present for T-cell subsets, but no treatment effects were observed. CD3⁻/CD4⁺ cell number was significantly reduced at 1.5 Post relative to Pre and Post time points (Table 4). In addition, CD3⁻/CD8⁺ cell numbers were significantly elevated at Post in comparison to Pre and 1.5 Post

Table 3. Leukocyte and leukocyte subset concentrations in whole blood before and after 1 h of treadmill exercise at 85% VO₂max

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pre</th>
<th>Post</th>
<th>1.5 Post</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukocytes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Con</td>
<td>5.60 ± 0.57⁺⁺</td>
<td>8.25 ± 0.88⁺⁺</td>
<td>7.79 ± 0.75⁺⁺</td>
</tr>
<tr>
<td>Indo</td>
<td>5.22 ± 0.50</td>
<td>8.58 ± 0.55</td>
<td>8.01 ± 0.85</td>
</tr>
<tr>
<td>Neutrophils</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Con</td>
<td>3.24 ± 0.51⁺⁺</td>
<td>4.59 ± 0.78⁺⁺</td>
<td>6.15 ± 0.80⁺⁺</td>
</tr>
<tr>
<td>Indo</td>
<td>3.16 ± 0.44</td>
<td>5.10 ± 0.60</td>
<td>6.33 ± 0.80</td>
</tr>
<tr>
<td>Monocytes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Con</td>
<td>0.31 ± 0.03*</td>
<td>0.31 ± 0.04</td>
<td>0.36 ± 0.03*</td>
</tr>
<tr>
<td>Indo</td>
<td>0.30 ± 0.02</td>
<td>0.38 ± 0.04</td>
<td>0.43 ± 0.02</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Con</td>
<td>1.83 ± 0.27⁺⁺</td>
<td>2.95 ± 0.41⁺⁺</td>
<td>1.26 ± 0.15⁺⁺</td>
</tr>
<tr>
<td>Indo</td>
<td>1.66 ± 0.13</td>
<td>2.75 ± 0.25</td>
<td>1.11 ± 0.13</td>
</tr>
<tr>
<td>B lymphocytes (CD19⁺)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Con</td>
<td>0.19 ± 0.03</td>
<td>0.19 ± 0.03*</td>
<td>0.16 ± 0.03*</td>
</tr>
<tr>
<td>Indo</td>
<td>0.19 ± 0.03</td>
<td>0.23 ± 0.03</td>
<td>0.18 ± 0.03</td>
</tr>
<tr>
<td>T lymphocytes (CD3⁺)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Con</td>
<td>1.38 ± 0.12⁺⁺</td>
<td>1.90 ± 0.29⁺⁺</td>
<td>0.95 ± 0.10⁺⁺</td>
</tr>
<tr>
<td>Indo</td>
<td>1.15 ± 0.10</td>
<td>1.87 ± 0.21</td>
<td>0.84 ± 0.11</td>
</tr>
</tbody>
</table>

Values are means ± SE given in ×10⁶ cells/l. Con, control (no treatment); Indo, indomethacin (150 mg/day for 2 days preceding trial). Pre, after 15 min of rest before the first run; Post, immediately after completion of the run; 1.5 Post, after 1.5 h of recovery. Like symbols denote significant time effect differences between the grand means of the indicated time points (P < 0.05).
(Table 4). The T helper-to-T suppressor (CD4+/CD8+) ratio was significantly lower at Post relative to Pre and 1.5 Post measurements (Table 4).

NCMC

Whole blood method. Analysis of NCMC in whole blood, measured as mean percent lysis as calculated from the formula described by Fletcher et al. (9) revealed no significant treatment (P = 0.823) or interaction (P = 0.335) effects (Fig. 1). However, a significant time effect (P < 0.001) was present such that Post (39.49 ± 2.26%) was greater than Pre (14.04 ± 2.07%) and 1.5 Post (7.14 ± 1.00%), and 1.5 Post was significantly lower than Pre. Data have been expressed as the means for the four target concentrations.

Similarly, when whole blood cytotoxicity was expressed per NK cell (1:1 effector-to-target ratio) as described by Baron et al. (1), no significant treatment (P = 0.917) or interaction (P = 0.798) effects were present (Fig. 2). A significant time effect (P < 0.001) was observed with Post (38.68 ± 2.89%) being greater than Pre (16.53 ± 2.08%) and 1.5 Post (11.31 ± 1.39%).

Isolation method. No significant treatment (P = 0.498) or interaction (P = 0.270) effects were found when percent cytotoxicity was determined by using the isolation assay method (Fig. 3). A significant time effect (P < 0.001) revealed that Post (22.24 ± 2.76%) was significantly greater than Pre (8.06 ± 1.39%) and 1.5 Post (5.86 ± 1.06%). Data are expressed as the means of the four target concentrations.

Whole blood vs. isolation method. A comparison of assay method (whole blood vs. isolation assay) revealed
significant method \( (P < 0.001) \) and time by method \( (P < 0.001) \) interaction effects for percent cytotoxicity (Fig. 4), but there were not significant method by treatment \( (P = 0.435) \) or time by method by treatment \( (P = 0.121) \) interaction effects present. The method interaction revealed that grand mean cytotoxicity values were significantly higher for the whole blood assay \((20.22 \pm 1.78\%) \) than the isolation assay \((12.12 \pm 1.73\%) \). The time by method interaction revealed that cytotoxicity \( (\% \text{lysis}) \) was greater for the whole blood assay at Pre \((14.04 \pm 2.07\% \text{ vs. } 8.06 \pm 1.39\% \) and Post \((39.49 \pm 2.26\% \text{ vs. } 22.24 \pm 2.76\% \) but not at 1.5 Post \((7.14 \pm 1.00\% \text{ vs. } 5.86 \pm 1.06\% \).}

**DISCUSSION**

The primary purpose of this investigation was to examine the effect of oral indomethacin, an inhibitor of prostaglandin synthesis, on the NCMC response to high-intensity endurance exercise by using both whole blood and isolation assay methods. Prostaglandin-induced inhibition of normal NK cell function has been proposed to be a causative factor in the transient suppression of NCMC during recovery from vigorous exercise (6, 25, 26). A secondary purpose of this investigation was to compare cytotoxic responses between the two common assay techniques.

Earlier investigators have reported enhanced NCMC during recovery from endurance exercise in response to in vivo or in vitro indomethacin administration (12, 25, 30). The data obtained in the present study fail to corroborate the findings of these investigators. Independent of the assay method used, 48 h of oral indomethacin were not found to enhance NCMC before or in response to 1 h of high-intensity treadmill running. It is important to note that this is the first study in which the effects of indomethacin dosing on NCMC have been examined by use of a whole blood procedure.

Pedersen et al. (25) reported improved NK activity during recovery from cycle ergometry when preceded by 48 h of oral indomethacin in comparison to a control condition. NK activity was significantly suppressed during recovery only with the control condition. In the same investigation, in vitro administration of indomethacin \((1 \mu g/ml) \) with blood mononuclear cells resulted in significantly greater cytotoxicity at all time points compared with control measurements (25). Other investigators have also shown elevated NK activity in response to in vitro indomethacin administration (12, 24, 30).

In contrast, Nieman et al. (15) did not report significant improvements in NK activity during recovery from 2.5 h of treadmill running when indomethacin was administered in vitro. Similarly, Baslund et al. (2) did not observe an effect of in vitro indomethacin on resting NK activity before and after 4 and 8 wk of an exercise training program. As a result, the link between prostaglandins and postexercise suppression of NCMC remains controversial.

It has been proposed that the suppression of NCMC during recovery from exercise may be related to a reduced number of NK cells in the circulation (15, 29). Significant reductions in NK cell concentration have been reported during recovery from moderate- and high-intensity endurance exercise (15, 16, 19, 29) and in response to exhaustive resistance exercise (17). We observed a 50% reduction in NK cell number during recovery in comparison to preexercise values. In conjunction with this large reduction in NK cell concentration, whole blood NCMC (expressed as percent cytotoxicity) was significantly lower during recovery relative to preexercise. Alterations in NK cell number were not affected by oral indomethacin treatment (Table 3).
In the present study, NCMC was found to be significantly lower during recovery relative to preexercise measurements only when expressed as percent cytotoxicity for the whole blood method (Fig. 1). At 1.5 h of recovery, NCMC had fallen to 51% of the preexercise measure. When whole blood NCMC was expressed as a 1:1 (effector-to-target) ratio, the recovery NCMC (1.5 Post) was 68% of Pre and was not found to be significantly reduced (Fig. 2). This finding lends support to the concept that suppression of NCMC may be closely related to a numerical redistribution of NK cells. At 1.5 Post, NK cell concentration was 51% of Pre; however, this did not represent a statistically significant reduction.

Nieman et al. (19) have also observed differences when cytotoxic activity was expressed per NK cell vs. unadjusted lysis. Using isolated peripheral blood mononuclear cells, these investigators found NK cell cytotoxic activity to be significantly higher than resting baseline measures after 2 h of recovery from high-intensity running when lytic units were expressed per NK cell (19). Shinkai et al. (29) reported a strong relationship between CD16+ cell numbers and NK activity in response to exercise and during recovery from exercise. They indicated that the exercise-induced change in NK activity could be explained by alterations in the concentration of CD16+ cells, without any need to implicate downregulation of NK cell function (29).

In the present investigation, during recovery, NCMC was 28% lower than preexercise values when determined by using the isolated PBMC assay method. However, this did not represent a statistically significant reduction in cytotoxicity. Many investigators have shown significant reductions in NCMC during recovery from moderate- and high-intensity endurance exercise (3, 5, 12, 24, 25, 29, 30) compared with preexercise measurements. Other investigators have found NCMC to return to baseline values during recovery from exercise (7, 8). Reasons for differences in these findings are not evident, but they may be related to a variety of factors, including mode, duration, and intensity of exercise and timing of blood sampling during recovery. Times of recovery measures have varied from 30 min postexercise (13, 29) to 24 h postexercise (28), although the most commonly reported times have been within 1–2 h of recovery from exercise (12, 19, 24, 30).

Whole blood vs. isolation method. The whole blood method was found to result in significantly greater cytotoxic activity than the isolation method when expressed as percent lysis. Before the investigation, laboratory data were analyzed to establish approximate effector-to-target ratios for whole blood samples. Comparable effector-to-target ratios were then calculated for use in the isolation procedure to yield ratios that would approximate those anticipated for the whole blood method. Despite these efforts, considerable limitations are evident in drawing conclusions about the effectiveness of one method over the other.

A strict comparison of the whole blood method and the isolation method was not possible because of the inability to establish equivalent effector-to-target ratios for the two methods. For example, while the number of targets may be held constant in both methods, the number of effectors could be controlled only with the isolated PBMC procedure. In the whole blood method, the volume of blood is held constant, but the distribution of cells within this volume may be quite variable based on external factors. As a result, the number of effectors added to each well could not be controlled by using the whole blood method.

Despite the limitations in drawing comparisons between the two methods for assessing NCMC, a number of factors appear to favor the whole blood procedure. For example, during the process of isolating the PBMCs, selective losses of CD8+ cells have been reported (8, 14, 29). Although we were unable to make these assessments both in whole blood and in the isolated PBMCs because of the expense, it is possible that a selective loss of CD8+ cells may explain the differences in the recovery NCMC between the two assay methods. That is, a reduced concentration of CD8+ cells in the isolated PBMC assay may have been instrumental in this procedure, showing a nonsignificant reduction in NCMC at 1.5 Post. Furthermore, the loss of these cells along with other factors present in the whole blood would modify the milieu of the effector-to-target environment. The repeated washing of the lymphocytes that occurs with the isolation procedure may result in losses of soluble, endocrine, and other factors that may affect the normal function of the effector cells and their interaction with the targets. In addition, the isolation method results in a lengthy delay before the effector cells are introduced to the target population. These issues require further attention to better understand the effects of the methodological differences in the determination of NCMC activity.

In summary, 48 h of oral indomethacin dosing were not found to affect NCMC before or after 1 h of high-intensity running exercise. Furthermore, indomethacin was found to have no effect on NCMC when determined by the whole blood or isolated PBMC assay methods. Finally, the whole blood method was found to result in significantly greater cytotoxicity than the isolation method. This last comparison requires further study to determine whether one method provides a better representation of the in vivo environment over the other.

Address for reprint requests and other correspondence: W. A. Braun, California State Polytechnic Univ., Dept. of Kinesiology and Health Promotion, Pomona, CA 91768 (E-mail: wabraun@csupomona.edu).

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