Sex-based effects on the distribution of NK cell subsets in response to exercise and carbohydrate intake in adolescents

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Sex-based effects on the distribution of NK cell subsets in response to exercise and carbohydrate intake in adolescents. J Appl Physiol 100: 1513–1519, 2006. First published January 12, 2006; doi:10.1152/japplphysiol.01125.2005.—Carbohydrate (CHO) supplementation and female sex independently influence the natural killer (NK) cell response to acute exercise. Consequently, this study sought to elucidate sex-based differences in the distribution of NK cell subsets (i.e., CD56dim and CD56bright) in response to exercise and CHO intake. Twenty-two healthy 14-yr-old girls (n = 11) and boys (n = 11) cycled for 60 min at 70% maximal oxygen consumption while drinking 6% CHO (CT) or flavored water (WT). Blood was collected at rest, during exercise (30 and 60 min), and during recovery (30 and 60 min) to identify CD3^+CD56^dim and CD3^+CD56^bright NK cells. The activation marker CD69 was also determined on CD3^-CD56^- cells. CD56dim responses, expressed as proportions or cell counts, were greater (P ≤ 0.01) in girls by 67 and 105%, respectively. CD56bright cell counts (P = 0.006), but not CD56bright proportions (P = 0.89), were greater in girls by 82%. Both CD56dim and CD56bright subset responses, expressed as proportions or cell counts, were lower (P ≤ 0.01) in CT vs. WT by 33–36%. The CD56bright-to-CD56dim ratio decreased at 30 min of exercise but increased during recovery (P < 0.001), with no effect of sex or CHO. Regardless of trial, CD3^-CD56^- cells expressed ~18% higher levels of CD69 during recovery in girls but not boys (P = 0.03), despite similar proportions and counts of CD69^- cells. These results demonstrate sex-based differences in the distribution of NK cell subsets and activation status in response to exercise, but not CHO intake, and further support the need to control for sex in exercise immunology studies.

A VARIETY OF PHYSIOLOGICAL RESPONSES to exercise are influenced by sex. The presence of, and fluctuations in, sex hormones appear to be important in regulating substrate utilization (43), muscle fatigue (18), temperature regulation (22), and endocrine responses (12) during exercise in humans. In addition, we (47, 49) and others (13) have reported sex-based differences in immunological, in particular lymphocyte, responses to endurance exercise. We have also shown that natural killer (NK) cells, which are the most responsive cell type due to their catecholamine sensitivity (36), display a significant sexual bias in their response to strenuous exercise among adolescents, with females experiencing a greater response (49). Given their potent cytotoxicity (11) and innate roles in antiviral (5) and anticancer (9) defenses, the sensitivity of NK cells to physiological stress and the influence of female sex on this response are of particular interest.

In both adults (33) and young children (48), exercise-induced increases in NK cell counts can be attenuated by carbohydrate (CHO) intake. Whether a nutritional blunting of NK cells in response to exercise is of significant health relevance is as yet unclear, but to date research into CHO effects on NK cell responses to exercise has been almost exclusively to adult men. Nieman et al. (35) did report lower postexercise NK cell counts in a CHO vs. water trial in female rowers. To our knowledge, however, no study has systematically investigated possible sex-based differences in NK cell responses to exercise with and without CHO intake under controlled experimental conditions. It has been proposed that CHO-mediated effects on NK cell redistribution are due to a blunted stress hormone (e.g., epinephrine) response mediated by maintained or elevated blood glucose levels (33). In this regard, it is noteworthy that epinephrine responses to endurance exercise are generally lower in females vs. males (10, 12, 20), suggesting that females may be less sensitive to the immune effects of CHO intake compared with males. Given the importance of NK cells in health and disease and the possibility of a sexual bias in the effect of CHO on NK cell responses to exercise, we were interested in providing further clarification on this issue.

Another shortcoming in descriptions of the NK cell response to exercise has been the phenotyping of this cell population. Traditionally, characterization of NK cells has been based on the coexpression of the cell surface markers CD16 and CD56 and lack of expression of the T-cell marker CD3 (11). Based on the intensity of CD56 expression, however, there exists two unique and functionally distinct NK cell populations (25) (Fig. 1). The CD56dim population constitutes ~90% of peripheral NK cells and is the more cytotoxic subset, whereas CD56bright cells have the capacity to produce abundant cytokines (11). Only a few investigations have reported CD56bright subset responsiveness to exercise (16, 19), but these studies did not distinguish between CD16 and CD56 expression (19), were delayed in collecting postexercise samples (16), and did not include females. The distribution of CD56dim and CD56bright subsets and factors that affect their relationship are of importance in aging (8) and some disease conditions (21). This ratio may also be of particular interest in females because the CD56bright-to-CD56dim ratio is positively associated with successful reproduction (23). To our knowledge, however, no study has determined the effects of acute exercise with or without energy supplementation on the distribution of NK cell subsets in females.

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In a previous publication (49) of the same adolescent cohort described herein, we reported a greater NK cell (i.e., CD3−CD16+CD56−) response to exercise without CHO intake in girls vs. boys. The purpose of the present investigation was to further elucidate the influence of sex on the distribution of NK cell subsets in response to exercise and CHO intake. Based on our earlier findings (49) we hypothesized that the response of both NK cell subsets to exercise would be greater in girls vs. boys, and that the attenuating effect of CHO on NK cells would be less pronounced in girls vs. boys because of their already lower epinephrine response. Given the involvement of stress hormones such as catecholamines, growth hormone (GH), and cortisol in exercise-induced immune changes, we also measured these factors to assess their involvement in the observed NK cell responses.

METHODS

Subjects. Twenty-two healthy 14-yr-old adolescent girls (n = 11) and boys (n = 11) volunteered for this study, which was approved by the McMaster University Research Ethics Review Board. Pubertal status of each subject was self-assessed based on breast (girls) or pubic hair (boys) development according to Tanner (42). Self-assessment of pubertal status has been shown to be valid and reproducible among girls and boys (28). Table 1 provides subject characteristics. None of the subjects were taking medication, and none of the boys among girls and boys (28). Table 1 provides subject characteristics.

Preliminary session. An initial visit was conducted to introduce and familiarize subjects with the testing procedures. During this visit, body height (SECA 216 Accu-Hite Stadiometer, Creative Health Products, Plymouth, MI), body mass (BW-BB-800, Tanita, Tokyo, Japan), fat-free mass (bioelectric impedance-101A, RJL Systems, Clinton, MI), and Tanner stage were determined. Each subject’s maximal O2 uptake (V\textsubscript{O2,max}) was then determined on a cycle ergometer (Ergomedic 818E, Monark, Varberg, Sweden) using a progressive, continuous exercise test as previously described (49). Subjects began cycling at either 30 or 60 W depending on estimated fitness level, with pedaling rate constant at 60 rpm and work rate increased by 30 W every 2 min. A test was considered maximal when pedaling rate dropped below 50 rpm for 3 s despite strong encouragement and the respiratory exchange ratio was >1.1. The highest 30-s O2 uptake was taken as the V\textsubscript{O2,max}.

Experimental sessions. All subjects recorded their nutrient intake and physical activity for 2 days before their first experimental session, which was completed at least 5 days following the preliminary session. Recorded food intake and physical activity were then repeated the 2 days before their next session. On arrival to the laboratory at either 0730 or 0830 on the day of testing, subjects voided their bladder, and a nude weight was taken. Subjects then rested supine for ~10 min, after which time an indwelling venous catheter (Becton Dickinson) was placed in either an arm or a hand. After a further 10 min of supine rest, a resting, preexercise blood sample was drawn, and within 5 min subjects consumed a small standardized breakfast, consisting of one piece of whole-wheat toast with sugar-free strawberry jam (~90 kcal), along with their first drink (12 ml/kg body mass). This was done to standardize preexercise nutrition. From this point forward, volumes of 4 ml/kg body mass were subsequently consumed at 15-min intervals throughout exercise and at 20 min into recovery. In one trial (CT), subjects consumed a 6% CHO-electrolyte solution (4% sucrose, 2% glucose, ~18 mM Na\textsuperscript{+}, ~3 mM K\textsuperscript{+}), and in another trial (WT), water identical in flavor, sweetness (sweetened with aspartame), and electrolyte concentration, but without CHO, was consumed. Forty minutes after the resting blood sample, subjects began cycling (Monark) at a power output equivalent to 70% of their predetermined V\textsubscript{O2,max}, with the target intensity achieved in the first 5 min by analysis of expired gas. Exercise consisted of two 30-min bouts separated by a 5- to 7-min rest period. Additional expired gas samples were collected throughout the session to ensure the proper work intensity, with the power output adjusted accordingly. At 30 and 60 min of exercise and at 30 and 60 min of recovery, additional blood samples were collected. Blood samples were drawn while subjects remained seated on the cycle ergometer or quietly in the laboratory. The catheter was kept patent by flushing with ~1.5 ml of sterile saline (0.9% NaCl) after each blood sampling. Consequently, the first 2 ml of blood at each sampling time were discarded. On completion of the exercise task and blood collection, subjects dismounted the cycle ergometer and sat quietly in the laboratory during the recovery period; they were allowed to empty their bladders if necessary. The two experimental trials were conducted at least 5 days apart in a double-blind and counterbalanced fashion.

Glucose analysis. Whole blood treated with EDTA was centrifuged at 2,000 g for 10 min, and the plasma was stored at −50°C until
analyzed. Plasma glucose was measured enzymatically (2300L STAT, Yellow Springs Instruments, Yellow Springs, OH), and concentrations were corrected for exercise-induced changes in plasma volume (see below). The intra- and interassay coefficients of variation for this assay were <1.5%.

Cortisol and GH analyses. Whole blood sampled at rest and at 60 min of exercise was allowed to clot and centrifuged at 2000 g for 10 min. Serum was stored at −70°C until analyzed in duplicate for cortisol and GH using commercially available RIA kits (catalog no. TKCO1 and KGHDI, respectively, Diagnostic Products, Los Angeles, CA). In our hands, the intra- and interassay coefficients of variation, respectively, are 2.5 and 8% for cortisol and ≤5 and 12% for GH, and postexercise concentrations were corrected for exercise-induced changes in plasma volume (see below).

Catecholamines analysis. Whole blood collected at rest and at 60 min of exercise was treated with EGTA and reduced glutathione and centrifuged at 2,000 g for 10 min, and the plasma was stored at −70°C until analyzed for epinephrine and norepinephrine. Plasma catecholamines were analyzed by high-performance liquid chromatography with electrochemical detection as previously described (27). The intraclass correlation of this procedure is 0.96, representing very high reliability, and the extraction efficiency from plasma ranges between 80 and 85%. All postexercise concentrations were corrected for exercise-induced changes in plasma volume (see below).

Lymphocytes and NK cell subsets. Total lymphocyte counts were determined in whole blood treated with EDTA using an automated Coulter counter. Hb and Hct were also assessed to calculate changes in blood and plasma volume according to Dill and Costill (14), and cell counts were corrected for exercise-induced changes in blood volume. Two- and three-color immunophenotyping of EDTA-treated whole blood was used to determine NK cells by CD3 (peridinin-chlorophyll protein [PerCP]), and CD56 [phycoerythrin (PE)] mixed (10 μl each) with 100 μl of whole blood. An additional 100 μl of blood were mixed with 10 μl each of CD3 (PerCP), CD56 (FITC), and CD69 (PE). CD69 was used as a marker of activation status of circulating NK cells. All blood samples were stained within 6 h of collection using standardized procedures. Following a 20-min incubation period at room temperature in the dark, red blood cells were lysed with 2 ml of BD Pharm Lyse during a further 10-min incubation at room temperature. Samples were then centrifuged (300 g for 5 min at room temperature), washed (2 ml of BD Pharmingen stain buffer), and fixed with 0.5 ml of BD Cytofix buffer. Samples were stored at 3°C for no more than 48 h before being run on a FACScan flow cytometer (Becton Dickinson, Mississauga, Canada) with CELLQuest software. A total of 10,000 events were collected in the lymphocyte gate based on forward- vs. side-scatter characteristics. The proportions of NK cell subsets (Fig. 1) were analyzed offline with WinMDI 2.8 software (Joseph Trotter, The Scripps Research Institute).

The lymphocyte population was gated using forward- vs. side-scatter characteristics, and a dot plot of CD3 and CD56 fluorescence was created from events within the lymphocyte gate. The expression of CD69 was determined in a similar fashion by creating a dot plot of CD3 and CD56 created from events within the lymphocyte gate. A histogram of CD69 fluorescence was created from events within the gated CD3−CD56− cell population. For each preexercise sample, one tube containing a cocktail of CD3 (PerCP), CD56 (FITC), and IgG1 (PE) served as an isotype control and was used to set the marker for CD69+ cells. The placement of this marker was held constant in the analysis of subsequent samples to detect shifts of events within the marked population over time (e.g., with exercise). Because the proportion of NK cells expressing CD69 was relatively low, no attempt was made to distinguish CD69 expression on CD56dim and CD56bright cells. Cell counts of each NK cell subset were calculated by multiplying the percentage of cells with appropriate fluorescence with the adjusted absolute lymphocyte count. Blood for CD69 analysis was collected at rest, after 60 min of exercise, and after 60 min of recovery only. The median fluorescence intensity (MFI) of CD69+ cells was also determined. To facilitate intersubject comparisons, postexercise and recovery CD69 MFI data were expressed as a percentage of the preexercise value.

Statistical analyses. Data are presented as means ± SE, unless stated otherwise. To determine whether training status of the girls influenced physical and fitness characteristics or NK cell-related responses to exercise, these variables were first analyzed by either independent t-test or two-way repeated-measures ANOVA, respectively. Given there were no training-related differences among the girls for these measures (results not shown), their data were pooled for subsequent analyses. Inter-sex differences in physical and fitness characteristics were analyzed by independent t-tests. Three-way mixed-factorial ANOVAs with one between factor (sex) and two within factors (trial and time) were used to analyze glucose, hormones, and immune cell proportions and counts. Where appropriate, a Tukey’s post hoc test was used to determine significance among means. Despite significant inter-sex differences in percent body fat, it was not associated with any immunological measures (results not shown), which is consistent with our adult findings (47). Pearson correlations were performed to determine associations between hormone concentrations and NK cell-related variables. Microsoft Office Excel 2003 (Redmond, WA) software was used to perform t-tests. STATISTICA 5.0 (StatSoft, Tulsa, OK) was used for ANOVAs, and GraphPad Prism 4.03 (GraphPad Software, San Diego, CA) was used for correlation analyses. The NK cell data were assessed for a normal distribution, which was confirmed using STATISTICA 5.0. For all statistical procedures, the threshold for significance was set at P ≤ 0.05.

RESULTS

There were no intersex or intertrial differences in exercise intensity, as a percentage of VO2max (P ≥ 0.26); the average exercise intensity was 67 ± 1% of VO2max, or 34.8 ± 0.8 ml·kg fat-free mass−1·min−1.

Plasma glucose. Glucose concentrations at rest were similar between sexes and trials (5.0 ± 0.1 mM). Postexercise glucose levels were higher in CT vs. WT (5.1 ± 0.2 vs. 4.5 ± 0.1 mM) and remained higher at 30 min of recovery (6.6 ± 0.3 vs. 5.0 ± 0.1 mM; trial × time interaction, P < 0.001).

Catecholamines, cortisol, and GH. There was a sex × trial × time interaction (P = 0.01) for epinephrine. In the girls, epinephrine increased with exercise more during CT (P < 0.001) than WT (P = 0.07). In the boys, epinephrine increased with exercise more during WT (P = 0.001) than CT (P = 0.12). Consequently, the increase in epinephrine during CT tended to be greater in the girls vs. boys (P = 0.08), but during WT it was significantly greater in the boys vs. girls (P = 0.02). Norepinephrine increased with exercise (time effect, P < 0.001), with no effect of sex or trial. Cortisol decreased over time (time effect, P < 0.001), with no effect of sex or trial. Postexercise GH levels were lower in CT vs. WT (Table 2).

Lymphocytes. There was a sex × time interaction (P = 0.001) for lymphocyte counts, with exercise values 29% (30 min) and 23% (60 min) higher in girls. Although the trial × time interaction did not achieve statistical significance (P = 0.23), the average lymphocyte count was lower during CT (2.19 ± 0.06 × 109cells/l) vs. WT (2.34 ± 0.08 × 109cells/l; trial effect, P = 0.046).

NK cell populations. At rest, the CD56dim subset comprised 88 ± 1% of total CD3−CD56+ cells, with no intersex or intertrial differences. As a proportion of total lymphocytes, a significant sex × time interaction, with exercise values higher in girls, was found for CD56dim (P = 0.01) but not CD56bright (P = 0.61) cells (Fig. 2, A and B, respectively). In contrast, significant sex × time
interactions, with exercise values being higher in girls, were found for both CD56dim (P < 0.001) and CD56bright (P = 0.006) cell counts (Fig. 2, C and D, respectively). Significant trial \times time interactions (P ≤ 0.01), with lower values during CT, were found for the proportions and cell counts of the CD56dim and CD56bright subsets. CD56dim cell proportions were lower at 60 min of exercise (12.2 ± 1.0 vs. 14.5 ± 1.2%; P < 0.001) and at 60 min of recovery (3.7 ± 0.2 vs. 5.3 ± 0.5%; P = 0.02) in CT vs. WT, respectively. CD56bright cell proportions were lower at 30 min of recovery (1.0 ± 0.1 vs. 1.2 ± 0.1%; P = 0.007) in CT vs. WT, respectively. CD56dim and CD56bright cell counts were also lower at 30 min (P ≤ 0.02) and 60 min (P < 0.001) of exercise in CT vs. WT. To highlight the influence of exercise on the relationship between the CD56bright and CD56dim subsets, the ratio of CD56bright to CD56dim cells was calculated (Fig. 3). This ratio

Table 2. Stress hormone concentrations before, during, and after exercise in carbohydrate and water trials in girls and boys

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<td>Girls</td>
<td>33 ± 7</td>
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<td>Boys</td>
<td>38 ± 8</td>
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<td>ND</td>
<td>51 ± 9</td>
<td>110 ± 10</td>
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<td>Norepinephrine, pg/ml</td>
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<td>Girls</td>
<td>259 ± 38</td>
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<td>Boys</td>
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<td>259 ± 38</td>
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<td>Girls</td>
<td>616 ± 57</td>
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<td>397 ± 48</td>
<td>643 ± 57</td>
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<td>Boys</td>
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<td>296 ± 19</td>
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<td>Girls</td>
<td>2.8 ± 1.0</td>
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<td>Boys</td>
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<td>14.5 ± 3.1</td>
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Values are means ± SE. CT, carbohydrate trial; WT, water trial; Pre, 40 min before exercise; Post, after 60 min of exercise; Recovery, 60 min after exercise; ND, not determined. *Main effect for time (Post > Pre), P < 0.05. "Main effect for time (Post < Pre; Recovery < Pre), P < 0.05. *Main effect for trial (CT < WT), P < 0.05. Interaction effects. **significantly different from girls at same time point within same trial, P < 0.05. †significantly different from CT at same time point, P < 0.05. §trial \times time interaction (CT < WT at Post), P < 0.05.

Fig. 2. NK cell subset responses to exercise in boys and girls. A: CD3 \cdot CD56dim cell proportions. B: CD3 \cdot CD56bright cell proportions. C: CD3 \cdot CD56dim cell counts. D: CD3 \cdot CD56bright cell counts. Values are means ± SE. Shaded box represents exercise disregarding rest. *Significant difference between boys and girls at same time point, P < 0.05.

Fig. 3. Ratio of CD3 \cdot CD56bright to CD3 \cdot CD56dim cells before, during, and after exercise in boys and girls. Values are means ± SE. Shaded boxes represent exercise disregarding rest. Main effect time (30 < −40; 90 and 120 > −40), P < 0.05.
decreased at 30 min of exercise but increased at 30 and 60 min of recovery (time effect, $P < 0.001$), with no effect of sex or trial.

$CD69^+$ cells. CD69 levels were undetectable in one of the boys. There was no effect of sex or CHO intake on the proportion of CD3$^+$CD56$^+$ cells expressing CD69, but values during recovery were lower than at rest (time effect, $P < 0.001$; Fig. 4A). Given the increase in total lymphocyte counts, however, the number of circulating CD3$^+$CD56$^+$CD69$^+$ cells increased with exercise and returned to resting levels by 60 min of recovery (time effect, $P < 0.001$), with no effect of sex or CHO intake (Fig. 4B). A significant sex $\times$ time interaction ($P = 0.03$) was found for the MFI of CD69$^+$ cells as a percentage of the preexercise value (Fig. 4C). In the girls, values during recovery were greater than at postexercise ($P = 0.001$) and rest ($P = 0.03$), whereas in the boys values remained stable over time ($P \geq 0.83$). The absolute MFI of CD69$^+$ cells at rest, postexercise, and during recovery were $18.5 \pm 1.2$, $16.7 \pm 1.1$, and $21.3 \pm 1.9$ and $15.3 \pm 0.9$, $15.9 \pm 0.7$, and $16.4 \pm 1.4$ fluorescence intensity units in the girls and boys, respectively (sex $\times$ time effect, $P = 0.03$).

Correlations. Postexercise GH concentrations from both trials correlated with CD56$^{dim}$ cell counts in boys ($r = 0.53$, $P = 0.01$) but not in girls ($r = 0.04$, $P = 0.86$). There was also a trend for a correlation between postexercise GH and CD56$^{bright}$ cell counts in boys ($r = 0.41$, $P = 0.06$) but not in girls ($r = -0.05$, $P = 0.84$). Postexercise epinephrine concentrations from both trials correlated with CD3$^+$CD56$^+$CD69$^+$ cell counts in girls ($r = 0.59$, $P = 0.005$) but not in boys ($r = -0.10$, $P = 0.67$), whereas postexercise norepinephrine concentrations from both trials correlated with CD3$^+$CD56$^+$CD69$^+$ cell counts in both girls ($r = 0.57$, $P = 0.005$) and boys ($r = 0.73$, $P < 0.001$).

**DISCUSSION**

This study investigated the distribution of NK cell subsets in response to exercise and CHO intake in healthy adolescent boys and girls. We hypothesized that the response of both CD56$^{dim}$ and CD56$^{bright}$ subsets would be greater in the girls. As a proportion of the total lymphocyte pool, however, only the response of CD56$^{dim}$ cells was greater in the girls, whereas exercise-induced increases in cell counts of both subsets were greater in the girls. We also investigated a possible interaction of sex and CHO intake on the distribution of NK cell subsets and hypothesized that any CHO effects would be less pronounced in the girls. Contrary to our hypothesis, the effects of CHO intake on the NK cell response to exercise were not different between boys and girls, as indicated by a lack of three-way ANOVA interactions.

Compared with the adult literature (39), few studies (6, 7, 31, 32, 34) have investigated NK cell responses to exercise in adolescents, and none have specifically addressed an effect of female sex. Although Nieman et al. (34) did not find an effect of female sex on CD3$^+$CD16$^+$CD56$^+$ cell responses to a tennis practice in adolescents who consumed a CHO beverage, the findings of more recent studies suggest a possible sex difference insofar as CD3$^+$CD16$^+$CD56$^+$ cells increased by $-238\%$ following a wrestling practice in boys (31) but by only $-154\%$ following a water polo practice in girls (32). These results (31, 32, 34) contrast with the findings in our subjects, whereby girls’ responses were greater than those of the boys. Sex differences in our study demonstrated an effect size of 1.04 for CD56$^{dim}$ and 0.74 for CD56$^{bright}$ responses, demonstrating high statistical power (45). Because the former studies were conducted in the field using different exercise tasks and allowed for varied exercise intensities, differences in the methodological approach likely contribute to the contrasting results.

Why girls experienced larger NK cell responses to exercise is unclear. A larger NK cell response in the girls is somewhat paradoxical given the current and previous findings (10, 12, 20) of a lower epinephrine response to exercise in females vs.

![Fig. 4. Expression of CD69 on circulating CD3$^+$CD56$^+$ cells before, during, and after exercise in boys and girls. A: proportion of CD3$^+$CD56$^+$ cells expressing CD69. B: CD3$^+$CD56$^+$CD69$^+$ cell counts. C: median fluorescence intensity (MFI) of CD69 on CD3$^+$CD56$^+$ cells as a percentage of the preexercise value. Values are means ± SE. Pre: 40 min before exercise; Post, after 60 min of exercise; Recovery, 60 min after exercise. Main effect time for CD3$^+$CD56$^+$CD69$^+$ cell proportions (Recovery < Pre) and counts (Post > Pre; Recovery < Post), $P < 0.001$. †Significantly different from Pre, $P < 0.05$. §Significantly different from Post, $P < 0.05$.](http://jap.physiology.org/)
males. It may be that NK cell sensitivity to catecholamines during exercise is greater in females given their greater lymphocyte β2-adrenergic receptor density (50) and post-receptor activity (17, 30) compared with men. From a functional perspective, NK cell cytotoxicity, as determined in whole blood, is less in women than in men (4, 51), although this same difference was not confirmed in adolescents (3). It remains possible, however, that to compensate for relatively low NK cell cytotoxicity, females respond to physiological stress (i.e., acute exercise) with an overall greater influx of NK cells to the peripheral circulation, in particular the CD56dim subset, which is the more cytotoxic population (11).

To our knowledge, only one previous study of Olympic-caliber rowers assessed the NK cell response to exercise with and without CHO intake in females (35). However, no information was provided as to the rowers’ menstrual status or use of oral contraceptive therapy. In the present study, no interaction of sex and CHO intake was observed, suggesting that the effect of CHO on NK cells is similar between males and females, at least during adolescence. Because CHO effects on NK cell responses may be due to a blunted stress hormone (e.g., epinephrine and cortisol) response, it was surprising to find a significant sex × trial × time interaction for epinephrine. Consistent with previous adult literature (10, 12, 20), the epinephrine response to exercise during WT was greater in females, with no apparent effect of CHO intake.

CD56bright cells (23). A recent study (41) found that, during sports training in healthy women, the lowest measured NK cell cytotoxicity occurred at a time when the blood CD56bright-to-CD56dim ratio was highest. Given the depression in NK cell cytotoxicity that occurs during recovery from high-intensity exercise (37), our findings of an elevated CD56bright-to-CD56dim ratio during the recovery period suggests that disproportionate changes in NK cell subsets may be responsible for reduced NK cell function, given the lower cytotoxicity of CD56bright cells (11). However, we did not measure NK cell function per se and suggest that future exercise studies should account for the distribution of CD56dim and CD56bright cells in assays of NK cell cytotoxicity.

Sex-based differences in the expression of CD69 on circulating CD3−CD56+ cells during recovery from exercise was another important and novel finding in this study. CD69 is a sensitive marker of lymphoid activation (40) and correlates with NK cell cytotoxicity (24). In addition, exercise performed with and without CHO intake increases CD3−CD56+CD69+ cell counts in adults (29). We also show in girls that, despite a slight reduction in the proportion of CD3−CD56+ cells positive for the CD69 antigen during recovery, circulating CD3−CD56+ cells at this time expressed higher levels of CD69 than at rest or postexercise. In contrast, the 14-yr-old boys did not experience a significant time effect on CD69 expression (<8%). More work is required to elucidate the biological significance of these age- and sex-related differences, but given the previously reported sex-based differences in NK cell cytotoxicity (4, 51), a greater activation of these cells in the face of stress may represent another compensatory mechanism used by the girls.

In light of the novel observations presented in this study, two important issues require acknowledgement. First, the more advanced pubertal status of the boys compared with the girls who were at the same chronological age could be considered a surprising finding, given the generally earlier sexual maturation of females. It is important to note that the two groups were assessed on different sexual characteristics (i.e., pubic hair for boys and breast development for girls). Had we used the pubic hair criterion for both groups, for example, the girls would most likely have been more advanced than the boys, and by not applying the same criterion to both groups our pubertal comparison is weakened. Although the lower pubertal status of the girls may have been a confounding factor in our study, we feel this is unlikely because less mature individuals would be expected to experience smaller NK cell responses to exercise (46), not a larger response, as was the case with the girls. Second, we found that the fitness levels of the five trained and six untrained girls were not different, which may also be considered a surprising finding. This lack of fitness difference may be a result of a selection bias in untrained girls who were quite active and participated in physical education programs. Indeed, it is likely that children and adolescents become interested in an exercise research project because they are healthy and active. However, we feel it is important to note that the VO2max values for our untrained 14-yr-old girls and boys are strikingly similar to published values derived from large numbers of 14-yr-old adolescents from around the world (1, 2, 15, 26, 38). In addition, aerobic power expressed relative to fat-free mass was identical between the male and female groups, and this is an important prerequisite in exercise studies that compare sexes (44).

In summary, the results of this study highlight sex-based differences in the response of CD56dim and CD56bright NK cells to high-intensity exercise in healthy adolescents. However, female sex did not influence the effects of CHO intake on the redistribution of NK cell subsets. This study also highlighted a significant increase in the CD56bright-to-CD56dim ratio following exercise and sex-based differences in the expression of CD69 on CD3−CD56+ cells during recovery from exercise. The redistribution of CD56dim and CD56bright cells in response to exercise and an increase in NK cell activation status could be of clinical relevance. More research is required to understand the impact of physiological stress on the immunoregulatory properties of the CD56dim and CD56bright subsets.

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