Effects of Sex and Intensity of Exercise on Circulating Leukocyte Counts after Exercise in Trained Subjects

Sakdarin Thammawong¹,², Nantaya Krasuaythong¹,² Yupaporn Kanpettha¹,², Orathai Tunkamnerdthai²,³, Naruemon Leelayuwat²,³

¹Graduate School, Khon Kaen University, Khon Kaen, Thailand, ²Exercise and sport Sciences Development and Research Group, Khon Kaen University, Khon Kaen, Thailand, ³Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand

ABSTRACT

Thammawong S, Krasuaythong N, Kanpettha Y, Tunkamnerdthai O, Leelayuwat N. Effects of Sex and Intensity of Exercise on Circulating Leukocyte Counts after Exercise in Trained Subjects. JEPonline 2017;20(4):11-23. We investigated the effects of sex and exercise intensity on the circulating total and differential leukocyte counts after exercise in trained participants. Ten men and 10 women randomly cycled at 30, 70, or 90% maximum workload for 30 min and then rested for 30 min, with 7 days apart. Blood samples were collected immediately before, after, and 30 min after exercise to measure leukocyte counts. Men and women had the same changes in total leukocyte and subtype counts, which returned to baseline at the end of recovery. Compared with low-intensity exercise, total leukocyte counts showed a greater increase immediately after higher intensities exercise (P<0.05 for all). The increase in the neutrophil count immediately after high-intensity was greater than that after lower intensities exercise (P<0.05). Lymphocyte count showed a greater increase at the end of low- to high-intensity exercise (P<0.05 for all). Monocyte count showed a greater increase at the end of high-intensity versus low-intensity exercise (P<0.05 for all). Changes in total leukocyte and subtypes counts depended on the exercise intensity, with the lowest values seen with low-intensity exercise; there was no effect of sex. It appears that 30 min recovery is sufficient time at all intensities for all leukocyte subtypes to return to baseline levels.

Key Words: Gender, Physical Activity Level, White Blood Cell
INTRODUCTION

Circulating total leukocytes (LE) and the differential leukocyte count including neutrophils (NE), lymphocytes (LY), and monocytes (MO) are recognized markers of inflammation (4). The NEs represent approximately 90% of all granulocytes. They are the first cells recruited from the blood to sites of injury or infection (5). Acute exercise increases the inflammatory response to help repair tissue damage induced by exercise that is associated with NE infiltration followed by macrophage infiltration several hours later (9). Thus, exercise-induced LE describes inflammation associated with training adaptations that result from exercise in healthy humans. Training with incomplete recovery contributes to poor physical performance (24). Evaluation of biomarkers, including circulating total LE and the differential leukocyte count, in responses to acute exercise may be beneficial in improving a coach's ability to assess recovery status after an exercise period and to determine the intensity of subsequent training periods for athletes.

Intensity of endurance exercise is a factor influencing the LE count in response to exercise in trained individuals (25). In a previous study, aerobically trained, eumenorrheic females in the early follicular phase of the menstrual cycle performed 30 min of exercise at three intensities: 90% lactate threshold (LT), LT, and, 110% LT (14). They found that after the exercise, the subjects showed an increase in total LE and LY counts, depending on intensity. More specifically, high-intensity exercise induces an initial pro-inflammatory response during exercise and a subsequent anti-inflammatory response after exercise (20). A 24-hr ultraendurance exercise bout contributed to an increase in blood LE count immediately after the exercise, which decreased after 28 hrs of recovery (19). Moreover, moderate-intensity exercise also increases LE count (2,12,31). The authors showed that moderate-intensity exercise (75% of maximal heart rate) for 2 hrs by 10 elite female national team soccer players also showed a significant 78% increase in total LE counts up to 4 hrs of recovery, and that the LE was caused primarily by neutrophilia without significant changes in LY after the end of exercise (2).

In addition, Nieman and coworkers (26) compared the effect of 45 min of high-intensity (80% VO₂ peak) and moderate-intensity (50% VO₂ max) treadmill exercise on circulating LE and LY subpopulations in well-conditioned, young males. They found that both exercise conditions resulted in significantly greater increases in lymphocytosis immediate after exercise that decreased at 1 hr and 2 hrs post-exercise relative to baseline levels following both exercise conditions. Exercise-induced leukocytosis seen in both men and women appears to be associated with the increased circulating catecholamines (27). However, Plaisance et al. (29) did not identify changes in LE count 24 to 120 hrs after a single aerobic exercise session expending 500 kcal at 70% VO₂ peak in moderately fit men, which may be due to the long duration of the recovery period. Positive results were found in the studies with recovery periods less than 4 hrs (31). Of note, a previous study (31) reported that short-term, low-intensity exercise showed a significant increase in total LEs and circulating LY in 2-month-old Wistar rats. No research has explored the effect of low-intensity exercise on these immune cell responses in humans.

The effects of sex on circulating LEs in response to exercise in trained subjects are still unclear. To our knowledge, the data on sex differences in LE after exercise are equivocal.
(1, 12, 13, 21, 23, 33, 34). While most studies suggest that there are no sex differences after exercise in terms of LE, LY, MO, and NE counts (3,23), Stupka et al. (34) reported higher numbers of circulating NE after 90 min of cycle ergometry in women taking oral contraceptive (OC) compared to men and non-users. It seems that when the menstrual phase and OC are controlled, males and females showed marked differences in immune response to exercise (12). However, Gleeson and colleagues (13) indicated that researchers probably do not need to consider sex differences in future mixed gender studies on exercise, infection, and immune function with the exception of research on mucosal immunity or natural killer (NK) cells (13).

To our knowledge, there has been no study investigating the effects of sex and three levels of exercise intensity on the circulating total and differential LE counts after a single bout of exercise in trained subjects. A greater knowledge of the effects of sex and exercise intensity on post exercise circulating total and differential LE counts may be beneficial to coaches planning appropriate exercise intensity to prevent overtraining. This may result in an increase in physical performance in male and female athletes. Therefore, the purpose of this study was to investigate the effects of sex and exercise intensity on the circulating total and differential LE counts after exercise in trained subjects. We hypothesized that these trained subjects will show different circulating total and differential LE counts after exercise at different intensities with a difference between the male and female subjects.

METHODS

Study Design

All subjects separately visited the laboratory on three occasions to perform acute, randomly assigned exercise, at least 7 days apart to prevent carry over effect. The protocol was similar each time, except the exercise intensity was 30% (low-intensity), 70% (moderate-intensity), or 90% maximum workload (Wmax) (high-intensity), respectively.

Subjects

Twenty healthy subjects (10 men and 10 women) participated in this study, which was approved by the Khon Kaen University ethics board. The study conformed to the standards set by the Declaration of Helsinki 2010 (HE531276). All subjects signed an informed consent document that explained the benefits and risks of the experiment. Each subject underwent a medical history, anthropometric measurements, and a 12-lead ECG and blood pressure measurements. Blood samples were collected from the subjects for medical screening. Body composition was measured with dual-energy X-ray absorptiometry (software version 14.10, LUNAR Prodigy, USA). Most of the subjects played endurance sports (cycling, basketball, middle and long distance running, or football). Their training intensity was moderate to high. All subjects had no physiological limitation that could have affected exercise performance. All female subjects were tested during the follicular menstrual phase.

Power Calculation

The difference in LE counts between men and women in a previous study (1) during high-intensity exercise was used to calculate the sample size of the present study. We decided to require 80% power at a significance level of 0.05. The calculated sample size was 8. The dropout rate was set at 20%. Thus, 10 subjects were required for this study.
Procedures

**Wmax and VO\textsubscript{2} Max Test**
Seven days before the first trial, the subjects’ Wmax and VO\textsubscript{2} max were measured on an electronically braked ergometer (Corival, Lode B.V., Groningen, Netherlands) during an incremental exhaustive exercise test. On that day, the gas analyzer (AD Instrument, Australia) was calibrated using gases provided by VIASYS Healthcare, Inc. Pennsylvania U.S.A). The subjects performed the test, which was demonstrated in our previous study (30). The maximal aerobic power (Wmax) was defined as the workload that corresponded to maximal oxygen consumption (VO\textsubscript{2} max). Heart rate was monitored with an oscilloscope monitor (Diascope type DS 521, Simonsen and Weel, Denmark) throughout the test.

**Diet and Physical Activity Prior to Testing**
A week before each visit, the subjects were asked to keep a 3-day food and physical activity diary for 2 weekdays and 1 weekend day. INMUCAL software (Mahidol University, Thailand) was used to analyze the dietary records. On every experimental day after an overnight fast, each subject arrived in the laboratory, having abstained from caffeine, alcohol, smoking, and heavy exercise for 48 hrs before the day of the study.

**Experimental Protocols**
After an overnight fast, subjects arrived at the laboratory and rested for 30 min and subsequently cycled for 30 min on the ergometer at a randomly chosen intensity of 25%, 65%, or 85% VO\textsubscript{2} max or 30%, 70%, or 90% Wmax, respectively, and then rested for 30 min. An electrocardiogram was recorded throughout every visit to the lab. Humidity and ambient temperature of the laboratory were 48 ± 1.8% and 25°C, respectively. Blood samples (2 mL) were obtained from an antecubital vein immediately before and after exercise and 30 min after exercise to measure LE counts.

**Blood Analysis**
Each 2 ml blood sample was analyzed for routine complete blood count including total LE count and differential LE subfractions (Pentra 60, ABX Diagnostics, France). The quantity of each LE subfraction (LY, MO, NE; cells/mL) was calculated by multiplying the measured fraction of each by the total LE count. All blood parameters were analyzed in the chemistry laboratory of Srinagarind hospital, Faculty of Medicine, Khon Kaen University, Thailand.

**Statistical Analyses**
All data are expressed as mean ± standard deviation unless stated otherwise. The Kolmogorov-Smirnov test was used to test the normal distribution of changes in the data. Two-way repeated-measures analysis of variance (ANOVA) was used to compare changes in the dependent variables over time between sexes. Duncan and Scheffe’s post hoc tests were applied in cases of a significant (P<0.05) F ratio to locate the differences. Paired Student’s t-test was used for non-time-dependent variables. A P value less than 0.05 was considered significant, and reliability of the dependent measures was shown with intra-class correlations.
RESULTS

Subject Characteristics
The subjects’ characteristics are reported in Table 1. Men had higher VO$_2$ max per kg body mass and Wmax than females (P<0.05).

Table 1. Anthropometric and Physiological Characteristics of Trained Male and Female Subjects.

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Men (n = 10)</th>
<th>Women (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>21.6 ± 4.84</td>
<td>20.3 ± 1.64</td>
</tr>
<tr>
<td>Body Mass (kg)</td>
<td>61.1 ± 4.79</td>
<td>52.9 ± 6.01*</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.72 ± 0.03</td>
<td>1.62 ± 0.06</td>
</tr>
<tr>
<td>BMI (kg·m$^{-2}$)</td>
<td>20.7 ± 1.50</td>
<td>20.2 ± 1.61</td>
</tr>
<tr>
<td>Waist Circumference (cm)</td>
<td>76.6 ± 4.16</td>
<td>64.3 ± 5.19*</td>
</tr>
<tr>
<td>Hip Circumference (cm)</td>
<td>88.9 ± 5.15</td>
<td>73.7 ± 4.57*</td>
</tr>
<tr>
<td>W/H Ratio</td>
<td>0.87 ± 0.04</td>
<td>0.87 ± 0.02</td>
</tr>
<tr>
<td>Body Fat (%)</td>
<td>11.5 ± 6.52</td>
<td>23.1 ± 7.08*</td>
</tr>
<tr>
<td>Fat Mass (kg)</td>
<td>6.6 ± 4.25</td>
<td>11.4 ± 4.48*</td>
</tr>
<tr>
<td>Lean Body Mass (kg)</td>
<td>50.6 ± 4.29</td>
<td>37.3 ± 2.71*</td>
</tr>
<tr>
<td>VO$_2$ Max (mL·kg$^{-1}$·min$^{-1}$)</td>
<td>54.0 ± 5.33</td>
<td>46.2 ± 1.76*</td>
</tr>
<tr>
<td>VO$_2$ Max (mL·kgLBM$^{-1}$·min$^{-1}$)</td>
<td>65.5 ± 7.66</td>
<td>64.7 ± 6.67*</td>
</tr>
<tr>
<td>Wmax (watts)</td>
<td>204 ± 34.7</td>
<td>140 ± 19.4*</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD; BMI = Body Mass Index; W/H = Waist to Hip Circumference Ratio; VO$_2$ max = Maximal Oxygen Consumption; LBM = Lean Body Mass; Wmax = Maximal Workload; VO$_2$ max = Maximal Oxygen Consumption; *Significantly different from men (P<0.05).

Circulating Leukocyte Counts
Both men and women had the same changes in circulating total LE and subtype counts, which returned to baseline levels at the end of recovery. Compared with low-intensity exercise, the total LE count showed a greater increase immediately after moderate-intensity and high-intensity exercise (P<0.05 for all) (Figure 1). The NE count immediately after high-intensity exercise was greater than that seen with moderate-intensity and low-intensity exercise (P<0.05 for both) (Figure 2). The LY count showed a greater increase at the end of low-intensity to high-intensity exercise (P<0.05 for all) (Figure 3). Moreover, the MO count was greater at the end of high-intensity versus low-intensity exercise (P<0.05) (Figure 4).
Figure 1. Leukocyte Counts (x$10^3$cells/µL) Before, Immediately, and 30 Min after Exercise at Low-Intensity, Moderate-Intensity, and High-Intensity in Men and Women. The data are expressed as mean ± SD; (N = 10 men, 10 women). *Significantly different from immediately after exercise on the same visit (P<0.05), @Significantly different from low-intensity exercise (P<0.05).

Figure 2. Neutrophil Counts (x$10^3$cells/µL) Before, Immediately, and 30 Min after Exercise at Low-Intensity, Moderate-Intensity, and High-Intensity in Men and Women. The data are expressed as mean ± SD; (N = 10 men, 10 women). *Significantly different from immediately after exercise on the same visit (P<0.05), @Significantly different from low-intensity exercise (P<0.05), $Significantly different from moderate-intensity exercise (P<0.05), §Significantly different from moderate-intensity exercise (P<0.05).
Figure 3. Lymphocyte Counts ($x10^3$ cells/µL) Before, Immediately, and 30 Min after Exercise at Low-Intensity, Moderate-Intensity, and High-Intensity in Men and Women. Data are expressed as mean ± SD; (N = 10 men, 10 women). *Significantly different from immediately after exercise on the same visit (P<0.05), @Significantly different from low-intensity exercise (P<0.05), $Significantly different from moderate-intensity exercise (P<0.05).

Figure 4. Monocyte Counts ($x10^3$ cells/µL) Before, Immediately, and 30 Min after Exercise at Low-Intensity, moderate-Intensity, and High-Intensity in Men and Women. The data are expressed as mean ± SD; (N = 10 men, 10 women). *Significantly different from immediately after exercise on the same visit (P<0.05), @Significantly different from low-intensity exercise (P<0.05).
DISCUSSION

This is the first study to demonstrate that in trained male and female subjects, total LE counts increased from baseline in responses to moderate-intensity and high-intensity exercise. The NE, LE, and MO counts correlated with exercise intensity but not sex.

All subjects in the present study had normal circulating total LE and differential counts before the experiment (35). This might demonstrate that they did not have any inflammation from their training before participation in this study, though they normally trained at the level of moderate-intensity to high-intensity (data not shown). In contrast to a previous study that showed high-intensity aerobic training session in competitive swimmers was associated with neutrophilia and lymphopenia lasting for at least 2 hrs (22). The observed lymphopenia suggests a lower immune response at the end of the exercise that may reduce the immunity of athletes, which suggests the need for extra care. Also, this finding might demonstrate that the subjects in the present were not prone to infection. In fact, the subjects in the present study showed no increased inflammation from the low-intensity exercise. This finding indicates that low-intensity exercise may be used by athletes during recovery from an injury. Furthermore, the absence of inflammation at 30 min after all exercise bouts in the present study indicates that the subjects could perform subsequent exercise bouts after 30 min without any inflammatory effect from the prior exercise.

To our knowledge, no study has investigated the effects of three exercise intensities on LE counts in the same subjects. This methodology may alleviate confounding covariates because each subject served as her or his own control. However, the study needs to be performed on different days since it was important to know the effect of exercise intensity on the LE counts. Of note, we did not find any significant differences among the pre-exercise data. This seems to confirm that there were no day-to-day variations of the baseline parameters in the present study. Thus, the results are expected to be sufficiently valid to allow for conclusions concerning the effects of sex and exercise intensity on total and differential LE counts in responses to exercise in trained individuals.

The results of the effect of exercise intensity on LE and differential LE counts in the present study support our hypothesis. The subjects showed different increases in the circulating total and differential LE counts immediately after exercise at different intensities, which is in agreement with earlier studies (2,12,19,14,20,25,31). Only one study found effects of three intensities of 30 min of exercise on total LE and LY counts in aerobically trained, eumenorrheic females in the early follicular phase of the menstrual cycle. No study has explored this in male athletes. Therefore, further study is required to investigate these effects in male athletes.

It is important to point out that changes in LE, NE, and LY with high-intensity exercise are equivocal. Hoffman-Gometz and Pedersen (17) pointed out that the blood LE count following high-intensity exercise dropped below normal. In contrast, Hansen et al. (15) demonstrated that immediately after high-intensity aerobic exercise, total LE count, represented evenly by NE and LY with a small contribution of MO, was higher than values obtained during resting pre-exercise. Other studies by Marklund et al. (19) and Markovitch et al. (20) on high-intensity exercise have reported that it induces an initial pro-inflammatory response during the exercise and a subsequent anti-inflammatory response 28 hrs after exercise.
Neiman and colleagues (26) indicated that moderate-intensity exercise (vs. high-intensity exercise) results in a much smaller level of post-exercise leukocytosis, lymphocytosis, and neutrophilia and a less-pronounced lymphocytopenia during recovery. Other studies (2,12, 31) found that moderate-intensity exercise (75% of maximal heart rate or 50% VO2 max) increased the number of LE in elite female soccer players and well-trained males (26). The authors showed that moderate-intensity exercise for 2 hrs also resulted in a significant increase in total LE after 4 hrs of recovery (26). Avloniti et al. (2) suggested that the leukocytosis was caused primarily by neutrophilia without significant changes in LY after the end of exercise.

However, Plaisance et al. (29) did not find changes in the LE count 24 to 120 hrs after a single aerobic exercise session expending 500 kcal at 70% VO2 peak in moderately fit men. This may be due to the long recovery period. Of note, a previous study reported that short-term, low-intensity exercise resulted in a significant increase in total LE and circulating LY counts in Wistar rats (31). However, no research has explored the effect of low-intensity exercise on these immune cell responses in humans. Hence, it is reasonable to conclude that the results in the present study regarding the effect of low-intensity exercise on these immune cell responses compared to moderate- and high-intensity exercise in each trained individual is the first of its kind. The discrepancy of the effects of intensity of exercise on the subjects' immune cell responses during recovery after exercise may be due to differences in fitness level of the subjects as well as the research methodology.

The increase in total LE, NE, and LY counts immediately after moderate- and high-intensity exercise appear to be explained by the effects of catecholamines (16) and cortisol (18) concentrations. The increase in epinephrine and cardiac output associated with exercise are thought to contribute to the exercise-induced leukocytosis through de-margination from vascular pools and immune organs (10,11). Nieman et al. (26) investigated the effect of 45 min of high-intensity (80% VO2 max) versus moderate-intensity (50% VO2 max) exercise on circulating LE and LY subpopulations in well-trained young men. They reported greater increases in the subjects' cortisol and epinephrine concentrations at high-intensity versus moderate-intensity, resulting in lymphocytosis immediately after the exercise. The findings of increased cortisol during and after high-intensity continuous exercise (70% VO2 max) is supported by the study of Cabral-Santos and colleagues (6), which is in agreement with the findings of Hötting et al. (18). However, Zschucke et al. (36) reported that the increases in TL, NE, and LY with moderate-intensity continuous exercise may not be explained by the increase in cortisol. They reported that walking/running on a treadmill for 30 min at 60 to 70% of the subjects' VO2 max did not change saliva cortisol concentration.

The absence of sex differences in the immune cell counts following the different exercise intensities in the present study is consistent with earlier research (3) that found no sex differences in exercise-induced increases in LE, LY, MO, and NE counts. One previous study (12) suggested that this might be because the prior research did not control for the subjects' menstrual cycle period, OC, and/or the matching of the male and female subjects for activity or fitness level. This study examined immune cell changes and controlled for menstrual phase, OC, and fitness, and found that women taking OC had a greater post-exercise increase in LY and NE compared to men and non-OC users after 90 min of cycling at 65% of VO2 max (34). Timmons et al. (32) reported that female participants in their study did not use
OC and had regular menstrual cycles, but they still did not show differences in immune cell counts in response to exercise when compared with males.

Another explanation may be related to epinephrine release in response to submaximal exercise, which has been shown to be sex dependent (28), with males demonstrating a greater release compared to mid-follicular females (7,8). Unfortunately, we did not measure epinephrine concentration in the present study. The evaluation of epinephrine levels in response to three levels of exercise intensity should be performed in trained men and women in a future study. Moreover, the fitness level of the subjects in the present study seems to be equivalent, since we matched the sex groups based on training quantity, which was not significantly different and has been shown to be more appropriate than matching based on body composition and VO$_2$ max (7). It is noted that when we considered the VO$_2$ max per kg of lean body mass the difference between men and women disappeared. This may reflect a similar level of maximal oxygen extraction by the muscle fibers of both sexes. Hence, the sex differences in LBM and VO$_2$ max in the present study are not limitations of the sex difference.

**Limitations of this Study**

The limitation of this study is the training status of the subjects, which showed that they were not well-trained. The results of this study may not be appropriate to apply to the well-trained subjects. In addition, immune function was not measured in the present study even though it provides more accuracy of immune response to exercise. Therefore, research exploring the effects of sex and intensity of exercise on immune cell counts and function should be further investigated in well-trained subjects.

**CONCLUSIONS**

In fasted, trained male and female subjects, circulating total LE, and NE, LY, and MO counts increased at the end of both the moderate-intensity exercise and the high-intensity exercise. The increase in these counts correlated with exercise intensity but not sex.

**ACKNOWLEDGMENTS**

This work was supported by an Incubation Research Grant from Khon Kaen University. Also, this study was partially supported by the Exercise and Sport Sciences Development and Research Group. We thank the subjects for their kind cooperation.

**Address for correspondence:** Associate Professor Naruemon Leelayuwat, PhD, Department of Physiology, University of Khon Kaen, Nai Muang, Muang Khon Kaen, Khon Kaen 40002, Thailand, Tel: +66-043-363263, +66-043-363185, Fax: +66-043-348394, Email: naruemon.Leelayuwat@gmail.com

**REFERENCES**


**Disclaimer**
The opinions expressed in *JEPonline* are those of the authors and are not attributable to *JEPonline*, the editorial staff or the ASEP organization.