ResearchGate

See discussions, stats, and author profiles for this publication at: https://www.researchgate.net/publication/326674254

Elevation of leukocyte counts is associated with an increase in the intensity and duration of exercise

Article *in* Sport Sciences for Health · July 2018

DOI: 10.1007/s11332-018-0486-6

CITATIONS
0

READS
26

3 authors, including:

Dayang maryama Daud
Universiti Malaysia Sabah (UMS)
13 PUBLICATIONS **17** CITATIONS

SEE PROFILE

Some of the authors of this publication are also working on these related projects:

stroke rehab View project

All content following this page was uploaded by Dayang maryama Daud on 03 April 2020.

The user has requested enhancement of the downloaded file.

Sport Sciences for Health
https://doi.org/10.1007/s11332-018-0486-6

ORIGINAL ARTICLE

Elevation of leukocyte counts is associated with an increase in the intensity and duration of exercise

D. Maryama Ag. Daud[1,2] · Aza Sherin Mohammad Yusoff[3] · Wan Zurinah Wan Ngah[4]

Received: 29 March 2018 / Accepted: 14 July 2018
© Springer-Verlag Italia S.r.l., part of Springer Nature 2018

Abstract

Purpose The aim of this study is to investigate the effect of intensity and duration of exercise related to the leucocyte count.
Methods 25 male subjects completed all nine cycling sessions at 55 ± 5 rpm on a cycle ergometer for 10, 20, and 30 min at workloads that corresponded to 50, 60, and 70% of an individual's pre-determined peak oxygen consumption ($\dot{V}O_2$peak), in random order. Heart rate and $\dot{V}O_2$peak were monitored each minute during the exercise to ensure that the subjects were exercising at the given relative intensity. Blood samples were taken before and after the exercise.
Results The overall leucocyte counts and its subtypes including lymphocytes, monocytes, and neutrophils were significantly elevated immediately after exercise at all intensity and duration of exercise. ANOVA showed that the main effect of time (T) on leucocyte, neutrophil, lymphocyte, and monocyte counts increases over time. ANOVA analysis also showed that only exercise duration has a significant effect in overall leucocyte counts, including its subtypes. Additionally, this study also revealed that the overall leucocyte counts and its subtypes had a positive correlation with the duration of exercise using Pearson's correlation coefficient test. However, only lymphocytes were positively correlated ($r = 0.178$) with exercise intensity.
Conclusions This study strongly recommends a re-evaluation of current views about the intensity and duration of physical exercise. A precise definition of an individual's workload that consists of intensity and duration of exercise is crucial as it will affect blood viscosity and blood flow during and immediately after exercise.

Keywords Exercise intensity · Exercise duration · Leucocyte count · Peak oxygen consumption

Introduction

Moderate physical exercise is beneficial for an individual's health but vigorous exercise may show the opposite effect. Generally, the total leucocyte count in the peripheral blood increases during and immediately after an exercise [1], indicating that physical exercise can trigger an elevation in blood viscosity. Previous studies reported that an increase in blood viscosity resulting from the elevated leucocyte count was associated with a higher risk of cardiovascular diseases and atherogenesis [2–4]. The sensitivity of leucocytes and their subtypes towards the intensity and duration of exercise is varied [5]. Studies revealed that the changes in leucocyte counts are linked with the exercise intensity and duration. For example, Abdossaleh et al. [6] reported that high-intensity exercise increased the total leucocyte, lymphocyte, monocyte, and neutrophil counts in college judoists. These findings are further supported by a more recent study in which a greater increase in the leucocyte count after high-intensity exercise in physically healthy and active young males compared to low-intensity exercise and no exercise [7]. McCarthy and Dale [8] also reported that leucocytosis is approximately proportionate to the intensity and duration of the exercise performed.

✉ D. Maryama Ag. Daud
dmaryama@ums.edu.my

1 Sports Science Department, Faculty of Psychology and Education, Universiti Malaysia Sabah, UMS Road, 88400 Kota Kinabalu, Sabah, Malaysia

2 Physical Fitness Rehab Centre, Faculty of Psychology and Education, Universiti Malaysia Sabah, UMS Road, 88400 Kota Kinabalu, Sabah, Malaysia

3 Medical Education Department, Faculty of Medicine and Health Science, Universiti Malaysia Sabah, UMS Road, 88400 Kota Kinabalu, Sabah, Malaysia

4 Department of Biochemistry, Faculty of Medicine, UKM Medical Centre, Universiti Kebangsaan Malaysia, Level 17, Pre-Clinical Building, Jalan Yaacob Latif, Bandar Tun Razak, Cheras, 56000 Kuala Lumpur, Malaysia

Published online: 28 July 2018

To date, most studies investigating the exercise intensity and duration only focused on a single variable at a time, which often limited with a subjective description of the duration and intensity of exercise [6, 7, 9, 10]. Additionally, a previous study investigated the effect of high and low exercise intensities on the leucocyte count with a limited number of participants [7], but the influence of a shorter duration and moderate-intensity exercise towards the leucocyte counts, as recommended by the American College of Sport Medicine [11], is inconclusive. Therefore, this study aimed to determine the association between moderate intensity (50, 60, and 70% of peak oxygen uptake) and short duration (for 10, 20, and 30 min) exercise on the leucocyte count, including its subtypes such as the monocytes, lymphocytes, and neutrophils. We hypothesised that moderate-exercise intensity for a short duration may increase the leucocyte count in an individual.

Methods

Subjects and pre-study screening

Research was conducted using a quasi-experimental design with a repeat measure of a single experimental group. 60 healthy male volunteers between 20 and 30 years of age were recruited in the Kuala Lumpur area for this study. Each volunteer provided written informed consent. The ethics approval for this study was obtained from the Universiti Kebangsaan Malaysia Medical Research Ethics Committee (reference no.: UKM 1.5.3.5/244/PPP2).

The volunteers underwent a pre-study screening test, which included a physical examination by a qualified medical officer, lung function test using a peak flow meter (Clement Clarke, UK), body fat analysis using a body fat monitor (Omron HBF-302, Japan), an electrocardiogram test (Mortara Rangoni, Italy), blood pressure measurement (Digital BP monitor, Model T8, Intellsense, Japan), and height and body weight measurement using the SECA (Germany).

Volunteers were excluded based on the following criteria: (i) taking antioxidant supplements (e.g. vitamin C, vitamin E, selenium, and carotenoids), other supplement use (performance enhancing or herbal-type products), and any medications that might interfere with the test; (ii) abnormal cholesterol, triglyceride, or glucose levels, anaemia, haemoglobin level < 12, systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≤ 90 mmHg; (iii) drinking alcohol, smoking, or being a vegetarian or having other restrictive dietary requirements; or (iv) presence of a medical illness such as cardiovascular disease, respiratory problems, cancer, and diabetes mellitus.

Volunteers underwent a symptom-limited maximal exercise test to confirm their sedentary status, as recommended by Wasserman et al. [12]. The subject was classified as sedentary if their measured peak oxygen uptake ($\dot{V}O_2$) was less than the predicted peak $\dot{V}O_2$ or if they performed any physical activity 1 or 2 days per week for less than 20 min each time.

Determination of $\dot{V}O_2$peak

The $\dot{V}O_2$peak was determined with a 1-min incremental exercise protocol using a cycle ergometer (Ergometrics 900, Ergoline, Germany), as previously described [12]. Subjects exercised on a cycle ergometer while measurements of gas exchange data were obtained breath-by-breath using the Cortex Metamax 3B (Germany). Heart rate was recorded using a heart rate monitor (Polar Electro, Inc., Woodbury, NY, USA). Briefly, subjects were requested to cycle for 3 min with unloaded pedalling at the beginning. An increment was then added at the start of each minute. The subject was encouraged to continue and maintain the cycling frequency between 50 and 60 revolutions per minute (rpm) until volitional exhaustion (i.e., the subject could not sustain the workload for a period of > 30 s), when the cycling frequency could not be maintained, or when the subject decided to terminate the incremental exercise. A respiratory exchange ratio > 1.1 was used as an additional criterion that maximal O_2 uptake had been reached. The results were used to estimate a workload corresponding to 70, 60, and 50% of $\dot{V}O_2$peak for each subject based on the regression line of the O_2 uptake compared to the workload from the incremental exercise test. The subjects were classified as sedentary if the measured peak $\dot{V}O_2$ was less than the predicted peak $\dot{V}O_2$.

Exercise schedule and haematological profile

All the maximal exercise test and exercise sessions were performed in a temperature-controlled room (21–23 °C). The test workload that corresponded to their relative exercise intensity (%$\dot{V}O_2$peak) and the exercise target heart rate (HR_{test}) was determined based on the results of the maximal exercise test. HR_{max} during maximal graded exercise testing was used. The HR_{test} for each subject was calculated using HR_{max} formula (HR_{test} = exercise intensity $\times HR_{max} \times 1.15$). The $\dot{V}O_2$ and HR were monitored each minute to ensure that the subject was exercising at the given intensity.

The exercise task consisted of a 3-min warm-up on a cycle ergometer, immediately followed by the exercise tests at the given workload. Subjects cycled with the estimated workload for a given duration while $\dot{V}O_2$ and HR were measured using the Metamax 3B (Cortex, Germany) and a polar heart monitor, respectively. Gas exchange data were obtained breath-by-breath and were recorded. Calibration of the system was performed before each test. The subjects

were encouraged to continue and maintain their HR at the HR_{test} for the given duration.

To ensure that the observation represents the differences resulting from the effect of different exercise intensity and duration instead of the training effects, the subjects were given at least 3 days of rest after the maximal exercise test, and between the exercise tests. Subjects were required to maintain their activity level throughout the experimental period. The subjects were requested to refrain from exhaustive physical activity, eating, and drinking for at least 2 h prior to exercise and to dress in attire suitable for all tests including the maximal exercise test.

This was a repeated measures study, where each subject acted as their own control, and all groups underwent pre- and post-testing, with no control group exposed to the intervention. Therefore, qualified subjects visited the lab 11 times inclusive of the maximal oxygen uptake test. During visits 3–11, subjects were asked to cycle for 10, 20, and 30 min on a cycle ergometer at the given workload that corresponded to 50, 60, and 70% of their $\dot{V}O_2$peak (individually) in a random order, and the tests were conducted at the same time of the day.

Peripheral blood samples were obtained from the forearm vein of each subject using a BD Vacutainer® tube coated with K_2EDTA before and immediately after each exercise session. A full blood cell count, haemoglobin, and haematocrit values were obtained using an automated cell counter (Becker Coulter, Coulter Gen_s Sys, USA). Cell counts were adjusted for changes in plasma volume according to the methods of Dill and Costill [13].

Table 1 Subject characteristics ($N=25$)

	Mean ± SE	Skewness ± SE
Age (years)	20.4 ± 0.32	0.73 ± 0.464
Body mass (kg)	58.8 ± 2.39	0.83 ± 0.464
Body mass index (kg/m^2)	20.5 ± 0.64	0.41 ± 0.464
Absolute value of measured $\dot{V}O_2$ peak (ml/min)	2152.3 ± 70.52*	− 0.926 ± 0.464
Absolute value calculated $\dot{V}O_2$ peak (ml/min)	2771.1 ± 60.68	− 0.172 ± 0.464
Relative value measured $\dot{V}O_2$ peak (ml/min/kg)	36.6 ± 1.17*	− 0.023 ± 0.464
Relative value calculated $\dot{V}O_2$ peak (ml/min/kg)	48.1 ± 1.10	− 0.702 ± 0.464

*Difference between measured and calculated $\dot{V}O_2$ peak significant at $p<0.05$

Table 2 Changes in hematocrits, red blood cell and haemoglobin concentrations after cycling at 50, 60, and 70% $\dot{V}O_2$ peak for 10, 20, and 30 min

Intensity (% $\dot{V}O_2$ peak)	Duration (min)	Hematocrits (% total blood volume) Mean ± SE		Red blood cell concentration (× 10^3 cells/µl) Mean ± SE		Haemoglobin (g/dl) Mean ± SE	
50	10	Pre	0.46 ± 0.005	Pre	5.44 ± 0.098	Pre	15.42 ± 0.207
		Post	0.47 ± 0.006*	Post	5.52 ± 0.509	Post	15.66 ± 1.081
60	10	Pre	0.47 ± 0.006	Pre	5.49 ± 0.124	Pre	15.51 ± 0.188
		Post	0.48 ± 0.006*	Post	5.61 ± 0.566	Post	16.01 ± 1.038
70	10	Pre	0.47 ± 0.006	Pre	5.46 ± 0.100	Pre	15.48 ± 0.194
		Post	0.49 ± 0.006*	Post	5.71 ± 0.509	Post	16.16 ± 0.931
50	20	Pre	0.45 ± 0.006	Pre	5.30 ± 0.090	Pre	15.12 ± 0.198
		Post	0.47 ± 0.007*	Post	5.44 ± 0.091	Post	15.50 ± 0.223
60	20	Pre	0.46 ± 0.005	Pre	5.34 ± 0.081	Pre	15.15 ± 0.188
		Post	0.48 ± 0.007*	Post	5.58 ± 0.106	Post	15.89 ± 0.214
70	20	Pre	0.47 ± 0.006	Pre	5.44 ± 0.115	Pre	15.47 ± 0.209
		Post	0.50 ± 0.006*	Post	5.73 ± 0.117	Post	16.37 ± 0.227
50	30	Pre	0.46 ± 0.006	Pre	5.39 ± 0.117	Pre	15.29 ± 0.161
		Post	0.47 ± 0.006*	Post	5.53 ± 0.101	Post	15.73 ± 0.170
60	30	Pre	0.45 ± 0.007	Pre	5.25 ± 0.112	Pre	15.10 ± 0.196
		Post	0.48 ± 0.007*	Post	5.53 ± 0.099	Post	15.91 ± 0.205
70	30	Pre	0.46 ± 0.006	Pre	5.44 ± 0.106	Pre	15.60 ± 0.187
		Post	0.48 ± 0.007*	Post	5.64 ± 0.125	Post	16.19 ± 0.234

$N=25$; $df=24$

*Significantly different from baseline at $p<0.05$

Statistical analyses

All data are expressed as the mean ± standard error (SE). Data were tested for normality of the distribution using skewness, and the data sets showed a normal distribution. Therefore, further analysis was performed using parametric tests. Analysis of variance (ANOVA) with repeated measures and paired *t* tests were performed for all exercise sessions, with time (pre- and post-exercise), exercise intensity, and exercise duration as the within-subject factors. Homogeneity of covariance or sphericity assumption of the data was verified using the Mauchly's test.

Because the sample size for each factor level was equal, the concern about homogeneity of the variance was eliminated. When significant interactions for intensity by duration over time occurred, the simple main effects of intensity and duration were assessed using paired *t* tests. Pearson's correlation coefficient was used to express the relationship between all parameters measured for the exercise intensity and duration. A probability level of 0.05 was set for significance. All statistical analysis was performed using the Statistical Packages for Social Sciences (IBM, USA).

Results

After a pre-study screening test, physical activity level analysis, and medical screening by a medical doctor, a total of 11 participants were excluded. Only 49 participants were eligible for the next screening phase, which is the maximal exercise test. After further assessment, 31 volunteers remained and were confirmed to have sedentary status, qualifying as a subject in this study.

Table 3 Leucocyte count after cycling at 50, 60, and 70% $\dot{V}O_2$ peak for 10, 20, and 30 min

Intensity (% $\dot{V}O_2$peak)	Duration (min)	Leucocytes ($\times 10^3$ cells/μl)		Repeated measures ANOVA				% changes (mean ± SE)
		Status	Mean ± SE	$I \times D \times T$	I	D	T	
50	10	Pre	7.41 ± 0.394	$F = 0.58$	$F = 2.21$	40.45*	154.71*	8.6 ± 2.22
		Post	7.99 ± 0.456	$df = 3.23$	$df = 2.0$	$df = 1.4$	$df = 1.0$	
		Post_Adj	8.04 ± 0.457*	$p = 0.65$	$p = 0.12$	$p = 0.00$	$p = 0.00$	
60	10	Pre	6.71 ± 0.219					19.2 ± 2.79
		Post	7.95 ± 0.319					
		Post_Adj	7.98 ± 0.322*					
70	10	Pre	6.76 ± 0.228					26.9 ± 3.31
		Post	8.47 ± 0.304					
		Post_Adj	8.52 ± 0.310*					
50	20	Pre	6.76 ± 0.244					128.2 ± 32.94
		Post	7.21 ± 0.246					
		Post_Adj	16.08 ± 2.938*					
60	20	Pre	6.63 ± 0.285					131.7 ± 26.20
		Post	8.25 ± 0.354					
		Post_Adj	15.56 ± 1.734*					
70	20	Pre	6.97 ± 0.288					183.7 ± 16.73
		Post	9.29 ± 0.287					
		Post_Adj	19.16 ± 0.946*					
50	30	Pre	6.66 ± 0.347					233.1 ± 35.97
		Post	7.23 ± 0.360					
		Post_Adj	22.15 ± 2.555*					
60	30	Pre	6.41 ± 0.234					264.6 ± 36.40
		Post	7.57 ± 0.298					
		Post_Adj	22.61 ± 2.130*					
70	30	Pre	6.98 ± 0.258					309.8 ± 51.71
		Post	8.53 ± 0.242					
		Post_Adj	27.72 ± 3.166*					

I exercise intensity, *D* exercise duration, *Post_Adj* corrected for plasma volume changes using Dill and Costill (1971) method, $\dot{V}O_2$ *peak* peak oxygen consumption

*Significant at $p < 0.05$

This study used a repeated measures within-subject design (3 × 3 × 2) on a single experimental group, to determine the effect of exercise intensity and duration on leucocyte and subtype counts. Of the 31 subjects, only 25 subjects completed all nine cycling sessions at three different exercise durations (10, 20, and 30 min) and three exercise intensities in a random order. Exercise intensity was an individually defined workload that corresponded to 50, 60, and 70% of their $\dot{V}O_2$peak. The power in this study was $1-\beta=0.95$, calculated based on 25 subjects.

Table 1 shows the demographic data of 25 subjects, which were normally distributed. Therefore, subsequent data analysis was performed using a repeated measures ANOVA, paired *t* test, and Pearson's correlation. Paired *t* test analysis confirmed the sedentary status of the 25 subjects, as indicated by a measured $\dot{V}O_2$ peak that was significantly lower than the predicted $\dot{V}O_2$ peak for both relative (36.6 ± 1.17 vs. 48.1 ± 1.10 ml/min/kg) and absolute (2152.3 ± 70.52 vs. 2771.1 ± 60.68 ml/min) values (Table 1).

Since exercise was performed in a control cold room temperature (air-conditioned lab), this study was applying an exercise-induced hemoconcentration, and a hyperthermia. As indicated in Table 2, hemoconcentration occurred after cycling at 50, 60, and 70% $\dot{V}O_2$peak for 10, 20, and 30 min. Therefore, leukocytes and its subtype cell count were corrected for plasma volume changes using a method by Dill and Costill [13].

This study shows that mean total leucocyte count increased significantly ($p<0.05$) immediately after exercise at all intensities and durations (Table 3), including the neutrophil, lymphocyte, and monocyte subtypes

Table 4 Neutrophil cell count after cycling at 50, 60, and 70% $\dot{V}O_2$ peak for 10, 20, and 30 min

Intensity (% $\dot{V}O_2$peak)	Duration (min)	Neutrophils (× 10^3 cells/μl)		Repeated measures ANOVA				% changes (mean ± SE)
		Status	Mean ± SE	*I* × *D* × *T*	*I*	*D*	*T*	
50	10	Pre	4.08 ± 0.403	*F* = 0.35	*F* = 0.90	29.04*	98.75*	5.8 ± 1.71
		Post	4.31 ± 0.438	*df* = 3.1	*df* = 1.6	*df* = 1.5	*df* = 1.0	
		Post_Adj	4.34 ± 0.439*	*p* = 0.79	*p* = 0.40	*p* = 0.00	*p* = 0.00	
60	10	Pre	3.42 ± 0.185					13.6 ± 2.04
		Post	3.88 ± 0.240					
		Post_Adj	3.90 ± 0.242*					
70	10	Pre	3.44 ± 0.228					23.1 ± 5.65
		Post	4.08 ± 0.249					
		Post_Adj	4.10 ± 0.252*					
50	20	Pre	3.29 ± 0.177					135.2 ± 35.83
		Post	3.59 ± 0.200					
		Post_Adj	8.05 ± 1.501*					
60	20	Pre	3.50 ± 0.272					128.0 ± 27.01
		Post	4.28 ± 0.333					
		Post_Adj	8.17 ± 1.049*					
70	20	Pre	3.61 ± 0.229					156.5 ± 12.07
		Post	4.40 ± 0.260					
		Post_Adj	9.06 ± 0.609*					
50	30	Pre	3.53 ± 0.301					233.4 ± 36.90
		Post	3.82 ± 0.315					
		Post_Adj	12.00 ± 1.724*					
60	30	Pre	3.25 ± 0.194					258.4 ± 33.00
		Post	3.81 ± 0.240					
		Post_Adj	11.22 ± 1.025*					
70	30	Pre	3.59 ± 0.227					283.8 ± 44.21
		Post	4.16 ± 0.240					
		Post_Adj	13.68 ± 1.811*					

I exercise intensity, *D* exercise duration, *Post_Adj* corrected for plasma volume changes using Dill and Costill (1971) method, $\dot{V}O_2$ *peak* peak oxygen consumption

*Significant at $p<0.05$

(Tables 4, 5, 6). These findings were further supported by the repeated measures ANOVA analysis, which showed that the main effect of time (*T*) on leucocyte, neutrophil, lymphocyte, and monocyte counts was highly significant (Tables 3, 4, 5, 6).

Additionally, this study also observed that the overall leucocyte counts were increased across the duration of 10, 20, and 30 min with an exercise intensity of 50, 60, and 70% $\dot{V}O_2$peak (Fig. 1). When stratified to subtypes, similar data were obtained for the lymphocytes, monocytes, and neutrophils (Fig. 1). Moreover, Fig. 2 also demonstrates a similar pattern, where the percentage increment of leucocytes, neutrophils, lymphocytes, and monocytes increases with an increase in exercise intensity from 50 to 60 to 70% $\dot{V}O_2$peak at a 10-, 20-, or 30-min duration of exercise, respectively. However, the percentage increment of leucocytes, neutrophils, lymphocytes, and monocytes differed significantly from 50% only for the 10-min exercise duration, indicating that there was no significant difference in the percentage increment of leucocyte, neutrophil, lymphocyte, and monocyte cells count between the intensity after exercising for 20- and 30-min durations.

The ANOVA analysis showed that the intensity and duration are independent factors that caused an increase in overall leucocyte counts and its subtypes (Table 3). However, there was no significant evidence to support that the interaction of intensity and duration with the elevation in the overall leucocyte counts, including the subtypes ($p > 0.05$). Similarly, the main effect of exercise intensity on leucocytes and neutrophils was also not significant, but it had a significant effect on lymphocyte and monocyte counts. However, the main effect of exercise duration was significant on leucocyte,

Table 5 Lymphocyte cell count after cycling at 50, 60, and 70% $\dot{V}O_2$ peak for 10, 20, and 30 min

Intensity (% $\dot{V}O_2$ peak)	Duration (min)	Lymphocytes ($\times 10^3$ cells/μl)		Repeated measures ANOVA				% changes (mean ± SE)
		Status	Mean ± SE	*I*×*D*×*T*	*I*	*D*	*T*	
50	10	Pre	2.39 ± 0.097	*F* = 1.08	*F* = 4.95*	43.40*	166.74*	13.8 ± 4.00
		Post	2.66 ± 0.099	*df* = 4.0	*df* = 2.0	*df* = 1.5	*df* = 1.0	
		Post_Adj	2.68 ± 0.101*	*p* = 0.37	*p* = 0.01	*p* = 0.00	*p* = 0.00	
60	10	Pre	2.39 ± 0.088					28.5 ± 5.15
		Post	3.00 ± 0.111					
		Post_Adj	3.01 ± 0.111*					
70	10	Pre	2.40 ± 0.108					41.9 ± 6.45
		Post	3.30 ± 0.134					
		Post_Adj	3.31 ± 0.136*					
50	20	Pre	2.57 ± 0.095					122.8 ± 30.48
		Post	2.70 ± 0.091					
		Post_Adj	5.90 ± 1.029*					
60	20	Pre	2.26 ± 0.096					141.1 ± 27.75
		Post	2.95 ± 0.153					
		Post_Adj	5.56 ± 0.668*					
70	20	Pre	2.43 ± 0.104					231.8 ± 23.90
		Post	3.73 ± 0.151					
		Post_Adj	7.77 ± 0.504*					
50	30	Pre	2.29 ± 0.086					238.2 ± 35.36
		Post	2.54 ± 0.102					
		Post_Adj	7.57 ± 0.751*					
60	30	Pre	2.34 ± 0.093					278.2 ± 42.08
		Post	2.84 ± 0.128					
		Post_Adj	8.61 ± 1.042*					
70	30	Pre	2.47 ± 0.109					364.4 ± 68.81
		Post	3.32 ± 0.143					
		Post_Adj	10.73 ± 1.270*					

I exercise intensity, *D* exercise duration, *Post_Adj* corrected for plasma volume changes using Dill and Costill (1971) method, $\dot{V}O_2$ *peak* peak oxygen consumption

*Significant at $p < 0.05$

neutrophil, lymphocyte, and monocyte counts, which means that the increase in leucocyte, neutrophil, lymphocyte, and monocyte counts is dependent of exercise duration.

Pearson's correlation coefficient analysis showed that the overall leucocyte ($r=0.585$), neutrophil ($r=0.599$), lymphocyte ($r=0.539$), and monocyte ($r=0.596$) counts were significantly and positively correlated with the exercise duration (Table 7). However, only lymphocytes were positively correlated ($r=0.178$) with exercise intensity.

Discussion

Leucocytosis was the most obvious and consistent change that occurred during or after exercise. This study revealed that the overall leucocyte counts and that of its subtypes were significantly higher after a short duration and a moderate-exercise intensity among healthy and sedentary adults. This finding was consistent with previous studies where the number of leucocytes was increased in a group of children and in young male subjects after exercise was performed [7, 14].

The strongest point of the present study is that it is the first to analyse the effects of exercise intensity and duration on leucocyte counts and that of its subtypes in a systematic manner, and bias was minimised by having each subject act as their own control. The current study, which used moderate intensity (50–70% $\dot{V}O_2$peak) and short duration (10–30 min), revealed the percent increase of leucocyte counts and that of its subtypes were dependent on the exercise duration, but not dependent on intensity. Conversely, Pedersen and Hoffman-Goetz [15] reported that the changes in leucocyte counts and that of its

Table 6 Monocyte cell count after cycling at 50, 60, and 70% $\dot{V}O_2$ peak for 10, 20, and 30 min

Intensity (% $\dot{V}O_2$peak)	Duration (min)	Monocytes ($\times 10^3$ cells/μl)		Repeated measures ANOVA				% changes (mean ± SE)
		Status	Mean ± SE	*I*×*D*×*T*	*I*	*D*	*T*	
50	10	Pre	0.45 ± 0.033	*F* = 1.08	*F* = 4.95*	43.40*	166.74*	9.5 ± 3.57
		Post	0.47 ± 0.028	*df* = 4.0	*df* = 2.0	*df* = 1.5	*df* = 1.0	
		Post_Adj	0.48 ± 0.028*	*p* = 0.37	*p* = 0.01	*p* = 0.00	*p* = 0.00	
60	10	Pre	0.39 ± 0.020					14.8 ± 3.32
		Post	0.45 ± 0.029					
		Post_Adj	0.45 ± 0.029*					
70	10	Pre	0.39 ± 0.018					24.4 ± 4.83
		Post	0.47 ± 0.022					
		Post_Adj	0.48 ± 0.022*					
50	20	Pre	0.41 ± 0.017					116.2 ± 28.99
		Post	0.42 ± 0.020					
		Post_Adj	0.91 ± 0.161*					
60	20	Pre	0.38 ± 0.015					140.9 ± 28.24
		Post	0.48 ± 0.022					
		Post_Adj	0.88 ± 0.099*					
70	20	Pre	0.46 ± 0.028					156.5 ± 17.98
		Post	0.54 ± 0.023					
		Post_Adj	1.10 ± 0.056*					
50	30	Pre	0.40 ± 0.027					221.4 ± 33.05
		Post	0.42 ± 0.022					
		Post_Adj	1.23 ± 0.120*					
60	30	Pre	0.39 ± 0.016					257.0 ± 36.93
		Post	0.45 ± 0.021					
		Post_Adj	1.33 ± 0.126*					
70	30	Pre	0.40 ± 0.018					286.4 ± 42.47
		Post	0.48 ± 0.027					
		Post_Adj	1.48 ± 0.143*					

I exercise intensity, *D* exercise duration, *Post_Adj* corrected for plasma volume changes using Dill and Costill (1971) method, $\dot{V}O_2peak$ peak oxygen consumption

*Significant at $p < 0.05$

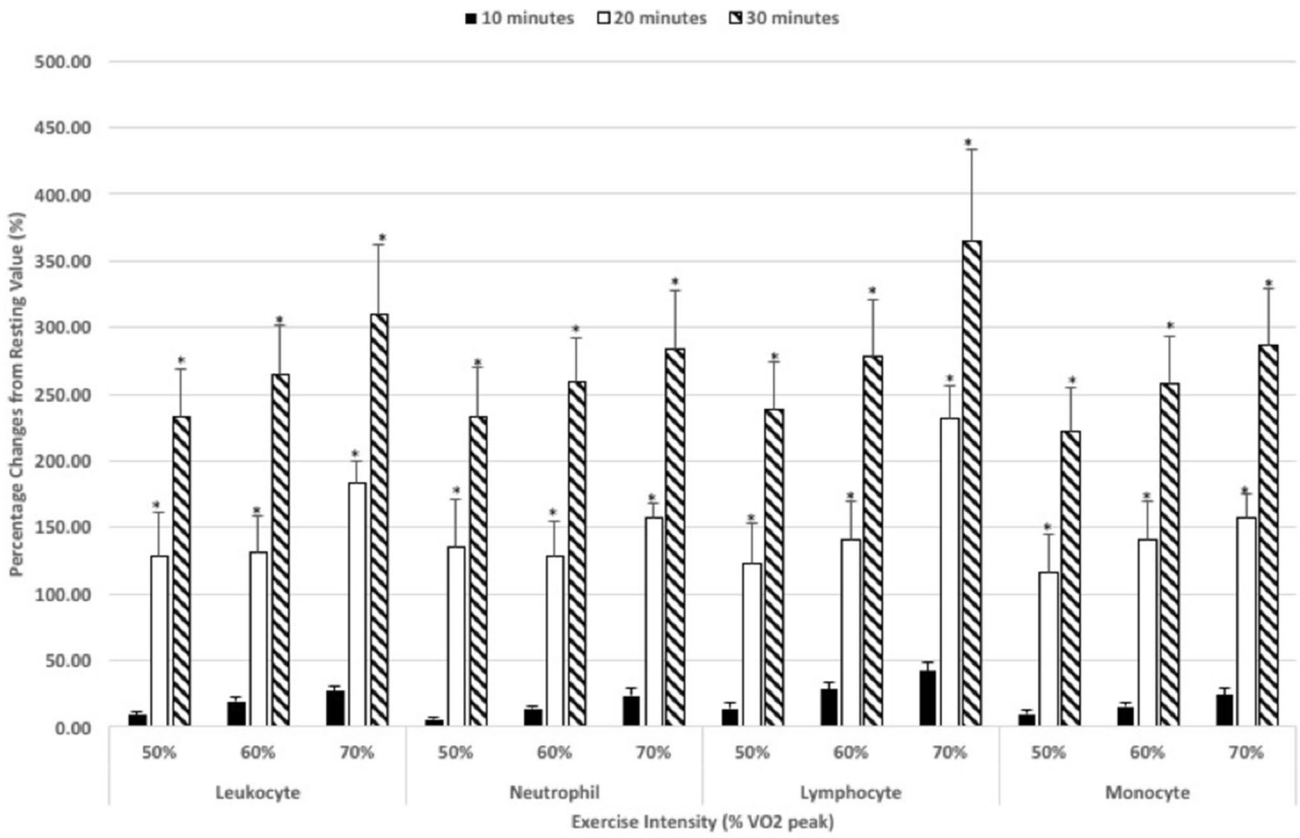

Fig. 1 Percentage changes of total leucocytes, neutrophils, lymphocytes, and monocytes (% resting value) with exercise duration after exercising at 50, 60, and 70% $\dot{V}O_2$ peak. *Significantly different from 10 min ($p < 0.05$); Mean ± SEM; $N = 25$

subtypes are dependent on the intensity and duration of the exertion. A study by Neves et al. [7] revealed that increase in leucocyte counts was higher after high-intensity exercise (80% $\dot{V}O_2$peak) compared with low-intensity exercise (40% $\dot{V}O_2$peak).

Additionally, our results demonstrated that the acute effect of moderate-intensity exercise for a short duration on leucocyte counts is neutrophil, lymphocyte, and monocyte dependent. A previous study also showed that exercise can elevate the circulating neutrophil levels and this increase was directly proportional to the intensity and duration of exercise [16]. Moreover, Hack et al. [17] also showed that heavy exercise increased the number of leucocytes, lymphocytes, monocytes, and neutrophils in athletes. Thus, these findings indicate that exercise can elevate leucocyte counts and that of its subtypes in the blood.

Fehrenbach and Schneider [18] reported that hyperthermia, hypoxia, oxidative stress, hormonal stress, and mechanical stress resulting from exercise could also increase cytokine secretion that may induce an immune response and subsequently elevate leucocyte counts in the blood. However, because the increase in leucocytes after exercise in this study did not exceed the normal range of 12×10^{-3}/µl, the response demonstrated in this study was not an overall inflammation response. The increase in leucocyte counts and that of its subtypes in this study could be caused by demargination, which occurs in response to adrenaline and noradrenaline hormonal actions [8].

Exercise stimulates the sympathetic nervous system by releasing the adrenaline and noradrenaline. Foster et al. [19] reported that the increase in sympathetic activity subsequently increases the heart's output and the speed of blood flow, which may contribute to leucocytosis. Moreover, the secreted adrenaline and noradrenaline may trigger vasoconstriction and venoconstriction. Vasoconstriction increases the blood flow to skeletal muscles and lungs while venoconstriction allows more blood to flow from the peripheral veins into the main circulation [20]. Both conditions cause leucocyte, lymphocyte, neutrophil, and monocyte cells to leave the marginal pool, thereby elevating their counts in the blood [15].

The increase in leucocyte count and that of its subtypes after an exercise has been reported to be beneficial against infection or inflammation [21]. The number of leucocytes and that of its subtypes that circulated in the blood after the exercise in this study remained within the normal

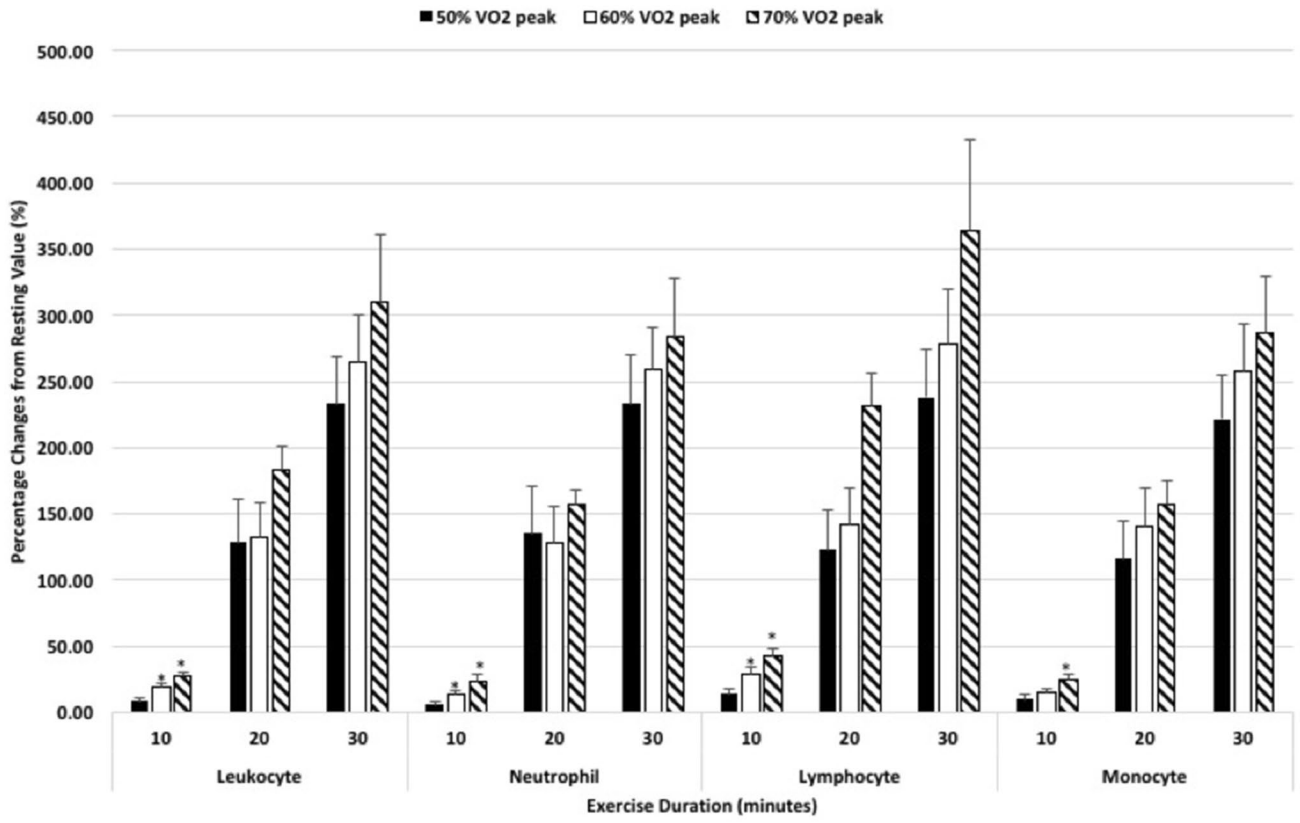

Fig. 2 Percentage changes of total leucocytes, neutrophils, lymphocytes, and monocytes (% resting value) with exercise intensity after exercising for 10, 20, and 30 min. *Significantly different from 50% $\dot{V}O_2$ peak ($p < 0.05$); Mean ± SEM; $N = 25$

Table 7 Correlation between the percentage changes of leucocytes and its subtypes with the intensity and duration of exercise

	Correlation coefficient (r)			
	Leucocytes	Neutrophils	Lymphocytes	Monocytes
Intensity	0.117	0.073	0.178*	0.096
Duration	0.585*	0.599*	0.539*	0.596*

*Statistically significant ($p < 0.05$)

range, indicating that exercise at a moderate intensity (50, 60, and 70%) for a short duration of 10–30 min could trigger the immune response and enhance the efficiency of the body's immune system. However, a prolonged resistance force exercise or repetitive heavy exercise could cause inflammation and that could disrupt the efficiency of the body's immune system as well as cause an increase in blood viscosity, which has been associated with several health disorders including cardiovascular diseases and atherogenesis [2–4].

We also found that leucocyte counts and that of its subtypes increased more than 200% following continuous exercise for 30 min at all intensities, suggesting that there is a lower risk of heart attack when sedentary subjects in our study exercised for less than 30 min at a moderate intensity. Similarly, a significant increase in leucocytes was also found among endurance and sprint athletes [22–26]. A previous study also reported that the number of leucocytes is increased even 3 h after exercise [7]. Nevertheless, leucocytosis is temporary among highly trained male adults, with a subsequent drop 1–3 h after exercise [22].

Leucocytosis led to an increase in blood viscosity after exercise, which is associated with a risk of sudden cardiac death [27]. As the blood becomes more viscous, it will disrupt blood flow to the heart [28]. Thus, exercise can sometimes increase the risk of a heart attack during and after exercise [29, 30], which can lead to sudden cardiac arrest [31]. This will increase the mortality risk with exercise among sedentary individuals, older people, and patients with chronic diseases especially heart disease patients. Additionally, non-continuous exercise, such as 10-min interval training at a moderate intensity, will provide the same benefit as continuous exercise [32, 33]. Therefore, the duration and intensity of exercise should be prescribed accurately, systematically, and specifically according to the individual's capacity.

Conclusion

In conclusion, a precise definition of the individual workload is important in exercise prescription to enhance the immune response in the body and to prevent development of health disorders because of a prolonged increase in blood viscosity. The findings of this study strongly suggest that current views about the intensity and duration of exercise should be re-evaluated, and that the data are beneficial for establishing a putative protective threshold for exercise.

Acknowledgements We would like to thank all the volunteers who participated in this study. This study was supported by the Universiti Kebangsaan Malaysia under the UKM Fundamental Research Grant Scheme (Grant No.: FF-050-2004) and the Ministry of Higher Education, Malaysia (Grant No.: TRGS0004-SG-12/2014).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Ethical approval The ethical approval of this study was obtained from the National Medical Research Register with reference no.: NMRR-16-38-28777(IIR) and Universiti Kebangsaan Malaysia Medical Research Ethics Committee, with reference no.: UKM 1.5.3.5/244/PPP2.

Informed consent All participants provided written informed consent to participate in this study.

References

1. Sand KL, Flatebo T, Andersen MB, Maghazachi AA (2013) Effects of exercise on leukocytosis and blood hemostasis in 800 healthy young females and males. World J Exp Med 3:11–20
2. Lowe GDO (1993) Blood viscosity and cardiovascular risk. Curr Opin Lipidol 4:283
3. Li J, Flammer AJ, Reriani MK, Matsuo Y, Gulati R, Friedman PA, Thomas RJ, Sandhu NP, Lerman LO, Lerman A (2013) High leukocyte count is associated with peripheral vascular dysfunction in individuals with low cardiovascular risk. Circulation J 77:780–785
4. Kim JH, Lim S, Park KS, Jang HC, Choi SH (2017) Total and differential WBC counts are related with coronary artery atherosclerosis and increase the risk for cardiovascular disease in Koreans. PLoS One 12:e0180332
5. Gabriel H, Kindermann W (1997) The acute immune response to exercise: what does it mean? Int J Sports Med 18:S28–S45
6. Abdossaleh Z, Fatemeh A, Frozan K, Mohhammad Amin S (2014) Leukocytes subsets is differentially affected by exercise intensity. Int J Sport Stud 4:246–253
7. Neves PRDS, Tenório TRDS, Lins TA, Muniz MTC, Pithon-Curi TC, Botero JP, Do Prado WL (2015) Acute effects of high- and low-intensity exercise bouts on leukocyte counts. J Exerc Sci Fit 13:24–28
8. McCarthy DA, Dale MM (1988) The leucocytosis of exercise. A review and model. Sports Med 6:333–363
9. van Eeden SF, Granton J, Hards JM, Moore B, Hogg JC (1999) Expression of the cell adhesion molecules on leukocytes that demarginate during acute maximal exercise. J Appl Physiol 86:970–976
10. Tuan TC, Hsu TG, Fong MC, Hsu CF, Tsai KK, Lee CY, Kong CW (2007) Deleterious effects of short-term, high-intensity exercise on the immune function: evidence from leukocyte mitochondrial alternations and apoptosis. Br J Sports Med 42:11–15
11. Pescatello LS, Arena R, Riebe D, Thompson PD (2014) ACSM's guidelines for exercise testing and prescription, 9th edn. Lippincottt Williams & Wilkins, Philadelphia
12. Wasserman K, Hansen JE, Sietsema K, Sue DY, Stringer WW, Whipp B, Sun XG (2015) Principles of exercise testing and interpretation: including pathophysiology and clinical applications, 5th edn. Wolters Kluwer Health, Philadelphia
13. Dill DB, Costill DL (1974) Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. J Appl Physiol 37:247–248
14. Perez CJ, Nemet D, Mills PJ, Scheet TP, Ziegler MG, Cooper DM (2001) Effects of laboratory versus field exercise on leukocyte subsets and cell adhesion molecule expression in children. Eur J Appl Physiol 86:34–39
15. Pedersen BK, Hoffman-Goetz L (2000) Exercise and the immune system: regulation, integration, and adaptation. Physiol Rev 80:1055–1081
16. Pyne DB (1994) Regulation of neutrophil function during exercise. Sports Med 17:245–258
17. Hack V, Strobel G, Rau JP, Weicker H (1992) The effect of maximal exercise on the activity of neutrophil granulocytes in highly trained athletes in a moderate training model. Eur J Appl Physiol Occup Physiol 65:520–524
18. Fehrenbach E, Schneider ME (2006) Trauma-induced systemic inflammatory response versus exercise-induced immunomodulatory effects. Sports Med 36:373–384
19. Foster NK, Martyn JB, Rangno RE, Hogg JC, Pardy RL (1986) Leukocytosis of exercise: role of cardiac output and catecholamines. J Appl Physiol 61:2218–2223
20. Hall JE (2016) Guyton and Hall textbook of medical physiology, 13th edn. Elsevier, Philadelphia
21. Fehrenbach E (2005) Cellular responses to environmental stress. In: Mooren FC, Volker K (eds) Molecular and cellular exercise physiology. Human Kinetic, Champaign, pp 199–217
22. Avloniti AA, Douda HT, Tokmakidis SP, Kortsaris AH, Papadopoulou EG, Spanoudakis EG (2007) Acute effects of soccer training on white blood cell count in elite female players. Int J Sports Physiol Perform 2:239–249
23. Cipryan L, Tschakert G, Hofmann P (2017) Acute and post-exercise physiological responses to high-intensity interval training in endurance and sprint athletes. J Sports Sci Med 16(2):219–229
24. Gabriel H, Brechtel L, Urhausen A, Kindermann W (1994) Recruitment and recirculation of leukocytes after an ultramarathon run: preferential homing of cells expressing high levels of the adhesion molecule LFA-1. Int J Sports Med 15:S148–S153
25. Nielsen HG, Lyberg T (2004) Long-distance running modulates the expression of leucocyte and endothelial adhesion molecules. Scand J Immunol 60(4):356–362
26. Žákovská A, Knechtle B, Chlíbková D, Miličková M, Rosemann T, Nikolaidis PT (2017) The effect of a 100-km ultra-marathon under freezing conditions on selected immunological and hematological parameters. Front Physiol 8:638
27. Hitosugi M, Kawato H, Nagai T, Ogawa Y, Niwa M, Iida N, Yufu T, Tokudome S (2004) Changes in blood viscosity with heavy and light exercise. Med Sci Law 44:197–200
28. Sloop GD (2017) Blood viscosity: its role in cardiovascular pathophysiology and hematology. Nova Science Publishers, New York
29. Morris JN, Clayton DG, Everitt MG, Semmence AM, Burgess EH (1990) Exercise in leisure time: coronary attack and death rates. Heart 63:325–334

30. Lowe GDO, Lee AJ, Rumley A, Price JF, Fowkes FGR (1997) Blood viscosity and risk of cardiovascular events: the Edinburgh Artery Study. Br J Haematol 96:168–173
31. Josephson ME (2014) Sudden cardiac arrest. Ind Heart J 66:S2–S3
32. Burgomaster KA, Howarth KR, Phillips SM, Rakobowchuk M, MacDonald MJ, McGee SL, Gibala MJ (2008) Similar metabolic adaptations during exercise after low volume sprint interval and traditional endurance training in humans. J Physiol 586:151–160
33. Guimarães GV, Ciolac EG, Carvalho VO, D'Avila VM, Bortolotto LA, Bocchi EA (2010) Effects of continuous vs. interval exercise training on blood pressure and arterial stiffness in treated hypertension. Hypertens Res 33:627–632

View publication stats