

Acute Effects of Prolonged Endurance Training with Moderate Intensity on Hematological Parameters among Young Men

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Abstract: Running is a popular type of endurance exercise, but clinical evidence of its health benefits and acute consequences is lacking. Previous studies focus on muscle functions, and only a few studies examine hematological parameters. The current study investigates the acute effects of prolonged endurance training on hematological parameters. This is a quasi-repeated measures design; ten healthy males were voluntarily involved (19 ± 1.00 yrs, BMI 23.0 ± 2.50). The protocol was approved by the institutional health research ethics committee. The 10 Km distance must be accomplished with an intensity of 70-80% of MHR. Venous blood samples were collected before, 1-d, and 2-d post-running. Cyanide-free hemoglobin spectrophotometry was used to measure Hb and electrical impedance for erythrocytes, MCV, MCH, MCHC, and RDW. One-way repeated measures ANOVA was performed to elucidate the differences in means with $p < 0.05$. All data are expressed as mean $\pm$ SEM. Hb declined at 1-d (15.93 ± 0.35), significant at 2-d (15.64 ± 0.51) from pre-running (16.30 ± 0.38) as $p < 0.05$. Erythrocytes declined at 1-d (5.54 ± 0.34), remarkable at 2-d (5.42 ± 0.39) from pre-running (5.67 ± 0.37). There were no differences in changes in MCV, pre-running (82.21 ± 5.42), 1-d (82.14 ± 5.40), and 2-d (82.77 ± 5.39). The changes in MCH were not significant in pre-running (28.85 ± 1.86), 1-d (28.86 ± 1.87), 2-d (28.96 ± 1.87), as well as in MCHC, pre-running (35.13 ± 0.44), 1-d (35.11 ± 0.38), 2-d (34.98 ± 0.29). Remarkably, RDW was increased both at 1-d (13.17 ± 0.80) and at 2-d (13.13 ± 0.67) from pre-running (13.07 ± 0.77) as $p < 0.05$. We conclude that prolonged endurance training significantly affects changes in Hb and erythrocyte levels and RDW in young healthy males. Prolonged endurance training can considerably induce pseudo anemia at 48 h post-workout and initiate the inflammation process. This phenomenon is considered as a normal physiological adaptation of training.

Keywords: physical activity, pseudo anemia, delayed-onset muscle soreness.

中等强度长时间耐力训练对青年男性血液学参数的急性影响

摘要：跑步是一种流行的耐力运动，但缺乏其健康益处和急性后果的临床证据。以前的研究侧重于肌肉功能，只有少数研究检查血液学参数。目前的研究调查了长时间耐力训练对血液学参数的急性影响。这是一个准重复测量设计；十名健康男性自愿参与（ 19 ± 1.00 岁，

体重指数 23.0 ± 2.50)。该协议得到了机构健康研究伦理委员会的批准。必须以最大心率的70-80%的强度完成10公里的距离。在跑步前、跑步后1天和2天收集静脉血样。使用无氰化物血红蛋白分光光度法测量红细胞的血红蛋白和电阻抗、平均红细胞体积、平均红细胞血红蛋白、平均红细胞血红蛋白浓度和红细胞分布宽度。进行单向重复测量方差分析以阐明均值差异, $p < 0.05$ 。所有数据均表示为平均值 $\pm$ 结构方程建模。血红蛋白在第1天(15.93 ± 0.35)下降, 在第2天(15.64 ± 0.51)比运行前(16.30 ± 0.38)显著下降, $p < 0.05$ 。红细胞在1-d (5.54 ± 0.34)下降, 在2-d (5.42 ± 0.39)与预运行(5.67 ± 0.37)相比显著下降。平均红细胞体积、跑前(82.21 ± 5.42)、1-d (82.14 ± 5.40)和2-d (82.77 ± 5.39)的变化没有差异。平均红细胞血红蛋白在跑前 (28.85 ± 1.86)、1-d (28.86 ± 1.87)、2-d (28.96 ± 1.87)以及平均红细胞血红蛋白浓度、跑前 (35.13 ± 0.44), 1-d (35.11 ± 0.38), 2-d (34.98 ± 0.29)。值得注意的是, 红细胞分布宽度在运行前(13.07 ± 0.77)的第1天(13.17 ± 0.80)和第2天(13.13 ± 0.67)均有所增加, $p < 0.05$ 。我们得出结论, 长时间的耐力训练会显著影响年轻健康男性的血红蛋白和红细胞水平以及红细胞分布宽度的变化。长时间的耐力训练可以在锻炼后48小时显示出假性贫血并引发炎症过程。这种现象被认为是训练的正常生理适应。

关键词：体力活动、假性贫血、迟发性肌肉酸痛。

Introduction

Health conditions can be improved by engaging in physical activities. In adults aged 40-65 years, regular physical activity maintains their muscle strength, balance, and endurance [1]. Specific physical training programs could be created with specific aims related to physical fitness components. Endurance training with intensity between 65-75% of maximum heart rate (MHR), 40-50 min, 3-4 days/week, 26-40 weeks can improve serum lipids, glycemic control, body composition, and overall physical fitness [2, 3]. Furthermore, an endurance training program for the elderly effectively decreased the unified parkinson's disease rating scale part III scores [4].

Running as a model of simple endurance training has been the favorite workout among the urban population. Long-term effects of running could improve muscle function, postural balance, bone mineral density, and cardiac adaptations (which might also enhance cardiovascular health) in adult women, potentially lowering the risk of falls and fractures [5]. Additionally, from psychological benefits, running could improve mental and emotional health including relief of tension, improved self-image, and better mood. Thus, it is suggested to be used as a

psychotherapy treatment [6].

The chronic effects of training have been well documented and show beneficial advantages for overall health status. People who engage in running programs are believed to have a longer life expectancy compared to the general population. However, a previous study showed that during the workout session, there is an association between body adaptations and sudden cardiac attack [7].

All heavy sports have risks of injury, including long-distance running. All runners have the risk of being injured. Unluckily, novice runners are believed to be the most vulnerable to injury compared to other runners [8]. Moreover, running-related musculoskeletal injuries such as medial tibia stress syndrome, Achilles tendinopathy, and plantar fasciitis are common cases in runners, and ultramarathon runners have the highest risk of Achilles tendinopathy and patellofemoral syndrome [9].

From different forms of endurance training, a long-term aquatic training program (28 weeks) increased remarkably the mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and hemoglobin (Hb) in the elderly [10]. Furthermore, a chronic effects study (pre-season phase) stated that there are relationships between blood

biomarkers and fitness status in professional soccer players.

The adaptative changes in blood parameters can be considered as changes in the whole organism and metabolism after the training program. Thus, proper management of recovery can be warranted to diminish illness and injury risks and to follow the direction and dynamics of adaptative changes [11].

Adaptation is one of the main traits of individuals who engage in sports; it is the determination time to whether they can improve or fail. Everybody must remember about post-training sessions where the bodies need time to recover, and it is a crucial part of the whole training program. Recovery will suppress the risk of injuries by replenishing all the substances used during activity. It could be completely achieved by managing nutrition intake, hydration, and sleep [12, 13].

Based on the elucidation above, the chronic effects of endurance training have been documented and understood very well. However, there are still plenty of studies that investigate its acute effects on hematological parameters, whereas it is crucial as a key to coping with the crisis time during the adaptation period, and this study aims to investigate the acute effects of prolonged endurance training with moderate intensity on hematological parameters (anemia related variables) among young men to provide an additional understanding on sports medicine issues, so the prevention and care could be more appropriate.

1. Materials and Methods

1.1. Participants

The population in this study was young men in Banyumanik District, Indonesia. The purposive sampling technique was applied to obtain the subjects and ten young men (aged 17-25 years) were voluntarily registered as subjects in this study. The inclusion criteria are as follows: 1) Willing to be the subject of study and follow every step of protocol, 2) Not a smoker, and 3) Not under medical treatment. Meanwhile, the exclusion criteria are: 1) Hesitating to perform all processes and defecting as a volunteer, 2) An elite athlete, and 3) Under medical treatment.

1.2. Research Design

This is a quasi-experiment study with a one-group repeatedmeasures design. The independent variable in this study was prolonged endurance training with moderate intensity, and the dependent variables were hematological parameters including Hemoglobin (Hb), erythrocytes, mean corpuscular volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), and red blood cell distribution width (RDW).

1.3. Procedures

This research procedure is divided into three phases:

1) The initiation phase consists of subject recruitment, instrument selection and preparation, selection of treatment location, and explanation (to be clear about what is expected to be done and what is not) of all the study procedures to subjects and to data collectors;

2) Implementation phase, when the subjects' hydration status was warranted. Further, venous blood sampling was performed as a baseline sample from subjects. Afterwards, subjects were asked to perform 10 km as prolonged endurance training. Then, on the first day and second day post training, venous blood samples were drawn again (Fig. 1);

3) At last, the evaluation phase consisted of data tabulation, presentation, interpretation, discussion, and reporting. All procedures have been approved by the Institutional Health Research Ethics Committee of UNNES.

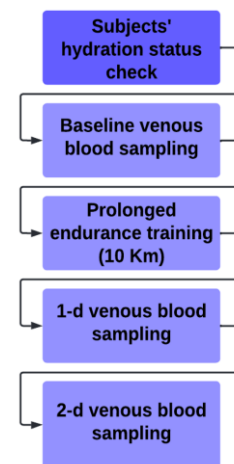


Fig. 1 The main processes of the study (Developed by the authors)

1.4. Prolonged Endurance Training with Moderate Intensity

Ten kilometers of continuous running was chosen as the prolonged endurance training. Moderate intensity was defined as 70-80% of maximum heart rate, and Xiaomi Mi Band 3 was used to track the subject's heart rate (to ensure all the subjects in their training zone). Training was performed outdoors at a public facility in Semarang. During the training, some data collectors were posted at several points to revive subjects. Subjects were asked to focus on finishing the whole distance rather than the finish time. Subjects were advised to take 7 days off from any heavy physical activity.

1.5. Data Collection

Venous blood sampling was collected before the endurance training and one and two days post training. Then, hematological parameters related to the anemia variables such as Hb, erythrocytes, MCV, MCH, MCHC, and RDW were measured from the venous

blood sample using Hematology Auto Analyzer (Zybio Z3 by Roche). The measurements for all variables were conducted at Klinik CITO by a professional laboratory analyst.

1.6. Statistical Analysis

To determine the acute effects of prolonged endurance training with moderate intensity on changes in hematological parameters (Hb, Erythrocyte, MCV, MCH, MCHC, and RDW), a one-way repeated measures ANOVA was used with a statistical level of $p < 0.05$. All data are presented as Mean $\pm$ SEM. All data analyses were carried out using IBM SPSS Statistics 26.

2. Results

Ten willing subjects aged eighteen to twenty years participated in this study, with body weights ranging from fifty-seven to seventy-one kilograms, heights ranging from one hundred sixty-one to one hundred seventy-two, and body mass indexes ranging from twenty point five to twenty-five point five kilograms per meter square.

2.1. Acute Effect of Endurance Training on Hemoglobin and Erythrocytes

Hemoglobin (gr/dl) decreased by ten kilometers as a form of endurance training both at one day after workout (15.93 ± 0.35), and remarkably at two days after (15.64 ± 0.51) compared to baseline data (16.30 ± 0.38), as $p < 0.05$ (Fig. 2). Hence, in this study, erythrocytes ($10^6/\mu\text{L}$) declined post endurance training both at one day post workout (5.54 ± 0.34), and significantly at two days (5.42 ± 0.39) compared to pre workout data (5.67 ± 0.37), as $p < 0.05$ (Fig. 3).

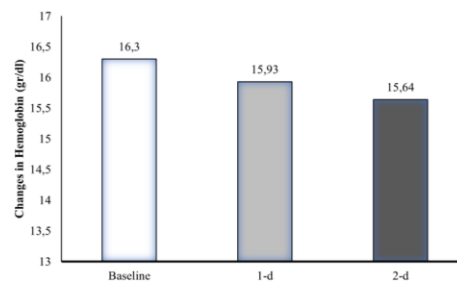


Fig. 2 Acute effect of endurance training on hemoglobin
(Developed by the authors)

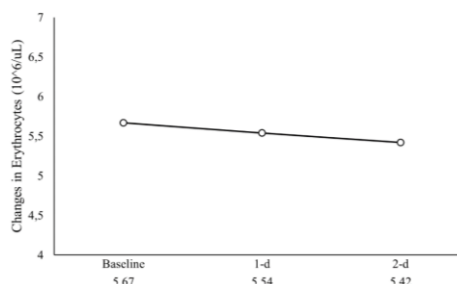


Fig. 3 Acute effect of endurance training on erythrocytes
(Developed by the authors)

2.2. Acute Erythrocytes Morphology Responses as Acute Effect of Endurance Training

Three variables of erythrocyte morphology, including MCV, MCH, and MCHC, were not significantly different compared to their baseline data (pre-endurance training). MCV baseline (82.21 ± 5.42) versus 1d post workout (82.14 ± 5.40); 2d (82.77 ± 5.39) as $p > 0.05$. MCH baseline (28.85 ± 1.86) versus 1d post workout (28.86 ± 1.87); 2d (28.96 ± 1.87) as $p > 0.05$. MCHC baseline (35.13 ± 0.44) versus 1d post workout (35.11 ± 0.38); 2d (34.98 ± 0.29) as $p > 0.05$. Impressively, RDW showed significant differences both one day (13.17 ± 0.80) and two days (13.13 ± 0.67) after the endurance training compared to baseline (13.07 ± 0.77) as $p < 0.05$ (Table 1).

Table 1 Acute erythrocyte morphology responses as acute effect of endurance training (Developed by the authors)

Variables (Erythrocytes Morphology)	Pre-ET	1d post-ET	2d post-ET
MCV (fl)	82.21 ± 5.42	82.14 ± 5.40	82.77 ± 5.39
MCH (pg)	28.85 ± 1.86	28.86 ± 1.87	28.96 ± 1.87
MCHC (gr/dl)	35.13 ± 0.44	35.11 ± 0.38	34.98 ± 0.29
RDW (%)	13.07 ± 0.77	$13.17 \pm 0.80^*$	$13.13 \pm 0.67^*$

* $p < 0.05$; Pre-ET - pre-endurance training; 1-d post-ET - one day after endurance training; 2-d post-ET - two days after endurance training

3. Discussion

An iron-containing protein found in erythrocytes that is required for oxygen transport from the lungs to all the body's tissues in animals and humans is known as Hemoglobin [14]. Furthermore, Hb is a part of the healthy erythrocyte structure. Erythrocytes, also known as red blood cells (RBC), are the properly functioning

components of blood that transport gases and nutrients throughout the body [15]. In this study, we found that prolonged endurance with moderate intensity could decrease the Hb $\Delta -0.66$ (gr/dl) and erythrocytes number $\Delta -0.25$ ($10^6/\mu\text{L}$). The reference range of Hb for males is 13.8 to 17.2 (gr/dl). Generally, anemia is defined as a decrease in hemoglobin (Hb), hematocrit (HCT), or RBC count, and it is usually due to iron

deficiency [16]. Although in females there is no correlation between Hb concentration and red blood cell morphology on the third day of menstruation [17].

Blood loss is the most common mechanism of anemia. Excessive bleeding and menstruation in females causes the body to lose RBCs faster than they can be replaced. Bone marrow needs approximately 120 days to produce new erythrocytes. Furthermore, there is a condition in which erythrocytes are destructed, accelerated Hb catabolism, and declined Hb concentration, but the attempt of bone marrow to reproduce Hb is called hemolytic anemia [18]. Finally, erythropoiesis can be reduced by an imbalance throughout erythroid proliferation and differentiation within certain conditions, such as high demands for erythrocyte generation and tissue oxygenation [19].

In athletes, an elevation of about 10-20% of plasma volume due to heavy physical training could lead to a decrease of Hb concentrations under the reference range, which is known as pseudoanemia [20]. Although pseudoanemia could return to normal physiological conditions, it could worsen once individuals do not know the strategies to be fully recovered as soon as possible. Some basic anemia symptoms include weakness, exhaustion, lethargy, restless legs, shortness of breath, chest tightness, and decreased capacity for exercise. These symptoms vary depending on the rate of blood loss [21].

Although a study conducted by Fazal and Mirza in 2016 stated that although there are changes in pseudoanemia markers, anemia was not detected in individuals who engaged in regular exercise for at least past three years [22] but either moderate or high volume of physical training caused the great blood parameters changes. A badminton training session with moderate intensity for 90 min causes physiological changes in elite para-badminton athletes' blood parameters. These changes, which vary by gender, can have an impact on an athletes health and performance [23]. Hematological responses on high-volume training were cluttered even in male elite athletes; thus, proper recovery post heavy training is highly recommended [24]. Regardless of the sex, physical training increased hematologic, hematopoietic, and cytokine criterion in healthy and young individuals, and it is assumed that exercise can have a physiological impact by mobilizing stem cells and thus enhancing tissue repair mechanisms through a process called inflammation [25]. Furthermore, long-term effects of exercise program could maintain optimal levels of hematological, immunological, and coagulation criterion in normal physiological range in untrained young males [26].

In this study, MCV, MCH, and MCHC were changed but not significant. The mean corpuscular volume (MCV) value could be used to justify the amount of Hb per erythrocyte. Its measurement to define the anemia phenomenon based on morphology.

The normal value of MCV is between 82 and 92 femtoliters (fl). MCV declined in microcytic anemia, iron deficiency, rheumatoid arthritis, thalassemia, sickle cell anemia, Hb C, lead poisoning, and radiation. In contrast, it will be inclined in aplastic anemia, hemolytic anemia, hypothyroidism, side effects of vitamin B12 consumption, anticonvulsants, and antimetabolic drugs. Further, the amount of Hb per erythrocyte in picogram (pg) is also known as mean corpuscular hemoglobin (MCH). Its normal value is between 27 and 31 (pg), and MCHC is the concentration of hemoglobin obtained per erythrocyte expressed in grams per deciliter (gr/dL). The normal value of MCHC is between 30 and 35 gr/dL. Both MCH and MCHC values drop in conditions such as microcytic anemia and hypochromic anemia, and increase in iron deficiency. MCV but not MCH remarkably increased right after running but returned to baseline three hours afterwards [27]. In pathophysiology, MCV and MCH levels were independent predictors of long-term vital unfavorable cardiovascular incidents and firmly correlated with blood homocysteine concentration in nonanemic but not anemic patients with acute coronary syndrome [28].

Red Blood Cell Distribution Width (RDW) increased significantly in this study after quarter-marathon (10 km) running. A previous study stated that post half-marathon (21 km) constantly increased RDW, its peak at 20 hours after workout [27]. These phenomena may provide an explanation for the association between endurance training and mortality. In individuals with cardiovascular diseases, high levels of RDW might be related to unpleasant outcomes [28]. The prevalence of carotid plaque was observed to be linked to the association of RDW with carotid intimal-medial thickness in people with hypertension [29]. RDW is linked with the inflammatory response and sports are well known as the physical stress to challenge our body adaptations [30]. Although the increased RDW is strongly linked to cardiovascular disease in medical history, in this case the increased RDW may be involved in the first stage of acute inflammation post exercise, which is termed delayed-onset muscle soreness.

4. Conclusion

We conclude that decreased Hb and the number of erythrocytes confirm that pseudoanemia as a physiological response can be induced by prolonged endurance training with moderate intensity. Hence, there is no meaningful change in morphological variables (MCV, MCH, MCHC) of erythrocytes. Meanwhile, the significant inclined in RDW might be a positive sign for inflammation as the first stage of the muscle adaptation. This scientific finding could be used to explain how prolonged endurance exercise changes the physiological condition of the body acutely,

especialmente desde una perspectiva hematológica. Sin embargo, la futura investigación debe ser conducida con variables relacionadas a marcadores de inflamación, grupos de control, y sexo diferente.

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