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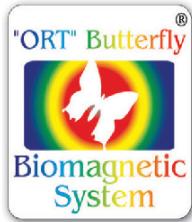
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# BIOMAGNETOTERAPIA W WYROBACH MEDYCZNYCH „ORT BUTTERFLY”

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# Short-term effects of milk (both unpasteurized and pasteurized) on active adolescence hemoglobin

*Krótkoterminowy wpływ spożycia mleka (niepasteryzowanego i pasteryzowanego) na aktywną hemoglobinę w okresie dojrzewania*

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## Abstract

Milk consumption is one of the beverages options for the recovery phase. On the basis of the manufacturing procedure, raw and pasteurized cow's milk are distinguished, but no one has investigated the effect of milk consumption on the hemoglobin levels of active people; some studies only analyze the effect of milk consumption on fitness and recovery advantages. Objective: The aim of this study is to compare the hemoglobin levels of active adolescents who consumed fresh cow's milk versus pasteurized milk for three consecutive weeks. It is intended that the three-week treatment will have a good effect on hemoglobin levels, hence enhancing physical performance. Methods: Using a quasi-experimental study with a Pretest-Posttest Randomized-Groups Design for three consecutive weeks of milk feeding twice daily per group. Twenty-four university football players were divided into three groups using the approach of purposive sampling: the fresh cow's milk (SSS) group, the pasteurized milk (SP) group, and the mineral water control group (K). The tool utilized to measure body composition is the Karada Scan Body Fat Omron HBF-356, while the Easy Touch GCHb is used to collect data on hemoglobin. Subjects participated voluntarily and were willing to have blood tested through capillaries before, immediately after, and 30 minutes after exercise. The analysis data uses paired t-test and ANOVA calculations to find differences within and between groups. Results: Hemoglobin immediately after exercise (0) in the post-test (three weeks after treatment) increased significantly ( $0.020 < 0.05$ ) compared to hemoglobin before exercise (-) in the pre-test (before three weeks of intervention) in all three groups (fresh milk, pasteurized milk, and mineral water). Upon further examination, it was determined that there was a highly significant difference in hemoglobin levels ( $0.010 < 0.05$ ) between fresh cow's milk and pasteurized milk immediately after exercise (0) in the post-test (after three weeks of treatment). In contrast, there were no significant differences ( $p\text{-value} > 0.05$ ) for the remaining calculations, both within and between subgroups. Conclusions: After three consecutive weeks of milk consumption, there was no significant variation in the amount of hemoglobin in the blood. To optimize the different elements that can affect the acceleration of the increase in hemoglobin in the blood, it is recommended that further study be conducted to intervene with milk and additional meal intake alongside physical activity.

## Keywords

fresh cow milk, pasteurization milk, physical activity, hemoglobin, active people

## Streszczenie

Mleko jest jednym z napojów spożywanych w okresie rekonwalescencji. Ze względu na sposób wytwarzania wyróżnia się mleko krowie surowe i pasteryzowane. Do tej pory nie zbadano wpływu spożycia mleka na poziom hemoglobiny u osób aktywnych fizycznie; niektóre badania analizują jedynie wpływ spożycia mleka na korzyści związane z kondycją i regeneracją. Cel: Celem badania jest porównanie poziomu hemoglobiny u aktywnych nastolatków, którzy spożywali świeże mleko krowie w porównaniu ze spożyciem mleka pasteryzowanego przez trzy kolejne tygodnie. Zakłada się, że trzytygodniowa interwencja będzie miała korzystny wpływ na poziom hemoglobiny, a tym samym na poprawę wydolności fizycznej. Metody: Wykorzystanie quasi-eksperymentalnego badania z randomizowanymi grupami poddanymi badaniom przed i po badaniu przez trzy kolejne tygodnie spożywania mleka dwa razy dziennie. Dwudziestu czterech piłkarzy uniwersyteckich podzielono na trzy grupy, stosując podejście celowego doboru próby: grupę spożywającą świeże mleko krowie (SSS), grupę spożywającą mleko pasteryzowane (SP) i grupę kontrolną, która piła wodę mineralną (K). Narzędziem służącym do pomiaru składu ciała było urządzenie Karada Scan Body Fat Omron HBF-356, natomiast urządzenie Easy Touch GCHb wykorzystano do zbierania danych o poziomie hemoglobiny. Badani uczestniczyli w badaniu dobrowolnie i poddali się badaniu krwi przez naczynia włosowate przed, bezpośrednio po i 30 minut po wysiłku. Aby określić różnice w grupach i między grupami wykorzystano dane analityczne uzyskane w sparowanym teście t i obliczeniach ANOVA. Wyniki: Poziom hemoglobiny bezpośrednio po wysiłku (0) (trzy tygodnie po interwencji) istotnie wzrósł ( $0,020 < 0,05$ ) w porównaniu do poziomu hemoglobiny przed wysiłkiem (-) (przed trzytygodniową interwencją) we wszystkich trzech grupach (mleko świeże, mleko pasteryzowane i woda mineralna). Po dalszych badaniach ustalono, że istniała wysoce istotna różnica w poziomach hemoglobiny ( $0,010 < 0,05$ ) pomiędzy grupami spożywającymi mleko świeże a mleko pasteryzowane bezpośrednio po wysiłku (0) (po trzech tygodniach interwencji). Natomiast nie zaobserwowano istotnych różnic (wartość  $p > 0,05$ ) dla pozostałych obliczeń, zarówno w obrębie podgrup, jak i pomiędzy nimi. Wnioski: Po trzech kolejnych tygodniach spożywania mleka nie stwierdzono istotnych różnic w poziomie hemoglobiny we krwi. Aby zoptymalizować różne elementy, które mogą wpływać na przyspieszenie wzrostu poziomu hemoglobiny we krwi, zaleca się przeprowadzenie dalszych badań w zakresie spożywania mleka i dodatkowych posiłków obok aktywności fizycznej.

## Słowa kluczowe

świeże mleko krowie, mleko pasteryzowane, aktywność fizyczna, hemoglobina, ludzie aktywni

## Introduction

Hydration is critical for everyone who is actively exercising in order to meet fluid needs and avoid dehydration. According to the Indonesian Ministry of Health, a person should drink at least 8 glass of water each day [1]. When someone exercises more energetically, there must be a greater rise in consumption than usual. Furthermore, if the activities are particularly energetic, such as exercising three times a week or more, with a suggested duration of 50 minutes each session (World Health Organization, 2020), it is preferable to increase fluid intake before, during, and after exercise [2]. In order to meet fluid requirements specific to hydration.

After fluid intake is fulfilled, the following demand is to meet various physical and physiological activity needs. Physical exertion and water consumption might impact physiological responses. For optimal performance, the cardiovascular system must be in top shape throughout physical activity. During movement, the cardiovascular system, which includes the lungs, heart, and blood arteries, plays a crucial function. For instance, in football, when the bulk of motions are performed with the lower extremities, 75–80 percent of blood flow will flow to the muscles that act efficiently during exercise [3], requiring the need for strong blood vessels to maintain the body's performance. Hemoglobin serves a crucial role in the transport of oxygen to sustain performance. The amount of hemoglobin definitely affects the body's performance; the more hemoglobin, the greater the oxygen supply, and the quicker the synthesis of energy sources may continue [4].

Hemoglobin must constantly bind oxygen and transmit it to cells for energy production. The action of hemoglobin is obviously not simple, therefore amount of assistance is required; the higher the hemoglobin content in the blood, the greater the body's capacity to accelerate a chemical reaction in cells. As a result, it is essential to ingest nutrients that can improve a performance when exercising and engaging in physical activity. The critical factor to consider is iron-rich intake, which may assist boost the quantity and is also linked to greater fitness [5]. Previous study has demonstrated that the time of nutritional intake is critical for optimal adaptation to the kind of exercise [6]. Because the body does not have much time to absorb nutrients properly when exercising, it is advisable to take nutrient-rich beverages rather than meals. It is interesting to note that the other minerals included in cow's milk, such calcium, salt, or potassium, may help with fluid recovery after exercise, which in turn may aid in the regeneration of skeletal muscle [7]. As a result, the aforementioned protein ratio may encourage sluggish digestion and absorption of amino acids, which may result in an elevated blood amino acid content (mainly branched amino acids) (Juvonen et al., 2011).

Most consumers pick beverages based on the notion that they promote stamina, low prices with minimal nutritional content, trainer recommendations, convenient availability, and skill-enhancing beverages. Even though nutritionists are needed to establish portion, quality, and amount of food to increase productivity as long as players eat every day, many clubs or parent organizations do not have their own nutrition budget [1]. Athletes that neglect eating due to training and competition

are another concern [9]. Athletes have less energy, and a progressive reduction in performance affects achievement [10]. Healthy food is hard to find, especially with catered meals that athletes sometimes ignore. Thus, multiple media reports describe athletes at a national training center eating simply packaged rice, which cannot cover their daily calorie demands. However, excessive supplement use without medical or scientific basis might harm athletic performance. Athletes and coaches believe various supplement and nutritional myths. Coaches and data teams seldom report lactate or hemoglobin levels. Some of these difficulties and behaviors treats coaches and team which seldom report lactate and hemoglobin level during on and off pitch [1].

There is still a misalignment between existing theory and realization in daily life. Because similar studies were conducted about ten years ago and were not specific to the fitness component, there are still few who discuss specifically how fluid intake may be after exercise through milk intake with differences in the production process in groups of active individuals correlated with hemoglobin levels. When it comes to physical exercise, milk has a significant impact in both performance and physiological elements. Numerous previous studies have focused on the benefit of milk consumption during the recovery stage after physical exercise by comparing several types of milk [11], comparing different types of liquids for recovery [12]. Additionally, understanding the advantages of milk in lowering the risk of muscular injury after physical exercise [13], the influence on sleep patterns [14] and appetites [15, 16].

This study is relevant since there are still a substantial number of individuals who rely solely on milk consumption as a substitute energy source if they exercise in the morning, but the effect is unknown based on the available literature. Despite several literature review, little is known about the relationship between milk consumption, hemoglobin issues, and exercise. Only one study completed a decade ago examined the benefits of iron-fortified milk, which resulted in greater physical fitness scores and exercise duration from baseline compared to regular milk intake [5]. Unfortunately, no one has examined in greater detail how consuming milk twice a day (with different processes) for three consecutive weeks affects hemoglobin levels before and after exercise. Therefore, the aim of this research was to evaluate the impact of milk with different production methods, both fresh cow's milk and pasteurized milk, with mineral water as a control, if it can considerably improve hemoglobin levels or not. The expectation is that it can contribute to a rise in hemoglobin, allowing athletes and sports enthusiasts to consume milk as an alternate method of encouraging progressive physical work and affordable substitution to an expensive post-work out supplements.

## Methods

### Research design

Single blind study and parallel subject treatments, with Pretest-Posttest Randomized-Groups for the investigation [17]. This study focuses on milk consumption for three consecutive weeks, twice a day, involving participants with a student-athletes background. Participants were divided into three groups including fresh cow's milk, pasteurized milk and mineral water.

## Study participants

This study comprised all 50 members of the football student activity unit, of which 24 football players were obtained at the university level, with an age range of 18–21 years, with a mini-

mum of five years experience in regional football competitions, and who were not allergic to milk. Purposive sampling was used to choose subjects, who were then randomly assigned to one of three groups: raw cow's milk, pasteurized milk, or mineral water.

**Table 1. Description of the participants of study**

Characteristics	Average	± SD
Age [years]	18.61	0.70
Height [cm]	168.25	5.90
Weight [kg]	60.63	5.34
BMI	21.40	1.29
VO2Max [ml/kg/min]	46.48	3.76
Age of training [years]	5.29	1.79
Nutrition status (Cal/day)	2503.32	155.87

The milk content used in this study was determined using the findings of the Health Laboratory Test. The production processes of fresh cow's milk and pasteurized milk differ; fresh cow's

milk is only cooled to a temperature of 200°C after which it can be drunk immediately, but pasteurized milk is cooled at 20°C and then heated for 15 seconds at a temperature of 72°C.

**Table 2. Fresh cow's milk and pasteurized milk content summaries**

Characteristics	Quantity of Milk present (150 ml)	
	Fresh cow milk	Pasteurized milk
Fats (%)	4.58	6.02
Proteins (%)	5.06	4.32
Carbohydrates (%)	5.48	15.26
Lactic acid (%)	0.57	0.33
Iron (ppm)	0.28	2.1
Calories (Cal)	83.29	132.43

## Data Analysis

Blood data sampling using Sysmex XN1000 using flowcytometry laser method to measure hemoglobin, with 6 cc blood sample were collected. On the day of the test, data gathering occurred both before and after the 2.4 km Cooper Test. Three blood samples were taken during the pre-test (before consuming beverages for three weeks) and post-test (after giving beverages for 3 weeks). In this study, the participant was informed that the data collected would only be used for research purposes. All identities are kept anonymous, and this research adheres to the Bandung Health Polytechnic's research ethics code. Subjects participated voluntarily and were willing to have blood taken through capillary blood vessels before, as soon as practical, and 30 minutes after activity. The Kolmogorov-Smirnov test is used to assess the normality of the obtained data, and all of the acquired data are normal. All data collection analyzed by paired t-test and ANOVA calculations, differences within and between groups were determined through the examination of the data.

## Ethical Approval

The research related to human use has complied with all the relevant national regulations and institutional policies, has follo-

wed the tenets of the Declaration of Helsinki, and has been approved by the Medicals and Health Research Ethics Committee (MHREC), Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada – Dr. Sardjito General Hospital with ref. no: KE/FK/0637/EC/2022.

## Informed consent

Informed consent has been obtained from all individuals. If a participant is unwell or allergic, they cannot continue. Furthermore, volunteers are disqualified if they missed more than one exercise session or blood sample test without providing an acceptable explanation to the researchers.

## Results

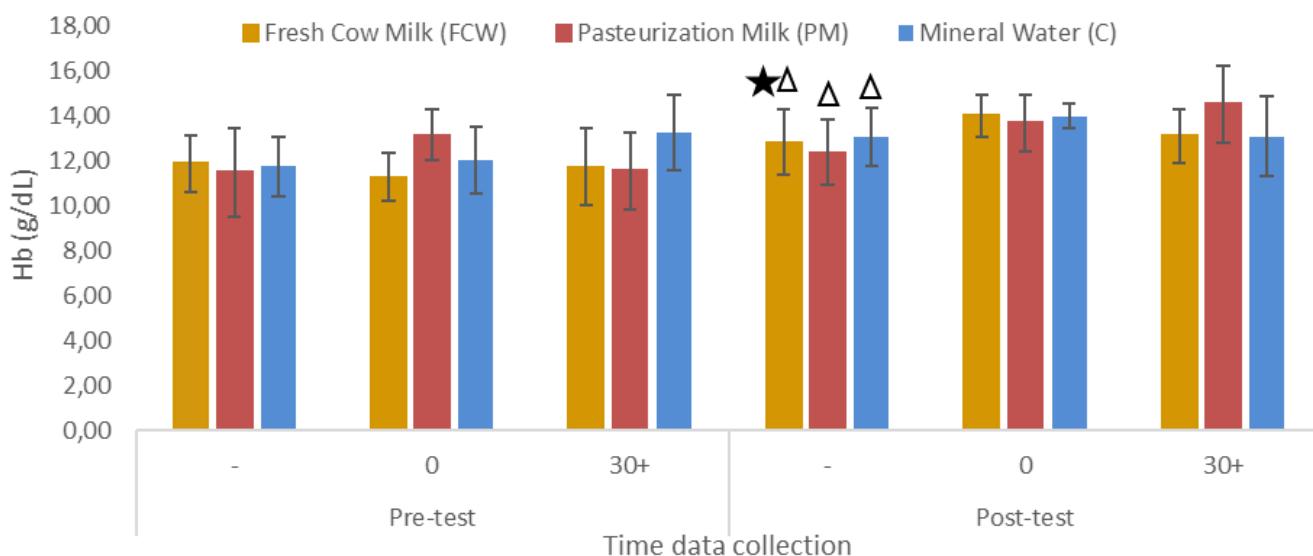
The data obtained provides a comparison of hemoglobin levels between groups before (pre-test) and after three weeks of treatment (post-test). When comparing pre- and post-treatments, there was a very significant difference between each group immediately after exercise ( $p$ -value < 0.05). Regarding to substantial differences between groups should be obtained as rapidly as feasible after exercise ( $p$ -value < 0.05).

**Table 3. Hemoglobin levels in different groups**

Group	Hemoglobin (g/dL)					
	Pre-test			Post-test		
	Before (-)	Immediately after (0)	30 minutes following exercise (30+)	Before (-)	Immediately after (0)	30 minutes following exercise (30+)
Fresh cow milk	11.9 ± 1.27	11.3 ± 1.09	11.7 ± 1.73	12.84 ± 1.46	14.01 ± 0.93Δ*	13.11 ± 1.20
Pasteurized milk	11.5 ± 1.98	13.2 ± 1.12	11.6 ± 1.72	12.39 ± 1.46	13.69 ± 1.26Δ	14.53 ± 1.72
Mineral water	11.8 ± 1.33	12.1 ± 1.48	13.3 ± 1.70	13.06 ± 1.31	13.99 ± 0.55Δ	13.09 ± 1.79

\* Sig. ( $p$ -value < 0.05) within groupΔ Sig. ( $p$ -value < 0.05) between group

## HEMOGLOBIN

**Figure 1. Hemoglobin pre-test and post-test comparison based on blood collecting time**

### Discussion

Hydration intake is crucial for supporting performance during exercise. Before, during, and after exercise, adequate fluid intake is important to sustain movement and prevent dehydration. Prior study has demonstrated that breakfast in the morning is crucial for sustaining daily activities. Unfortunately, adolescents tend to forget this. Teenagers frequently skip meals or drinks [9] and substitute it with nutritionally deficient alternatives like energy drinks and sports bars [18]. This unpleasant behavior can be impacted by adolescents' childhood behaviors. Several research on the drinking habits of adolescents indicate that the majority of teenagers whose parents are busy or who work live alone and prepare their own food if their parents are busy or work [19], thus typically have minimal food and beverage options. This issue is perpetuated by the tendency of adolescents to be picky eaters or to engage in so-called picky eating [20], [21]. In accordance with the findings of a number of these research and the behavior of today's adolescents, it is recommended that adolescents increase their nutritional intake to meet their energy requirements and enhance their nutritional status [22]. Milk is one of the highly suggested fluids to consume when exercising [23, 24].

Milk has a distinct nutritional profile compared to other beverages since it contains carbohydrates, lipids, and proteins. As a liquid component, only milk has good nutritional value to present. According to numerous studies, milk is currently very impressive for the recovery time following exercise [23, 25]. In an effort to increase blood hemoglobin levels, this study proposes that milk be drunk for three consecutive weeks during the recovery period following exercise. Milk was selected as a component that can enhance hemoglobin levels for good reason. Several earlier research have demonstrated that fluid consumption can affect glucose levels, which play a crucial role in energy generation [26]. Taste is the primary attraction of the milk option, with chocolate, strawberry, and vanilla being the three most common varieties offered in supermarkets [13]. Milk is unique in what it contains. Another alternatives to whey protein supplements, which is more inexpensive but nonetheless effective, is milk. If individual absolutely must engage in exercise or physical work with just a little time of rest in between, the full content of milk will greatly assist in cell regeneration and the ability to recover [27, 28]. The included macronutrients can help restore energy levels and performance following strenuous physical activity [28, 29]. Sweat loss of electrolyte fluids can be compensated for by absorbing micro-

nutrients milk's contents [30], particularly sodium and potassium, prior, during, and after strenuous physical activity [31]. Beyond that end, milk can stand in for more expensive and less effective supplements by giving you satiety [8]. Therefore, it may minimize the possibility of post-workout weight gain [16, 32] and decrease electrolyte excretion [33]. Milk is being marketed as the "new sports drink" [26]. To increase blood hemoglobin levels, this study proposes that milk be drunk for three consecutive weeks during the recovery period following exercise. Milk was selected as a component that can enhance hemoglobin levels for good reason. Several earlier research have demonstrated that fluid consumption can affect glucose levels, which play a crucial role in energy generation [26].

Cow's milk has been shown to significantly improve workout performance and muscle recovery in a few previous scientific investigations [7]. One research that is indeed similar to recent ones focuses at how ingesting cow milk for a short period of time affects lactate, finding that it drops significantly lower than average [34]. The contents of pasteurized cow's milk and fresh cow's milk have a tendency to possess an effect that is vastly different from one another. Milk that has been pasteurized has a tendency to resist insulin and improves lipid oxidation, both of which serve to induce lactate synthesis. As a result of the process of pasteurization, milk includes carbohydrates, proteins, and lipids, in contrast to fresh cow milk, which did not contain any of these beverages categories. In other research, data gathering was only conducted while the treatment was being provided. One of these investigations, which examined the efficacy of milk in the recovery of climber individuals, found that the treatment drastically improved the athletes' ability to recover. But, unfortunately, this was not accomplished by administering treatment for a significant amount of time [34]. No research were discovered during the authoring of this study that measured the effect of milk consumption on hemoglobin, a marker of energy production during strenuous exercise. Meanwhile, the iron in cow milk is a helpful addition.

Hemoglobin, also known as an iron-rich transport protein, is a structural component of the blood's sustaining components, specifically red blood cells. Hemoglobin has an active role as a transport channel for oxygen, but it also transports carbon dioxide. Directly formed by red blood cells, hemoglobin has an active function [35]. Hemoglobin is an important element in all conditions of physical exercise due to its role as a blood and oxygen transporter throughout the body. Without adequate hemoglobin levels, an athlete's limbs and motor abilities will be unable to function efficiently, resulting in a severe decline in physical performance [36]. Iron intake is essential for the synthesis of hemoglobin. A sufficient iron intake will not inevitably lead in enough hemoglobin production if it is not balanced with the presence or participation of other nutrients [37]. Nutrients have a significant role in anemia, with iron being the most important of the nutrients involved. Although iron deficiency is the most common cause of anemia in developing countries, deficiencies of B-complex vitamins, particularly folate and vitamin B12, may also play a role [38].

The role of Hemoglobin (Hb) throughout the preparation stage  
The transfer of oxygen from the respiratory organs to the peripheral tissues and the transport of carbon dioxide with different

protons from the peripheral tissues to the respiratory organs for further excretion are two important activities of hemoglobin in the human body [39]. The essential of hemoglobin's function in the human body and the frequency of regular physical exercise are interconnected. In a study examining the association between physical activity and hemoglobin levels, it was found that when a person engages in physical activity, such as exercising, there is an increase in metabolic activity and acid production (hydrogen ions, lactic acid), resulting in a reduction in pH. The attraction between oxygen and hemoglobin is diminished when the pH level is low. This increases oxygen delivery to the muscles by prompting the hemoglobin to release more oxygen [40].

The higher a person's hemoglobin level, the better the respiratory system since the muscles store a lot of oxygen reserves through the blood, the lower a person's hemoglobin level, the less oxygen supply is stored in the muscles [41]. Previous research [42] found that hemoglobin levels, as a measure of an athlete's maximum aerobic capacity in a team sport or game category, were required to transport oxygen throughout the body. Hemoglobin levels are also required to assure the oxidation process associated with this sport [43]. Hemoglobin levels are one of the components that determine physical fitness, and hemoglobin levels play a part in defining physical fitness in players. Furthermore, the study found a substantial link between blood hemoglobin and cardiovascular athletes [41].

Athletes have a high level of exhaustion and are at risk for anemia during the preliminary phase due to the high volume of exertion in daily exercise activities. One of the causes of anemia is a lack of hemoglobin, which creates difficulties in the circulation of blood and oxygen throughout the body. A lack of hemoglobin guarantees that the athlete's endurance will deteriorate, causing athletes to become easily fatigued [44]. The athlete's concentration level diminishes, the quality of movement coordination decreases, and the capacity to move aerobically has issue endurance. When it comes to football, it requires high focus and endurance to be able to carry out passing, shooting activities effectively and precisely. In addition, every player needs the endurance ability to sprint from their various positions and then explore the field with repetition [45].

High-intensity exercise appears to lower hemoglobin levels, implying an inverse connection with internal load [46]. Meanwhile, heavy volume and high-intensity exercise for a specific period often occurs during the preparatory period for the purpose of forming the athlete's physical condition during preparation in general, so that in this phase a high need for hemoglobin and nutritional intake is necessary to support the creation of sufficient blood requirements as a source of energy and effort to facilitate oxygen in the body to flow properly throughout the body, especially for football players.

### **Hemoglobin's role during competition**

The long and intense competitive season gives football players insufficient time to recover. Players need optimal physical condition throughout the season [47]. Hemoglobin delivers oxygen throughout the body during activity and rest. In the optimal level of  $\text{VO}_2\text{Max}$  for each player, the hemoglobin level is also good since oxygen distribution can be channeled properly for optimal recovery time and physical condition with a very long level of activity in one match [48]. Good physical health improves the

heart's circulation. Athletes need endurance, for example. Endurance is a key component of the main physical condition. When an athlete wants to improve speed, strength, flexibility, and essential capacity, endurance is key. To support players' performance during a long season, it's important to monitor their endurance [49].

Football athletes must be muscular and strong. Passing, shooting, dribbling, jumping, and sliding involve endurance. This sport requires strength and balance endurance of the upper and lower muscles because it repeats the movement of passing or shooting under pressure. The endurance of leg strength also plays a crucial role in sprinting speed with and without the ball, also shooting. Good dietary balance can help boost endurance and VO<sub>2</sub>Max. Iron-rich meals can help avoid anemia caused by iron deficiency and B vitamin deficiencies, especially folate and vitamin B12. With nutritional intake that supports excellent hemoglobin, it is hoped that the athlete's performance would continue at its finest despite the season's high competition density. Baseball players' hemoglobin levels must be maintained effectively to ensure peak performance [50]. The competition phase

has a high level of physical stress, considering the intensity of the effort exerted during the match. In order to retain performance on the field throughout the competition phase, one of the absolute requirements for a baseball player is to keep a correctly filled hemoglobin level by eating a balanced diet and consuming the necessary nutrients for hemoglobin.

### Conclusion

The extra content of pasteurized milk had no effect on the level of significant variation in hemoglobin content in the blood after three weeks of milk intake intervention. More study is needed to intervene with milk and additional food intake, along with physical activity, to maximize numerous aspects that can effect the accelerated growth in hemoglobin in the blood.

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