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### Effect of Chocolate Milk Consumption on Cardiorespiratory Endurance of Central Java BPPLOP Games Athletes

### Pengaruh Konsumsi Susu Coklat terhadap Daya Tahan Kardiorespirasi Atlet Cabang Olahraga Permainan BPPLOP Jawa Tengah

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#### ABSTRACT

**Background:** Sports games with aerobic and anaerobic energy systems require muscular and cardiovascular endurance, as well as high dietary intake. Carbohydrate loading before training can maximize glycogen storage and carbohydrate oxidation, thereby increasing performance. Chocolate milk powder, as much as 45 g, contains 55 g of carbohydrates, contributing to 6-8% of total carbohydrate needs.

**Objectives:** Analyzing the effect of chocolate milk consumption on muscle endurance and maximum oxygen volume capacity (VO2 Max) in sports athletes at the BPPLOP Central Java Student Sports Education and Training Center.

**Methods:** Research with pretest-posttest control design with the same sample with a washout period. A sample of 47 athletes was taken using a total sampling technique, all used as a control group and treated for 6 days with 200 ml 2 hours before training. Measurement of endurance using the multistage fitness test. Test the difference in effect before and after treatment using the Paired Samples T-Test, while testing the difference between control and treatment groups using Mann Whitney.

**Results:** There was a significant effect between before and after consuming chocolate milk on increasing muscle endurance by  $2150.64 \pm 420.72$  to  $2314.47 \pm 400.14$  m and VO2 Max capacity by  $53.95 \pm 5.96$  to  $56.30 \pm 5.62$  ml/kg/minute with a p-value=0.00. There was a significant difference between the consumption of placebo and chocolate milk in increasing muscle endurance by 163.83 m and VO2 Max capacity by 2.34 ml/kg/minute with a p-value=0.00.

**Conclusions:** Carbohydrate loading by consuming 45 g of chocolate milk in 200 ml containing 55 g of carbohydrates before training for 6 days can affect increasing muscle endurance and VO2 Max capacity in sports athletes.

#### INTRODUCTION

Physical condition in the world of sports is the foundation for achieving optimal performance and achievement<sup>1</sup>. Endurance, both muscular endurance and cardiovascular endurance, is a physical condition needed in game sports<sup>2,3</sup>. Football, sepak takraw, and basketball are game sports with changing intensity, intermittent match forms, repetition of explosive movements, and game patterns that often change places<sup>4–6</sup>. This condition requires muscle endurance as an anaerobic capacity, which is the ability of muscles to work continuously for a long period and a certain load<sup>7</sup>. Muscle endurance can occur by using anaerobic energy systems.

Aerobic energy systems dominate playing sports due to the long duration of matches. This condition requires cardiovascular endurance, which is the ability to collect oxygen that will be channeled to the muscles through the blood circulation to be used in aerobic metabolism as energy fuel<sup>7</sup>. Cardiovascular endurance as aerobic capacity is measured by maximal oxygen volume capacity (VO<sub>2</sub> Max). VO<sub>2</sub> Max is the maximum amount of oxygen that can be consumed during Intense physical activity until exhaustion<sup>8</sup>.

The multistage fitness test or MFT is one of the tests used to measure endurance by running 20 m back and forth<sup>9</sup>. The efficiency of heart and lung function, as well as muscle endurance to continue to contract expected in the MFT, will produce a total level and feedback. The results of this total level and feedback will be converted into VO<sub>2</sub> Max capacity as an indicator of cardiovascular endurance, and the total mileage of running back and forth in a 20-m track is a result of muscle endurance to contract, which is an indicator of muscle endurance<sup>1,10</sup>.

The pre-research study was conducted by analyzing the endurance of athletes from the Central Java Student Sports Education and Training Center (BPPLOP) using the MFT method. Based on the results of the analysis, the average VO<sub>2</sub> Max values (ml/kg/minute)

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## Amerta

and mileage (m) are 53.7 and 2290 football athletes, 50.5 and 1911 sepak takraw athletes, and 50.2 and 1886 basketball athletes, respectively. The average value compared to the VO<sub>2</sub> Max standard set by the physical trainer of each game sport indicates a good category for football athletes (53.4-62.7) and sepak takraw (47.1-53.7), while basketball athletes are classified as sufficient (48.7-53.1). This condition can be maintained and improved through one of the factors that affect athlete endurance, namely food intake.

Endurance athletes require a higher dietary intake to help meet energy and nutrient requirements during training or competition by emphasizing the availability of the body's energy reserves, especially glycogen reserves, for use over a long period of time<sup>11</sup>. Moreover, adolescent athletes require nutrient intake by their needs to optimize growth and development. However, often, the regulation and monitoring of nutrient intake have not been carried out optimally, resulting in the fulfillment of inappropriate nutrient intake<sup>12</sup>. This is supported by previous research in 2019<sup>13</sup> and has been observed by researchers in preresearch, where there are no meal arrangements based on athlete training periodization a BPPLOP Central Java.

Carbohydrate loading before training or competition can maximize liver and muscle glycogen storage, thereby Increasing endurance<sup>14</sup>, glycogen storage, thus increasing endurance<sup>15,16</sup>. Carbohydrates have high energy efficiency and can be metabolized both aerobically and anaerobically<sup>17</sup>. This condition will inhibit fatigue by about 20% and increase performance by 2-3%<sup>17–19</sup>. Based on literature studies, within 1-2 hours before training or competing, athletes can consume foods or drinks with a carbohydrate content of 6-8%<sup>20,21</sup>. Athletes can consider choosing carbohydrate loading that is consumed with a composition of complex carbohydrates, carbohydrates and protein, and fluids<sup>22</sup>. Milk contains carbohydrates with protein in liquid form which is easily absorbed by the body.

Chocolate milk is one of the carbohydrate loading food products with nutritional content that is considered better than commercial sports drinks<sup>22</sup>. Chocolate milk contains carbohydrates in the form of lactose, which is the main carbohydrate in milk and sucrose. Lactose is a disaccharide consisting of glucose and galactose, while sucrose consists of glucose and fructose. Lactose will be metabolized by the enzyme lactase and sucrose will be metabolized by the enzyme sucrose in the small intestine. Glucose metabolized from lactose and sucrose will be released directly to the whole body, while galactose and fructose will be channeled to the liver. In the liver, simple sugars other than glucose will be converted into glucose. Glucose can then be stored as glycogen in the liver or released into the bloodstream<sup>22</sup>.

Chocolate milk contains proteins that include mostly branched-chain amino acids (BCAAs), namely casein and whey with important roles in protein synthesis and muscle metabolism<sup>23</sup>. Research shows that protein intake combined with carbohydrates before training can improve protein balance and stimulate protein synthesis during the training or recovery phase<sup>16,24</sup>. Protein synthesis in the training phase contributes to muscle mass gain leading to increased strength and endurance, while in the recovery phase, it leads to muscle repair<sup>16,25</sup>. Protein intake can increase amino acid availability, reduce exercise-induced protein catabolism, and minimize muscle damage.

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Chocolate milk powder of 45 g dissolved in 200 ml of water contains 55 g of carbohydrates and 7 g of protein. The content of chocolate milk contributes 6-8% of the total carbohydrate needs of Central Java BPPLOP game athletes. Research in 2015 on football athletes stated that there were significant differences in VO<sub>2</sub> Max values and better running distances in the chocolate milk group compared to commercial sports drinks<sup>23</sup>. Another study conducted in 2022 on badminton athletes found that chocolate milk increased time to exhaustion (TTE) by 24%, increased VO<sub>2</sub> max, and decreased delayed onset muscle soreness (DOMS) in the lower extremities<sup>26</sup>. In addition to the better nutritional content chocolate milk is a readily available product at an affordable cost, so it can be implemented at the youth athlete population level<sup>27</sup>. This is based on the results of pre-research study interviews with Central Java BPPLOP game athletes who stated that all athletes did not have allergies to milk and liked chocolate milk.

Based on this analysis, chocolate milk's high carbohydrate content can increase athletes' muscle endurance and VO<sub>2</sub> Max capacity. However, the diversity of research is still very limited and needs further testing. Therefore, researchers are interested in determining the effect of chocolate milk consumption on muscle endurance and VO<sub>2</sub> Max capacity of Central Java BPPLOP game athletes. The results of this study are expected to add to the literature and become reference material in the scope of sports nutrition science, primarily related to the effect of chocolate milk consumption on muscle endurance and VO<sub>2</sub> Max capacity of game sports athletes.

#### METHODS

This quantitative study used the quasi experimental method with a pretest and posttest control group design with the same subject and washout period for three days. This research was designed to analyze the effect on research subjects, where each subject gets more than one treatment in the same experiment<sup>28</sup>. The washout period is defined as a break between two intervention periods intended to eliminate the influence of the previous intervention<sup>29</sup>.

The research was conducted at the Central Java Student Sports Education and Training Center (BPPLOP) for athletes of game sports consisting of football, sepak takraw, and basketball. A total sampling technique was employed with the criteria of athletes who are willing to become research subjects after being given directions about a series of stages of research implementation and have signed informed consent. The total sample in this study was 47 athletes consisting of 18 football athletes, 13 sepak takraw athletes, and 12 basketball athletes. The dropout criteria in this study aims to cancel the subject as a sample in the selected population if the subject does not follow each stage of the study and the subject experiences illness or injury during the study.

The treatment group was given an intervention of chocolate milk consumption. The chocolate milk used

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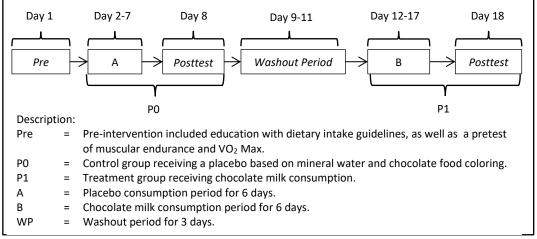
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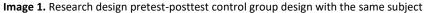
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was 45 g of powder dissolved in 200 ml of water. The content contained in chocolate milk was the energy of 180 kcal, 7 g of protein, 3 g of total fat and 2 g of saturated fat, 32 g of total carbohydrates, and 23 g of total sugar consisting of 10 g of sucrose and 12 g of lactose. The control group in this study consumed a placebo in the form of 200 ml of mineral water which was given unflavored brown food coloring as a dye to produce a color similar to chocolate milk.

This study consisted of two intervention periods, namely treatment and control, which were given at 13.00 WIB or 2 hours before training for 6 consecutive days. The difference in intervention periods was separated by a washout period of 3 days so that the total implementation of the study was carried out for 18 days to the control and treatment groups. On the first day, athletes were assessed through anthropometric characteristics data collection, which included measurements of body weight, height, and nutritional status based on body mass index according to age (BMI/U). Assessment to see the characteristics of athletes' food intake on training days was carried out using a 1x24-hour food recall form. The choice of training day was related to the benefits of carbohydrate loading before training/competing to increase glycogen synthesis. Assessment to measure total energy needs was carried out using the Harris Benedict formula plus sports activity energy using the Metabolic Equivalent formula. Sports activities are known through secondary data on training programs obtained from the coach of each sport. Then, researchers conducted interviews with athletes using the sports activity form to find out the suitability of the training program during training time and training duration. Furthermore, an endurance pretest was conducted using the multistage fitness test (MFT). The level and feedback results from the running test within 20 m back and forth will be accumulated and then converted into mileage which is an indicator of muscular endurance and converted into VO<sub>2</sub> Max capacity as an indicator of cardiovascular endurance.





Chocolate milk consumption was the independent variable, while muscular endurance and VO<sub>2</sub> Max capacity of athletes were the dependent variables. This study had confounding variables, namely food intake and gender. Food intake was controlled with food intake guidelines to provide a reference in meeting nutrient intake, as well as calculating and analyzing food intake data with 24-hour food recall. The food guide was given to the entire population by educational methods before the treatment was carried out. Gender was controlled by selecting research subjects based on gender, specifically selecting male athletes in game sports as research subjects.

Univariate analysis aims to describe the characteristics of each research variable. This analysis will produce a frequency distribution and percentage of each variable. Bivariate analysis is conducted on two variables that are believed to be related or correlated. Bivariate analysis begins with a normality test and homogeneity test to determine the statistics test that must be used to determine the relationship between variables. The normality test uses the Shapiro-Wilk test to determine whether the research data comes from a normally distributed population<sup>30</sup>. The homogeneity test

with the Levene test is used to determine whether the research data has a homogeneous population variant<sup>31,32</sup>. A homogeneity test was conducted on subject characteristics and confounding variables of food intake.

Analysis to determine the effect of chocolate milk consumption on muscle endurance and VO<sub>2</sub> Max capacity between before and after treatment in this study using the parametric test, namely Paired Sample T-Test. Meanwhile, the analysis test to determine the difference in the effect of chocolate milk consumption between the treatment group and the control group with normally distributed data will be carried out with a nonparametric test, namely Mann Whitney. This research can be carried out by obtaining approval with ethical clearance from the Semarang State University Health Research Ethics Commission No. 242/KEPK/EC/2023.

#### **RESULTS AND DISCUSSION**

The subjects in this study were football, sepak takraw, and basketball athletes at the Central Java Student Sports Training Education Center, totaling 47 athletes. Some of the characteristics used to describe

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the subjects of this study include age, weight, height, nutritional status based on IMT/U, TEE, and food intake before the intervention using 1x24-hour food recall

consisting of energy, protein, fat, and carbohydrate. The complete characteristics of the research subjects are presented in Table 1.

|--|

Characteristics	Mean±SD (n=47)	Min	Max	p#
Age (years)	16.28±1.05	14	18	0.227*
Body weight (kg)	64.13±8.03	45	83	0.171*
Height (cm)	173.22±7.53	154,.8	188	0.735*
BMI/U (z-score)	0.10±0.71	-1.89	1.23	0.254*
TEE (kcal)	3532±323	2732	4289	0.702*
Energy (kcal)	3090±773	1610	5971	0.004
Protein (g)	103.84±29.47	55.3	233.9	0.132*
Fat (g)	100.2±37.63	44	277.5	0.102*
Carbohydrate (g)	440.5±114.5	137.38	767.5	0.001

Description:

BMI/U: body mass index by age

TEE: total energy expenditure

SD: standard deviation

<sup>#</sup>Homogeneity test with Levene's test

\*Significance at p-value>0.05 level

All subjects in the study were in the subject age range between 14-18 years with an average of 16.25±1.05 years. The subject's body weight was at a minimum of 45 kg and a maximum of 83 kg, with an average of 64.13±8.03 kg. The average height of the subjects was 173.22±7.53 cm, with a minimum of 154.8 cm and a maximum of 188 cm. The average nutritional status according to BMI/U was 0.10±0.71 SD (standard deviation) with a minimum value of -1.89 SD and 1.23 SD. The average total energy requirement of the subjects was 3532+323 kcal, with a minimum total energy requirement of 2732 kcal and a maximum total energy requirement of 4289 kcal.

Characteristics of athletes' food intake with 1x24 hour food recall conducted before the intervention found that the average energy intake consumed by the subjects was 3090+773 kcal with a minimum total energy intake of 1610 kcal and a maximum of 5971 kcal. The average yield was 103.84+29.47 g, with a minimum intake of 55.3 g and a maximum intake of 233.9 g. The subject's minimum fat intake was 44 g, and maximum intake was 277.5 g, with an average intake of 100.2±37.63 g. The average carbohydrate intake of the subjects was 440.5+114.5 g, with a minimum intake of 137.38 g and a maximum intake of 767.5 g. Based on the homogeneity test of the subject's food intake characteristics before the intervention, protein and fat intake showed homogeneity with p=0.132 and p=0.102 (p>0.05), while energy and carbohydrate intake did not show homogeneity. This is supported by the statement that athletes often fail to meet the recommended amount of energy and carbohydrates, so strategies to replenish carbohydrate stores may be prioritized to prepare for maximum performance in the next match<sup>33</sup>.

During the study, the subject characteristics based on the subject's food intake with the 3x24-hour food recall method were not consecutive in each treatment group. The average energy intake in the control group was  $3249 \pm 1016$  kcal, and the treatment group was 3485 + 952 kcal. The average protein intake of the control group was  $103.46 \pm 34.03$  g, while the treatment group was  $114.45 \pm 35.03$  g. The average fat intake of the control group subjects was  $89.76 \pm 34.39$  g, and the treatment group was  $109.31 \pm 40.32$  g. The average carbohydrate intake of the control group subjects amounted to  $489.33 \pm 170.05$  g, while in the treatment group subjects amounted to  $500.97 \pm 157.31$  g. The characteristics of the subjects' food intake during the study are presented in Table 2 below.

**Table 2.** Characteristics of study subjects based on food intake during the study

Group	Energy (kcal)	Protein (g)	Fat (g)	Carbohydrate (g)
Control	3249±1016	103.46±34.03	89.76±34.39	489.33±170.05
Treatment	3485±952	114.45±35.03	109.31±40.32	500.97±157.31

This difference in total food intake indicates an increase in energy and nutrient intake in the treatment group. Chocolate milk can increase carbohydrate intake, although, in this study, there was no 6-8% increase when viewed from the total food intake. This condition may be due to the different food menus consumed by the study subjects in the control and treatment groups. This causes differences in food preferences, where athletes may prefer the menu cycle during the control group research period compared to the treatment group

research period, affecting the amount of food consumed. However, the treatment group with chocolate milk consumption before training still experienced an increase in carbohydrate intake aimed at carbohydrate loading to maintain adequate muscle and liver glycogen stores.

Statistical test analysis to determine the increase and difference in muscle endurance before and after the intervention is presented in Table 3. The results of the analysis showed that there was a difference in muscle

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endurance assessed by the increase in the average mileage between before and after the intervention in the treatment group with chocolate milk consumption with p-value=0.000 (p<0.05). Based on this treatment, it can be seen that the consumption of chocolate milk has

an effect on increasing muscle endurance by 163.83 m. This increase occurred when before the treatment the athlete's muscle endurance was  $2150.64 \pm 420.72$  m and after treatment  $2314.47 \pm 400.14$  m.

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Group	Pretest (Mean±SD)	Posttest (Mean±SD)	Δ Posttest-Pretest (Mean±SD)	р#	p^
Control	2150.64±420.72	2182.98±434.75	32.34±119.45	0.070	0.000*
Treatment	2150.64±420.72	2314.47±400.14	163.82±141.21	0.000*	0.000

Description: SD: standard deviation

<sup>#</sup>Parametric test with Paired T Test

<sup>^</sup>Non-parametric test of  $\Delta$  posttest-pretest comparison with Mann Whitney

\*Significance at p-value<0.05 level

Increased muscle endurance in terms of running mileage was also carried out in 2015 research<sup>23</sup> in adolescent athletes in football sports which states that the mileage value of athletes is higher in the treatment with low-fat milk consumption compared to commercial sports drinks. The increase in mileage caused by carbohydrate loading is aligned with the 2016 research citation. Increased carbohydrate intake is proven to increase running mileage in football athletes of the Football Association of Sleman (PSS) Yogyakarta by 26%<sup>34</sup>.

The increase in muscular endurance that occurs due to the consumption of chocolate milk may be due to the nutrient content in chocolate milk, particularly carbohydrates. Carbohydrates in chocolate milk consist of lactose, sucrose, and commercial added sugars such as glucose-fructose syrup<sup>35</sup>. These carbohydrates are converted into glucose in the liver and then become a source of energy in the body. Glucose in the human body serves as fuel for metabolic processes and is one of the main sources of energy. Muscles consume glucose as fuel and store glycogen, which is an energy source for muscle contraction<sup>15,36</sup>. Carbohydrate intake at 3-4 hours before exercise increases endogenous glycogen stores while helping maintain blood glucose levels to prevent hypoglycemia<sup>18,33</sup>. In addition, pre-workout carbohydrate consumption has been shown to extend cycle time to fatigue by 20% and improve exercise performance by 2-3%<sup>18,37</sup>.

When the body is deprived of carbohydrate stores during exercise, it will look for other sources of carbohydrates, such as converting protein into gluconeogenesis. carbohydrates or During gluconeogenesis, proteins in the muscles are broken down into amino acids and converted into glucose in the liver to be used as an energy source. This is one of the reasons why carbohydrate intake is crucial for athletes. If carbohydrate intake is sufficient to meet energy needs, then protein does not need to be converted into carbohydrates, and muscle protein is spared from catabolism. In other words, sufficient carbohydrate intake in the diet will reduce muscle protein catabolism<sup>22</sup>.

Protein intake can increase amino acid availability, reduce exercise-induced protein catabolism, and minimize muscle damage. Combining small amounts of protein with carbohydrates before exercise can improve protein balance and stimulate protein synthesis during the training or recovery phase. Protein synthesis in the training phase plays a role in increasing muscle mass, which will increase muscle strength and endurance, while in the recovery phase, it will impact the muscle repair process<sup>16,25</sup>.

The results of the statistical test analysis of muscle endurance after the intervention presented in Table 3 show that there is a significant difference between the control and treatment groups with a p-value=0.000. The muscle endurance value of the treatment group has a higher mileage value compared to the control group. Based on this, it can be seen that the treatment group with chocolate milk consumption increased muscle endurance by 163.83 m.

Carbohydrate consumption before to exercise of a certain intensity and duration may improve athlete performance. Conversely, inadequate intake may impair performance and physiological adaptations associated with high-intensity exercises, such as increased carbohydrate oxidation<sup>38</sup>. Thus, muscular endurance is closely related to the nutrients consumed by athletes. The greater the amount of glycogen stored in the muscle, the longer the endurance. The higher the carbohydrate consumption, the higher the muscle glycogen storage. Another study mentioned that marathon athletes who were on a high-carbohydrate diet had muscle endurance in running for 240 minutes, athletes with a normal diet for 120 minutes, and athletes with a high-fat diet for 85 minutes<sup>39</sup>.

This increase in muscular endurance can occur with the support of fat oxidation as an energy supply<sup>38</sup>. Training for long periods with long-term periods performed by athletes can increase the capacity of muscles to use fat to protect glycogen usage and improve physical endurance capacity<sup>23</sup>. Good exercise forms good quality and number of mitochondria. This can occur because aerobic activity can help increase biogenesis or mitochondrial formation. Good mitochondria are needed in the process of adenosine triphosphate recycling (ATP recycling) which is the process of releasing energy from nutrients to form ATP. Physical exercise can also increase membrane folds (cristae) in mitochondria that function as a place where the process of ATP formation from ADP and phosphate groups occurs. The connection between mitochondria and exercise is vital as a process of adaptation to energy

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needs and availability. Mitochondria are known to increase the amount of ATP through the process of ATP recycling resulting from oxidative phosphorylation and beta oxidation of fatty acids<sup>37</sup>. Chocolate milk contributes to fat intake by containing 5 g of fat

consisting of 3 g of total fat and 2 g of saturated fat. This condition caused an increase in the average fat intake in the treatment group to  $109.31 \pm 40.32$  g compared to the control group of  $89.76 \pm 34.39$  g.

Table 4. Effect and difference of Maximal Oxygen Volume Capacity (VO <sub>2</sub> Max) of subjects before and after the intervention
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Group	Pretest (Mean±SD)	Posttest (Mean±SD)	Δ Posttest-Pretest (Mean±SD)	p#	p^	
Control	53.95±5.96	54.40±6.21	0.44±1.76	0.089	0.000*	
Treatment	53.95±5.96	56.30±5.62	2.34±2.05	0.000*	0.000*	
Decentrations.						

Description:

SD: standard deviation

<sup>#</sup>Parametric test with Paired T Test

^Non-parametric test of  $\Delta$  posttest-pretest with Mann Whitney

\*Significance at p-value<0.05 level

Table 4 shows a significant difference in the average VO2 Max capacity between before and after the intervention with a p-value=0.000 (p<0.05). In this study, it is known that chocolate milk consumption increases VO2 Max capacity by 2.34 ml/kg/minute. This increase occurred before the treatment the athlete's muscle endurance was 53.95 ± 5.96 ml/kg/minute and after treatment 56.30 ± 5.62 ml/kg/minute. This increase in VO<sub>2</sub> Max capacity occurred in research on adolescent athletes in football in 2015 which stated that the value of VO2 Max capacity of athletes was higher in the treatment with low-fat chocolate milk consumption compared to commercial sports drinks with an average value of 58.57 ± 4.39 and 55.82 ± 4.33 ml/kg/minute respectively<sup>23</sup>. An increase in VO<sub>2</sub> Max capacity also occurred in a 2022 study on badminton athletes. There was a significant increase between the pretest and posttest in the low-fat chocolate milk consumption treatment group which amounted to  $38.6 \pm 0.32$  and 39.1 ± 0.56 ml/kg/minute respectively<sup>26</sup>.

The increase in VO<sub>2</sub> Max capacity that occurred after the chocolate milk consumption treatment for 6 days can cause an increase in the availability of glucose and glycogen in the body. Consuming carbohydrates before exercise can increase carbohydrate oxidation in the body. Oxidized carbohydrates will increase O2 saturation in the blood so that increased O2 consumption is distributed to tissues. The availability of O2 in the body can increase muscle work which will lead to improved athlete performance<sup>23</sup>. This condition can result from changes in the cardiovascular system to the amount of oxygen consumption which will correlate to improved skeletal muscle function through oxygen transfer and utilization<sup>26</sup>. Proper total energy intake before training, such as from the consumption of chocolate milk, will ensure that protein is not used as an energy source during training. Adequate carbohydrate intake will decrease amino acid oxidation and avoid muscle protein catabolism which will lead to improved athlete performance<sup>22</sup>.

The content of chocolate milk in this study can act as bioactive components such as growth factors, immunoglobulin (Ig), lactoperoxidase, lysozyme, lactoferrin, cytokines, nucleosides, vitamins, peptides, and oligosaccharides. The immunoglobulin content in 30 g/L cow's milk protein reaches 1 g/L. The content of immunoglobulin G (IgG) in cow's milk can contribute to preventing gastrointestinal and respiratory infections in humans through proteolytic processes and increasing the active capacity to bind to receptors. Receptors that play a role in immunity are Fc receptors (FCRs). FcRs have important immunological functions including phagocytosis, degranulation, antibody-dependent cellular cytotoxicity (ADCC), cytokine formation, lipid mediators, and superoxide production<sup>40</sup>.

The protein content in chocolate milk can be one of the causes of an increase in VO<sub>2</sub> Max capacity. This is in line with previous research<sup>23</sup> which says that giving protein drinks 1-3 hours before training can increase the VO<sub>2</sub> Max levels of athletes when compared to consuming only water. Cow's milk has good nutritional quality because it has an essential amino acid composition, namely the protein fraction called whey protein and casein. Whey protein has characteristics as a protein that has high digestibility and absorption or is often referred to as a fast protein. Whey protein also has a higher content of branched chain amino acids (BCAA) and has a high biological value consisting of  $\beta$ lactoglobulin,  $\alpha$ -lactalbumin, and glycomacropeptide with the main content being leucine. Research shows that consuming whey protein and casein in cow's milk can increase muscle protein synthesis and gain after resistance exercise. Whey protein in this case takes a role in stimulating muscle protein synthesis while casein takes a role in prolonging the effect of protein synthesis<sup>41</sup>. The highest proportion of casein found in milk protein is  $\alpha$ s1,  $\alpha$ s2,  $\beta$ , and  $\kappa$ -casein. Casein can trigger a decrease in gastric emptying so that casein is digested more slowly than whey protein and helps delay the use of amino acid reserves in the blood after eating. Another study showed that feeding 2x15 g of milk for 24 weeks accompanied by progressive resistance-type exercise training (2 sessions/week) was able to increase lean body mass gain in young people. This study also showed that milk feeding accompanied by resistance exercise was able to influence muscle fiber hypertrophy while increasing muscle mass and muscle strength compared to carbohydrate and isoenergetic soy consumption<sup>42</sup>. Chocolate milk in this study has contributed to protein intake by 7 g so there is an increase in the average protein intake in the treatment group to 114.45+35.03 g compared to the control group

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of 103.46±34.03 g. The average protein intake in the treatment group was 114.45±35.03 g compared to the control group.

The results of the statistical test analysis of VO<sub>2</sub> Max capacity after the intervention presented in Table 4 show that there is a significant difference between the control and treatment groups with a p-value=0.000. This condition indicates a significant difference between the control and treatment groups. The VO<sub>2</sub> Max capacity of the treatment group has a higher VO<sub>2</sub> Max value compared to the control group. Based on this, it can be seen that the treatment group with chocolate milk consumption has an effect in increasing VO<sub>2</sub> Max capacity by 2.34 ml/kg/minute. This condition can occur because VO<sub>2</sub> Max capacity is influenced by several factors including training, daily activities, rest and recovery time, and psychological factors when testing is carried out<sup>23</sup>. Based on these factors, the control group without chocolate milk consumption still affects the increase in VO<sub>2</sub> Max capacity. Physical exercise results in several changes in the cardiorespiratory system that correlate to an increase in VO<sub>2</sub> Max capacity. These changes include 1) an increase in the volume of blood pumped by the heart due to hypertrophy of the heart muscle and an increase in the contraction ability of the heart muscle, 2) an increase in total lung capacity, and 3) an increase in blood volume and hemoglobin as essential factors in the oxygen transportation system and strongly correlated with VO<sub>2</sub> Max<sup>12</sup>.

The results of this study are in line with research in the year that compared the control group, physical activity treatment group, and physical activity treatment group with chocolate milk consumption<sup>24</sup>. The physical activity treatment group can be assumed to be like the control group in this study. The average pretest of the control group, physical activity treatment group, and physical activity treatment group with chocolate milk consumption were 38.86, 38.80, and 38.14, respectively, and with posttest values of 38.07, 50.40, and 53.93, respectively. The results showed that there was a significant difference in mean VO<sub>2</sub> max between the adjusted pretest and posttest means in each group at the 0.05 level.

Exercise activities with high intensity and long duration will cause the formation of reactive oxygen species or ROS. The ROS produced will act with several molecular signals and induce molecular changes. One of the positive effects of ROS production is mitochondrial biogenesis. However, excessive and uncontrolled ROS production will lead to prolonged inflammation<sup>37</sup>. ROS will cause complaints such as muscle damage, fatigue, and slow recovery that are detrimental to the sport<sup>7</sup>. Athletes who have done long-term training can improve the body's adaptation to ROS because they can produce antioxidant enzymes that can suppress the formation of excess ROS, such as superoxide dismutase (SOD) and glutathione peroxidase (GPX). In addition, consuming foods rich in antioxidants can be done to help control ROS production<sup>37</sup>.

Specifically, the consumption of chocolate milk containing cocoa powder has benefits as an antioxidant. This nutraceutical strategy has been increasingly proposed as a potential tool suitable for controlling ROS

and associated inflammation during intense physical exercise. Chocolate is a source of antioxidant polyphenols that have been shown to have healthpromoting effects through their antioxidant, antiinflammatory, and metabolic properties<sup>43</sup>. The benefits of chocolate polyphenols as antioxidants were demonstrated in a previous study with the consumption of dark chocolate containing 200 mg polyphenols in cyclists for two weeks hours before a 1.5-hour cycling workout. The study found a significant increase in total antioxidant activity capacity, although there was no improvement in athlete performance43. Meanwhile, a study on the benefits of chocolate polyphenols to improve performance in football athletes revealed significant improvements in antioxidant defense and exercise performance after administration of polyphenols at a low dose of 68 mg for two weeks and a high dose of 1050 mg for five days<sup>43</sup>.

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Chocolate milk, besides being a food product with a high carbohydrate content, also contains chocolate powder in it. In each serving of chocolate milk, 45 g of milk powder contains about 2.27 g. Based on literature studies, chocolate powder contains 12-18% polyphenols of dry weight<sup>43</sup>. Therefore, it can be seen that each serving of chocolate milk contains 272.4-408.6 mg. However, according to experts, the cocoa powder contained in chocolate milk has gone through an alkalization process, thus reducing its antioxidant potential<sup>44</sup>.

Beyond the regulation of increasing muscular endurance,  $VO_2$  max capacity, muscle recovery, and the appropriate amount of carbohydrate intake, at any given time carbohydrate consumption has not been shown to negatively impact performance or recovery. Strategically timed carbohydrate consumption may accelerate muscle glycogen resynthesis and provide favorable hormonal responses. However, studies in professional athletes with high training intensity have shown that glycogen levels are maximally restored within 24 hours if athletes consume a diet containing 28 g/kg/day of carbohydrate<sup>33,45</sup>.

This study has several limitations during the implementation of the study. The total food intake of athletes in a day outside of the chocolate milk intervention that affects muscle endurance and VO<sub>2</sub> Max capacity cannot be controlled properly. This is related to the possibility of athletes not complying with the food intake guidelines provided by researchers as a form of control of food intake, while researchers cannot supervise food intake directly at every meal. Therefore, more appropriate instruments and methods are needed to control food intake outside of the intervention provided to assess the effect of carbohydrate loading before training/competition on muscle endurance and VO<sub>2</sub> Max capacity of game sports athletes.

The differences in training programs and training periodization that exist in each game sport cause differences in training programs. This can allow differences in the type, composition, intensity, volume, and duration of training that can increase greater endurance between sports. In addition, selecting MFT to measure muscular endurance and VO<sub>2</sub> Max with a large

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number in a short time gives athletes a sense of boredom.

#### CONCLUSIONS

The results of this study obtained the conclusion that there was a significant effect on the consumption of chocolate milk with muscle endurance and maximum oxygen volume capacity (VO<sub>2</sub> Max) of athletes in BPPLOP Central Java between before and after treatment, and there is a significant difference in muscle endurance and maximum oxygen volume capacity (VO<sub>2</sub> Max) of athletes in BPPLOP Central Java between the control group and the treatment group. This effect is also supported by the total food intake consumed by athletes outside the chocolate milk consumption intervention, which helps increase athletes' carbohydrate adequacy and muscle and liver glycogen. Meanwhile, the training program of each game sport can increase muscle endurance and VO<sub>2</sub> Max capacity through the formation of a good number and quality of mitochondria to increase ATP from oxidative phosphorylation and beta oxidation of fatty acids.

The suggestions that can be made in future research are that other measurement methods can replace the measurement of muscle endurance focusing on specific muscle endurance such as push ups, sit ups, and squad jumps. More interesting measurement methods can be used to measure maximum oxygen volume capacity (VO<sub>2</sub> Max), such as selecting running test methods or using a static bicycle or treadmill.

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All authors in this study have no conflict of interest. This research was funded independently by the authors.

#### **Author Contributions**

VMBJ: responsible for all scientific content of the article, formulating problem formulation, collecting, analyzing, and interpreting data, preparing draft manuscripts, revising manuscripts, and revising manuscripts. interpreting data, preparing manuscript drafts, revising; NDP: conduts supervision and mentoring in all contents of scientific articles scientific articles, provide criticism, input and suggestions for writing manuscripts, revising manuscript discussions.

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