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ORIGINAL RESEARCH

Efficacy of chocolate milk in facilitating post-workout regeneration of young male skiers

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Abstract

The use of nutritional strategies has become more widespread in recent years. This study investigates the effect of chocolate milk (CM) consumption on the recovery rate of elite male adolescent alpine skiers after intense training. Twelve male elite alpine skiers aged 13-16 years were randomly divided into two groups: chocolate milk (CM; n = 6) and placebo (PLA; n = 6). After exercise, the CM group was given 400 ml (200+200) of chocolate milk, and the PLA group took water (placebo) in the same regimen. Venous blood samples were taken before and 12 hours after drinking the fluids to determine the activity of selected enzymes, insulin, testosterone, glucose, and some minerals. It was observed that in the CM group, serum AST (Pre: 110.33±126.62 U/L vs. Post: 83.17±100.42 U/L) and CK (Pre: 2393.12±2542.22 U/L vs. Post: 1556.33±1401.45 U/L) activity, as well as testosterone (Pre: 21.12±3.59 ng/mL vs. Post: 15.19±4.96 ng/mL; p=0.028) and iron (Pre: 163.00±14.76 µg/dL vs. Post: 109.00±23.91 µg/dL; p=0.028), decreased significantly, but potassium (Pre: 4.38±0.16 mmol/L vs. Post: 4.64±0.09 mmol/L) and magnesium (Pre: 1.82±0.09 mg/dL vs. Post: 2.07±0.04 mg/dL) levels increased after supplementation compared to baseline levels ($p<0.05$). After taking into account the changes that occurred in the PLA group and significant differences in some variables in terms of between-group comparisons, it was found that the decrease in AST and CK activity, along with the presence of positive ionic changes in the serum after CM supplementation indicates a reduction in negative changes after training and improving the process of post-training regeneration in young male athletes.

Keywords: Chocolate milk, exercise physiology, recovery, ski, sports drinks, sports nutrition.

Introduction

It is documented that exercise performance is affected by the amount of glycogen contained in skeletal muscles, and vigorous exercise reduces these reserves, which leads to lower physical performance (Karp et al., 2006). Alpine skiing is a winter sport with varying degrees of muscle contractility, alternating between high and low-intensity efforts (Seifert et al., 2005). Under such conditions, muscle glycogen becomes the primary substrate that drives muscle contractions during various evolutions. With such an intense load, muscle glycogen stores may be depleted by more than 50% of the initial concentration (Tesch, 1995). In addition, alpine skiers' training programs include long duration and varying intensity (Gilgien et al., 2018), which also deplete muscle glycogen stores. Therefore, replenishing depleted glycogen stores is crucial to maintaining full performance during the subsequent training session.

One of the key factors to replenish muscle and liver glycogen reserves, as well as fluid and electrolytes lost during exercise, is post-exercise nutrition (Amiri et al.,

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2019). Glycogen resynthesis occurs fastest between training sessions if carbohydrate (CHO) is consumed immediately after exercise (Karp et al., 2006). Moreover, delaying the intake of CHO after the training session will reduce the rate of glycogen resynthesis (Ivy, 2001). To increase the rate of resynthesis in the next few hours, it is recommended to consume 50–75 g of CHO in the first 30–45 minutes after exercise (Karp, 2006). Moreover, adding protein (PRO) to the CHO consumed after exercise can also improve the recovery rate (Burke et al., 2017).

It has also been shown that compared to CHO alone, CHO + PRO supplementation lowers the level of markers of muscle membrane disorders, such as creatine kinase (CK) and myoglobin (MB), reduces muscle soreness, and improves muscle function (Gilson et al., 2010). The latest guidelines for optimizing glycogen resynthesis suggest that protein co-ingestion does not affect glycogen synthesis when adequate CHO intake (1 g/kg/h) is attained. The addition of 0.3 g/kg/h of protein to a carbohydrate supplement, however, might accelerate muscle glycogen resynthesis through a synergistic increase in insulin secretion and muscle glucose uptake under specific conditions, including low daily energy consumption or when the amount of carbohydrates is insufficient (especially in the first 4 h after exercise) (Burke et al., 2017). While solid and liquid supplements are equally effective, liquid supplements are preferred. Fluids also keep the body hydrated. Therefore, in post-exercise recovery, it is recommended to take liquid supplements (Spaccarotella & Andzel, 2011).

Various drinks have been used for regeneration purposes, including those containing only CHO (Temesi et al., 2011; El-Sayed et al., 1995), CHO + PRO, or carbohydrates and electrolytes (CHO + E) (Jeukendru, 1997). It has been suggested that CHO + E drinks can improve the efficiency of repeated exercises (Davison et al., 2008), and supplementation may be effective in restoring fluid balance during short-term recovery after exercise, especially after exercise-induced dehydration (Wong & Chen, 2011). Moreover, CHO + PRO drinks can optimize the time to exhaustion (TTE) (Saunders et al., 2004; Saunders et al., 2007), reduce the increase in CK activity (Skillen, 2008), enhance glycogen resynthesis (Ivy et al., 2002; Berardi et al., 2006), and thanks to the protein content, provide additional energy compared to CHO drinks (Saunders et al., 2004). Nutrient supplementation has been widely shown to improve endurance in CHO and CHO + PRO forms. Consuming 600 mL of the sports drink CHO + PRO per hour reduces muscle tension and allows 3 hours of skiing (Saunders et al., 2007). However, due to the intense nature of alpine skiing racing, it is debatable whether competitors could tolerate the consumption of sports drinks in amounts expected to improve performance during training (Seifert et al., 2012).

Defined as functional food, milk has a direct and measurable effect on the body (Keri Marshall, 2004). The high concentration of electrolytes in milk helps to replace those lost during exercise by sweating. Studies comparing the effectiveness of milk, water, and CHO drinks in rehydrating the body after cycling in a hot environment showed that participants maintained a positive balance of fluid and potassium only after consuming milk (Shirreffs et al., 2007; Watson et al., 2008). Other studies confirmed these observations indicating that milk is a preferred supplement (Karp et al., 2006; Pritchett et al., 2009; Thomas et al., 2009).

Chocolate milk (CM) has been studied as a possible recovery beverage, as it contains comparable carbohydrate and protein levels to CHO+PRO beverages associated with improved post-exercise recovery. Compared to many commercial CHO+E drinks, CM contains more CHO in the same volume. It also supplies fluids and sodium (sodium has a crucial role in rehydration) (Clapp et al., 2000), which need to be replaced due to sweat loss during training. CM also contains alkali-treated cocoa, which contains flavanols that decay oxidative stress markers. The flavanol content in cocoa-based drinks and chocolates varies and highly depends on the processing (Pritchett et al., 2013). In addition to its other benefits, CM has also been noted for its good taste, wide availability, low price, and accessibility, which could make it a popular alternative to commercial sports drinks (Gilson et al., 2010). In accordance with the information above, our study analyzes the effects of CM consumption on the recovery rate in alpine skiers after high-intensity training. Therefore, we hypothesized that chocolate milk consumption would attenuate exercise-induced muscle damage and accelerate recovery.

Methods

Research Group

The children's parents or legal guardians voluntarily consented to participate in this study. In addition, Sinop University's Human Research Ethics Council has approved this study (number: 55317723-604.01.01-E).

Twelve volunteers, male elite alpine skiers aged 13 to 16, competing in the Turkish national team, participated in the study and trained regularly six times a week for four hours a day during a training camp. Participants were randomly divided into chocolate milk (CM; n= 6) and placebo (PLA; n= 6). Both groups were subjected to the same training programs and recovery methods during the camp period. Hence, the reliability of the supplementation effect on the participants' parameters, whether positive or negative, was determined. The same recovery methods were applied to athletes with the same high-volume and intensity training program. Later, participants were instructed to divide the training into two sessions a day, morning and afternoon. The athletes looked for health requirements, the absence of chronic or acute disease, and the absence of mobility limitations due to disability for any reason.

Table 1
Descriptive statistics for participants (Mean±SD).

Variables	CM	PLA
Age (year)	14.50±1.00	14.50±1.73
Height (cm)	169.25±4.57	173.00±15.68
Weight (kg)	61.75±12.01	63.00±9.20
BMI (kg/m^2)	21.70±5.08	20.32±.60
Total supplement (CM) consumed (mL)	200+200	-
Total water consumed (mL)	-	200+200

Blood Measurements

The athletes' venous blood was drawn twice, before training (basic intake) and 12 hours after consumption of the first portion of chocolate milk/water, by a qualified nurse (Saunders et al., 2004). Blood samples were centrifuged at 3500 x g for 15 min. The obtained serum samples were aliquoted and stored in Eppendorf tubes at -80 °C for later analysis. The following variables were determined in these samples: total protein, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT), lactate dehydrogenase (LDH), creatine kinase (CK), insulin, testosterone, glucose, sodium, potassium, calcium, chlorine, magnesium, and iron. Biochemical and hormonal analyses were performed on serum samples using an Abbott Architect c16000 automated chemistry analyzer and a Siemens Advia Centaur XP hormone (hormone) analyzer.

Nutrition Protocol

The study was conducted as a single-blind test. A total of 400 mL of chocolate milk in the CM group was administered in two portions of 200 mL each. The first was given immediately after the end of exercise and the second was given 2 hours later (Amiri et al., 2019). The energy value of this supplement along with its approximate composition is presented in Table 2. The PLA group received a placebo (water) in a similar amount and at the same time as the CM group. Athletes were not informed about what they were drinking. In this way, possible psychological effects that could negatively affect the study results were eliminated in the first place.

Table 2
Averaged composition of the drink for the chocolate milk (CM) group.

Nutrient Content	200 mL	400 mL
Energy (kcal)	128	256
Carbohydrates (g)	21	42
Protein (g)	5.6	11.2
Fat (g)	2.4	4.8

Experimental Design

First, the height and weight of the athletes were measured (Table 1). The 12 participants were randomly divided into two groups: the chocolate milk (CM; n = 6) and the placebo (PLA; n = 6). Similar nutrition, sleep and fluid consumption programs were applied to the athletes as a diet plan since they were in the camp period. Immediately after the last training session, the athletes were given the first supplementary dose, i.e., chocolate milk in the CM group and water as a placebo in the PLA group. Two hours later, subjects were administered the same second supplementation doses. Venous blood samples were collected from athletes for biochemical analyses for 12 hours (the next day, immediately after waking up) after administering the first supplementary dose (Saunders et al., 2004). The same research test protocol was used twice, before and after supplementation. The study was conducted without any interference with the athletes' daily routines, training systems, or nutritional plans. Measurements and tests were carried out under the same physical conditions for both CM and PLA groups.

Statistical Analyses

The results are presented as mean ± standard deviation and 95% confidence intervals (CI). We assessed the

distribution of the analyzed variables using the Shapiro-Wilk test. The results showed that the obtained values differed from the normal distribution. Therefore, detailed statistical analyses were applied using non-parametric tests. The Mann-Whitney U test was used to assess the differences between the CM and PLA groups. However, the Wilcoxon test was used to assess statistical differences before and after supplementation in each group. Statistical significance was accepted as $p<0.05$. The software package SPSS 22.0 was used for statistical analysis.

Results

According to Table 3, in the pretest comparisons of the CM and PLA groups, no significant difference was detected in T. Protein (g/dL) values (U=6.000; p=0.327), a significant difference was found in posttest comparisons (U=8.000; p=0.624). A significant difference was observed in ALT (U/L) values in pretest comparisons (U=2.000; p=0.015), whereas there was a significant difference in ALT (U/L) values (U=3.000; p=0.041) in posttest comparisons. Statistical significance was observed in the pretest comparisons of the AST (U/L) variable (U=4.000; p=0.026). No significant difference was found in posttest comparisons in AST (U=4.000; p=0.142). No significance was found in the pretest comparisons of the GGT (U/L) variable (U=4.000; p= 0.459), while a significant difference was observed in the posttest comparisons (U=4.500; p=0.026). LDH (U/L) variable was not significant in the pretest comparisons (U=8.000; p=0.624); likewise, there was no significant difference in the posttest comparisons (U=8.000; p=0.624). No significant difference was found in the pretest values of the CK (U/L) (U=4.000; p=0.142). Similarly, no difference was observed in posttest comparisons (U=8.000; p=0.624). No significant difference was found in the pretest values of the insulin (μIU/mL) (U=7.000; p=0.462). Likewise, no statistically significant difference was found in posttest comparisons (U=6.000; p=0.327). In the pretest comparisons of the testosterone (ng/mL), a significant difference was found (U=.000; p=0.002); accordingly, a statistically significant difference was observed in the posttest comparisons (U=2.000; p=0.026).

In the within-group pretest and posttest comparisons of the CM group, no significant difference was detected in T. Protein (Z=-.948; p=0.248), ALT (Z=-.962; p=0.458), GGT (Z=-.680; p=0.246), LDH (Z=-.405; p=0.917) and Insulin (Z=-.674; p=0.345) values. It was determined that the AST value of the CM group decreased in the posttest (Z=-2.023; p=0.028). The CK values were found to be low in the posttest (Z=-2.023; p=0.028), while the Testosterone values were decreased (Z=2.023-; p=0.028). In the within-group pretest and posttest comparisons of the PLA group, no significant difference was detected in T. Protein (Z=1.826-; p=0.345), ALT (Z=1.342-; p=0.066), AST (Z=-.921; p=0.461), Insulin (Z=-1.095; p=0.173) and Testosterone (Z=-1.826; p=0.075) values. Also, it was found that GGT values increased (Z=-1.633; p=0.034), LDH values increased (Z=-1.826; p=0.028), and similarly, CK values increased (Z=1.461; p=0.046).

Table 3

Changes in mean metabolic, enzymes, and hormonal values of CM and PLA groups (Mean±SD).

Variables	CM		PLA	
	Pre	Post	Pre	Post
T. Protein (g/dL)	72.83±2.03	74.53±3.51	71.82±2.70	73.30±1.80
ALT (U/L)	45.17±13.00	43.67±13.46	24.33±5.47*	25.83±7.14#
AST (U/L)	110.33±126.62	83.17±100.42†	41.33±19.16*	39.33±18.30
GGT (U/L)	18.00±4.98	19.33±3.08	14.67±2.34	15.67±2.94†#
LDH (U/L)	289.50±73.56	291.33±57.74	258.67±61.12	268.67±58.54†
CK (U/L)	2393.12±2542.22	1556.33±1401.45†	810.20±1087.78	1072.62±1120.04†
Insulin (μIU/mL)	6.71±1.37	8.27±2.47	8.55±2.67	9.79±1.61
Testosterone (ng/mL)	21.12±3.59	15.19±4.96†	10.74±5.41*	8.81±4.77#

*CM: Chocolate milk group; PLA: Placebo group; Pre: Pre-supplementation period; Post: Post-supplementation period. Values are mean±SD. † Significant difference compared with pre- and post-supplementation values (p<0.05). * Significant difference in pre-supplementation between CM and PLA groups (p<0.05); # Significant difference in post-supplementation between CM and PLA groups (p<0.05).*

Table 4
Changes in mean metabolic and mineral concentration of CM and PLA groups.

Variables	CM		PLA	
	Pre	Post	Pre	Post
Glucose (mg/dL)	82.50±4.51	80.83±3.76	82.17±6.01	79.83±2.93
Sodium (mmol/L)	139.17±1.17	140.83±1.17	138.83±0.75	140.67±1.37†
Potassium (mmol/L)	4.38±0.16	4.64±0.09†	4.47±0.22	4.55±0.21
Calcium (mg/dL)	9.48±0.25	9.80±0.30	10.17±0.17*	9.76±0.24†
Chlorine (mmol/L)	107.00±1.79	107.85±1.72	104.50±1.05*	107.33±1.86†
Magnesium (mg/dL)	1.82±0.09	2.07±0.04†	1.95±0.07*	2.29±0.05#
Iron (µg/dL)	163.00±14.76	109.00±23.91†	127.83±12.25*	105.17±24.8

*CM: Chocolate milk group; PLA: Placebo group; Pre: Pre-supplementation period; Post: Post-supplementation period. Values are mean±SD. † Significant difference compared with pre- and post-supplementation values (p<0.05). * Significant difference in pre-supplementation between CM and PLA groups (p<0.05); # Significant difference in post-supplementation between CM and PLA groups (p<0.05).*

According to Table 4, no significant difference was found in Glucose (mg/dL) in the pretest comparisons of the CM and PLA groups (U=8.000; p=0.903), and no significant difference was detected in the posttest comparisons (U=5.500; p=0.266). No significant difference was observed in the pretest comparisons of the Sodium (mmol/L) variable (U=4.500; p=0.150); similarly, no significant difference was found in the posttest comparisons (U=8.500; p=0.706). No statistically significant difference was found in the comparison of potassium (mmol/L) pretest (U=9.500; p=0.902) and posttest (U=7.000; p=0.462) values. On the other hand, a significant difference was detected in Calcium (mg/dL) pretest comparisons (U=0.000; p=0.002), whereas no significant difference was observed in posttest comparisons (U=8.000; p=0.624). While there was a significant difference in the pretest comparisons of the chlorine (mmoL/L) variable (U=3.000; p=0.026), no significant difference was found in the posttest comparisons (U=8.000; p=0.802). A significant difference was found in the comparison of pretest (U=3.000; p=0.026) and posttest (U=.000; p=0.002) values in Magnesium (mg/dL). Lastly, a statistically significant difference was found in the pretest comparisons of the Iron (µg/dL) variable (U=1.000; p=0.002), while no difference was found in the posttest comparisons (U=7.000; p=0.462).

In the within-group pretest and posttest comparisons of the CM group, no statistically significant difference was found in Glucose (Z=-1.095; p=0.136), Sodium (Z=-1.518; p=0.068), Calcium (Z=-1.483; p=0.075), Chlorine (Z=-.736); p=0.414) values. It was observed that Potassium (Z=-2.023; p=0.028) and Magnesium (Z=-2.023; p=0.028) values increased in the posttest while Iron (Z=-2.023; p=0.028) values decreased. There was no statistically significant difference in Glucose (Z=-1.095; p=0.207), Potassium (Z=-.730; p=0.249), Magnesium (Z=-;1.826 p=0.270) and Iron (Z=-1.461; p=0.075) values. While Sodium (Z=-1.604; p=0.024) and Chlorine (Z=-1.841; p=0.027) values increased in the posttest, Calcium (Z=-1.826; p=0.028) values decreased.

Discussion

This current study aimed to investigate CM consumption's effects on the recovery rate in alpine skiers after high-intensity training. In competitive sports, among many adaptation changes, there is muscle damage and their regeneration, reduction of the body's energy reserves (glycogen), significant dehydration of the body with electrolyte changes, or increased protein metabolism. The results of this study showed that the proposed mechanism for promoting post-exercise recovery by chocolate milk supplementation might be twofold: a decrease in the activity of enzymes associated with damage to body cells (CK, AST) with a decrease in testosterone and changes in potassium, magnesium and iron levels.

Enzymatic Changes

Based on the decrease in the level of AST, and especially CK in the CM group, it can be clearly stated that the consumption of chocolate milk after exercise reduces muscle damage caused by this type of intervention and improves the process of muscle regeneration after exercise. This beneficial change is very convincing, the

more so that the serum CK level increased after the supplementation in the PLA group. Thus, all these findings supported our hypothesis. There are a limited number of studies assessing the change in CK levels under the conditions of chocolate milk consumption. However, the results of these studies are consistent with ours because after 4 days of intense soccer training and isocaloric consumption of drinks containing chocolate milk and CHO, there was a favorable reduction in serum CK activity (Gilson et al., 2010). Moreover, in a study involving cyclists and triathletes, researchers investigated the effects of chocolate milk and an isocaloric commercial drink containing CHO on the recovery rate after intense, intermittent exercise. The results showed that both drinks positively affect CK levels, but chocolate milk is more beneficial than commercial drinks. However, the study groups had no significant difference in time to exhaustion (TTE) (Pritchett et al., 2009). (Most modern field studies use TTE to measure the rate of post-exercise recovery.) The opposite results were obtained in two other studies where it was observed that TTE at an exercise intensity of 70% of VO_2peak was longer in the CM group than in the CHO group (Karp et al., 2006; Thomas et al., 2009). These data indicate that CM supplementation improves post-training regeneration of the body. The above-mentioned studies' recovery time was relatively short (~4 hours). Thus, drinks containing only complex CHO may take a long time to digest. Chocolate milk, on the other hand, contains only monosaccharides and disaccharides and, therefore, can cause effective regeneration sooner. Due to the fat content in chocolate milk, it may also increase the concentration of circulating free fatty acids and delay the loss of glycogen (Pritchett et al., 2009). Thus, the low proportion of complex carbohydrates and the content of fats in the composition of chocolate milk may be the reason for more favorable conditions for the post-training regeneration process in the CM group.

In the literature, plasma/serum CK activity is often used as an indicator of exercise-induced muscle damage (Lilleng et al., 2011; Kyriakides et al., 2010). On the other hand, reports are showing that CK levels may be weakly associated with direct damage to muscles or muscle function (Warren et al., 1999; Beaton et al., 2002). Therefore, the practical importance of lower serum CK levels with CM supplementation conditions is not an obvious indicator of improvement in post-exercise recovery. Accordingly, it is postulated that the extent of post-exercise muscle damage and the pace of the regeneration process should not be estimated only by the serum CK level. For example, the increase in strength developed during maximum voluntary contraction (MVC) during four days of increased training duration was slightly greater with chocolate milk than with CHO. In a study with judo athletes, chocolate milk consumption after exercise was shown to help improve recovery in athletes performing vigorous exercise. Contrary to other studies, our experiment assessed the effect of chocolate milk on post-exercise regeneration not in laboratory tests, but in sports conditions, increasing their utilitarian value (Papacosta et al., 2015).

ALT, AST, and GGT are liver enzymes. AST and ALT are enzymes of transaminases found mainly in the liver and involved in the metabolism of amino acids. Lower AST levels after consumption of chocolate milk in elite male adolescent alpine skiers are probably the result of decreased amino acid metabolism, including in skeletal muscle (Fragala et al., 2017). Given the fact that supplementation with chocolate milk did not change the concentration of total protein in the serum in our studies, it should be concluded that this dietary intervention had a positive effect on the process of post-exercise regeneration of the body, considering that the administration of placebo did not change the level of AST (Table 3). Thus, the determination of ALT, GGT, and LDH levels was of little use in tracking the rate of post-workout regeneration in elite male adolescent alpine skiers.

Electrolyte Changes

It seems evident that the correct level of electrolytes is essential for proper body regeneration after exercise. In our study, serum potassium and magnesium levels increased significantly, and iron levels significantly decreased in the post-training recovery period with chocolate milk supplementation compared to the initial levels. An increase in serum potassium was also observed after complete 8-day fasting and accompanying dehydration (Letkiewicz et al., 2021). In our study, no symptoms of athlete dehydration were observed, so another factor must have caused this effect.

Although a significant increase in magnesium concentration was observed after supplementation with chocolate milk, the levels of this mineral in the PLA group before and after exercise were higher than in the CM group. Therefore, it can be clearly stated that consuming chocolate milk after exercise has no greater positive effect on magnesium levels than drinking water. Maintaining the correct concentration of magnesium in the serum after supplementation with

chocolate milk is vital because this mineral plays an important role in the body. Magnesium acts as a counter ion for the energy-rich ATP and nuclear acids. It is a cofactor in over 300 enzyme systems that affect, among other things, protein synthesis, neuromuscular conduction, cell signal transduction, blood glucose control, and blood pressure regulation (Gröber et al., 2015).

There is no other study on iron deficiency after supplementation with chocolate milk in regeneration conditions after exercise. The decrease in serum iron levels observed in our research may result in anemia and other diseases. Therefore, it may have serious health consequences, especially in adolescents (Zimmermann & Hurrell, 2007).

Epidemiological data show that as much as 25% of the world's population is characterized by a reduced iron level in the body (McLean et al., 2009). On the other hand, in societies with easy access to food, there is a problem of increased iron concentration in the body and its toxicity (Kell, 2009). Therefore, some researchers promote the concept of maintaining a low level of iron in the body as a prophylaxis in the development of neoplastic diseases [39] or as a way to achieve longevity (Forte et al., 2014). Low blood iron levels are often found in competitive sports (Lakka et al., 1994; Casado et al., 2015).

In light of the above data, the decrease in iron levels in our study does not seem to be a negative metabolic effect; it is only an apparent change because there was a significant difference between the baseline iron concentrations in the CM and PLA groups.

The CM group had higher basal iron levels, which may explain the reduction in iron levels after supplementation in the CM group. This issue should be investigated in detail in future research.

However, there were no differences in the concentrations of chlorine, calcium, and sodium in the serum after supplementation with chocolate milk. In the comparative group of PLA, changes in the concentrations of these electrolytes occurred after supplementation. These changes were mainly caused by a tendency to lower sodium and chlorine concentrations and higher calcium concentrations in the basic study before supplementation. The influence of the type of water consumed on these changes in the PLA group cannot be ruled out, as the ionic composition of the consumed water may significantly differ (Casado et al., 2015; Ouattrini et al., 2016). Moreover, Shieffe et al. (2007), postulate that milk and chocolate milk contain similar amounts of sodium, potassium, and chlorine as water. In light of the results of these studies and unclear reasons for changes in sodium, potassium, and chlorine concentrations in the PLA group, it can be concluded that the consumption of chocolate milk does not have an apparent beneficial effect on the levels of these electrolytes in the recovery period after exercise. It should also be noted that the elite male adolescent alpine skiers studied by us have an intense sweating process that affects water and electrolyte changes in the body (Shirreffs & Maughan, 1998).

Hormonal and Metabolic Changes

In our study, serum testosterone levels in the CM group unexpectedly decreased significantly after the supplementation session. However, in another study, after a week of intensive training with chocolate milk supplementation, the testosterone level in athletes' saliva remained the same (Papacosta et al., 2015). Such different results in these two studies may be due to the significant age difference of the people participating in them or of making measurements in two different biological materials, i.e., serum and saliva. Also, Letkiewicz et al. (2021) found a significant reduction in testosterone levels induced by 8 days of water-only fasting. These data indicate that many factors can modify testosterone levels in the body. In addition, the observation of significantly higher basal serum testosterone levels among CM athletes compared to the PLA group also indicates the fluctuation of the serum concentration of this hormone. It suggests a cautious interpretation of this reduction following supplementation with chocolate milk.

Moreover, it was found that the concentrations of insulin, glucose, and total protein did not differ within or between groups. This means that CM supplementation was safe and did not affect the hormonal and metabolic changes mentioned here, and there were no pathological changes in this regard in the tested athletes. The fact that there were no intra- or intergroup differences in the range of serum sodium concentrations is another indication that the iso-osmolality of body fluids is maintained, which is related to the body's water-mineral balance. It should be remembered that the blood glucose levels, natremia, and urea concentration in the body are the main factors determining plasma osmolality (Giuliani & Peri, 2014; Rasouli, 2016; Refardt et al., 2020). These three variables appear to be stable in our study. Although serum urea

levels were not studied, it can be assumed that serum urea concentration was probably stable, as the level of total serum protein did not change after supplementation with chocolate milk. It was previously observed that consuming chocolate milk suppressed the breakdown of muscle proteins during post-exercise regeneration (Papacosta et al., 2015; Lunn et al., 2012). These data prove the existence of a positive protein balance in the period of post-exercise restitution of young downhill skiers.

Although the mechanisms explaining the effect of chocolate milk on the body are not entirely clear, the combination of CHO, protein, fats, fluids, and electrolytes in the composition of this supplement promotes the resynthesis of muscle glycogen and hydration (Spaccarotella & Andzel, 2011; Shalan et al., 2019), limiting the degradation of muscle proteins [30], reducing muscle soreness and increasing the body's exercise capacity (Potter & Fuller, 2015), in the recovery period after exercise.

Increased fat content in chocolate milk may delay the reduction of glycogen content, especially in low and moderate-intensity efforts (Karp et al., 2006; Thomas et al., 2009). The combination of only carbohydrates and electrolytes had a less favorable effect on the body, although it was a good regenerative drink, having a relatively weak diuretic effect (Jeukendrup et al., 1997), and delayed muscle fatigue during exercise and increased exercise capacity (Davison et al., 2008).

All the biomarkers assessed in this study were combined for the first time in this unique cohort of young athletes. However, the present study has certain limitations that should be considered. The recovery rate was the primary outcome we looked for, but blood was only taken once twelve hours after the chocolate milk or placebo administration to determine the recovery rate. Another limitation can be said as the number of subjects was 12. The last limitation was that the athletes were not informed about what they digested.

Conclusion

Chocolate milk consumption after high-intensity training sessions in elite male adolescent alpine skiers has beneficial effects on recovery. Based on the present study findings, it can be claimed that, thanks to the decrease in AST and especially CK activity and maintenance of physiological electrolyte conditions, chocolate milk consumption attenuates exercise-induced muscle damage and accelerates recovery. It should be added that most of the studies about the effect of chocolate milk on recovery have been conducted on adult males. Thus, there is a lack of evidence about the effects of chocolate milk on females at different ages, and therefore, in order to better understand the effects of this supplement on the body, such research should be undertaken.

Authors' Contribution

Study Design: AM, MCB, SAA; Data Collection: MCB, HM; Statistical Analysis: KA, FK; Manuscript Preparation: MCB, MCB, MCE; Funds Collection: MCB, AM.

Ethical Approval

The study was approved by the Sinop University of Human Research Ethics Council (number: 55317723-604.01.01-E and it was carried out in accordance with the Code of Ethics of the World Medical Association also known as the Declaration of Helsinki.

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Conflict of interest

The authors hereby declare that there was no conflict of interest in conducting this research.

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