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The Impact of a Single Exhaustive Training Session with Protein Supplementation on Muscle Damage Markers in Young Cyclists

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A B S T R A C T

This study aimed to investigate the impact of a single exhaustive training session with protein supplementation on muscle damage markers in young cyclists. Twenty professional cyclists with experience at local and national championship levels were recruited using a convenience sampling method. They were divided into an experimental group (n=10; mean age 21.4±4.7 years; body mass index (BMI) = 20.56±2.12 kg/m2) and a placebo group (n=10; mean age 19.1±4.1 years; BMI = 21.48±3.96 kg/m2). Blood samples were collected in a fasted state to measure phospho creatine kinase (CPK) and lactate dehydrogenase (LDH) levels. The experimental group consumed a solution containing 400 mg of protein powder (100 mg per kilogram of body weight) with 400 cc of water, while the placebo group consumed a solution containing maltodextrin powder (100 mg per kilogram of body weight). A second blood sample was taken one hour after supplementation. Participants did not engage in any physical activity during the supplementation period. After a 10-minute warm-up, the Wingate test was performed, and blood samples were collected 24 and 48 hours after the test. The findings indicated that one exhaustive training session with protein supplementation did not have a significant effect on CPK (p=0.126) and LDH (p=0.526) concentrations in professional cyclists. Post-CPK and LDH concentrations were highest at 24 and 48 hours after strenuous activity. Based on this study, a single exhaustive training session with protein supplementation did not significantly impact muscle injury markers in young cyclists. Therefore, alternative nutritional recommendations and strategies should be considered.

Keywords: *cyclists, CPK, LDH, Recovery.*

1. Introduction

Exercise is a popular way to challenge human physiological systems, and cycling is a prevalent sport in the United States, Europe, and Asia. Cycling can have numerous physiological effects, including improving cardiovascular, pulmonary, and vascular function. Athletes often follow various nutritional strategies, including consuming sports supplements and energizers, to improve their performance (1). Many athletes use different dietary supplements, such as sports drinks, minerals, and caffeine, for a variety of reasons (2). However, exercise can also have negative effects, such as muscle damage from physical activity. (3) This damage occurs when the stress placed on the muscle during activity leads to a slight rupture of the muscle fibers, which stimulates the inflammatory process, resulting in further damage to muscle fibers (4). Today, many athletes use different

dietary supplements for a variety of reasons; among the most popular products are ergogenic aids such as sports drinks, minerals, and caffeine (5). Studies have shown that muscular damage results in a malfunction of plasma enzyme activity. Prolonged strenuous physical activity can result in increased exercise-induced muscle fatigue in skeletal muscles, leading to an increase in muscle damage markers, such as CPK, AST, ALT, and LDH, which can also be used as a marker of muscle stress (6). On the other hand, it seems that exercise causes muscle fiber damage along with rupture of myofibrils and Z lines. The term muscle damage refers to damage to the extracellular matrix and exercise-induced muscle cells that can ultimately lead to a normal decline in function. Muscle damage from exercise, first described by Huff (1902), is characterized by delayed onset muscle soreness (DOMS), disruption of the fibers, weakened maximal force production, and the appearance of muscle proteins in the blood (7). Researchers have found a link between sports-related muscle damage and abnormal disruption of muscle structure, as well as superficial ischemia (7, 8). Prolonged and intense physical activity can lead to increased exercise-induced muscle fatigue in skeletal muscles, which may cause an elevation of muscle damage markers such as creatine kinase (CPK), aspartate transferase (AST), alanine transferase (ALT), and LDH. These markers can also be used to indicate muscle stress (9). The body's response to the damage caused by exercise is not immediately visible, and pain may not progress for several hours. In fact, membrane damage or protein breakdown may persist for days or even weeks (10). CPK is an important enzyme that plays an important role in muscle cell metabolism and accelerates the conversion of creatine to phosphate or vice versa (5); the plasma rate of this enzyme increases by high physical activity intensity. Researchers consider CPK to be the most important enzyme for muscle damage (11), particularly given that it plays an important role in the conversion of pyruvic acid to lactic acid or vice versa in the anaerobic glycolysis pathway. Changes in this enzyme occur with a delay than CPK and, usually, increase gradually 24 to 48 hours after stimulation. The cellular mechanism of secretion of this enzyme is still unknown, but it is often attributed to structural changes in muscle tissue following intense activity (12). Moderate-to-severe exhaustive training causes changes in muscle and blood, some of which include decreased muscle creatine phosphate and ATP stores, decreased muscle glycogen, and increased lactic acid in muscle and blood (5). Various strategies have been studied to reduce the rate of muscle destruction and contusion, including flexibility, massage, chiropractic, ultrasound, homeopathy, and the use of anti-inflammatory drugs, such as acetaminophen, and Vitamin C, E, and *L-carnitine* (13). It is believed that low-fat milk, due to its relatively high carbohydrate and protein content and low-fat content, may be considered as useful and complementary food for athletes who seek to reduce the negative effects of muscle damage. Cookburn et al. (2020) reported that consuming two different amounts of low-fat milk (500 ml and 1 liter) after an intense workout reduced the risk of muscle damage, while one liter of milk elicited heaviness and fullness of the stomach (14). Athletes seek adequate nutrition after exercise to prevent muscle damage, replace lost fluids and nutrients, maintain bone integrity , and recover quickly after exercise (14). It has been indicated that the simultaneous consumption of carbohydrates and protein can reduce muscle damage through exercise by altering protein metabolism (15); protein intake will increase the availability of amino acids, whilst carbohydrate intake, by increasing blood insulin, creates a favorable hormonal environment to increase amino acid absorption (16). The combination of these two factors will increase protein synthesis (17). In addition, the combination of this two factors inhibits increasing protein breakdown by reducing cortisol release (2). In addition to supplying amino acids, milk proteins also possess significant biological properties. In fact, certain milk-digesting peptides play a role in nutrient absorption, post-meal hormone release, and immune system function (18). Milk proteins are divided into two main groups: caseins and its proteins. Proteins have been found to possess antioxidant properties, and caseins, which are macromolecular masses present in cow's milk, are believed to be the primary components with these antioxidant properties (19). To the best of our knowledge, there is a lack of research investigating the impact of domestically available and cost-effective MPC supplementation on the rate of muscle damage. Considering the significance of sports supplements and their frequent usage among cyclists, we aimed to investigate the effects of protein supplementation during an exhaustive exercise session on muscle damage markers in young cyclists.

2. Methods and Materials

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2.1. Study Design and Participants

The study included a total of 20 professional cyclists, who had achieved championships at local and national levels. The participants were selected using a convenience sampling method and were divided into two groups: the experimental group (n=10; mean age 21.4±4.7 years; body mass index [BMI] = 20.56±2.12 kg/m2) and the placebo group (n=10; mean age 19.1±4.1 years; BMI = 21.48±3.96 kg/m2).

2.2. Measure

Body composition was assessed using the ZEUS body composition measuring device (model 9.9, manufactured in South Korea). Anaerobic performance was measured using the Wingate test on a Monark cycle (Model, E 894). Prior to their participation, all participants provided informed consent. They were familiarized with the laboratory and environment and were instructed to abstain from exercise for 48 hours before the test, as well as avoid any dietary or pharmacological stimulants. Participants were also asked to record their dietary intake on the day preceding the test. Following a 12-hour fasting period, the subjects arrived at the Sports Science Laboratory of Imam Khomeini International University at 8:00 AM. After a 30-minute rest, the first blood sample was collected to measure LDH and CPK levels. Subsequently, participants in the supplementation group consumed a solution containing MPC powder (at a dosage of 400 mg/kg body weight), while the placebo group took capsules containing maltodextrin in the same dosage (100 mg/kg body weight). Throughout the experiment, participants refrained from engaging in any physical activity. Following a 10-minute warm-up, the Wingate test was conducted, and immediately after the test, a third blood sample was collected. Finally, subsequent blood samples were collected at 24 and 48 hours after the test, respectively.

2.3. Data Analysis

All statistical analyses were conducted using IBM SPSS Statistics for Windows, version 26 (IBM Corp., Armonk, N.Y., USA). The data are presented in figures as means ± standard error (SE). The normality of data distribution was assessed using the Kolmogorov-Smirnov test (K-S). The results of the Kolmogorov-Smirnov test indicated that all research variables exhibited a normal distribution ($p \geq 0.05$). Repeated measures analysis of variance (ANOVA) and Bonferroni post hoc tests were employed, where appropriate, for data analysis. Statistical significance was predetermined at $P \leq 0.05$.

3. Findings

The subjects' characteristics are presented in Table 1.

Table 1

Characteristics of participants

Group	N	Age (yrs)	Height (Cm)	Body mass (Kg)	BMI (Kg/m^2)	BF (%)
Supplement	10	5.07±21.45	5.93±178.90	6.28±65.59	2.12±20.56	4.86±15.68
Placebo	10	4.11±19.11	3.95±174.60	13.70±68.75	3.96±21.48	6.56±17.72
Sig	-	0.142	0.065	0.214	0.194	0.89

The measures of CPK and LDH variables in five blood sampling are presented in Table 2.

Table 2

CPK and LDH variables in two groups of professional cyclists)

Variables	Group	Blood Sampling Intervals				
		Day1			Day 2	Day 3
		8:30 a.m.	9:00 a.m.	10:00 a.m.	8:30 a.m.	8:30 a.m.
CPK	Supplement	47.84±112.60	61.71±136.20	87.55±152.70	81.16±187.40	81.27±149.90
Units/Lit	Placebo	47.04±110.30	63.08±130.50	93.87±143.60	78.05±201.20	114.45±164.60
LDH	Supplement	60.70±209.70	75.47±280.00	70.37±309.90	185.55±427.50	78.95±361.00
Units/Lit	Placebo	50.51±246.40	111.33±289.00	157.28±336.00	69.14±328.40	157.111±454.40

According to the findings presented in Table 3, there was no significant difference observed between the supplement and placebo groups ($F_{(36, 1)} = 28.01$, $p = 0.126$). However, a significant difference was found between the

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different blood sampling times ($F_{(36, 4)}$ = 7.28, p < 0.001). Figure 1 displays the pairwise comparison of means for CPK across the five blood sampling phases. Further analysis using Bonferroni post-hoc testing revealed a significant increase in LDH concentration (p = 0.013) in the supplement group between the first and fourth blood sampling. Additionally, there was a significant increase in CPK levels (p = 0.023) in the placebo group, with the highest levels observed after 24 hours of intense physical activity among the professional cyclists.

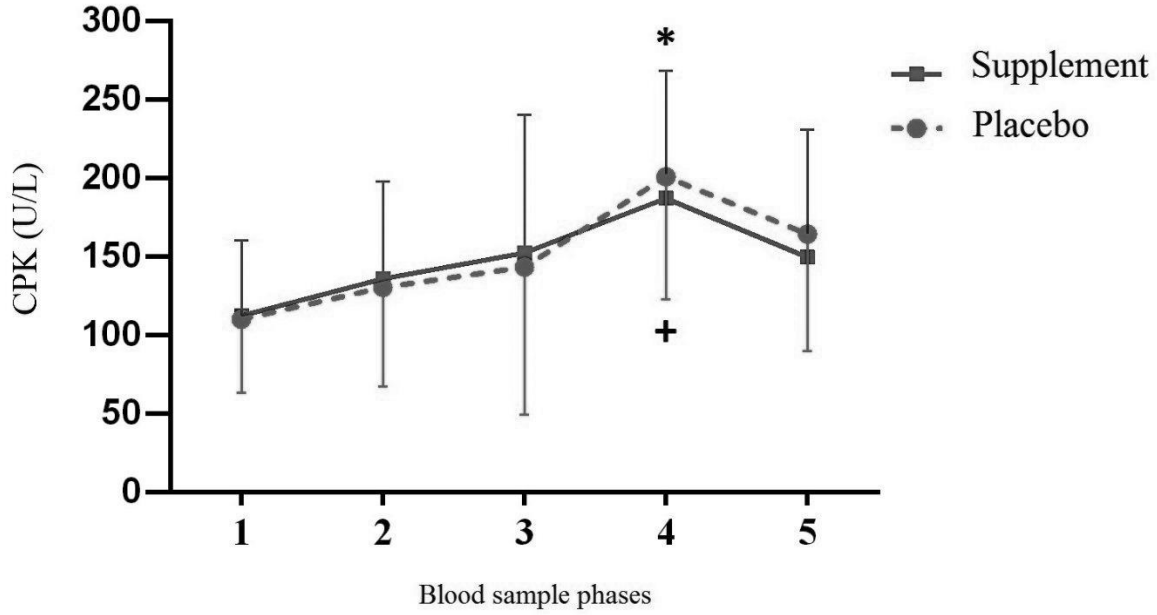
Table 3

Intragroup and intergroup effects testing for creatine kinase

Source		sum of squares	Df	Average squares	F	Sig	Eta coefficient
CPK (Units/Lit)	group	14.33	1	14.33	28.01	0.126	0.80
	time	75151.00	4	75515.01	7.28	0.001<	0.45
	Error	93331.20	36	2592.53	-	-	-

Figure 1

Bonferroni test results to compare the means of creatine kinase in the two groups for 5 blood samples.



*Significant difference with the first blood draw in the supplement group
+Significant difference with the first blood draw in the placebo group

The results of the intergroup effects test are summarized in Table 4. No significant difference was observed between the supplement and placebo groups [(p = 0.526 and F = 0.42 (36, 1)]. However, a significant difference was found between the blood sampling sessions [p < 0.001 and F = 8.54 (36, 4)]. The pairwise comparison of means for CPK across the five blood sampling phases is illustrated in Figure 2. Further analysis using Bonferroni post-hoc testing revealed a significant increase in LDH concentration in the supplement group during the fourth and fifth blood sampling periods, when compared to the first (p = 0.01). In the placebo group, there was a significant increase in LDH concentration during the fifth blood sampling compared to the first (p = 0.03). Notably, the concentration of LDH in professional cyclists reached its highest level after 24 and 48 hours of intense physical activity, when compared to the resting state.

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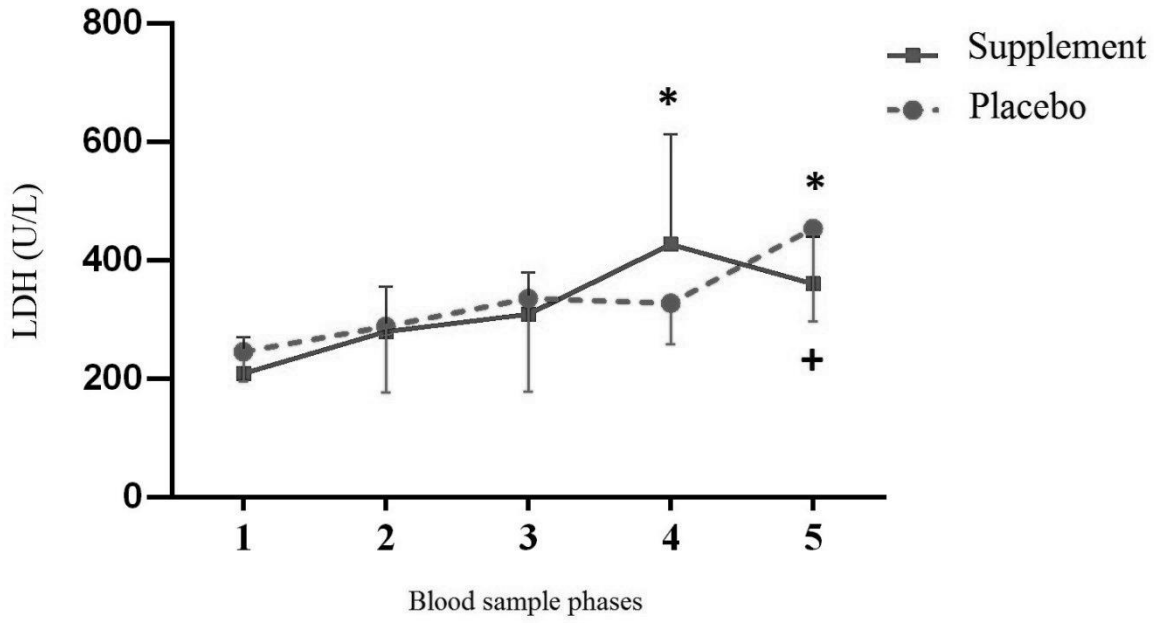
Table 4

Test of intergroup and intragroup effects for lactate dehydrogenase

Source		sum of squares	df	Average squares	F	sig	Eta coefficient
LDH U/L	group	10.01	1	10.01	0.42	0.526	0.76
	time	413675.44	4	10341.92	8.54	0.001<	0.49
	Error	435993.34	36	12110.93	-	-	-

Figure 2

Bonferroni test results to compare the means of lactate dehydrogenase in the two groups for 5 blood samples.



*Significant difference with the first blood sampling in the supplement group
+Significant difference with the first blood sampling in the placebo group

4. Discussion

The use of supplements to enhance sports performance and prevent injuries has become a contemporary area of interest in the pursuit of athletic success (20-22). Cycling, in particular, is a sport that is highly susceptible to sports injuries due to prolonged periods of high-intensity exercise (23). Therefore, this study aimed to investigate the impact of protein supplementation on muscle damage markers in young cyclists following exhaustive training. The findings of this study revealed that protein supplementation did not have a significant effect on the level of LDH in professional cyclists. However, post-CPK and LDH concentrations were found to be at their highest levels 24 and 48 hours after intense physical activity. Previous research by Podgórski et al. (2021) has reported a significant increase in muscle damage and inflammatory markers (LDH and CK) throughout the training cycle. Exercise is known to challenge the body's homeostasis, leading to disturbances in various cells, tissues, and organs as a response to increased metabolic activity in the skeletal muscles (24). However, a study by Matsus et al. (2006) did not observe an increase in enzyme levels after a load-free resistance training session with 10 repetitions and one minute of rest (25). The discrepancy in results may be attributed to factors such as the type of exercise, recovery time, and exercise intensity, which can affect the release of these enzymes (25). It seems that the type of exercise, recovery time, and intensity of exercise affect the release of these enzymes, which may be a reason for the discrepancy between the results of the present study and previous research (26). Contrary to the present study, Buckley et al. (2010) reported that whey protein supplements consumed after eccentric exercise resulted in faster recovery of

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muscle strength (27). Additionally, the ingestion of whey protein was found to significantly reduce post-exercise serum CPK and myoglobin levels compared to water, which contradicts the findings of this study (28). Based on the current study's findings, LDH and CPK enzyme levels increase after physical activity. However, it is important to note that these enzymes are also present in cells of other organs, such as the liver and heart. Therefore, daily exercise sessions for athletes at this fitness level may not only impact their performance but also pose a risk to other organ cells, particularly muscle cells. Proper planning of training sessions, appropriate adjustment of training load, and the use of supplements with established effectiveness should be advocated to minimize delayed muscle soreness, enhance physiological performance, reduce injuries, and lower medical costs for societies (9). Although this study is believed to be the first to investigate the effects of exhaustive training with protein consumption on muscle damage markers in young cyclists, it has certain limitations. The sample size was small, and precise control over subjects' diets was not possible. Therefore, future studies should consider using larger sample sizes based on a priori power calculations and implement more accurate laboratory controls on individuals.

5. Conclusion

One exhaustive training session with protein supplementation did not show a significant effect on muscle injury markers in young cyclists. As a result, it is important to consider other nutritional recommendations and strategies.

Authors' Contributions

K.I: Conducted the systematic search, reviewed the literature, and drafted the manuscript; KI; M.B: critical review of the paper; KI: Provided guidance, reviewed and revised the manuscript.

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Declaration of Interest

The authors declare no competing interests.

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Ethics considerations

The present study was conducted in accordance with the ethical guidelines outlined in the code IR.QUMS.REC.1400.315. Ethical considerations were taken into account in all aspects of the research, including participant recruitment, informed consent, confidentiality, and data handling. Participants were provided with detailed information about the study, and their voluntary participation was ensured.

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