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The Effect of Whey Protein Supplementation after Eccentric Resistance Exercise on Glutathione Peroxidase and Lactate Dehydrogenase in Non-Athletic Young Men

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Abstract

Background: *Laboratory studies have revealed that intense exercise is associated with increased production of free radicals, and weakened capacity of antioxidant. The aim of the study was to determine the effect of short-term whey protein supplementation on some oxidant and antioxidant indices following an intense exercise session among non-athletic men.*

Methods: *In this double blind randomized clinical trial study, 24 non-athlete male students (21.9 ± 1.19 kg of weight) without any chronic and metabolic diseases were randomly allocated into two groups: males who received whey protein supplement (0.4 g / kg body weight for 3 consecutive days), and those who received placebo. Blood samples were collected before, immediately, 24, 48, and 72 hours after an intense resistance exercise session, and were analyzed for glutathione peroxidase (GPx) and Lactate dehydrogenase (LDH). The repeated measure ANOVA was used to compare GPx and LDH between these two groups.*

Results: *Significant differences were observed in the GPx change pattern between the two groups. On the other hand, at each stage of the sampling, there was a significant increase in the amount of GPx compared to placebo group (1.164 ± 0.166 vs 0.924 ± 0.054 for 24 recovery; 1.111 ± 0.104 vs 0.896 ± 0.105 for 48 hours recovery; 1.036 ± 0.131 vs 0.873 ± 0.083 for 72 hours recovery) (Pvalue < 0.05). However, no significant difference was observed in the LDH change pattern between the two groups (Pvalue = 0.99).*

Conclusions: *Whey protein supplementation is associated with the improvement of GPx activity after an intense resistance exercise among non-athletic young men.*

Keywords: Antioxidant enzyme, Resistance exercise, Whey protein, Stress oxidative.
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Introduction

Several studies have shown that intense exercise trainings are associated with the formation of oxidative stress condition.[1] Researchers have frequently reported that intense muscular exercises, especially those that involve intense resistance and eccentric muscle contractions, lead to delayed onset muscle soreness (DOMS), particularly in beginners or non-athletes.[2,3] Due to the increase of oxygen consumption by 100 times after intense exercise, the production of free radicals or oxidants also significantly increases.[4] Intense resistance and extrinsic muscle contractions appear to result in the formation of free radicals or oxidants, especially among non-athletes, and reduction of the capacity of antioxidant indices.[5] The findings of some studies have shown that one session of intense resistance exercise increases indices of lipid peroxidation and reduces the activity of antioxidant enzymes or antioxidants such as glutathione peroxidase (GPX) in mice.[6] Intense muscle contractions are also associated with increased levels of damage indices to muscle cells and tissues.[7] It has been shown that those exercises that are accompanied with intense muscle contractions such as resistance or eccentric contractions, are associated with increased production and release of muscle damage indicator enzymes like creatine phosphokinase (CK), lactate dehydrogenase (LDH), some liver enzymes such as alanine aminotransferase (ALT), and aspartate aminotransferase (AST).[8,9] Muscle damages caused by intense exercise are associated with membrane dysfunction, leakage of extracellular fluid, and increase in enzymes such as LDH and CK in the blood.[10] LDH is found with varying concentrations and high levels in cytoplasm of most of the body tissues, and the destruction of Z lines and sarcolemma damage increase its release as a muscular damage indicator into the interstitial fluid and blood circulation.[11]

On the other hand, the antioxidant system of the body has the responsibility of providing enzymatic or non-enzymatic antioxidants to reduce the production of chain reactions resulted from free radicals. Although some studies have emphasized that regular exercise trainings reduce the production of free radicals and strengthen the antioxidant system of the body through higher activity levels and releasing of oxidative enzymes or total antioxidant capacity (TAC),[6] some other studies have proposed the use of nutritional materials or antioxidant supplements to improve and also strengthen the antioxidant system of the body.[12] Compounds in milk especially proteins with sources of branched amino acids, have been shown to act not only as nutrients; but also as a potent antioxidant against the invasion of free radical.[13] Among them, whey protein is rich in essential and branched amino acids with higher antioxidant properties.[14] Scientific sources have supported the high levels of cysteine and methionine sulfuric amino acids in whey protein, which are able to convert glutathione as an antioxidant and anti-cancer tripeptide, and enhance the immune function.[15]

Scientific evidence points to increased production of free radicals and cellular damage after performing an intense exercise, such as resistance training or intense eccentric contractions.[16] On the other hand, strengthening the antioxidant

system through antioxidant supplements or regular long-term exercise training by increasing the total antioxidant capacity or other enzymatic and non-enzymatic antioxidants, leads to a reduction of free radicals production.[16] Scientific sources have shown that antioxidant levels decreased immediately after exercise, and that antioxidant supplementation has a protective effect against exercise-induced oxidative stress. On the other hand, whey protein has antioxidant properties due to having high amounts of leucine, which is one of the most important free radical scavengers in intracellular glutathione precursors,[17] as their antioxidant properties during exercise were highlighted in some studies.[18] On the other hand, despite its effects on the levels of creatine phosphokinase following isokinetic, isotonic, or extrinsic exercises,[19,20] its role as an antioxidant supplement has been less investigated regarding antioxidant indices, as well as other indicators of muscle damage following intense muscle contractions, especially among non-athlete participants. Hence, the present study aimed to determine the GPx response as one of the most potent antioxidants, along with LDH as one of the inflammatory markers of muscle damage indicator regarding supplementation of this protein in delayed periods after an intense extrinsic resistance exercise session in young non-athlete men.

Materials and Methods

The statistical population of this double-blind study included non-athletic and non-smoker male students with the ages ranged from 18 to 24 years old. At first, 24 subjects who met the inclusion criteria in the double-blind design were selected through convenience and purposive sampling, and then were allocated into experimental (n = 12) and placebo (n = 12) groups based on the random sampling using a table of random numbers. The experimental group refers to the group which underwent whey protein supplementation at a daily average of 30 g (0.4 g per kg of body weight for 3 consecutive days).[18]

Inactivity or not participating in a regular training program over the past 6 months was one of the main inclusion criteria of this study. The participants were non-smokers and non-alcoholic. Their weight fluctuation in the past 6 months was less than a kilogram. Lack of specific diet and chronic and metabolic diseases such as asthma, diabetes, metabolic syndrome, cardiovascular diseases, etc. were among the inclusion criteria. Lake of illnesses by which a person should not have intense exercise was another inclusion criterion.

Anthropometric indexes were measured in both of the whey protein and placebo groups. The measurement of height was done using a wall-mounted height gauge, without shoes, and with an accuracy of 0.5 cm. Body weight was measured with minimum clothing by Seca scale with 0.5 kg accuracy. To measure the abdomen circumference in the thickest region, the researchers used non-resilient strip meter. The body mass index was calculated from the numerical values of height and weight in meters. To reduce the individual error rate, all anthropometric measurements were performed by one person.

This study aimed at determining the effect of whey protein supplementation on LDH and GPx activity after an intense resistance exercise during 24, 48, and 72 hour intervals after the test. Both groups performed intense resistance tests in the form of going up and down the stairs (height: 50 cm), and their blood samples were collected at 5 stages (before, immediately, and 24, 48, and 72 hours after the test); which has the goal of measuring the LDH and GPx activity.

The test duration was 20 min, which took place in the form of four 5-min stages with 1-min intervals; in each minute, the participant was supposed to perform 24 cycles of going up and down the stairs (height: 50 cm). One run cycle had four parts 1) up with the right foot, 2) up with the left foot, 3) down with the right foot, 4) down with the left foot. The number of steps per minute was 96 beeps per minute which was determined using the metronome software, so that the participant could complete 24 entire steps of going up and down the stairs. From these four stages, the participants began two steps of going up with their right leg and the other two with their left foot. The right leg constricted as the body went up and eccentrically constricted as it went down. During the test, to exert excess load, the subject hold 2 weight (dumbbells) with his hands each one being 7% of his body weight (totally 14% of the body weight). In addition, at any time of the test, when the participant reached the fatigue peak and could not continue the test, the test was stopped for him.[21,22]

Whey protein and placebo supplementation were performed at three stages (days 1, 2, and 3).

Day 1: All participants were in the laboratory by passing 10-12 hours from overnight fast. Fasting blood samples were collected (baseline levels), and then the exercise test was performed. Immediately after discontinuation of the test, blood sampling was done (acute response), followed by supplementation of whey protein (first supplementation).

Day 2: Blood sampling was repeated in fasting conditions (24 hours of recovery), followed by repeating the whey protein supplementation (second supplementation).

Day 3: Blood sampling was repeated in fasting conditions (48 hours of recovery), followed by repeating the whey protein supplementation (third supplementation).

Day 4: Blood sampling was repeated in fasting conditions (72 hours of recovery).

The control group also performed all of the above mentioned steps. Accordingly, the difference was that subjects in this group took placebo instead of whey protein. At each stage, whey protein supplementation was carried out 20 to 30 min after blood sampling. Blood samples were centrifuged for serum separation at 2000 rpm at 10 min. LDH was measured using spectrophotometry, and GPx by Optical spectroscopy method.

Anthropometric measurement was measured in baseline. Mean and SD of anthropometric and blood indexes and other statistical analysis were conducted using SPSS software version 15.0. Shapiro-Wilk test was used to determine the normal status of the obtained data. A repeated measure analysis of variance (2-way ANOVA) was used to evaluate time-course change in variables for LDH and GPx, and Bonferroni post hoc test was used to determine significant values between specific means. Then, independent sample T-test was used to compare each variable between 2 groups at each stage. Significant level was set at 0.05.

Results

Table 1 shows the descriptive anthropometric features of the study groups. Based on the independent T test, there were no differences in the age, body weight, BMI, and body fat percentage between the two groups (see table 1).

Table 2 presents the changes in GPx and LDH during the study for the subjects. Significant differences were not found in LDH and GPx activity at pre-exercise (baseline) between 2 groups.

The statistical results of 2-way ANOVA showed no significant change in serum LDH at sampling stage (Pvalue = 0.998). Based on this data, compared with placebo, whey protein supplementation is not associated with acute and recovery changes in LDH as muscle damage marker response to eccentric resistance exercise in non-athlete's young men.

In contrast, results by 2-way ANOVA showed significant changes regarding the GPx activity (Pvalue < 0.0001). In addition, data analysis by Bonferroni post hoc test was also showed significant increase at 24, 48 and 72 hours' recovery by whey protein supplementation in comparison with placebo subjects (figure 1). Actually, compared to pre-exercise, GPx activity was increased at each stage of blood sampling for 2 groups, separately. However, data analysis by ANOVA showed significant change between 2 groups. On the other hand, comparison of GPx between the two groups indicated that the activity of this antioxidant index was significantly higher in experimental, compared to the placebo subjects at each stage of blood sampling (Pvalue < 0.05).

Data shows whey protein supplementation can increase the GPx activity after severe eccentric contraction in non-athlete's young men.

Table 1. Mean and standard deviation of anthropometric characteristics of the studied groups (mean ± SD)

Variables	Experimental group	Placebo group	Pvalue
Age (year)	22.50 ± 1.17	21.33 ± 1.16	0.22
Height (cm)	176.00 ± 2.31	174.70 ± 3.14	0.25
Weight (kg)	89.90 ± 6.73	89.30 ± 6.96	0.81
Body mass index (kg/m^2)	29.01 ± 1.82	29.28 ± 2.45	0.77
Body Fat (%)	30.14 ± 2.27	30.63 ± 2.71	0.31

Table 2. Mean and standard deviation of GPx and LDH in stage of blood sampling of 2 groups

Variable	Group	Pre-exercise	Post-exercise	24 hours recovery	48 hours recovery	72 hours recovery	Sig (ANOVA)
LDH (IU/L)	Experimental	0.873 ± 0.083	0.625 ± 0.046	0.631 ± 0.040	0.873 ± 0.083	0.612 ± 0.050	0.99
	Placebo	0.602 ± 0.054	0.641 ± 0.063	0.650 ± 0.058	0.664 ± 0.050	0.620 ± 0.076	
	Sig (Independent T test)	0.659	0.513	0.343	0.533	0.765	
GPx (IU/L)	Experimental	0.821 ± 0.070	0.837 ± 0.072	1.164 ± 0.166	1.111 ± 0.104	1.036 ± 0.131	
	Placebo	0.838 ± 0.061	0.769 ± 0.055	0.924 ± 0.054	0.896 ± 0.105	0.873 ± 0.083	
	Sig (Independent T test)	0.53	0.17	< 0.0001	< 0.0001	0.001	< 0.0001

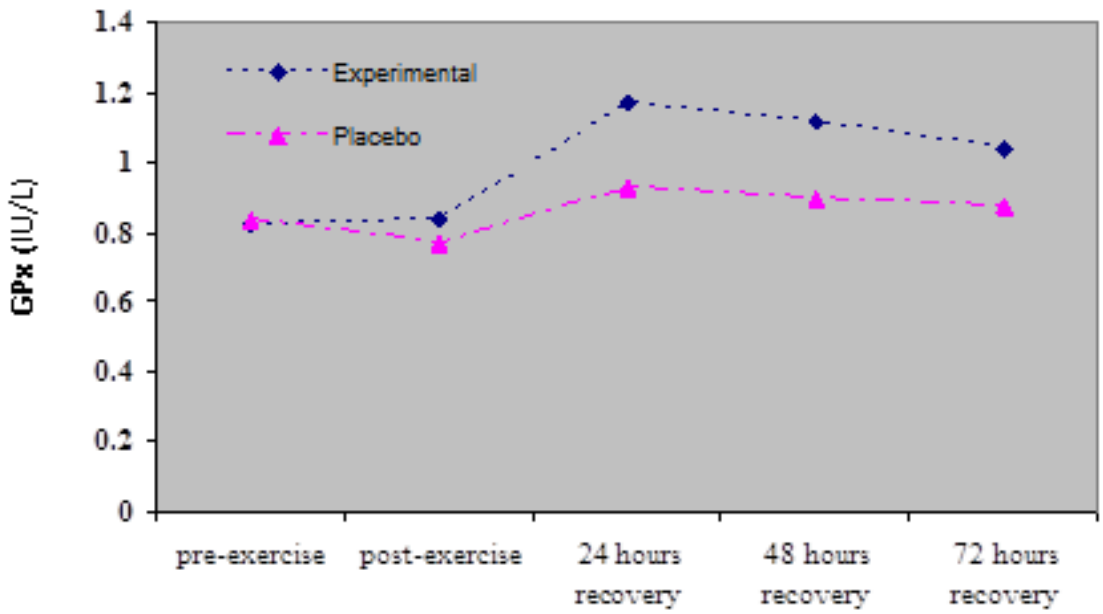

Figure 1. The changes pattern of GPx activity in experimental or placebo subjects

Discussion

The main finding of the present study was indicating the increase in the level of GPx activity in response to whey protein supplementation. In other words, in contrast to the placebo group, whey protein supplementation significantly increased the GPx activity at 24, 48, and 72-hour intervals after eccentric resistance exercise in inactive men. However, whey protein supplementation did not affect LDH levels compared to the placebo group. In other words, at each interval (24, 48, and 72 hours), no significant difference was observed in LDH levels between the placebo and whey protein groups, which can be resulted from low sample size of the study. It should be noted that, immediately after the exercise test, the levels of GPx and LDH in both groups showed a significant reduction and increase, respectively, compared to the pre-test. Concerning the acute or delayed response of GPx and LDH to whey protein supplementation after an intense eccentric exercise session, though the studies are limited. Ahmadi Kani et al. (2011) reported that using whey protein along with resistance training significantly increased TAC in overweight young men; however, GPx activity was not significantly changed compared to the placebo group.[23]

Although the GPx activity, by passing 24, 48, and 72 hours from the exercise test elevated in both groups, the level of its activity was significantly higher in the group supplemented with whey protein in each step of the post-test exercise sampling (48, 24 and 72 hours) in comparison with the placebo group. These findings confirm the antioxidant effects of whey protein in intervals following intense resistance exercise test in non-athlete individuals. Studies have supported the body's ability to respond to higher levels of lactoferrin, beta-lactoglobulin, alpha-lactalbumin, glycomacropeptide, and immunoglobulins, which constitute the biological function of his protein, all of which contribute to its potent role as an antioxidant.[24,25]

In addition, intracellular conversion of cysteine amino acid to glutathione is one of the other mechanisms representing the antioxidant properties of this protein.[24] In an earlier study, the antioxidant effect of whey protein was demonstrated by the free radical scavenging properties of rats.[26] Another study found that although resistance training alone leads to an antioxidant system strengthening in overweight young men, the combination of resistance exercise combined with whey protein brings more beneficial effects on the antioxidant system with emphasis on TAC.[23] In this area, Zavorsky et al. (2007) also pointed out that in response to 2 weeks of whey protein supplementation, a significant relationship was observed between the amount of this antioxidant supplement and increase in GPx.[27] In addition, Gad et al. (2010) confirmed the scavenging effect of free radicals and antioxidant effects of whey protein;[18] however, Cornish et al. (2009) emphasized on the lack of antioxidant property of whey protein during resistance trainings.[28] Coombes et al. (2000) pointed out that concentration of LDH in the whey protein group significantly decreased 5 days after exercise 29 compared with the placebo group.[29] Eizadi et al. (2018) demonstrated that whey protein supplementation, in comparison to the placebo group, significantly reduced the activity of alanine aminotransferase (ALT) 24 to 72 hours after an intense eccentric exercise session in non-athletic male students.[30] In contrast, Asjodi et al. (2013) reported no change in LDH levels by passing 24 and 48 hours from resistance exercise in response to whey protein supplementation.[31] In general, muscular or liver damages after exercise, especially intense eccentric resistance exercises,[32] can be developed in response to the destruction of muscular structure or local ischemia due to exercise and manifest through changes in LDH, AST, or CK levels.[33] However, the main mechanisms responsible for this process are not well defined. In this regard, Nameny et al. (2004) reported significant increases in CK and LDH, 24 and 48 hours after intense eccentric contractions.[34] It is noteworthy that, changes in LDH in response to intense muscular contractions appear slightly after the changes in CK, gradual increase of which occurs about 24 to 48 hours after stimulation.[35] Since regarding the response to stimulation, LDH is less sensitive compared to CK, LDH activity may alter with more intensity or time in response to exercise. The lack of measurement of CK is one of the main limitations of this study.

The lack of difference in LDH between the two groups was reported, while whey protein consumption after muscular damage following intensive muscular activities leads to more absorption of proteins with rapid recovery, and also with repair of damaged muscle fibers.[36] Clinical studies have revealed that whey protein supplementation leads to inhibition of DNA damages caused by iron increase in leukocytes and colonocytes.[37] Some other studies have indicated that during exercise, there is a correlation between oxidative stress markers and some cellular damage indicators such as CK and LDH.[38] In this regard, Seifi et al. (2008) reported that 30-minute running with 75% VO_2max performed by non-athlete participants significantly increased both CK and malondialdehyde (MDA).[39] Lack of measurement of other antioxidant or oxidative stress indicators such as MDA or TAC is also one of the limitations of the present study.

In conclusion, despite the antioxidant effect of whey protein with an emphasis on GPx, lack of LDH change may be attributed to the dispersion of scores or small number of samples, in comparison to the control group. Of course, not all of the previous studies, supported the antioxidant effects and repair of muscular damages. The beneficial effects of whey protein have also been reported regarding inhibition or prevention of increase in enzymes indicating muscular or liver damage such as LDH following muscular damages caused by severe extrinsic contractions among healthy people.[19] On the other hand, Buckley et al. (2010) found that whey protein consumption could accelerate the recovery period and repair the muscular damage via reducing the muscle damage indices such as CK and LDH after eccentric contractions.[40] Researchers believe that supplementation of amino acids and proteins may be associated with reduced muscle damage and delayed exercise-induced fatigue.[41] On the basis of this evidence, the potential role of branched amino acids has always been raised as a useful adjunct to muscle recovery in stroke

injury following intense resistance exercise. Also, one study found that supplementation of branched amino acids before and after exercise had beneficial effects while reducing muscle protein synthesis induced by muscle injury.[42]

Moreover, the reduction effect on muscle damage biomarkers through supplementation of this protein along with long-term aerobic exercises has already been observed.[43] Some researchers have attributed the beneficial effects of this protein supplement to some of its bioactive factors such as beta-lactoglobulin, alpha-globulin, albumin, lactoferrin, lactose peroxidase, and phospholipoproteins.[44] Some other studies, concentrated on its biological components such as glycomacropeptide, and some immunoglobulins, have introduced whey protein as a potent antioxidant, antihypertensive, anti-tumor, triglyceride-lowering agent, antivirus, and antibacterial protein which also improves the immune system function.[45,25] Researchers believe that the effective mechanism of whey protein in reducing free radicals is perceived as a strong antioxidant through intracellular conversion of cysteine amino acid to glutathione.[25]

Kim et al. (2013) have also shown that whey protein intake can decrease the oxidative stress and lipid peroxidation, due to excess of iron intake in laboratory mice.[37] Also, Whey protein can significantly reduce apoptosis induced by H_2O_2, which is one of the most important indicators of oxidative stress, and increase the antioxidant activity through mitochondrial pathways.[46] Oral intake of whey protein and intravenous injection may also increase the activity of antioxidant enzymes in oxidative stress conditions induced by paracetamol in laboratory mice.[47]

Determination of acute and recovery response of GPx and LDH to eccentric resistance exercise after whey protein supplementation in non-athletic young men are the main objectives of study. Compared with the placebo group, whey protein supplementation revealed a significant increase in GPx at 24, 48, and 72-hour intervals following eccentric resistance exercise in non-athletic male students. On the other hand, lack of change in LDH may be attributed to the consumption dose, dispersion of scores, or the small sample size. However, its anti-inflammatory or anti-oxidant effects cannot be attributed only to LDH changes. Because lack of measuring of other inflammatory markers or muscle damage indicators such as MDA, CRP and CK are the limitations of the present study. Therefore, further studies are needed to clarify possible mechanisms by which whey protein supplementation on anti-oxidant or muscle damage markers during exercise.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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