

## Whey Protein Supplementation is Associated with Antioxidant Markers Following Severe Eccentric Contractions in Obesity

Laleh Behboudi<sup>1</sup>, Mojtaba Eizadi<sup>2\*</sup>, Homa Masrour<sup>3</sup>

1. Assistant Professor of Exercise Physiology, Islamshahr Branch, Islamic Azad University, Tehran, Iran.
2. Assistant Professor of Exercise Physiology, Saveh Branch, Islamic Azad University, Saveh, Iran.
3. Assistant Professor of Nephrology, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

### \*Correspondence:

Mojtaba Eizadi, Department of Exercise Physiology, Saveh Branch, Islamic Azad University, Saveh, Iran  
**Tel:** (98) 919 355 1960  
**Email:** izadimojtaba2006@yahoo.com

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### Abstract

**Objective:** Intense muscle contractions are associated with oxidative stress and immune system deficiency, especially in the presence of obesity. This study aimed to determine the effect of whey protein supplementation following eccentric resistance contractions on some determinants of oxidative stress in non-athletic obese students.

**Materials and Methods:** In this double blinded randomized clinical trial study, 24 non-athlete obese male students were divided into two groups: experimental (whey protein supplementation, 0.4 g / kg body weight for 3 consecutive days) or placebo groups by simple random sampling. Blood samples were taken before, immediately, 24, 48 and 72 hours after an intense resistance exercise session with regard to measure Superoxidasedismutase (SOD) and Malodaldoeide (MDA). Subsequently, a two-way repeated measure ANOVA was performed to compare data between groups. *P*-value of less than 5 percent was considered statistically significant.

**Results:** No significant differences were observed between two groups in MDA (*P*-value: 0.211) and SOD (*P*-value: 0.222) at post-exercise. Based on ANOVA data, significant changes were observed between two groups with regard to MDA (*P*-value: 0.001) and SOD (*P*-value: 0.001). On the other hand, MDA activity was significantly lower in experimental than placebo subjects in each stage of study (24 hours recovery, 48 hours recovery and 72 hours recovery with *P*-value: 0.001). Also, SOD activity was significantly higher in experimental than placebo subjects in each stage of study (24 hours recovery, 48 hours recovery and 72 hours recovery with *P*-value: 0.001).

**Conclusion:** Based on this study, it is concluded that whey protein supplementation can be improve antioxidant capacity after intense exercise in non-athletes obese male.

**Keywords:** Eccentric exercise, Whey protein, Antioxidant

### Introduction

Oxidative stress refers to an imbalance between oxidants and antioxidants, which is associated with increased free radicals and weakening of the immune system (1,2). Epidemiological evidences also support the increase of oxidative stress in the presence

of obesity (3). Laboratory studies have revealed that intense exercise trainings or intense muscle contractions are associated with increased oxidants or free radicals and reduced antioxidants (1). These conditions are especially evident in the first training sessions of non-athletes. In this condition, muscular injuries and oxidative stress caused by resistance trainings which are also associated with eccentric muscle contractions are found to be more than those caused in other exercise methods and would last several days after exercise (4). Also, the production of free radicals exceeds the potency of the antioxidant system, which will be accompanied with oxidative pressure and an increase in lipids such as malondialdehyde (MDA) and a reduction in antioxidants such as glutathione peroxidase (GPX) or superoxide dismutase (SOD) on days after the exercise (5).

In this regard, the findings of some studies showed that an intense resistance training session increases MDA as a lipid peroxidation indicator and decreases antioxidant enzymes activity such as glutathione synthetase and SOD in mice (6). Regarding the higher resting level of oxidative stress and the reduction of antioxidant function in the presence of obesity, intense muscle contractions in untrained individuals are associated with higher oxidative stress than individuals with normal weight.

Hence, the use of antioxidant drugs or supplements has recently been prevalent to reduce oxidative stress and to increase the antioxidant capacity among athletes, especially during intense resistance exercises, and is often suggested by athletic coaches. In this regard, some studies have shown that the use of some nutritional or antioxidant supplements can increase antioxidant capacities against the production of free radicals or oxidants following intense muscle activities (7,8). Compared with other proteins such as soy or casein, whey protein is composed of more essential amino acids especially branched-chain amino acids with antioxidant properties (9).

Whey is a protein that is present in milk and its supplementation is common among athletes after resistance exercises (10). It has been introduced far more than other protein supplements due to its high absorption (11). Scientific sources have pointed out that the change in oxidative stress markers observed in mice supplemented with whey protein is probably due to the rapid and high absorption levels of this protein (12).

Although the beneficial effects of whey protein supplementation as a protein rich in amino acids with antioxidant properties have been confirmed in reducing liver enzymes introduced by some researchers as indicative of muscle damage enzymes (13,14), and several studies have pointed to its antioxidant properties and protective effect against oxidative damages (15-17), its effect on enzymatic oxidants such as MDA and SOD has not been studied after intense or eccentric resistance exercise, especially in untrained obese individuals. Hence, this study was conducted to evaluate the effect of whey protein supplementation on the activity of MDA and SOD after an intense eccentric exercise in non-athletic obese male.

## Materials and Methods

The statistical population of this double-blinded study included non-athletic obese male students aged 18-24 years old. Twenty-eight participants who met the inclusion criteria in the double-blinded design were selected first through convenience and purposive sampling and then divided into experimental (n= 12) and placebo (n= 12) group based on random sampling using a table of random numbers. (Islamshahr, Iran, Fall 1396). 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 one kilogram. Lack of specific diet and chronic and metabolic diseases such as asthma, diabetes, metabolic syndrome, cardiovascular diseases, etc. were the inclusion criteria. The use of edible or nutritional medications and supplements during the study was the exclusion criteria.

Anthropometric indexes were measured in both 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 individual error rate, all anthropometric measurements were performed by one person.

This study aimed to determine the effect of whey protein supplementation after an intense resistance exercise on MDA and SOD activity 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); the goal was to measure MDA and SOD activity.

**Resistance test:** 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 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 the right leg and the other two with the left foot. The right leg constricted as the body

went up and eccentrically constricted as it went down. During the test, in order to exert excess load, the subjects 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 (19,20).

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

**Day 1:** All participants were in the laboratory after 10-12 hours of overnight fasting. Fasting blood samples were collected (pre-exercise), and then the exercise test was performed. Immediately after discontinuation of the test, blood sampling was done (post-exercise), followed by supplementation of whey protein (first supplementation).

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

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

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

The control group also performed all of the mentioned steps. At each stage, whey protein supplementation or placebo was taken 20 to 30 min after blood sampling. Blood samples were centrifuged for serum separation at 2000 rpm at 10 min. SOD (Nasdox™-Superoxide Dismutase Assay Kit- Non Enzymatic) and MDA (Nalondi™-Lipid Peroxidation Assay Kit-MDA) measured by Optical spectroscopy method.

After calculation of the mean and the standard deviation (SD), the statistical analysis was conducted using the SPSS software version 15.0. Shapiro-Wilk test was used to determine of normal status of the data. A repeated measure analysis of variance (ANOVA) was used to evaluate time-course change in variables for SOD and MDA and Bonferroni

post hoc test used to determine significant values between specific means. Then independent sample T-test was used to compare each variable between 2 groups at each stages. A criterion alpha level of  $P$ -value  $< 0.05$  was used for all statistical comparisons.

### Ethical considerations

This study was approved by committee of Ethics Standard in Research of the Institute of Physical Education and Sport Science, Ministry of Science, Research and Technology, Tehran, Iran, with number of IR.SSRC.REC.1398.663.

### Results

Anthropometrical indexes of two groups are shown in table 1. Based on independent T-test, there were no statistically significant differences between experimental and placebo groups with regard to all anthropometrical indexes (Table 1).

Table 2 presents data of MDA and SOD activity at each blood sampling of studied groups. At baseline, there was no difference in MDA and SOD activity between the two groups.

Based on data of repeated measure analysis, significant changes were observed in serum MDA in sampling stages. Bonferroni post-hoc

test showed significant decrease in MDA at 24, 48 and 72 hours recovery by whey protein supplementation compared with placebo subjects (Figure 1). In addition, in each stages of blood sampling, MDA concentration in experimental group was significantly lower than placebo subjects.

In addition, significant changes were observed with regard to SOD activity ( $P$ -value  $< 0.001$ ). Bonferroni post-hoc test also showed significant increase at 24, 48 and 72 hours recovery by whey protein supplementation compared with placebo subjects (Figure 2). Also, comparison of SOD between the two groups showed that the activity of this antioxidant index was significantly higher in experimental than placebo subjects at each stage of blood sampling.

### Discussion

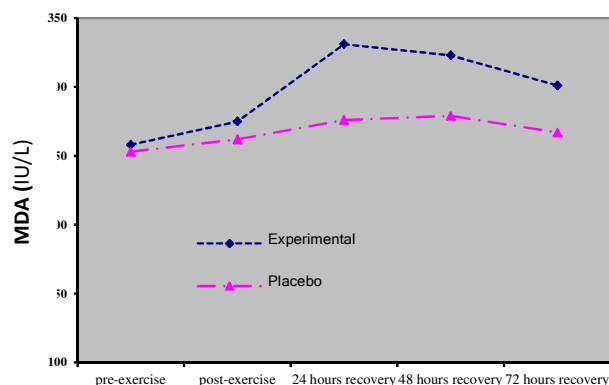
Reduction of MDA along with an increase in SOD activity is the main findings of this study. In other words, whey protein supplementation can decrease MDA and increase SOD activity in recovery times (24, 48 and 72 hours) after resistance exercise compared with placebo group in obese non-athletic male. These findings supported the antioxidant effects of this protein supplement after severe eccentric contractions.

**Table 1. Mean and standard deviation of anthropometric characteristics of studied groups (mean  $\pm$  SD).**

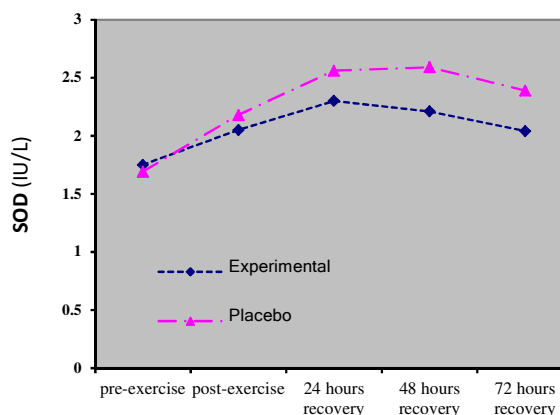
Variables	Experimental group	Placebo group	$P$ -value
Age (year)	22.5 ( $\pm$ 1.17)	21.33 ( $\pm$ 1.16)	0.222
Height (cm)	176 ( $\pm$ 2.31)	174.7 ( $\pm$ 3.14)	0.248
Weight (kg)	89.9 ( $\pm$ 6.73)	89.3 ( $\pm$ 6.96)	0.814
Body mass index (kg/m <sup>2</sup> )	29.01 ( $\pm$ 1.82)	29.28 ( $\pm$ 2.45)	0.767
Body Fat (%)	30.14 ( $\pm$ 2.27)	30.63 ( $\pm$ 2.71)	0.311

**Table 2. Mean and SD of biochemical variables of the subjects**

Variable	Group	Pre-exercise Mean ( $\pm$ SD)	Post-exercise Mean ( $\pm$ SD)	24 hours recovery Mean ( $\pm$ SD)	48 hours recovery Mean ( $\pm$ SD)	72 hours recovery Mean ( $\pm$ SD)	$P$ -value (ANOVA)
MDA (IU/L)	Experimental	258 ( $\pm$ 16.5)	275 ( $\pm$ 13.8)	331 ( $\pm$ 18.9)	323 ( $\pm$ 19.2)	301 ( $\pm$ 23.6)	$< 0.001$
	Placebo	253 ( $\pm$ 12.1)	262 ( $\pm$ 9.32)	276 ( $\pm$ 13.3)	279 ( $\pm$ 16)	267 ( $\pm$ 16.1)	
	$P$ -value (Independent T-test)	0.384	0.211	$< 0.001$	$< 0.001$	0.001	
SOD (IU/L)	Experimental	1.75 ( $\pm$ 0.118)	2.05 ( $\pm$ 0.183)	2.30 ( $\pm$ 0.112)	2.21 ( $\pm$ 0.098)	2.04 ( $\pm$ 0.160)	$< 0.001$
	Placebo	1.69 ( $\pm$ 0.135)	2.18 ( $\pm$ 0.147)	2.56 ( $\pm$ 0.144)	2.60 ( $\pm$ 0.156)	2.39 ( $\pm$ 0.091)	
	$P$ -value (Independent T-test)	0.258	0.222	$< 0.001$	$< 0.001$	$< 0.001$	



**Figure 1.** The changes pattern of MDA in experimental or placebo subjects. Data shows whey protein supplementation can be decrease serum MDA after severe eccentric contraction in non-athletes young men.



**Figure 2.** The changes pattern of SOD activity in experimental or placebo subjects. Data shows whey protein supplementation can be increase SOD activity after severe eccentric contraction in non-athletes young men.

Although similar studies were limited, especially in obese participants, several studies have reported controversial findings on other athletic and non-athletic populations. Kerasioti et al (2012), demonstrated that compared with carbohydrate alone, using whey protein plus carbohydrate does not affect oxidative stress markers and TAC at 30 min, 1, 24, and 48 hours after an exhaustive exercise (21). Nevertheless, Xu et al (2011) suggested that whey protein intake during intensive exercise would lead to an improvement of antioxidant capacity in response to acute oxidative stress and proposed the use of this protein supplement as one of the strongest antioxidants in the prevention of exercise damage resulting from invasion of free radicals (22). However, in a study by Deminice et al (2005), 4 weeks of whey protein supplementation did not lead to a change in GPX activity in experimental mice (23). Eizadi et al (2018) found that although whey protein supplementation was not associated with a change in aspartate aminotransferase (AST) at delayed intervals after a session of severe eccentric contractions compared to the placebo group in non-athletic men, the activity of alanine aminotransferase (ALT) significantly decreased compared with the placebo group (24).

Despite the mentioned contradictory results, in our study, although the SOD activity at 24, 48,

and 72 hour intervals after the exercise test showed an increasing trend in 2 groups, its activity was significantly higher in the whey protein supplement group at each stage of study after exercise test (at 24, 48, and 72 hour intervals) compared with the placebo group. These findings confirm the antioxidant effects of whey protein during the intervals after intense resistance test in non-athletic obese people. Mosoni et al (2014) reported that high absorption of whey protein resulted in delay in the reduction of fat body mass in healthy mice during a gradual increase in age (25). Sousa et al. (2012) pointed out the proactive role of whey protein in weight gain and obesity and type-2 diabetes through increasing the release of anti-appetite hormones such as leptin and reducing hunger hormones such as ghrelin, as well as lowering blood pressure, inflammation, and oxidative stress in obese people (26). Zhang et al (2015) suggested that whey protein has antioxidant properties and protective effects against oxidative damages (27). It should be noted that antioxidant enzymes secreted from different tissues in response to oxidative stress possess destructive characteristics of free radicals; they reduce the destructive property of radicals through decreasing their energy or electrons and preventing the initial formation of oxidative reactions (28). Among these enzymes, SOD is a metalloprotein and acts as the first and most



important defense line against free radicals produced in the cell (29,30). The highest level of SOD activity is in the liver, heart, and slow-twitch skeletal muscle and the lowest activity is in fast-twitch muscle fibers (31). Based on the findings, its appearance as an antioxidant in intense resistance exercises which involve a larger percentage of fast-twitch muscle fibers is far lower compared to low intensity exercises.

Apart from the increase in SOD, whey protein supplementation in the present study was associated with a significant decrease in MDA in each interval after the resistance exercise test compared to the placebo group. In other words, despite the reduction trend of MDA after exercise test in both groups, its levels were significantly lower in the whey protein group at each stage following exercise test sampling (24, 48, and 72 hour intervals) compared with the placebo group. The close relationship between levels of MDA as one of the strong oxidants and creatine kinase as one of the muscle damage indicators has already been reported (32). Accordingly, the relationship between free radicals and muscle damage indicators has been evaluated in some studies. Some of them have reported a close and significant relationship between MDA as a lipid peroxidation indicator and creatine kinase as an indicator of muscle damage after exercise (33). Studies have argued that some exercise activities are associated with excessive increase in release of free radicals and oxidants such as MDA, decrease in antioxidant capacity, reduction in antioxidant agents, and increase in oxidative damage to bio-macromolecules including proteins and lipid membranes (34). In this regard, scientific sources pointed to the increase of oxidative stress indicators in athletic men (35) and decrease of antioxidant enzymes activity in mice after an intense resistance training session (4). It has also been reported that the only factor that can stop the destruction trend of free radicals during intense muscle contractions is enhance antioxidant system (36).

The antioxidant effect of whey protein as a protein rich in amino acids has been confirmed by most studies (37,38). Whey protein plays a major role in neutralizing toxins in the body via a wide range of important amino acids such as glutamine, cysteine, and glycine. This protein is also the precursor of glutathione, which is the most important defensive compound of the body against cancer and age-related diseases such as Alzheimer, Parkinson disease, and arteriosclerosis (37,39). In a study on rats, it was determined that whey protein had a free-radical-scavenging effect and increased antioxidant activity (40). Researchers have also indicated that whey protein, through multiple pathways, improves the antioxidant capacity against acute oxidative stress and can be used as a rich antioxidants source in preventing damages resulting from the accumulation of free radicals in athletes (22).

## Conclusions

Whey protein supplementation is associated with antioxidant effects after one session of intense resistance exercise in non-athletic obese males. In the present study, its supplementation reduced the MDA activity and increased SOD activity at 24, 48, and 72 hour intervals after resistance exercise compared to the placebo group. The antioxidant effects of whey protein after intense resistance exercise are remarkable from the clinical prespective. Hence, whey protein can be described as an antioxidant supplement resulting in an increase in immune system function followed by intense muscle contractions in obese untrained individuals. However, recognizing the molecular and cellular mechanisms responsible for the importance of whey protein in oxidative stress conditions caused by intense exercise requires further studies.

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## Conflict of Interest

The authors report no conflicts of interest.

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