Research Article

# The Effect of Consuming 500 mL Low-Fat Milk on Cortisol Response and Salivary CRP After Resistance Training Among Young Healthy Women

Parisa Poorabedi Naeini [1] and Farzaneh Taghian [1, *]

[1]Department of Physical Education and Sport Science, Isfahan (Khorasgan) Branch, Islamic Azad University, Isfahan, Iran

*Corresponding author: Department of Physical Education and Sport Science, Isfahan (Khorasgan) Branch, Islamic Azad University, Isfahan, Iran. Email: f_taghian@yahoo.com

## Abstract

**Background:** During acute severe muscular activities, the levels of inflammatory markers, including cortisol and C-reactive protein (CRP), significantly increase, which can be the underlying factor and the initiator of atherosclerosis and cardiovascular diseases. Given the complications and high prices of sports supplements consumed to neutralize these undesirable effects following acute severe muscular activities.

**Objectives:** The purpose of this study was to evaluate the effect of consuming 500 mL of low-fat milk on cortisol and salivary CRP response after resistance training in young healthy women.

**Methods:** In this study, two groups with the age range of 20 - 25 years and the body mass index (BMI) from 20 - 24.9 kg/m$^2$ were recruited. Then, a group of 10 individuals performed two protocols at two intervals and were then compared together. The resistance training program included three sets with 10 reps and 75% intensity of a maximum rep in two one-hour sessions. On the first day, salivary sampling was carried out in fasting conditions, immediately before and after training, and also at the 60th and 120th minutes following it. One week later, in the second phase, the above-mentioned steps were fulfilled using 250 mL of low-fat milk in two sessions during sports activities. Descriptive statistics were also used to determine the mean and the standard deviation; and repeated measures analysis of variance (ANOVA) was employed at the inferential level. This test was employed to examine the main and the combined effects within four different times (immediately before and after training as well as one and two hours after training) in the experimental group in two different situations. The significance level of the tests was also considered as P < 0.05.

**Results:** The results of the study did not reveal significant changes in the between-group comparisons of low-fat milk consumption in terms of cortisol and salivary CRP response after one-session resistance training program (P > 0.05).

**Conclusions:** Based on the findings of this study, it was concluded that low-fat milk consumption was not able to adjust cortisol and CRP levels as inflammatory markers following one session of resistance training. Although milk consumption had reduced the CRP levels after training, this was not reported as a significant decrease. Definitely, measurement times in this test were of utmost importance.

*Keywords:* Low-Fat Milk, C-Reactive Protein, Cortisol, Resistance Training

## 1. Background

Nowadays, individuals are under excessive pressure to attain better results in their workplace, sports activities, and personal life (1). Physical activities and psychological stress are one of the most powerful and effective stimulants on the hormones secretion levels. Resistance training, with regard to its impact on body composition and some biological markers like cholesterol and insulin resistance, has also become one of the interventions advised for lifestyle modification. However, cortisol levels, after resistance training, do not reach the initial levels for up to three hours and post-training cortisol stability in the blood can be correspondingly harmful (2). Moreover, cortisol secretion levels in normal individuals (with no prior practices) compared with professional athletes have been reported high after performing resistance training (3).

Mobilizing fatty acids from fatty tissues in the body in order to maintain storing of blood glucose, cortisol increases plasma free fatty acid concentration. Thus, a rising trend in gluconeogenesis and a modest reduction in blood glucose consumption by tissues lead to an increase in blood glucose concentration and consequently diabetes. High concentrations of cortisol can also decrease abnor-

mal sensitivity of most tissues; particularly, skeletal muscle and fatty tissues to the stimulatory effects of insulin on glucose absorption and consumption. Accordingly, high levels of cortisol can cause a growing trend in blood pressure, blood fat, and blood sugar, and subsequently reduce synthesis of proteins (1, 4).

In this regard, researchers have argued that not only immunosuppressive hormones such as cortisol but also pro-inflammatory cytokines increase during acute severe sports activities even for one training session, they cause suppression and weakening of the immune system as well (5).

C-reactive protein (CRP) is considered as one of the acute-phase reactants produced in response to infection, surgery, trauma, and muscle damage affected by sports activities. Moreover, CRP concentration after training has an increasing trend (6). In this respect, many researchers believe that the rise in inflammatory markers is the underlying factor and the initiator of atherosclerosis and cardiovascular diseases (7). Thus, one of the important strategies proposed to diminish inflammation and adverse effects of acute severe muscular activities is the consumption of nutrients and special supplements such as branched-chain amino acids (BCAAs) (8). In addition, some studies have attempted to use various supplements in three steps before, during, and after training to reduce the effects of cortisol on the body of athletes. These studies have demonstrated that the intake of carbohydrate supplements, along with amino acids during training, reduces cortisol levels and its catabolic effects on muscle protein breakdown (9, 10).

Naturally, there is a combination of several carbohydrate supplements such as, whey protein, casein, and BCAAs in milk (11). In the study by Farzanegi et al., a significant relationship was reported between daily milk consumption and reduction in the inflammatory marker of CRP as well as decreased prevalence of metabolic syndrome and cardiovascular diseases (5). Furthermore, the studies by Farzanegi et al. (5) and Drouin-Chartier et al. (12) showed that milk consumption could reduce post-training cardio-metabolic risk factors.

In a standard sample of natural milk, there is 65% of saturated fatty acids, mainly palmitic acid, stearic acid, and myristic acid. The palmitic and myristic acids increase total cholesterol and LDL. Considering the role of saturated fatty acids in the development of cardiovascular diseases, diabetes, inflammatory diseases, and many chronic illnesses (13), low-fat milk was used in the present study. It is believed that low-fat milk can be taken into account as a useful nutrient and a supplement for athletes due to having relatively high carbohydrate and low amount of fat, seeking to lower the negative impact of muscular damage after training. Consuming two different amounts of low-fat milk (500 mL and 1 L) immediately after extroverted sports activities can reduce markers causing damage to muscles; however, it should be noted that 1 L of milk leads to a full and heavy stomach. Investigations reporting positive results have revealed that a combination of carbohydrate and protein can result in metabolic changes in protein, which may be the result of the reduction in break down or increase in protein synthesis. Moreover, a combination of carbohydrate and protein can prevent the increase in protein breakdown through cortisol reduction. In addition, amino acids and carbohydrates can separately affect protein metabolism. Thus, consumption of nutrients containing both substances can be helpful for athletes (14).

Milk is one of the cheap, available, and energy-boosting drinks that can contribute to post-training recovery, even more effective than sports drinks manufactured for this purpose (15). With respect to the side effects of sports supplements and irrespective of their high prices (16), the present study aimed to investigate the effect of milk as a nutrient-rich supplement to stimulate proper hormonal response after training in order to create a better environment for anabolism, improve effectiveness of sports activities, and consequently prevent diseases. Despite research studies on the effects of other nutritional and supplementary factors on the secretion of inflammatory markers after training, the effect of milk consumption on reduction of inflammatory markers of cortisol and CRP has been less evaluated. In addition, since previous investigations have been more focused on long-term sports activities and the impact of low-fat milk consumption on inflammatory markers of cortisol and CRP within a one-session resistance training program in athletes has not been yet investigated.

## 2. Objectives

The purpose of the present study was to shed light on the impact consuming low-fat milk on cortisol and salivary CRP the levels after high-intensity resistance training in young healthy women who had not practiced before the test.

## 3. Methods

### 3.1. Research Method

This quasi-experimental study was conducted using an experimental group via a pretest-posttest design. To this end, 34 women referring to Omid-e-Noor Sports Complex in the city of Isfahan, Iran, and who volunteered to participate in the study were recruited.

The inclusion criteria of the study were age range of 20 - 25 years, BMI from 20 - 24.9 kg/m², no history of regular training; no history of bacterial and viral infections, active rheumatic fever, acute heart failure, and RA; no-use of steroidal and non-steroidal anti-inflammatory drugs (NSAIDs) and contraceptives; tobacco, caffeine, drug, and food supplement use affecting inflammatory processes; no surgery over the past year; lack of emotional stress or physical injury, and no allergy to dairy products or lactose intolerance. After completing the questionnaire, screening was done for the individuals who did not meet the mentioned criteria and they were excluded. Following the screening, 10 participants with a mean age of $22.3 \pm 1.63$ years, height of $162.4 \pm 3.34$ cm, weight of $58.5 \pm 7.96$ kg, and BMI of $22.15 \pm 2.73$ kg/m² were selected using convenience sampling method. The sample size was determined based on previous similar studies.

### 3.2. Research Procedure and Measurement of Variables

To collect the data, the necessary coordination was made with women and other colleagues to do the physical activities including stretching and resistance. First, the required explanations regarding research objectives, procedure, and potential risks of performing the activities were explained to the participants and then, full information regarding the study research was provided following the completion of the consent form. The participants also became familiar with the saliva test procedure. The whole project design and its implementation steps were also approved by the Ethics Committee of Islamic Azad University, Isfahan (Khorasgan) Branch, with the code no. IR.IAU.Khuisf.REC1396.29. In addition, since acute severe muscular activities could affect the immune system for several hours, the participants were asked to avoid performing sports and non-sports activities for 48 hours before sampling [17].

Besides, the participants were requested to abide by normal patterns of sleep (a minimum of eight hours) and also dietary patterns as well as avoid eating and drinking any substances influencing the implementation of the test [18]. Furthermore, to determine the better effect of the measured markers, the participants were advised to abstain from consuming any dairy products within three days prior to the implementation of the protocol in both phases. To ensure it further, the participants were asked to register the food consumed in these three days in the daily record of food intake form with accuracy and honesty. At this step, the weight and the height of the participants were measured via a height-measuring column scale with the brand name of Seca 700, made in Germany, with the weighing accuracy of 0.05 kg and height-measuring ac-

curacy of 0.001 meter by researchers. In addition, their BMI was calculated using the following formula:

$$BMI = weight\,(kg)\,/\,height\,(m) \times height\,(m)$$

Then, the participants became familiar with the correct principles and procedure as well as the main risk factors of weight-lifting; next, a maximum rep was determined. All of these steps were fulfilled accompanied by the presence of a bodybuilder and the following formula was employed to calculate a maximum rep for each participant [19]:

$$maximum\,rep = lifted\,weight\,/\,1.0278 - (0.0278 \times number\,of\,reps\,until\,getting\,fatigued)$$

The resistance training program used in this study included resistance exercises done in two one-hour sessions. These exercises included movements affecting the muscles of the thighs, the calves, the waist, the hands, the deltoid, and the abdomen (all the muscles in the upper and lower extremities).

In the test implementation session, the information collected from the daily record of food intake forms was examined by researchers and the participants' observance of their dietary patterns (without dairy) was ensured. To lower the effect of muscle soreness, there was a 7-day interval between the record-taking day and the initial test.

In the first phase, while all the participants had not eaten anything eight hours before the training, they were first asked to wash their mouth and pour some of their saliva in a non-stimulated manner into the specimen tube. Saliva sampling was performed at the test site under the supervision of the researcher. Samples of the saliva were then collected in laboratory tubes and immediately kept in a cold box containing ice until the end of sampling [20]. Then, stretching movements were practiced with a coach's help for warm-uplasting six minutes. The session was followed by performing resistance training according to the protocol. Each exercise was done in three sets with 10 reps and 75% intensity for one rep, the total exercise lasted for about 48 minutes. In the end, movements to back to the initial state were performed for six minutes. The rest intervals were considered to be about 30 seconds. The training time was also between 8 am and 9 am. The table of resistance training program, for the participants, was as follows:

After performing the training protocol, sampling was fulfilled immediately and also in minutes 60 and 120 after training in accordance with the mentioned procedure. The samples were then transferred without any delay to a specialist medical and pathological laboratory and frozen by an expert at -20°C until the measurement time of hormones. One week later, at the second phase, the participants in fasting conditions performed the given protocol, however, this time after consuming 250 mL of low-fat milk

**Table 1.** Resistance Training Program for the Study Participants

| Type of Exercise | Volume (Sets) | Reps | Time (Minute) |
|---|---|---|---|
| Warm-up and special stretching exercises | | | 6 |
| Chest press | 3 | 10 | 5 |
| Foot press | 3 | 10 | 5 |
| Front arm with barbell | 3 | 10 | 8 |
| Lateral stretching | 3 | 10 | 5 |
| Opening and bending knees | 3 | 10 | 6 |
| Back thighs | 3 | 10 | 5 |
| Shoulder press | 3 | 10 | 6 |
| Crunch-up with weights | 3 | 10 | 8 |
| Cool-down stage | | | 6 |

in two turns (two glasses) during physical activities. In the present study, pasteurized low-fat milk was used, which had been produced in the Teen Dairy Company with the trademark of Damdaranin the city of Tehran, Iran, with the health license number of 16.20107 from Iran's Food and Drug Administration. The weight of the milk fat used in the study was 2.76 g, its total carbohydrate was 7.24 g, and its sugar content was 0.62 g; moreover, the weight of the protein was 8.05 g in 250 mL of milk, which was consumed in the post-test stage in two turns during training in minutes 20 and 40. To examine the saliva samples, salivary cortisol ELISA kit (DBC: Diagnostics Biochem Canada Inc.), manufactured in Canada with the accuracy of 1 ng/mL, and Salivary CRP ELISA kit (Hangzhou EastbiopharmCo.LTD), made in the United States with an accuracy of 1 mg/L were used. To determine the cortisol and CRP concentrations, the ELISA method was used.

### 3.3. Statistical Analysis Method

Statistical analysis was done at descriptive and inferential levels. Normal distribution of the data was also investigated through Kolmogorov-Smirnov test. At the descriptive level, the values of mean and standard deviation, as well as a statistical diagram, were used to describe the cortisol and CRP levels in individuals measured in four different times and within two different situations (with a one-week interval). Repeated measures ANOVA and Bonferroni post-hoc test were further employed at the inferential level. To compare the markers in the first and second phases of the study, independent *t*-test was used. All the statistical processes were also fulfilled through SPSS Statistics Software (version 22). The significance level of the test was set at $P < 0.05$.

## 4. Results

The present study was conducted on 34 female volunteers with a mean age of $22.30 \pm 1.63$ years. The anthropometric characteristics of the participants were illustrated in Table 2.

**Table 2.** Anthropometric Characteristics of the Participants

| Variables | Values (Mean $\pm$ SD) |
|---|---|
| Age (y) | $22.30 \pm 1.63$ |
| Height (cm) | $162.40 \pm 3.34$ |
| Weight (kg) | $58.50 \pm 7.96$ |
| BMI (kg/m$^2$) | $22.15 \pm 2.73$ |

As can be seen, independent *t*-test was used in each phase of the study to examine the between-group differences (Table 3). The results indicated no significant difference in the phase with and without intervention in CRP levels in the comparisons of first step (P = 0.119), second step (P = 0.067), third step (P = 0.284), and fourth step (P = 0.865). In addition, in the case of cortisol variable, no significant differences were observed in comparisons in the first step (with and without intervention) (P = 0.822), second step (P = 0.861), third step (P = 0.793), and fourth step (P = 0.480). Then, within-group repeated measures, ANOVA was used for the variable of measurement steps to determine the impact of low-fat milk along with resistance training on the cortisol and salivary CRP response in the participants. The results (Table 3) showed that the consumption of low-fat milk accompanied by resistance training had no significant effect on cortisol and salivary CRP levels. For precise comparisons of these changes from one test to the subsequent one in the first phase, Bonferroni post-hoc test was used and its results were presented in Table 4.

Based on the results of the Bonferroni post-hoc test in Table 4, changes in the salivary CRP levels, as seen in all test times, were different in the first phase. According to the results of Bonferroni post-hoc test, variances in salivary cortisol response were not significant in all test times except for the second and the fourth steps (0.03) as well as the third and the fourth ones (0.03). Changes in salivary cortisol levels in the second phase were also different only between the first and the fourth steps (0.02) as well as the second and the fourth ones (0.04).

Furthermore, in the second phase of the study, fluctuations in cortisol levels at pre-training times and two hours after training (P = 0.023) as well as immediately after training and two hours after training (P = 0.047) were reported significant.

**Table 3.** Comparison of Cortisol and CRP Means Among Participants in Four Steps Within Two Phases of the Study[a]

| Variable/Study Phase | Sampling Times | | | | P Value Repeated Measures ANOVA |
|---|---|---|---|---|---|
| | Before Training | Immediately After Training | One Hour After Training | Two Hours After Training | |
| **CRP (mg/L)** | | | | | |
| First phase | 3.03 ± 0.77 | 3.53 ± 0.61 | 3.96 ± 0.58 | 4.74 ± 0.75 | ≤ 0.001 |
| Second phase | 3.69 ± 1.01 | 4.63 ± 1.60 | 4.86 ± 2.42 | 4.44 ± 2.16 | 0.12 |
| P value | 0.0119 | 0.067 | 0.284 | 0.865 | - |
| **Cortisol (ng/mL)** | | | | | |
| First phase | 37.19 ± 25.75 | 24.35 ± 10.78 | 25.16 ± 11.53 | 15.80 ± 5.27 | 0.026 |
| Second phase | 39.94 ± 27.93 | 23.43 ± 12.34 | 23.86 ± 10.25 | 13.58 ± 8.13 | 0.16 |
| P value | 0.882 | 0.861 | 0.793 | 0.480 | - |

[a]Values are expressed as mean ± SD.

**Table 4.** Results of Bonferroni Post-Hoc Test in Four Measurement Times of CRP and Cortisol Response in the First Phase of the Study

| Time | Cortisol | | CRP | |
|---|---|---|---|---|
| | Mean Difference | P Value | Mean Difference | P Value |
| **Before training** | | | | |
| Immediately after training | 16.50 | NS | - 0.50 | 0.014 |
| One hour after training | 16.07 | NS | - 0.93 | ≤ 0.001 |
| Two hours after training | 26.35 | 0.02* | - 0.71 | 0.001 |
| **Immediately after training** | | | | |
| One hour after training | - 0.43 | NS | - 0.43 | 0.04 |
| Two hours after training | 9.84 | 0.04* | - 1.20 | 0.006 |
| **One hour after training** | | | | |
| Two hours after training | 10.27 | 0.004 | - 0.77 | 0.01 |

## 5. Discussion

The results of the present study did not show significant changes in the between-group comparisons of low-fat milk as a source of protein, carbohydrate, and BCAA such as glutamine on salivary CRP and cortisol response after one-hour resistance training. According to the findings of the present study, consumption of milk did not demonstrate a significant effect on reducing CRP after one-session resistance training. Although the diagram for the comparison of the within-group CRP levels indicated the impact of resistance training on increasing the salivary CRP levels in the participants and considering that low-fat milk could lower the rising trend of CRP marker in intervals of 3T and 4T, the given decrease with attention to the time of sampling was not significant.

However, in the study by Farzanegi, a significant relationship was reported between daily milk intake and the reduction of CRP inflammatory marker, decreased prevalence of metabolic syndrome, and cardiovascular diseases

(5). According to Farzanegi et aL., the mechanism of the effect of milk on reducing the mentioned markers was that dairy consumption was inversely correlated with body weight, glucose homeostasis, and metabolic syndrome. Calcium inside the cells could also be used to regulate fat metabolism and even absorb and store insulin-dependent glucose having a direct effect on fat cells. The calcium inside dairy sources could further prevent lipogenesis and consequently facilitate lipolysis. Moreover, high calcium intake from dietary pattern could increase the fecal excretion of fat. CRP also plays a role in the incidence of chronic illnesses such as cardiovascular diseases, diabetes, and cancer. The results of the study by Farzanegi showed that aerobic exercises along with milk consumption could result in reduced CRP levels.

Moreover, research by Farzanegi et al. (5) and Drouin-Chartier et al. (12) indicated that milk intake had moderated cardio-metabolic risk factors after training. It seems that calcium metabolism and perhaps other components

of milk products could affect balancing energy and could play a role in weight control (21). Several studies have also found an independent relationship between dairy consumption and favorable fatty profiles (22), lowered risk of high blood pressure (23), insulin resistance, and type-2 diabetes (24).

In addition, Atashak et al., in their research reported the positive effect of the consumption of BCAA supplement on CRP levels after a one-session resistance training program in soccer players (25). The mechanism of the effect was that muscle breakdown could cause cellular damage, which would lead to inflammation and production of CRP after training. BCAA supplement can also stimulate the production of interleukin-2 and interferon, thus, preventing interleukin-4 production following acute severe sports activities. Consumption of BCAAs could also prevent glutamine concentration decrease of plasma. Maintaning the concentration of glutamine in plasma may have an effect on decreasing CRP levels. Therefore, it can be argued that the use of BCAAs can reduce the CRP inflammatory marker following resistance training.

The results of between-group comparison of cortisol response showed that low-fat milk consumption did not have a significant effect on salivary cortisol levels after one-session resistance training. In addition, within-group comparison at intervals of T2 and T3 revealed the effect of resistance training on increased cortisol levels; however, milk intake could not significantly affect the declining trend of cortisol response after training, which is probably due to the impact of the strong circadian cortisol regulation (26). Moreover, other research studies in this field have shown that the consumption of carbohydrate and amino acid supplements during training could diminish cortisol levels as well as its catabolic effects on muscle protein breakdown (9, 27). However, another study in this domain did not determine the effect of protein and carbohydrate supplements on reducing the amount of cortisol (1).

A dietary pattern containing carbohydrates and proteins may also result in more insulin secretion, which may lead to more glucose excretion as well as faster glycogen synthesis in the muscles. Protein intake helps to provide the essential amino acids needed for anabolism and tissue repair. This underlying muscle anabolism mechanism can be boosted through amino acid, protein, and carbohydrate supplements that can be accompanied by increases both in insulin and amino acids available. Therefore, the effect of carbohydrate and protein supplements may be attributed to higher levels of amino acids or higher plasma insulin concentrations (14).

Witbracht et al., in a study on overweight women, examined the effect of low-fat dairy consumption on reducing salivary cortisol and weight (28). The results of the given investigation indicated that the average consumption of low-fat dairy products, along with energy intake, could be effective in reducing salivary cortisol levels. In addition, in this test, the consumption of low-fat dairy having a potential metabolic effect on cortisol levels could cause weight loss, although the mechanism of this effect has not become clear yet.

However, Kazemzadeh and Gaeini concluded in his research that while 6% carbohydrate supplement might improve hormonal changes (increases in insulin concentrations and decreases in cortisol concentrations after training) compared with the use of proteins during resistance training, hormone status after resistance training was only part of the anabolic environment in the body and the conditions of other variables such as the amount of circulating amino acids, the level of synthesis, the body protein breakdown (net protein balance), and the level of activation of anabolic and catabolic enzymes could effectively shape the given environment under the influence of these hormones (29).

*5.1. Conclusions*

In the present study, consumption of low-fat milk did not result in a significant reduction in cortisol secretion and CRP inflammatory marker response after one-session resistance training. It seems that the intervals in the measurement of CRP and strong circadian rhythm of cortisol regulation could significantly influence the results. Accordingly, it was suggested to investigate the effect of low-fat milk on CRP marker with the measurement time of 6, 12, and 48 hours after training, and also examine cortisol response, being at different levels in the morning based on circadian rhythm regulation, in the afternoon. In addition, recruitment of more participants, precise control of nutritional conditions, as well as physical activities and stress in participants can be considered as factors contributing to achieving the desired results in this domain.

**Footnotes**

**Authors' Contribution:** Study concept and design: Parisa Poorabedi Naeini. Analysis and interpretation of

data: Farzaneh Taghian.

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