

Original Article

Effects of Vitamin C Supplementation on C-Reactive Protein, Lactate and Blood Pressure Following Resistance Exercise in Overweight Men

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ABSTRACT

Background and Objectives: Strenuous exercises have been shown to induce inflammation. However, consumption of products rich in antioxidants such as vitamin C may enhance this effect. The purpose of this study was to assess effects of vitamin C supplementation on C-reactive protein, lactate and blood pressure following resistance exercise in overweight men.

Materials and Methods: In a randomized, double-blinded placebo-controlled trial, 20 young men (age of 38.8 y ± 3.4 and BMI of 28.36 kg/m² ± 1.2) voluntarily participated and were randomly divided into vitamin C supplementation (n = 10) and placebo (n = 10) groups. After two weeks of receiving 500 mg/d of vitamin C or placebo, participants were involved in a session of resistance exercises (three sets of eight repetitions at 80% of one repetition maximum). To assess changes in C-reactive protein and lactate, blood samples were collected after blood pressure measurement at phases of baseline, pre exercise, immediately post resistance exercise and 24 h post exercise.

Results: Results using ANOVA with repeated measurement showed that levels of C-reactive protein, lactate and systolic and diastolic blood pressure were not significantly changed in participants after supplementation (p>0.05). Moreover, significant increases were seen in lactate and systolic and diastolic blood pressure immediately after exercise intervention in both groups (p < 0.05). However, C-reactive protein levels significantly increased only in placebo group immediately post resistance exercise and 24 h post exercise, compared to those at baseline (p = 0.008 and p = 0.021, respectively) and pre exercise (p = 0.007, and p = 0.019, respectively) times. The unique statistically significant difference between the two groups included a significant increase in level of C-reactive protein in placebo group immediately post resistance exercise (p = 0.031) and 24 h post exercise (p = 0.038) times, compared to supplement group.

Conclusions: Results from the present study have demonstrated the beneficial effects of vitamin C supplement on improved blood pressure and lactate tolerance as well as decreased inflammatory responses of C-reactive protein caused by resistance exercises.

Keywords: Physical activity, Inflammation, Blood pressure, Lactic Acid, CRP

Introduction

Overweight and excessive body fats are important relevant factors involved in advancement of diabetes mellitus, hypertension, inflammation and cardiovascular disease (CVD) (1). The CVD is one of the common causes of death in developed and developing countries. It is reported that inflammation plays a key role in progression of CVD (2). Currently, C-reactive protein (CRP) is considered as one of important inflammatory markers that is over expressed during inflammatory processes such as various diseases, infections and damaged tissues (3). The

levels of serum CRP is less than 2 mg/l in healthy men and less than 2.5 mg/l in healthy women, which increase dramatically in response to inflammations, infections and injuries (4). Studies have shown that CRP is secreted in liver, adipocytes, macrophages and endothelial cells in response to inflammatory conditions (5). Furthermore, CRP increases blood pressure (6) and increases reactive oxygen species (ROS) by stimulating release of angiotensin II (Ang II) (7). These can lead to endothelial dysfunction, decreases in vasodilation, increases in vascular contraction and

damages to vessels that increase blood pressure (5). Therefore, higher CRP levels are correlated with higher risks of developing hypertension (8). A solution to improve high blood pressure is to decrease anti-inflammatory biomarker of CRP and improve endothelial function (9).

Researchers believe that diets and regular physical activities can decrease inflammatory markers and their effects can improve human health (10). Studies have been carried out on effectiveness of physical activity inflammation, especially resistance interventions on exercise training. However, most of these studies have produced contradictory results. For example, Ramel et al. (2015) indicated that resistance exercise included no significant effects on CRP serum levels (11); as Cormie et al. (2016) did (12). Unlike these results, Bizheh and Jaafari (2011) have reported significant increases in CRP serum levels following resistance exercises (13). Poorabedi Naeini and Taghian (2018) reported similar results as well (14). However, lack of time is one of the most common reasons for people that fail to do regular exercises; thus, greater focuses have been reported on acute physical activities. However, inflammatory responses to acute resistance exercises vary, which mostly increase inflammations. High lactate concentrations during resistance exercises naturally induce increases in lactic acid accumulation in muscles that causes acidosis in muscle cells and hence leads to cell damages (15). This subsequently results in inflammation. In recovery period, body eliminates acidosis state using lactate discharging. Therefore, factors that are able to decrease production of lactate during physical activities accelerate the recovery process.

Recent studies have shown that use of oral antioxidant and anti-inflammatory supplements can decrease oxidative stress damages and inflammatory responses to exercises (16, 17). Relatively, the role of vitamin C supplementation is important. Vitamin C or ascorbic acid is one of the most important vitamins and water-soluble natural antioxidants. Furthermore, it is shown that vitamin C includes antiinflammatory properties (18) and its consumption is one of good solutions to alleviate inflammation, which can decrease atherosclerosis disease (18). Juraschek et al. (2012) reported that short-term vitamin C supplementations decreased systolic and diastolic blood pressure (19). Monica Rahardjo et al. (2013) showed that intravenous administration of 1000 mg of vitamin C could improve lactate levels in septic patients (20). Patlar et al. (2017) demonstrated that increases in lactate levels due to exhaustion exercises were relieved using vitamin C supplementation (21). Biniaz et al. (2013) reported that consumption of vitamin C supplementation modified levels of CRP in patients with hemodialysis (22). However, a few studies have been carried out on effects of vitamin C consumption on decreasing levels of inflammatory markers

such as CRP, which are mostly contradictory (23). Considering effectiveness of resistance exercises on inflammation and benefits of vitamin C in this area, it seems that consumption of vitamin C supplementation before resistance exercises can be a good strategy to achieve various goals such as fast recovery, improving exercise tolerance and decreasing inflammation in overweight individuals. Therefore, the aim of the present study was to assess effects of vitamin C supplementation on CRP serum, lactate and blood pressure following resistance exercises in young overweight men.

Materials and Methods

Experimental design and subjects

This study was a double-blind, randomized, placebocontrolled quasi-experimental study using repeatedmeasures design. The study protocol was approved by the Institutional Review Board of Islamic Azad University, Marivan Branch, Iran. Twenty overweight middle-aged men from Marivan City, Iran, voluntarily participated in the study (Table 1). The statistical sample size was one the limitations within the present study, estimated as 24 individuals with the power of 0.8 using G-Power Software. Of 41 volunteers, 24 volunteers were selected based on the entry criteria. However, four volunteers withdrew due to various reasons. Inclusion criteria were age (30–40 y), BMI (25–29.9 kg/m²), gender (male), non-smoking and lack of regular physical exercises in the past six months. Moreover, exclusion criteria were consumption of drug supplements during study, lack of regular participation in research studies and having no diseases and physical injuries during the study. Based on the health information questionnaires, participants were healthy with no drug uses in the past two months. Furthermore, all participants were advised to avoid supplements or foods containing vitamin C and intensive physical activities during the study. In The current study, participants received two weeks of supplementation or placebo after being randomly divided into two groups of supplement (n = 10) and placebo (n =10) groups and then participated in a session of acute resistance exercise. Before starting familiarization sessions were carried out to explain benefits and potential risks of the study for the participants, describing all programs and procedures. Then, participants signed consent forms. In these sessions, participants received guidance s for the use of bodybuilding machines and specific exercises. One repetition maximum (1RM) in all movements was recorded using Brzycki equation (24) of weight / $[1.0278 - (0.0278 \times number of repetitions)]$. In addition, Bruce test was used to estimate VO2max values. The VO₂max was calculated using the following formula:

 $VO_2max = 14.8 - (1.379 \times total \ time \ on \ the \ treadmill) + [0.451 \times (total \ time \ on \ the \ treadmill)^2] - [0.012 \times (total \ time \ on \ the \ treadmill)^3] \ (25).$

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Table 1. Baseline descriptive and functional characteristics of the participants

Group	Age (y)	Body Weight Height (cm) BMI (kg/m ²)		BMI (kg/m ²)	VO ₂ max	Vitamin C
		(kg)			(ml/kg.min)	(µmol/l)
Supplement	38.2±3.7	88.92±5.13	176.4±3.61	28.57±1.24	42.02±3.31	44.6±5.3
Placebo	39.4±3.6	87.67±4.52	178.6±4.34	27.51±0.84	41.18±2.09	46.1±4.8

Values are expressed as mean ±SD

Resistance exercise

Exercise session involved one session of resistance exercise, which was carried out after two weeks of supplementation. Resistance exercise included five exercises of three sets of eight repetitions with 80% of repetitions maximum (1RM) for bench press, leg extension, lateral pull down, lying leg curl and triceps pushdown. In general, 2 and 3 min of recovery periods were used between the sets and the exercises, respectively (26). Before starting resistance exercises, warm up sessions were carried out, including 10 min of two sets with 12 repetitions of the first exercise at 40% of 1RM. At the end, 5 min of cool down sessions were carried out by stretching movements. During exercise session, participants in resistance exercise were supervised by experienced fitness instructors.

Vitamin C supplement

Vitamin C supplement group received 500 mg.d-1 of vitamin C in capsules (Shahre Daru Pharmaceutical, Tehran, Iran) for 14 d. Moreover, maltodextrin was similarly prepared in capsules and provided to placebo group. Maltodextrin is a white powder made from starch with a chemical formula of $C_{6n}H_{(10n + 2)}O_{(5n + 1)}$. Maltodextrin is a compound produced from hydrolysis of starch or sweeteners by catalyzing an aqueous sucrose solution, which is odorless with low sweetness. According to a greater effectiveness, participants consumed supplement or placebo after breakfasts. Another limitation of this study included lack of precise control on the participants' diets. All participants completed validated food intake questionnaires one day before the study as well as 24-h food records to investigate if the two groups included similar consumed foods.

Blood pressure

Blood pressure was measured four times before supplementation (baseline, fasting), before resistance exercise (fasting), immediately post resistance exercise and 24 h post resistance exercise (fasting). In each phase, systolic and diastolic blood pressure were measured with 5-min intervals in sitting position using upper arm digital blood pressure monitor (Andon Health, Model KD-5917, Tianjin, China) and the mean values of these two measurements were recorded.

Blood collection and analysis

Blood samples (each 5 ml) were collected before supplementation (baseline), pre resistance exercise, immediately post resistance exercise and 24 h post resistance exercise. Blood samples were collected from antecubital forearm veins in blood tubes. Then, tubes were centrifuged at 3000 g for 20 min at 4 °C and sera were stored at -80 °C until use. Serum CRP was assessed using ELISA technique (Hangzhou Eastbiopharm, Hangzhou, Zhejiang, China). Intra and interassay coefficients of variation and sensitivity included 12%, 10% and 0.012 mg/l, respectively. The baseline levels of serum vitamin C were assessed using ELISA technique (MyBioSource, San Diego, California, USA) which intra and interassay coefficients of variation less than 15% and sensitivity of 2.0 µmol/l. Blood lactate was assessed using Lactate Scout Analyzer (SensLab, Leipzig, Germany). Left hand middle fingertip was washed and disinfected with distilled water and alcohol. Then, fingertip was punctured by lancet and the first drop of blood wiped off using cotton balls and the second drop was transferred onto a test strip and the lactate concentration was calculated.

Statistical analysis

Shapiro-Wilk and Levine tests were carried out to verify data normality and homogeneity of variances, respectively. Data were analyzed using two-way analysis of variance (ANOVA) with repeated measures (2×4) , followed by Bonferroni post hoc analysis or unpaired t-testing when appropriate. Data were analyzed using SPSS Software v.22 (IBM Analytics, USA) at a significance level of p < 0.05.

Table 2. Resistance exercise programs for the participants

Exercise	Volume (set)	Repetition	Recovery between sets	Recovery between exercises	Intensity
Bench press	3	8	2 min	3 min	80% of 1RM
Lateral pull down	3	8	2 min	2 min	80% of 1RM
Leg extension	3	8	2 min	2 min	80% of 1RM
Lying leg curl	3	8	2 min	2 min	80% of 1RM
Triceps pushdown	3	8	2 min	2 min	80% of 1RM

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Results

Serum C-reactive protein levels

In placebo group, CRP level significantly increased immediately post resistance exercise and 24 h post exercise, compared to baseline (p=0.008 and p=0.021, respectively) and pre exercise (p=0.007 and p=0.019, respectively) with no statistically significant changes by exercises in supplement group, indicating that vitamin C supplementation could prevent increased inflammation produced by acute resistance exercises. In addition, changes in CRP, as a measure of inflammation, was significantly lower immediately post resistance exercise [p=0.031, f=18.92 and effect size (EF) = 0.66] and 24 h post exercise (p=0.038, p=33.04 and EF = 0.83) in supplement group instead of placebo group. Changes in serum levels of CRP are shown in Table 3 and Figure 1.

Blood pressure

The mean changes in systolic and diastolic blood pressure for the two groups are shown in Table 4 and Figure 2. Results demonstrated that systolic blood pressure in both groups significantly increased immediately post exercise, compared to baseline (p = 0.000), pre exercise (p = 0.000) and 24 h post exercise (p = 0.000). In addition,

diastolic blood pressure was significantly higher immediately post exercise in both groups, compared to baseline (p=0.043 and p=0.016, respectively for supplement and placebo groups), pre exercise (p=0.036 and p=0.012, respectively for supplement and placebo groups) and 24 h post exercise (p=0.023, and p=0.009, respectively for supplement and placebo groups). The present findings showed no significant differences in systolic (p=0.121 and f=1.242) and diastolic (p=0.237 and f=0.507) blood pressure between the two groups at any time points.

Lactate levels

Blood lactate levels in the supplement and placebo groups were significantly higher immediately post exercise instead of baseline (p = 0.004 and p = 0.008, respectively), pre exercise (p = 0.002 and p = 0.005, respectively) and 24 h post exercise (p = 0.011 and p = 0.014, respectively). No differences were seen in changes between the groups (group effect, p = 0.489; interaction effect, p = 0.124 and f = 2.62). Mean values for both groups at any time points are presented in Table 5.

Table 3. C-reactive protein changes in the study groups

CRP	Group		Effect ANOVA	f	p	Partial eta squared
(mg/l)	Supplement	Placebo	Time	42.7	0.001	0.704
Baseline	0.79±0.16	0.67±0.13	Group	9.6	0.008	0.348
Pre- exercise	0.68 ± 0.14	0.65 ± 0.12				
Post-exercise	0.89 ± 0.29	1.35±0.23*	Interaction	23.1	0.002	0.563
24 h post-exercise	0.74 ± 0.11	1.14±0.15*				

Values are expressed as mean ±SD; *significant differences between groups

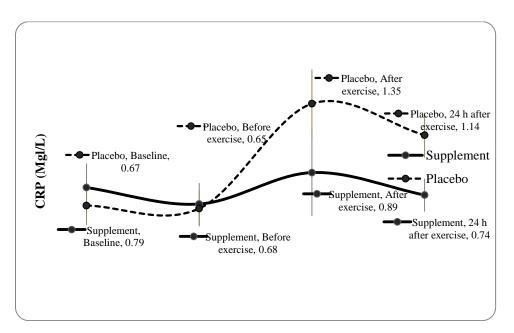


Figure 1. Changes of serum C-reactive protein levels in supplement and placebo groups at baseline, pre exercise, immediately post resistance exercise and 24 h post exercise times

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Table 4. Systolic and diastolic blood pressure changes in the study groups

Variable	Research stage	Group		Effect ANOVA	f	p	Partial eta squared
	•	Supplement	Placebo	Time	22.11	0.000	0.813
Systolic blood pressure	Baseline	115.1±4.6	111.3±3.7	Group	1.421	0.476	0.211
(mm/Hg)	Pre exercise	112.1±3.1	110.4 ± 3.4				
	Post exercise	151.2 ± 4.1	156.3±3.8	Interaction	0.812	0.121	0.135
	24 h post exercise	109.4±2.9	108.6±3.1				
Diastolic blood pressure	Baseline	84.2±2.7	82.6±1.5	Time	15.78	0.001	0.747
(mm/Hg)	Pre exercise	83.6±1.9	82.5±1.6	Group	0.507	0.237	0.124
	Post exercise	91.1±3.1	92.2 ± 3.4	Interaction	0.086	0.612	0.114
	24 h post exercise	83.1±2.1	82.2±1.7				

Values are expressed as mean ±SD

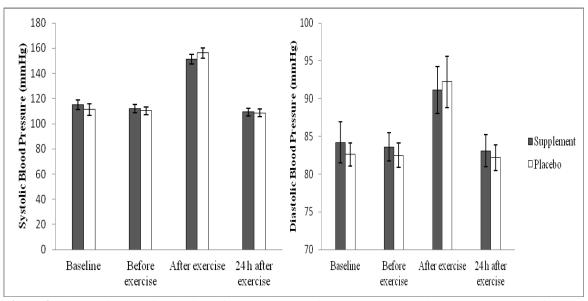


Figure 2. Changes in systolic and diastolic blood pressure in supplement and placebo groups at baseline, pre exercise, immediately post resistance exercise and 24 h post exercise times

Table 5. Changes in blood lactate levels in the study groups

Lactate (mmol/l)	Gro	Group		f	p	Partial eta squared
	Supplement	Placebo	Time	19.7	0.001	0.718
Baseline	3.82±1.12	3.95±1.74	Group	0.843	2.62	0.211
Pre exercise	3.69 ± 1.03	3.88 ± 2.39				
Post exercise	9.48 ± 3.14	9.08 ± 2.64	Interaction	0.921	0.124	0.433
24 h post exercise	4.56±1.69	4.26±1.29				

Values are expressed as mean ±SD

Discussion

The purpose of this study was to investigate effects of two weeks of vitamin C supplementation on serum CRP and lactate levels as well as blood pressure in young overweight men following one session of resistance exercises. Findings showed that 14 d of vitamin C supplementation (500 mg/day) decreased the resting levels of CRP and lactate as well as blood pressure in overweight men with no statistically significance. Similar to these findings, Church et al. (2003) reported that decreased CRP levels were most seen in adults who had increased chemical levels (more than or equal to 1.0 mg/L) at

baseline (27). In contrast, Block et al. (2009) demonstrated that vitamin C supplementation decreased CRP concentration, depending on baseline CRP levels and decreasing by 25% in those with CRP levels of 1 mg/L or greater (23). In the present study, the baseline CRP level was less than 1 mmol/L, which is one of the reasons for the insignificant effects of supplemental vitamin C on serum CRP concentration. In the present study, baseline levels of systolic and diastolic blood pressure and blood lactate levels of the participants were almost normal, which were possibly the reason of insignificant effects of vitamin C.

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Based on the results from this study, systolic and diastolic blood pressure was unaffected using various doses of vitamin C (28). Another study suggested that a 15-d vitamin C supplementation included no significant effects on resting blood CRP and lactate levels (29). Minor changes in blood pressure of healthy individuals could be attributed to patients with hypertension. Accordingly, patients with hypertension are most affected by vitamin C supplementation. Thus, this minimal lowering of blood pressure is mostly important from clinical perspectives. Considering associations between blood pressure and CRP increases (30), one of the possible reasons for lack of significant changes in serum CRP levels might be linked to minor changes in blood pressure.

Results showed that lactate level and blood pressure significantly increased in the two experimental groups immediately post resistance exercise, compared to baseline and pre exercise. However, no significant differences were shown between supplement and placebo groups. This lack of difference between the two groups was likely associated to the fairly high intensity of the exercise programs and similar volumes. Increases in systolic and diastolic blood pressure are normal immediately after acute resistance exercises and are usually based on the physiological demands and blood delivery to tissues such as muscles (31). Increases in blood lactate post exercise is resulted from the anaerobic energy system (glycolysis) involved in sustainable energy supplying (32). It has been reported that resistance exercise increases secretion of cortisol and thereby increases lactate levels because there is a positive correlation between the cortisol and blood lactate levels (33). Nevertheless, the present study included unmeasured cortisol changes. In addition, increases in lactate levels such as lactic acid accumulation in active muscles lead to acidosis at intracellular levels. In this study, CRP concentration significantly increased in placebo group immediately post resistance exercise, compared to baseline and pre exercise. Studies in this area include contradictory results. However, these results are similar to those by Bizheh and Jaafari (2011), who reported that serum CRP concentration increased immediately after one session of circuit resistance exercise (13). Nevertheless, no significant changes were observed in supplement group following the resistance exercise. The most important finding of this study is that short-term (two weeks) vitamin C supplementation before acute resistance exercises decreases post-exercise inflammation by attenuating increases in serum CRP levels. This has been verified by Atashak et al. (2013), who reported that dietary supplement could prevent increases in serum CRP levels due to acute resistance exercises (34).

The current results demonstrated that systolic and diastolic blood pressure significantly decreased in 24 h post resistance exercise group, compared to those in

immediately post exercise group. However, this decrease was not significant, compared to that of pre exercise and baseline. However, supplement group showed a greater relative decrease in systolic blood pressure 24 h post exercise, compared to the baseline. This change of systolic blood pressure was probably caused by vitamin C supplementation that was important pathologically. Furthermore, data showed that lactate concentration decreased 24 h post exercise in the two groups but did not return to its baseline level. As a possible reason, the participants' lactate tolerance might increase, compared to the baseline. Furthermore, serum CRP concentration decreased in the two groups 24 h post exercise, whereas CRP level was significantly higher in placebo group 24 h post exercise, compared to baseline and pre exercise groups. Furthermore, CRP level in placebo group was significantly higher than that in supplement group immediately post resistance exercise and 24 h post exercise. These data are similar to those by Phillips et al. (2003), who showed that CRP concentration decreased post eccentric exercise following dietary supplements (35). Although a greater CRP decrease in supplement group might be due to vitamin C supplementation, the exact mechanism; by which, vitamin C decreased CRP is still unclear. Nevertheless, vitamin C is able to increase antioxidant capacities and decrease cellular damages post resistance exercise, leading to decreased serum CRP levels in recovery periods (36). In addition, inhibition of transcription nuclear factor kappa B (NF-kβ) by vitamin C prevents over expression of inflammatory proteins (37). As a part of the inflammatory pathways, antioxidants affect CRP through their effects on upstream cytokines, such as tumor necrosis factor alpha (TNF-α), interleukin 1β (IL-1β) and interleukin 6 (IL-6), which are the major stimulators of acute-phase responses (38). Another limitation of the present study included that these indices were not assessed overall. Hartell et al. (2004) reported that vitamin C inhibited lipopolysaccharide (LPS) activity, resulting in the production of TNF-α and IL-6 (39). Based on the results of the present study, the supplement group included a slightly higher increase in resting lactate concentration post resistance exercise, compared to that the placebo group did. This increase in lactate tolerance was likely due to vitamin C intake, leading to improvements in capacity of acidosis tolerance, which decreased cellular damages. Hence, this process could decrease CRP levels, which could lead to decreases in blood pressure. Generally, these results need further studies for better verifications.

Conclusions

This study has revealed that a session of strenuous resistance exercise can induce inflammatory response of CRP and increase lactate concentration and blood pressure in overweight men. However, short-term (2 w) vitamin C supplementation may improve these markers following

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resistance exercises and can be a good strategy for minimizing exercise-induced cellular damages and inflammatory responses in post-exercise periods.

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Authors' Contribution: Mehdi Hakimi designed and supervised the study, carried out the experiments, processed the experimental data, carried out the analysis, drafted the manuscript and designed the figures. Araz Mohammadi contributed to the design and implementation of the study, analysis of the results and writing of the manuscript.

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