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The Effect of Acute Resistance Exercise on Serum Protein Carbonyl in Resistance-Trained and Untrained Collegiate Men

[1]Hossein Sharafi and [2]Rahman Rahimi

[1]Department of Physical Education & Sport Sciences,
Mahabad Branch, Islamic Azad University, Mahabad, P.O. Bax: 59135, Iran
[2]Department of Physical Education and Sport Science,
University of Kurdistan, Sanandaj, P.O. Bax: 416, Iran

Abstract: Acute exercise has been shown to induce oxidative stress and increase damage to lipid DNA and protein several investigations indicate chronic exercise may enhance antioxidant defenses. Therefore, the purposes of this study were to evaluate the effect of resistance exercise (RE) on the protein carbonyl (PC), marker of protein oxidation and whether resistance training status of the participants influences the magnitude of the RE-induced protein oxidation. Eighteen college-age men participated in this study which includes nine resistance-trained (RT) and nine untrained (UT) men. All subjects performed a RE protocol that included 4 sets of: bench press, leg press, seated bar shoulder press, arm curls and lat pull down exercises at 80% 1RM. Blood samples were collected at pre-exercise (Pre), immediately post (IP), 3h post (3h Post) and 24 h post (24h Post) RE for measurement of serum PC and lactate concentration. In addition, there were no significant differences in PC concentration between groups. PC was significantly elevated 3h Post as compared with Post exercise for UT. Lactate concentrations didn't differ between groups. Although, the changes in serum PC concentrations were not significant, but were slightly lower in the RT group. It seems that RE training status of the subjects having a little impact on protein oxidation.

Key words: Resistance exercise · Oxidative stress · Training status

INTRODUCTION

During resistance exercise, free radicals and reactive oxygen species (ROS) are produced via xanthine/xanthine oxidase pathway, respiratory burst of neutrophils, catecholamine auto-oxidation, local muscle ischemia/hypoxia and conversion of the weak superoxide to the strong hydroxyl radical by lactic acid [1]. In situation that, ROS generation overwhelms the physiological capacity of the antioxidant's system, oxidative stress occurs and resulting in oxidative damage to DNA, lipids and proteins [2].

Protein carbonyls have been employed as a measure of oxidative damage to proteins [for reveiwe see 3]. Protein carbonyls are considered to be mainly 2-aminoadipic semialdehyde formed from oxidative deamination of lysine and glutamic semialdehyde formed by oxidation of proline and arginine residues. The oxidation of proteins in physiological systems occurs by spontaneous autoxidation of cysteinyl thiols [3], and by interaction of proteins with oxidizing agents such as hydrogen peroxide H_2O_2, hydroperoxides ROOH, hypochlorite ClO^-, peroxynitrite and other oxidizing reactive intermediates (hydroxyl radical $HO^{\bullet}$, carbonate radical anion $CO_3^{\bullet-}$, and others) [4]. These processes may be catalyzed by trace redox active metal ions such as iron (III) or ferric ion Fe^{3+} and Cu (II) or cupric Cu^{2+} ion [5].

Proteins appear more susceptible to oxidation from reactive oxygen and nitrogen spices [6]. An increase in PC content in tissues was associated with a number of pathological disorders, including rheumatoid arthritis, Alzheimer's disease, respiratory distress syndrome, Parkinson's disease and atherosclerosis [6]. However, relatively few data are currently available in relation to PC concentration following RE, especially referenced to human subjects. Some studies reported that PC concentration increase following a single set of RE and 30 min intermittent dumbbell squatting at 70% of 1RM [7,8], while no change in PC after repeated barbell squats was also reported [9].

Corresponding Author: Hossein Sharafi, Department of Physical Education & Sport Sciences, Mahabad Branch, Islamic Azad University, Mahabad, P.O. Bax: 59135, Iran.

Although acute exercise has been shown to induce oxidative stress and increase damage to lipid, protein and DNA [7,8,10-13], several investigations indicate that chronic exercise may enhance antioxidant defenses [14]. For example, RE training for 6 months in older adults resulted in an attenuated MDA and hydroperoxide response following an aerobic exercise as compared to before training [14]. In addition, a 40% decrease, albeit non-significant, in basal oxidative damage to DNA following resistance training in older adults has been reported [15]. Recently, Parise, *et al.,* [16] reported that 14 weeks of resistance training induced decreases in oxidative damage to DNA and increased electron transport chain activity; in particular, an up-regulation of complex IV may be responsible for the reduction in oxidative stress. Other studies demonstrate that oxidative stress is dependent on training status, with DNA damage less evident in trained athletes [17].

Taken together, these findings suggest that chronic training enhanced the antioxidant defense system, thereby protecting cells from exercise-induced oxidative stress. To our knowledge, only two studies have examined training status on oxidative stress after RE [18,19]. However, to date, there have been no studies examining the effect of RE on protein oxidation in resistance trained and untrained men. Therefore, the purposes of this cross-sectional study were to determine the effects of acute RE on marker of protein oxidation and whether RE training status influences the magnitude of the RE-induced protein damage. Based on previous findings, we hypothesized that protein carbonyl levels would increase post-RE and that the magnitude of this response would be lower in the resistance trained subjects.

MATERIALS AND METHODS

Subjects: Eighteen college-age men who studied in the University of Guilan participated in this investigation. Nine of them were recreationally resistance trained (RT) with a minimum of 1 year continuous whole-body RE experience. Other student included nine untrained men (UT) with no regular resistance or aerobic experience within the past year (Table 1). The experiment procedure was explained in details to all subjects. Written informed consent was obtained from each subject prior to be recruited for this study, which was approved by the local institutional ethics committee. Subjects were on their ordinary diet, not permitted to use nutritional supplementation and did not consume anabolic steroids or any other anabolic agents known to increase performance.

Experimental Design: The subjects were familiarized with the experimental RE trial during a control day about 1 week before the actual measurements. Subjects were instructed not to train or be involved in strenuous activity for 48 hours before or after the experimental RE trial. One repetition maximum (1RM) for *the bench press, leg press, seated bar shoulder press, arm curls and lat pull down exercises* was determined a week before the experimental RE trial (Table 2) [20,21]. Also, body compositions of the subjects were determined by the body composition analyzer (InBody 3.0). Prior to RE protocol, all subjects performed warm-up, which consisted of 3 min running, 5-10 repetitions at 50% of perceived maximum strength and stretching period. The warm-up procedure was held constant throughout all the testing sessions. All the subjects completed a RE protocol that consisted of 4 sets

Table 1: General characteristics of the subjects

Groups	Age (year)	Height (cm)	Weight (kg)	Soft lean mass (kg)	Percent body fat	BMI (kg/m^2)	Basal metabolic rate (kcal)
Resistance Trained	22.37±1.99	174±5.04	71.32±5.57	59.46±5.43	16.29±2.55	23.58±2.05	1979.37±147
Untrained	22.25±2.13	171±3.4	68.45±3.23	56.03±3.13	18.14±2.79	23.41±1.08	1841.56±89

No significant difference between groups (P>0.05).

Table 2: 1RM value of the subjects

Exercise	Resistance Trained 1RM (kg)	Untrained 1RM (kg)
Bench press	100.66±22.15*	82.03±7.03
Leg press	245.33±54.31*	198.04±29.54
Sited bar shoulder press	74.11±14.53*	47.26±6.12
Arm curls	54.27±7.23*	43.51±1.24
Lat pull down	79.94±11.64*	60.93±5.28

*significant difference between groups (P<0.05).

of *the bench press, leg press, seated bar shoulder press, arm curls and lat pull down exercises* at 80% of 1RM with 2 min rest between sets and each set was performed to exhaustion [13]. The RE lasted from 09:00 to 11:00 hours. To ensure that all subjects were moving at approximately the same velocity for each repetition, each set was timed using a handheld stopwatch. The spotter called out a cadence for the eccentric and concentric phases of each repetition. The repetition velocity consisted of a 3-second eccentric phase followed by a 1-second concentric phase. The volume of each set was calculated as the number of complete repetitions completed × resistance used.

Blood and Urine Collection and Analysis: Prior to the experimental resistance exercise session (Pre), immediately post (IP), 3 h post (3h Post) and 24 h postexercise (24h Post), a 7-mL blood sample was taken via vacutainer from an antecubital vein. A 4 mL of blood samples for analysis of serum PC was collected into serum collection vacutainer tubes. The remaining blood samples were collected into vacutainer tubes containing Heparin solution for analysis of plasma lactate. Serum and plasma tubes were centrifuged at 6,000 rpm for 10 minutes at room temperature.

Serum protein carbonyl was measured by Colorimetric assay using Protein Carbonyl Assay kit (Cayman Chemical's ACE™ Colorimetric assay kit, Catalog No: CM10005020, USA). Plasma lactate was measured using Enzymatic-colorimetric method (*ELI TECH Kit, France*).

Statistical Analyses: Data are expressed as Mean ± SD. Statistical evaluation was performed with SPSS (SPSS, Chicago, IL) for windows. The data obtained for PC concentrations were analyzed using a 2 group x 4 times repeated measures MANOVA. The data obtained for plasma lactate concentrations were analyzed using 2×2 repeated-measures ANOVA. Multiple comparisons with confidence interval adjustment by the *Bonferroni* method were used as *post hoc* when ANOVA yielded significant results. Independent-samples *t*-test was performed to determine possible group differences for physical and anthropometrical characteristics, 1RM testing and dietary intake. The significance level was set at $p < 0.05$.

RESULTS

Changes in PC in response to the RE in RT and UT subjects are presented in Figure 1. There were no significant differences in PC concentrations between RT and UT subjects (P>0.05). PC was significantly elevated 3h Post as compared with Post exercise for UT (P<0.05). Lactate concentrations didn't differ either between groups or over time (P>0.05, Figure 2).

Dietary Data: Subjects were required to complete dietary record sheet3 days preceding the RE protocol. The daily calories, protein, carbohydrate, fat, vitamin C, vitamin E and vitamin A intake during the 3 days preceding the RE protocols were analyzed using *the Nutritionist IV computer program*. The mean daily calories are presented in Table 3. No statistically significant differences were noted between RE protocols for any measured dietary variable.

Table 3: Dietary intake assessed during the 3 d prior to each exercise session

	Group	*Mean±SD*
Energy intake (kcal)	RT	2910±198
	UT	2446±260
Protein (g)	RT	155.33±25.49
	UT	142±20
Carbohydrate (g)	RT	296.16±22.06
	UT	265±21
Fat (g)	RT	79.5±10.82
	UT	68.55±11.12
Vitamin E (mg)	RT	9.5±1.87
	UT	10.94±2.26
Vitamin C (mg)	RT	97.5±15.89
	UT	87.66±12.44
Vitamin A (RE)	RT	760.5±98.90
	UT	681.55±129.60

No significant difference between groups (P>0.05).

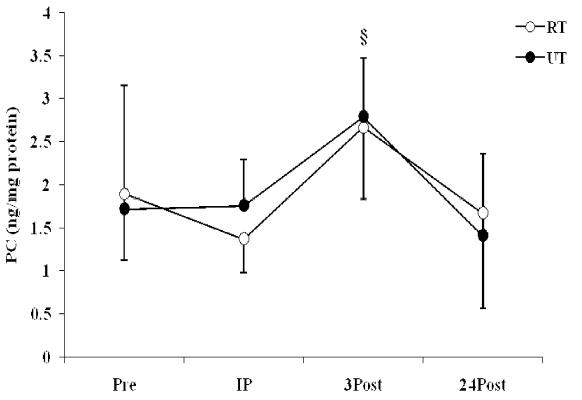

Fig. 1: Serum protein carbonyl concentration at pre-, immediately post- (IP), 3 hours post- (3h Post) and 24 hours postexercise (24h Post). Resistance trained (RT), Untrained (UT). § Different between post and 3 h post RE (P<0.05)

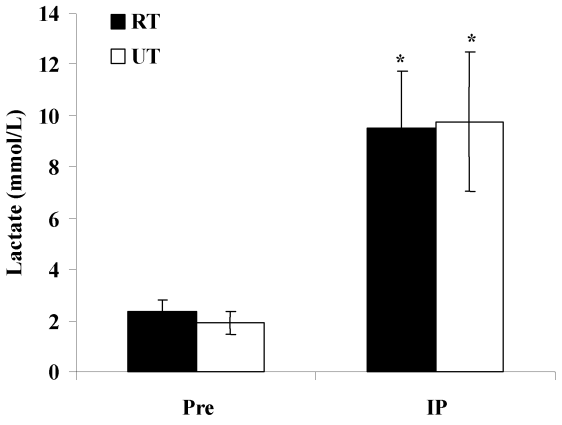

Fig. 2: Plasma lactate concentration at pre-, immediately postexercise (IP). Resistance trained (RT), Untrained (UT).
*Different from pre RE exercise (P<0.05).

DISCUSSION

In general, resistance exercise requires high contraction frequency and high power or force outputs of specific muscles such that fatigue (a decline in force/power output or inability to complete a repetition) occurs rapidly. Resistance exercise requires high rates of ATP turnover that much of this is supplied via anaerobic pathways from phosphocreatine, which is rapidly dephosphorylated to reform ATP at the onset of high-intensity exercise and the breakdown of glucose into pyruvate [22].

During high-intensity exercise, or high repetition sets of resistance exercise with short rest periods, lactate accumulation and the rising H^+ concentration ultimately lead to fatigue [22]. These can cause production of free radicals and ROS via conversion of the weak superoxide to the strong hydroxyl radical by lactic acid [1] and subsequently might lead to oxidative stress.

As mentioned earlier, protein carbonyls have been employed as a measure of oxidative damage to proteins. In this study, there were no significant changes in the levels of PC immediately after and 24h after the RE protocol in both groups compared to baseline. These findings are not in accordance with those of Bloomer *et al*. [7,8] who reported plasma PC was elevated following one set of 15 repetitions of squats using 70% of 1RM and 30 min of the intermittent dumbbell squatting at 70% of 1RM in RT men. However, our findings are in accordance with those of Bloomer *et al*. [9] who reported plasma PC was not affected by sets of squats carried to a point of momentary muscular failure using 70% of 1RM.

Furthermore, in our study, a significant increase only was observed in the serum of PC at 3h after RE compared with immediately after RE in UT group. This finding is in accordance with Hudson *et al*. [23] findings that reported elevated PC immediately after RE at 90 % of 1RM and 1 h post RE with 75 and 90% of 1RM. While PC in RT group was highest 3h post RE protocol, the elevation from baseline didn't reach statistical significance.

In addition, serum PC concentration was slightly greater in UT subjects compared with RT subjects but failed to reach statistical significance. While the oxidative stress response appears greater following higher volume aerobic [9] and sprint exercise [24] this may be not true for our results (during the immediately after and 24h after the RE protocol) with regard to changes in PC.

Exercise intensity is an important factor in the production of free radicals [10,25]. In our study, exercise intensity was indirectly measured by blood lactate concentrations, which significantly increased following RE protocol (RT=9.53 and UT=9.77 mmol/L). However, previous studies reported that blood lactate concentration reach to 18-20 mmol/L following high-intensity RE protocols [10,26].

Also, Waterfall *et al*. [27] reported that lactate accumulation that occurs following high intensity exercise and accompanying acidosis lead to lipid peroxidation. In addition, Lovlin *et al*. [25] reported that lactic acid accumulation may be lead to decrease in cofactors, which required for activity of a number free radical scavenging enzymes. However, blood lactate levels following the current RE protocol (4 sets of *5 exercises* at 80% of 1RM) were relatively low in RT and UT subjects, most likely was insufficient in stimulating free radical production by this mechanism.

Our findings reveled that training status had no effect on serum PC concentration following RE. To our knowledge, no study evaluated PC response to training status following RE. However, only two studies have examined the effect of training status on oxidative stress biomarker after [18,19]. Dixon *et al.* [19] reported that training status of the participants had no effect on serum MDA concentration following moderate-intensity whole-body RE. However, Ramel *et al.* [18] reported significant increase in plasma MDA concentration after a circuit RE bout (18 min of RE with 75% of 1RM in 10 exercises) in trained and untrained subjects. Although, studies that directly evaluated training status on protein oxidation following RE are rare. However, it could be mentioned that subjects in the Bloomer *et al.* [7] and Davis *et al.* [28] studies, were aerobically trained and experienced a decrease in PC compared with untrained subjects in the Goldfarb *et al.* [29] study.

CONCLUSION

An increase in oxidative stress, in tissues is associated with a number of pathological disorders, including rheumatoid arthritis, Alzheimer's disease, respiratory distress syndrome, Parkinson's disease, type 2 diabetic and atherosclerosis [6,30]. To our knowledge, the present study was the first to examine the effects of resistance training status on serum PC following acute whole-body RE. Taken together, this study indicates that resistance training status of the subjects had no effect on serum PC concentrations following high-intensity whole-body RE. It seems that RE training status of the subjects having a little impact on protein oxidation.

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