The effect of eight weeks occlusion strength training on oxidative stress responses to a graded maximal exercise test in overweight men

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ABSTRACT

Low-intensity strength training with vascular occlusion was reported to result in muscle hypertrophy and strength increases similar to high-intensity training without occlusion. The effects of occlusion strength training on oxidative stress still remain to be more elucidate. Therefore, the purpose of this study was to compare the effect of strength training with and without occlusion on oxidative stress biomarkers on basal and following to graded exercise test (GXT) in overweight men. Thirty volunteer subjects were randomized into occlusion strength training (OST), traditional strength training (TRT) and control (CON), were submitted to a GXT at baseline and after the 8-weeks of training protocol (30% 1-repetetion maximum for OST and 65-80% 1-repetetion maximum for TRT), with blood samples collected before, post, 1 and 24 h post-GXT test. Plasma malonaldehyde (MDA), and total antioxidant capacity (TAC) levels were evaluated. Following the eight weeks training, the OST and TRT groups displayed a significant decrease in MDA (in pre and 1-h post GXT), and increase in TAC (in 24-h post GXT) levels compared to CON group (P<0.05). For OST and TRT, MDA demonstrated a significant reduction and TAC demonstrated a significant increase in basal and 24-h post GXT than baseline (P<0.05). In conclusion, the OST similar to TRT protocol may be able to improve oxidative stress defense allowing overweight subjects to cope with the oxidative stress induced by an acute exercise more effectively.

KEY WORDS: oxidative stress, Antioxidant capacity, strength training, vascular occlusion
INTRODUCTION

Oxidative stress occurs when the generation of reactive oxygen species (ROS), also known as free radicals, in a system exceeds the system’s ability to neutralize and eliminate these molecules. Accumulation of excess free radicals can damage a cell’s lipids, protein, or DNA. Acute oxidative stress can manifest beneficial outcomes by activating signaling pathways leading to cellular adaptations whereas, chronic oxidative stress can be detrimental and result in adverse situations that may lead to cell damage or even cell death [1].

It has been reported that ROS production during acute exercise increases angiogenesis, mitochondrial biogenesis and muscle hypertrophy [2]. In addition, ROS production accompanies skeletal muscle injury regeneration and has been reported to be an essential activator of satellite cell-mediated skeletal muscle hypertrophy [3]. Therefore, the importance of maintaining a proper oxidant-antioxidant balance during exercise is essential for proper cellular signaling and adaptation of muscle.

Strength training (ST) with an intensity exceeding 65% of an individual’s one repetition maximum (1RM) is typically required for increasing muscle size and strength [4]. Strength exercise with intensity lower than 65% 1RM can often result in significant improvements in the muscle’s oxidative capacity without considerable effect on muscular size [5]. Recent studies have shown that when low-intensity strength training associated with partial vascular occlusion (PVO) is utilized both muscle hypertrophy and strength increases are similar to high-intensity training without PVO [6,7]. This type of training utilizes modified blood pressure cuffs to alter blood flow to certain areas of the body. The modified blood pressure cuffs are placed proximal to the exercising muscles and result in partial occlusion of the vasculature. This decreases the amount of oxygenated blood getting to the working muscle and can result in blood pooling distal to the partial occlusion [6].

However, antioxidant- and lipid-peroxidation-related adaptations after strength training have been poorly investigated, and studies on the effect of strength exercise on oxidative stress report contradictory findings [8-10]. This could be due to either differences in prescribed training intensity [11,12]. Limited research is available examining the effect of PVO on oxidative stress markers. Takarada et al (2000) examined the concentration of lipid peroxides following low-intensity exercise (20%1RM) with and without partial vascular Occlusion, and noted no significant increase over time or difference between groups in lipid peroxides concentration immediately post exercise up to 24 hours post exercise [13]. In contrast, Goldfarb et al. (2008) reported that protein carbonyl and glutathione status were significantly elevated immediately after exercise and 15 minutes post-exercise in the moderate resistance without PVO group and partial vascular occlusion only group when compared to pre-exercise values [14].

Furthermore, the effect of strength training with blood flow restriction on lipid-peroxidation and antioxidant capacity has not been studied. Additionally, no previous studies have compared the chronic effects of occlusion strength training on these factors. Therefore, the purpose of this study was to compare the effect of traditional strength training and occlusion strength training on cellular and biochemical oxidative stress markers at basal level and after a maximal exercise stress test (GXT) in overweight men.

METHODS

In this interventional trial, 30 overweight male (BMI over 25) aged 18 through 24, voluntarily participated in this study. All subjects were familiarized with the training program and were informed about the possible
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Risks and benefits involved with the study and all the subjects gave their written informed consent to participate in the study.

The subjects were randomly divided in three groups: occlusion strength training (OST, n=10), traditional strength training (TRT, n=10) and control (CON, n=10). Subjects in OST and TRT did regular strength exercises and subjects in CON did not do any exercises for 8 weeks.

The subjects performed strength exercises three times a week for 8 weeks. Strength training included the 5-minute warm-up period, strength training, and 5-minute cool-down period. Warm-up and cool-down periods included treadmill walking at constant speed and inclination, as well as stretching exercises.

The main workout program comprised of the movements of leg extension, biceps, back-leg, triceps and leg press. Selection of the movements were based on using the large muscles of the body, and the order of the movements were in a way that upper and lower body muscles were used frequently [15]. For all of movements, 1-repetition maximum (1RM) was measured for every subject at the beginning of the 8-week training program.

The OST group was exposed to a blood flow restriction stimulus and completed 30 repetitions followed by three sets of 15 repetitions (1×30+3×15) at an intensity of 30% 1RM. The TRT group were not exposed to blood flow restriction exposure and performed 3 sets of 10 repetitions at an intensity of 65-80% 1RM. For TRT group, training intensity was 65% of 1-RM for first 2 weeks and exercises were progressed with gradually increasing intensity of 5% of 1-RM in each 2-week period. A new 1-RM was measured for every subject at the beginning of each two-week period and then the training intensity were applied considering the new 1RM. The rest between the sets and between the stations were 1 to 1.5 minutes and 3 to 4 minutes, respectively [15].

In order to determine the individual cuff pressure, in pilot study, a number of OST subjects with different arm and thigh size completed a test to determine the maximum limb occlusion pressure (LOP). In order to establish maximum LOP participants were asked to lie in a supine position with a blood pressure cuff attached to the proximal thigh or arm [16]. A Doppler probe (Ultrasound Technologies Ltd, Caldicot, UK) was used to detect the ausculatory pulse at the medial malleolus of the tibia for the right leg. The blood pressure cuff was inflated to 50 mmHg for 30 seconds and then increased in increments of 40 mmHg until the arterial pulse was no longer detected. The pressure was then decreased in increments of 10 mmHg until the pulse was detected again. Maximum LOP was determined as the greatest pressure at which the arterial pulse was not detected [17]. The OST protocol placed the participants under 70% of LOP, a training methodology that has been shown to enhance strength and hypertrophy [16], as well as being safe and practical in the context of a training program [18].

Graded maximal exercise stress test (GXT):

The Ellestad test is the basic test for determining a person’s aerobic fitness. It consists of seven stages: the first four periods take 3, 2, 2, 3 min respectively. The grade is 10% for the first four periods with treadmill speed at 1.7, 3, 4, 5 mph respectively. The last three periods each has 2 min duration and the grade constantly increases 15% in these periods. At the last three periods treadmill speed increases 6, 7, 8 mph respectively [19]. The subjects were asked to walk on reverse incline treadmill until exhaustion. (mean time to failure means the most exhaustive point when the subjects are unable to proceed the activity).

Blood sampling

Blood samples were collected at rest (PRE) and 1 h and 24 h after GXT, at baseline and after the experimental time 8 weeks. Blood sample collection and tests were performed
early in the morning, after the night rest and overnight fasting. Test conditions were kept constant both for control and trained subjects. Blood samples were drawn from the antecubital vein while subjects remained in reclined position. Blood was centrifuged and separated for preparation of serum and plasma and was frozen at -80°C for later analysis. Samples were assayed for markers of malondialdehyde (MDA) and total antioxidant capacity (TAC) by assay kit and spectrophotometry [20]. Dietary requirements for this study, restriction of antioxidant and vitamin supplementation was very important. Subjects did not take vitamin supplements one week prior to testing. Subjects were asked to refrain from consuming the foods which contain high antioxidant and/or beverages for three days prior to testing [21]. Otherwise, participants were asked to follow their normal diet. Subjects were asked to keep a 3-day food intake journal prior to the GXT to ensure compliance. All statistical analyses were performed using IBM SPSS Statistics 21. After testing whether data were normally distributed (Shapiro–Wilk test), an analysis of variance (ANOVA) with repeated measures for time (pre-training and 8 weeks) and group (trained and control) was performed. Where significant main effects were observed, Bonferroni's post hoc correction (p≤0.05) was used to aid interpretation of these interactions.

**STATISTICAL RESULTS**

Table 1 shows the anthropometrical characteristics of participants in the three groups. The PRE-GXT and POST-GXT values of antioxidant status and cellular damage parameters were comparable between groups. Significant group * time differences were found both for MDA and TAC. MDA increased after GXT and reaching the maximum after 1 h in all groups. The response to GXT was similar after 8 weeks for OST and TRT. There were significantly decrease within OST (p=0.021) and TRT (p=0.007) groups than CON in 1hour. After 8 weeks training, MDA decreased for OST and TRT than baseline in pre-GXT and 1 hour after GXT (p≤0.05). (fig.1).

For TAC, Before the training period, GXT did not induce significant changes among the different time points of sampling. The level of TAC was not different between groups (trained vs. control) at any time point analyzed only between TRT and CON in 24 hours after GXT (p=0.043). However, following 8 weeks only OST and TRT showed a significant increase in 24-h and pre-GXT than baseline (p≤0.05). (fig.1).

<table>
<thead>
<tr>
<th>Variable</th>
<th>CON</th>
<th>OST</th>
<th>TRT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>91/7±7/3</td>
<td>93/8±6/2</td>
<td>92/4±5/1</td>
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<tr>
<td>Height (cm)</td>
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<tr>
<td>Age (yr)</td>
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<tr>
<td>Body Mass Index (Kg/m²)</td>
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<td>26/7±2</td>
<td>28/8±2</td>
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<tr>
<td>Basal MDA (nmol/ml)</td>
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<td>6/1±1/7</td>
<td>6/48±1/7</td>
</tr>
<tr>
<td>Basal TAC (mmol/l)</td>
<td>2/4±0/8</td>
<td>2/34±0/6</td>
<td>2/38±0/7</td>
</tr>
</tbody>
</table>

CON: control, OST: occlusion strength training, TRT: traditional resistance training, MDA: malonaldehyde, TAC: total antioxidant capacity

Table 1. subject's characteristics
DISCUSSION

The present study was planned to evaluate the effect of 8 weeks OST and TRT modulates the capacity of sedentary overweight subjects to cope with an acute maximal exercise able to disrupt redox homeostasis and induce oxidative damage. The results of the current study indicate that MDA amount after 8 weeks of OST and TRT decreases significantly in rest and 1 h after GXT and the amount of TAC increases in rest and 24 h after GXT. Our findings are in line with the results of previously conducted studies. In accordance with our results, the results of the Atabec et al. (2010) demonstrated that hypertrophy- and strength-intensity whole-body resistance training (RT) performed regularly for 6 weeks, decreased MDA concentration in rest conditions in previously untrained healthy young men. These decrements occurred independently of training intensity [22].

Vincent et al. (2002) demonstrated that RT performed for 6 months resulted in an attenuated MDA and hydroperoxide response after an acute aerobic exercise as compared with pre training status in older adults [23]. Furthermore, it has been demonstrated that conjugated dienes, a quantitative marker for free-radical interaction with cell membranes, significantly increased only in untrained men after circuit RT, and it has been suggested that regular RT partly prevents lipid peroxidation during exercise [10]. The studies showed that however, if strength training has been performed regularly for a long time, it has been reported that antioxidant enzyme activities increased [12]. Parise et al. (2005) demonstrated that CuZn– SOD and CAT enzyme activities significantly increased in vastus lateralis of the trained leg, 48 hours after the final exercise bout, in healthy elderly participants. They performed a progressive RT program with only 1 leg for 12 weeks [12].
addition, Peters et al. (2006) reported that after 6 weeks of isometric exercise training, oxidative stress markers were significantly decreased and whole blood GSH/oxidized GSH ratio increased in hypertensive adults [24]. This study is one of the first that assesses the chronic OST on oxidative profile at baseline, post, 1 and 24 hours after an acute session of exercise. The first study that demonstrated the effects of low intensity blood flow restriction training (LI-BFRT) on oxidative stress markers was the Takarada et al.’s (2000) study. In this study, the authors did not verify a significant difference for lipid peroxidation after the session with LI-BFR [13]. This corroborates with the study of Ramis et al. (2017). Ramis et al. compared the effects of LI-BFR (30% 1RM), high intensity training without BFR (HI, 80% 1RM) and low intensity training without BFR (LI, 30% 1RM). The oxidative profile was assessed in baseline, immediately after exercise, and 24 and 48 hours after the acute exercise. The results did not show statistical significance (p>0.05) among moments and among groups for the antioxidant activity and protein carbonyls. However, in the study of Takarada et al. (2000) [13] the protocol of exercise and sample (athletes) were different from the present study, hence the comparison became difficult. According to Loenneke et al. (2011), oxidative stress appears not to increase with LI-BFR in intensities below 30% 1RM, regardless of the exercise performed [26]. In accordance with this, the results of Ramis et al (2017) did not indicate changes in plasma concentrations of the sulfhydryl groups in response to acute exercise with BFR, which demonstrate maintenance of repair capacity as a response to oxidative stress. In addition, that response was not different from other groups indicating that LI-BFR showed the same repair capacity of the HI despite the lowest intensity [25].

Goldfarb et al. (2008) compared the effects of LI-BFR (30% 1RM), HI (70% 1RM) and without exercise (BFR). The authors verified that there was a significant increase in plasmatic concentration of protein carbonyls (PC) in HI and BFR groups in comparison with LI-BFR [14]. A study of Garten et al. (2015) also demonstrated a significant increase in PC with moderate intensity (MI 70% 1RM) in comparison with LI-BFR [27]. Taking into account the data presented above, it could be possible to state that LI-BFR does not generate higher protein damage than the same exercise performed without occlusion, even with a high intensity. Because of the not increased PC, it is possible that the LI-BFR reduces oxidative damage [27]. According to Garten et al. (2015) this appears to occur because the continued arterial flow may change circulating antioxidants, reducing the accumulation in ROS proceeding from vasculature and muscle [27]. However, this mechanism is debatable because the flow is constantly altered during the contractions with or without occlusion. Moreover, Goldfarb et al. (2008) suggested an explanation for the unchanged oxidative stress in OST [14]. According to the authors the muscle contractions during exercise with blood flow restriction will be able to overcome the resistance of venous return, and then the oxidative stress markers would be removed from circulation [28]. We do not fully agree with this statement because overcoming resistance to venous return could generate a greater turbulent of blood flow causing greater biochemical activity with greater oxidative stress response. The explanation for an oxidative stress less than or equal to the high intensity exercise without occlusion remains speculative, requiring studies that verify each mechanism in more detail. Exercise-induced ROS production can lead to redox-sensitive signaling pathways in muscle and may contribute to muscular remodeling [28]. Alterations in ROS concentration are rapidly sensed, neutralized and accompanied by adaptive increases in oxidant buffering capacity through key upregulation of antioxidant enzymes [29]. The inhibition of these cellular signals may have a detrimental
effect on improving redox muscle cell status [29], increasing blood flow and vasodilation of vessels and increasing mitochondrial respiration [2]. The blunting of oxidative stress revealed in this study during low-intensity exercise performed with PVO brings into question whether the diminished oxidative stress in this model further reduces the ensuing necessary cellular and muscular adaptation associated with resistance exercise or might suggest greater ROS still present in the muscle. It is currently unknown what the appropriate amount of exercise-induced ROS accumulation is that must occur to facilitate these skeletal muscle adaptations, and it could therefore be possible that both exercise intensities could have reached this threshold [30]. This aspect requires further research to examine the ROS signaling within the muscle directly after PVO. Another explanation could be that PVO resulted in an increase in pooled blood in the exercising arm and a subsequent increase in circulating antioxidants.

Finally, in present research, authors have studied only the activities of TAC and MDA in plasma. A better understanding of the effect of resistance training (in particular) on antioxidant defense would be gained by measuring the activities in skeletal-muscle tissue since this is the principle site of energetic flux.

In conclusion, the results of the current study indicated that that OST have protective effects against oxidative stress induced by intense acute exercise similar RET in overweight men.

ACKNOWLEDGEMENT

Gratitude is expressed to the subjects that participated in this study as well as to each of the assistants who were instrumental in the collection of the data. This study was funded by a product grant from the Islamic Azad University, Sari Branch, Sari, Iran.

REFERENCES


