Plasma protein carbonyl responses to anaerobic exercise in female cyclists

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ABSTRACT

Single bouts of aerobic exercise may lead to oxidative stress due to the use of oxygen for metabolism and the generation of reactive oxygen. In athletes, oxidative stress can lead to several deleterious performance effects, such as muscular oxidative damage, muscle soreness, loss of skeletal muscle force production and/or inflammation. However, little is known regarding the severity and duration of oxidative stress arising from intensive anaerobic modes of exercise in aerobically-trained athletes. The purpose of this study was to investigate the effect of a single bout of intensive anaerobic exercise on plasma protein carbonyl (PC) in aerobically-trained women. Aerobically-trained, provincial female cyclists [n = 18, age: 24.2±2.7 years; stature: 163.6±4.6 cm; body mass: 53.4±4.2 kg] were randomly assigned into either a non-exercising control (CON; n = 9) or experimental (EXP; n = 9) group that underwent a 30-second anaerobic (Wingate) cycle ergometer exercise session. Blood sampling took place before exercise, immediately after the exercise (IE), and 24 hours following the exercise (24HR) bout. In the EXP, results indicated significant (P ≤ 0.05) differences in PC levels between the pre-test and IE (0.010±0.0124 to 0.0149±0.0420 mmol/milt; P = 0.010), and IE and 24HR (0.0149±0.0420 to 0.0111±0.0183 mmol/milt; P = 0.013). No significant differences were observed between pre-test and 24HR (0.010±0.0124 to 0.0111±0.0183 mmol/milt; P = 0.371). These results indicate that oxidative protein damage, as indicated by PC levels, rises immediately with the onset of anaerobic exercise, but returns to resting levels within 24 hours following exercise in aerobically-trained women.

Keywords: Carbonylated protein; oxidative stress; Wingate cycle ergometer test
INTRODUCTION

Although physical activity is critical for human health, physical activity results in an increased generation of reactive oxygen. It is thought that the production of this reactive oxygen occurs during aerobic exercise due to the increased delivery of oxygen to active peripheral skeletal muscle tissue. Reactive oxygen is eliminated by the antioxidant system. However, if the antioxidant defenses are overwhelmed by the generation of reactive oxygen, oxidative processes dominate and result in “oxidative stress” [15]. This oxidative stress results in the increased oxidation of fatty acids in cellular membranes and the initiation of a chain of destructive reactions [20, 22] which, in turn, results in muscular oxidative damage [11], muscle soreness, loss of skeletal muscle force production [25], inflammation [9], and/or cellular necrosis [20, 22].

Direct measurement of oxidative stress is challenging due to the relatively short half-life and high reactivity of reactive oxygen species (i.e. 10^-9 and 10^-5 seconds for the superoxide radical and hydroxyl radical, respectively) [14]. Indirect assessments of oxidative stress include the measurement of molecular products originating from the reactivity of active species of oxygen and from specific biomolecules. Such common molecular products include inter alia malondialdehyde (MDA), oxidized DNA, oxidized amino acids, and oxidized proteins (such as plasma protein carbonyl (PC)). In this regard, carbonylated protein and chemical reactions that produce carbonyl groups have been accurately identified and are thus commonly used in the measurement of oxidative stress [27]. Carbonylated protein is a type of oxidized protein that can be converted to active forms of oxygen. Proteins are one of the main constituents of reactive oxygen species, and oxidation of these proteins can lead to their loss of function. Reactive range oxygen species also prevent alterations in amino acids, such as lysine, proline, and histidine [24]. Carbonyl formation is often produced by the reaction of 2, 4-dinitrophenylhydrazine (DNPH), and by its conversion to hydrogen, which is widely used in important structures of protein oxidation in skeletal muscles. As such, carbonyl groups produced by oxidized proteins are often used as a marker of systemic oxidative stress [7].

With regards to the oxidative stress arising from exercise, it has previously been demonstrated that as the duration and intensity of aerobic exercise increases, so does the production of free radicals, effectively leading to increased muscle fiber damage [19]. It is well documented that aerobic exercise is responsible for an increased oxidative stress as this exercise is responsible for the production of active oxygen species and changes in deoxyribonucleic acid (DNA), lipids, and proteins largely due to disruption of electron transport, leading to increased superoxide radicals [16]. As such, aerobic exercise has been the focus of much previous research on the effect of exercise on oxidative stress. However, physiologically, it appears that anaerobic activity may also lead to oxidative stress. This is so since, in addition to electron leakage, it has been suggested that anaerobic activity may result in oxidative stress via alternative mechanisms to that of aerobic exercise [3, 11]. However, in the case of both aerobic and anaerobic exercise, under exercise conditions, the amount of reactive oxygen due to physical activity may be determined by several factors such as the type of muscle contraction [5], availability of exogenous dietary oxidants [5, 10], and the duration and, possibly, the type of exercise [17], and the intensity of exercise since higher intensity exercise may lead to a greater degree of oxidative stress [20, 22]. As such, the production of reactive oxygen...
species and their concomitant increase can vary depending on anaerobic stress arising from various exercise design variables. In addition, increased PC following anaerobic exercise may further be attributed to the invading phagocytic cells in muscle tissue that occurs in the hours following an anaerobic exercise bout [25]. However, frequent and regular aerobic exercise has been shown to increase the resistance of tissues against lipid peroxidation and increase their antioxidant capacity in athletes [6, 10]. This is because increased anti-oxidative enzyme activities in response to regular exercise are considered to be as a result of the need to produce antioxidants to assist in the protection against the production of reactive oxygen species [5]. However, it appears that aerobic exercise training performed at low intensities may not result in this adaptability, due to the body’s inherent ability to eradicate the relatively low active oxygen species being produced at such low intensities. As such, such protective adaptations may only result from the collective effect of repetitive exercise of sufficient intensity and duration [4, 19]. This is because the oxidative stress caused by intense exercise may be reduced, but not completely eliminated and may result in an enhanced defensive system in the long-term [21, 22]. This defense against oxidative stress as a result of intense aerobic exercise is essential since without a sufficient increase in the antioxidant defense system, the possibility of cellular damage caused by reactive oxygen species production increases [5]. While it is known that intense aerobic exercise increases oxidative stress, little date is forthcoming regarding the severity and duration of oxidative stress, as measured using plasma protein carbonyl (PC), arising from intensive anaerobic types of exercise in already aerobically-trained athletes. Thus, the purpose of this study was to investigate the effect of a single bout of intensive anaerobic exercise on plasma PC in aerobically-trained women.

MATERIALS AND METHODS

Subjects: The study population included women cyclists’ city of Shiraz. Among them, 18 cyclists (age: 24.2±2.7 years; stature: 163.6±4.6 cm; body mass: 53.4±4.2 kg) with a minimum of three years competition at a provincial level, were selected. Prior to participation in this study, subjects were screened by a medical doctor for any relative or absolute contraindications to the exercise and/or testing and provided written informed consent before participating in this study [13]. Furthermore, no subject used oral contraception or anti-inflammatory drugs. They were informed of the purposes and methods of the study before they provided written consent. This study was conducted according to the principles of the Declaration of Helsinki for research on human subjects and the study was approved by the Institutional Review Boards of University of Birjand, Iran. Once sampled, subjects were randomly divided into either a non-exercising control (CON: n = 9) or experimental (EXP: n = 9) group.

Study design and time schedule: This was a randomized, single-blind study with both groups receiving no information about the existence and/or results of the alternative group.

Dietary assessment and stabilization: On the test day, to ensure equivalent levels of body water percentage across the subjects, ½ hour before blood sampling, all subjects were given 0.25 liters (L) of cool water (12 °C), which was consumed gradually. Following 10 minutes of quiet rest, a blood sample (5ml by venipuncture method) was taken from each subject at 10am. Subjects included in this study did not consume dietary supplements in the form of carbohydrates, proteins [26] and/or amino
acids, nor were any of the subjects taking anabolic steroids either prior to or during participation in this study. Each subjects’ schedule of daily activity and dietary intake were carefully evaluated by a qualified dietician two days prior to the measurements and during implementation of the experiment, until the final sample had been collected. The dietician instructed subjects on their food intake which composed 3-5 daily meals, containing an approximate fat content of 30%, carbohydrate content of 50%, and protein content of 20%, as this combination has previously been shown to have no demonstrable effect on the circadian rhythm of testosterone concentrations [2, 11, 21].

**Analysis of plasma protein carbonyl (PC):** 5ml of blood was sampled from each subject via venipuncture method prior to the 30-second anaerobic cycle ergometer exercise (at 10am), immediately after (IE), and 24 hours after the exercise (24HR). Measurement of PC was performed using Protein Carbonyl ELISA Kits (Cell Biolabs, INC, USA).

**30-second anaerobic cycle ergometer exercise:** Prior to the 30-second anaerobic (Wingate) cycle ergometer exercise [30], each subject’s body mass was measured in kilograms (kg) using a calibrated medical scale (SECA700, CA, USA). Subjects were required to wear minimal clothing and no shoes whilst a single technician completed this measurement. Subjects began the exercise session with a warm-up consisting of a warm-up that included 5-minutes of stretching and a cycle ergometer warm-up of 20 minutes. For exercise intensity, flywheel resistance was calculated using each subject’s body mass (in kg) multiplied by 0.08. At the start of the exercise anaerobic session, the technician gave each subject the “GO” command at which point, the subject began pedalling as fast as possible with no flywheel resistance. After 3 seconds, the individually-calculated resistance was applied to the flywheel and the subject continued to pedal as fast as possible for 30 seconds [30].

**Non-exercising control group:** The non-exercising control group was required to remain inactive during the experimental period and 5ml of blood was sampled at 10am, 25½ minutes later and 24 hours later to determine if an extraneous variable (i.e. time course) that could have influenced PC levels.

**Statistical analysis:** All statistical analyses were performed using SPSS for Windows software, version 19.0 (IBM Corporation, Armonk, NY). Data are presented as means±standard deviation (SD). Statistical significance was set at P ≤ 0.05. Levene's test was applied to the data to determine homo- or heterogeneity. Since this study had two main variables and anaerobic exercise session served as the independent variable and the dependent variable was PC, a K-S test was applied to ensure normality of the data and a repeated measures was applied to assess changes within groups. A Bonferroni post hoc test was also utilized to calculate pairwise alpha to keep the family wise alpha value at 0.05

**RESULTS**

Results demonstrated that the two groups were heterogeneous at pre-test (P = 0.283), at IE (P = 0.341) and at 24HR (P = 0.181) with regards to PC. In the EXP, results indicated significant (P ≤ 0.05) differences in PC levels between the pre-test and IE (from 0.010±0.0124 to 0.0149±0.0420 mmol/ml; P = 0.010), and IE and 24HR (from 0.0149±0.0420 to 0.0111±0.0183 mmol/ml; P = 0.013) (Table 1). No significant differences were observed between the pre-test and 24HR (from 0.010±0.0124 to 0.0111±0.0183 mmol/ml; P = 0.0371). In the CON, no significant differences were found between pre-test and
IE (P = 0.711), IE and 24 HR (P = 0.611), and pre-test and 24HR (P = 0.241). Whilst no significant difference was found between the two groups at pre-test, and IE and 24HR, the results of the variance analysis with repeated measures revealed a significant difference in the time factor (P = 0.002 and $F_{2 \text{ and } 28} = 11.162$), indicating that the intervention resulted in the difference between the pre-test and post-test data. Moreover, analysis of variance with the repeated measures demonstrated a significant interaction between time and group (P = 0.004 and $F_{2 \text{ and } 28} = 10000$).

Table 1. Plasma protein carbonyl levels following 30-second anaerobic cycle ergometer exercise

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Pre-test (mmol/mll)</th>
<th>Immediately after the exercise (IE) (mmol/ml)</th>
<th>24 hours after the exercise (24HR) (mmol/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein Carbonyl of Plasma</td>
<td>Experimental (EXP)</td>
<td>0.009±0.0013</td>
<td>0.0120±0.0089*</td>
<td>0.0100±0.0083**</td>
</tr>
<tr>
<td>Non-exercising control</td>
<td>(CON)</td>
<td>0.0082±0.0015</td>
<td>0.0083±0.0014</td>
<td>0.0092±0.0021</td>
</tr>
</tbody>
</table>

Data are presented as means± SD. *: Indicates significant (P ≤0.05) differences existed in PC levels between the pre-test and IE. #: Indicates significant (P ≤0.05) differences existed in PC levels between the IE and 24HR.

DISCUSSION

This study investigated the effect of a single bout of intensive anaerobic exercise on PC in already aerobically-trained women. The results revealed that 1) PC levels rise markedly from resting levels following a 30-second anaerobic cycle test, and 2) that PC levels return to resting levels within 24 hours following intense anaerobic exercise in aerobically-trained women. Interestingly, this study’s findings demonstrate that anaerobic exercise can lead to oxidative stress to the extent that it reaches levels comparable to those of aerobic exercise [4, 8]. This may possibly illustrate that the type of muscle contraction may have a larger effect on PC than whether the exercise engaged is aerobic or anaerobic in nature. This may be so since although dynamic exercise in general has been found to result in oxidative stress biomarkers [1, 12], no changes in biomarkers of oxidative stress have been reported in association with eccentric exercise [18]. This has been confirmed previously with increasing PC levels being found following concentric, but not eccentric training [15, 29]. This may indicate that when compared to eccentric exercise, concentric exercise may lead to greater increases in protein oxidation in muscles.

Previous research has also demonstrated increased lipid peroxidation in untrained subjects compared with trained subjects following exercise [28]. This is so since previous findings have indicated that trained subjects have higher anti-oxidative capacities than untrained subjects [28]. However, this cannot be confirmed by the present study and it may prove useful in future to determine if a difference exists in
oxidative stress between untrained, aerobically-trained, and anaerobically-trained athletes following anaerobic exercise. In addition, the mode of training that an athlete engages in may influence PC levels following exercise. In this regard, it has previously demonstrated that protein oxidation was increased following Scott exercise, but that DNA and lipid oxidation were much less affected and that increases in PC were observed following both Scott exercises and sprinting (74% and 111% above resting levels, respectively) [3, 4].

Further, changes, or lack thereof, in PC levels following exercise may also be related to exercise intensity, since the greater the exercise intensity, the greater the degree of oxidative stress [22]. This supposition is confirmed by the present study and previous studies demonstrating increased PC levels following intense aerobic and anaerobic physical activity, but not low-intensity exercise [4, 5, 23]. This is so since during any form of intense exercise, the body, due to increased production of free radicals, is put at an increased risk of oxidative stress resulting from an increased lipid peroxidation and/or protein oxidation.

In conclusion, the results of this study indicate that a single 30-second bout of anaerobic exercise does indeed result in increased PC levels. However, this increase was found to be transient in aerobically-trained women and PC levels returned to normal within 24 hours.

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REFERENCES


