Effects of acute high-intensity aerobic and anaerobic exercise on oxidative damage to lipids, proteins and DNA in untrained subjects

Serkan Revan

Department of Physical Education and Sports, School of Physical Education and Sports, Selçuk University, Aleaddin Keykubat Campus, Konya, Türkiye. E-mail: serkanrevan@gmail.com. Tel: +90-332-2233111. Fax: +90-332-2411608.

Accepted 16 August, 2011

The purpose of this investigation was to determine the effects of aerobic and anaerobic exercise on oxidative damage to lipids, proteins and DNA. Fourteen untrained men performed an incremental exercise test to exhaustion and Wingate anaerobic test on two different occasions. Blood samples taken prior to the exercise, immediately post-exercise and 30 min post-exercise were analyzed for plasma malondialdehyde (MDA), plasma protein carbonyls (PC) and plasma 8-hydroxy-2-deoxyguanosine (8-OHdG). There were significant main effects for time or exercise type for 8-OHdG levels. After anaerobic exercise, 8-OHdG levels of the 30 min post-exercise were lower than those of the pre-exercise. There was no significant interaction or main effects for plasma MDA and PC levels. These data indicated that acute high-intensity aerobic and anaerobic exercise did not result in either lipid or protein oxidation. However, DNA oxidation was affected by anaerobic exercise.

Key words: Acute exercise, lipid peroxidation, protein oxidation, DNA damage.

INTRODUCTION

Exercise is associated with increased ATP need and an enhanced aerobic and/or anaerobic metabolism, which results in an increased formation of reactive oxygen/nitrogen species (RONS) (Radak et al., 2008). Both aerobic and anaerobic exercise of sufficient intensity and duration has been reported to result in increased oxidative modifications to proteins, nucleic acids, and lipids (Bloomer and Goldfarb, 2004; Fisher-Wellman and Bloomer, 2009). In response to acute or single bout of exercise, the body can not adapt to the oxidative challenge due to the shortness of exercise duration and the physiological demands of intensity. Physical exercise under these conditions generates increased levels of reactive oxygen species (ROS), and results in oxidative damage to macromolecules. However, regular exercise leads to adaptation of the antioxidant and repair systems, which could result in a decreased base level of oxidative damage and increased resistance to oxidative stress (Davies et al., 1982; Radak et al., 2001). It has been assumed in several research papers and reviews that an increased electron flux through the mitochondrial electron transport system may lead to an enhancement of electron leakage and consequent oxygen species (ROS) production in aerobic exercise. However, in addition to electron leakage, it has been suggested that oxidative stress specific to anaerobic exercise (e.g., isometric, eccentric, resistance, and sprint exercise) may be mediated through several other pathways: Xanthine and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase production, prostanoid metabolism, ischemia /reperfusion, phagocytic respiratory burst activity, disruption of iron-containing proteins, and alteration of calcium homeostasis (Bloomer and Goldfarb, 2004; Leeuwenburgh and Heinecke, 2001; Sen et al., 2000; Viña et al., 2000).

It had been shown that maximal intensity of aerobic exercise ( Alessio et al., 1998, 2000; Ajmani et al., 2003; Benitez-Sillero et al., 2011; Quindry et al., 2003; Radak et al., 2003) and anaerobic exercise (Alessio et al., 1998, 2000; Cuevas et al., 2005; McBride et al., 1998) result in increased oxidative stress in different groups. However, several researchers indicate that exhaustive resistance exercise (McAnulty et al., 2005) and strenuous bouts of squat or cycle sprint exercise (Bloomer et al., 2006) did not lead to oxidative stress in trained subjects.

Several researches reveal that aerobic and anaerobic...
exercise may have different effects on oxidative stress. Nevertheless, the number of studies on how maximal intensity aerobic and anaerobic exercise affects oxidative damage in untrained subjects is limited. These studies have focused mostly on the effects of aerobic or anaerobic exercise of different intensity (supramaximal, maximal, submaximal etc.) and of different types (resistance sprints, cycling etc.) on trained individuals (Alessio et al., 1998; Bloomer et al., 2005; Bloomer et al., 2006).

This study aimed to investigate changes in blood markers of oxidative damage of lipid, protein and DNA induced by acute high-intensity aerobic and anaerobic exercise in untrained subjects.

**MATERIALS AND METHODS**

**Subjects**

Fourteen healthy, young adult male students from the School of Physical Education and Sports of Selçuk University volunteered for the study. All the participants were between the ages 18 and 25 years, they were nonsmokers, and had not taken any mineral or vitamin supplements. The subjects had not participated in regular exercise training for the 6 months preceding the study although they were moderately active. The participants were informed about the purpose of the study and potential risks and they signed the written informed consent before participating. The study protocol was approved by the Ethical Committee of the School of Physical Education and Sports of Selçuk University, Konya, Turkey. The characteristics of the subjects are shown in Table 1.

**Assessment of body composition and exercise tests**

Participants were required to attend the laboratory on three occasions. During the first visit, the body weight and height of all participants were recorded, and then body mass index (BMI) was calculated as weight (kg) divided by square height (m²). The body density of each individual was obtained, and then body fat was calculated from density using Siri’s equation.

\[
\text{Body density} = 1.1631 \times 0.0632 \times \log (\text{Biceps} + \text{Triceps} + \text{Subscapula} + \text{Subbrialiac})
\]

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Mean±SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>22.3±0.5</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>177.1±2.0</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>71.7±2.1</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>22.8±0.4</td>
</tr>
<tr>
<td>Percent body fat</td>
<td>13.9±0.7</td>
</tr>
<tr>
<td>VO₂max (ml/kg/min)</td>
<td>53.0±1.5</td>
</tr>
<tr>
<td>Peak power (W)</td>
<td>900.7±31.9</td>
</tr>
<tr>
<td>Peak power/Kg (W/kg)</td>
<td>12.5±0.3</td>
</tr>
<tr>
<td>Minimum power (W)</td>
<td>228.3±8.7</td>
</tr>
<tr>
<td>Minimum power/kg (W/kg)</td>
<td>3.2±0.1</td>
</tr>
</tbody>
</table>

The subjects performed a Wingate anaerobic test (WAnT) on a mechanically braked cycle ergometer (834 E, Monark) and an incremental exercise test on a cycle ergometer (835 E, Monark). Both tests were performed at the same times in the morning on separate days at least three hours after a light meal. The interval between the tests was one week. The subjects were instructed not to engage in any strenuous exercise for the 48 h period preceding the exercise tests.

The Wingate anaerobic test is generally used to evaluate anaerobic cycling performance. The WAnT session started with a standardized warming up of 5 min cycling at 50 W including two sprints, each lasting 3 s, performed at the end of the 3rd and the 5th minute as preparation for the sprint like WAnT. After a 10 min rest, the subjects were then instructed to pedal as fast as possible. A resistance corresponding to 7.5% of the body mass was applied after an acceleration phase lasting 3 s. The subjects were verbally encouraged to maintain as high a pedalling rate as possible throughout the 30 s duration of the test (Beneke et al., 2002).

One week after the WAnT, the subjects performed an incremental exercise test to exhaustion following a 5 min of warm-up by cycling at 20 W. The test began at 30 W and work rate was increased by 30 W every 2 min. Subjects were instructed to maintain the pedaling rate as close to 60 rpm as possible. The expired air was measured and analyzed breath by breath using an automated online system (K4 B² system, Cosmed Srl, Rome, Italy) and heart rate was monitored and recorded throughout the test. Before each test, the device was calibrated according to the manufacturer’s instructions. The criteria to reach VO₂max were as follows; a plateau in oxygen uptake must occur as the workload is increasing, a respiratory exchange ratio must exceed 1.15, and heart rate within 10 beats of the age-predicted maximal heart rate calculated as 220 bpm–age.

**Blood analysis**

Venous blood samples were collected at rest, immediately post exercise, and 30 min recovery for each exercise tests. Plasma was obtained by centrifugation of blood at 2.500 rpm for 10 min at +4°C, and stored at -80°C until analysis. Plasma MDA level, an indicator of lipid peroxidation in cells and tissues, was measured spectrophotometrically, according to instructions from Oxis Research kit (Biovtech MDA-586 Assay No 21044). Protein carbonyls were measured using the OxiSelect Protein Carbonyl ELISA kit (Cell Biolabs, San Diego, CA, USA) following the manufacturer’s protocol. 8-hydroxy-2-deoxyguanosine level was measured using the Cayman Chemical 8-hydroxy-2-deoxyguanosine EIA Kit (no. 589320) following the manufacturer’s protocol.

**Statistical analysis**

The results were analyzed by repeated-measures two factor (2x2) analysis of variance. Significant interactions and main effects were analyzed using Bonferroni’s post hoc tests. When the time effect p value was p<0.05, the changes from pre-exercise, post-exercise and recovery period values were compared one-way repeated measures analysis of variance. Results were reported as the mean±SEM of all observations, with the level of significance set at P<0.05.

**RESULTS**

The physical characteristics of the subjects are presented...
Table 2. Changes in the levels of malondialdehyde (MDA), protein carbonyl (PC) and 8-hydroxy-2-deoxyguanosine (8-OHdG) during aerobic and anaerobic exercise.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pre-exercise</th>
<th>Post-exercise</th>
<th>30 min post-exercise</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time</td>
<td>Exercise type</td>
<td>time</td>
<td></td>
</tr>
</tbody>
</table>

| MDA (MikroM) | AE | 1.75±0.24 | 1.53±0.14 | 1.71±0.17 | 1.38 | 0.39 | 0.66 |
| AnE         |    | 1.67±0.16 | 1.36±0.18 | 1.75±0.16 |      |      |      |
| PC (nmol/ml) | AE | 6.16±0.6  | 6.02±0.76 | 6.12±0.3  | 1.86 | 2.05 | 0.02 |
|             | AnE| 6.49±0.59 | 6.81±0.69 | 4.79±0.41 |      |      |      |
| 8-OHdG (pg/ml) | AE | 0.08±0.00 | 0.07±0.01 | 0.06±0.01 | 10.49 $^*$ | 1.44 | 5.79 $^*$ |
|             | AnE| 0.08±0.01 | 0.06±0.00 | 0.04±0.00 $^a$ |      |      |      |

*Difference between types of exercise, P<0.05. $^a$Time main effect, 30 min post different from all other time points, P<0.05. $^a$30 min post-exercise is significantly different than pre-exercise, P<0.05. AE= aerobic exercise, AnE= anaerobic exercise.

in Table 1. Plasma levels of MDA, PC and 8-OHdG data before and following the high-intensity aerobic and anaerobic exercise are presented in Table 2. There was no significant interaction or main effects for plasma MDA and PC levels. There was no significant exercise type X time interaction for plasma 8-OHdG levels. However, the main effects for time or exercise type were significant (F = 10.49, F = 5.79, P<0.05). Additionally, after anaerobic exercise, pre-exercise values of 8-OHdG levels were significantly lower than their values of 30 min post-exercise (P<0.05).

**DISCUSSION**

The present study investigated the effects of high-intensity aerobic and anaerobic exercise on oxidative damage of lipid, protein and DNA in untrained subjects. The main findings of the study were that MDA and PC levels were unaffected by either aerobic or anaerobic exercise. Surprisingly, 8-OHdG levels decreased after anaerobic exercise at 30 min of recovery compared with rest.

Malondialdehyde (MDA) is an end-product derived from peroxidation of polyunsaturated fatty acids and related esters. Thus, measurement of MDA levels in plasma or serum provides a convenient in vivo index of lipid peroxidation and represents a non-invasive biomarker of oxidative stress (Merendino et al., 2003). Most exercise studies typically use MDA or thiobarbituric acid-reactive substances (TBARS) as a marker of lipid peroxidation or oxidative stress. The present study findings indicate that plasma MDA levels were not significantly elevated at any time points post-exercise following either aerobic or anaerobic exercise. With regard to acute aerobic exercise, several researchers have reported an increase in MDA or TBARS levels after submaximal exercise (Ferré et al., 2009; Laaksonen et al., 1999; Ozbay and Dülger, 2002). However, Goto et al. (2007) indicated that high-intensity exercise (30 min, 75% VO$_{2\text{max}}$), but not mild-intensity (30 min, 25% VO$_{2\text{max}}$) or moderate-intensity (30 min, 50% VO$_{2\text{max}}$) exercise increased plasma concentration of 8-isoprostane, an index of oxidative stress. In addition, Lovlin et al. (1987) reported that exhaustive maximal exercise induced to increase plasma MDA levels while submaximal exercise (that is, less than 70% VO$_{2\text{max}}$) did not lead to lipid peroxidation. In other studies, MDA levels increased in plasma and erythrocytes after both the cycling stage (Sureda et al., 2005) and half-marathon (Child et al., 1998). Similar increases in MDA or TBARS levels were found following incremental cycle or treadmill exercise in untrained subjects (Brzeszczynska et al., 2008; El-Yassin et al., 2005; Jammes et al., 2004). In addition to these results, Jammes et al. (2004) observed that the TBARS levels also tended to decrease at the end of the 30-min recovery period. Conversely, other investigators showed that MDA levels in plasma or serum was unaffected by maximal aerobic exercise in trained or untrained subjects (Leaf et al., 1997; Niess et al., 1996). Anaerobic exercise is a type of exercise that includes a large variety of sport activities (e.g. sprints, jumps or resistance exercise) (Finaud et al., 2006). With regard to acute anaerobic exercise, previous studies have reported an increase in lipid peroxidation after maximal or submaximal resistance exercise in trained or untrained participants (Ramel et al., 2004; Viitala et al., 2004; Vincent et al., 2004). Similarly, Marzatico et al. (1997) found an increase in plasma MDA between 6 and 48 h following six sprints (150 m) in trained individuals. In contrast to these results, MDA was unaltered in muscle and plasma after high-force eccentric exercise in physically active but untrained young adults (Child et al., 1999). Additionally, Groussard et al. (2003) indicated that plasma TBARS levels decreased at 20 and 40 min of recovery compared with rest after short-term supramaximal anaerobic exercise (30-s Wingate test). The authors also suggested that supramaximal anaerobic exercise induced an oxidative stress and that the plasma
TBARS levels were not a suitable marker during this type of exercise. Similarly, Revan et al. (2010) noted that short duration exhaustive running exercise did not lead to lipid peroxidation and exhaustive exercise-induced oxidative stress might be related with exercise duration. In the present study, the fact that the MDA level is not affected by either exercise type can be explained through the fact that duration and/or intensity of exercise is not sufficient to increase it. In addition, this is possibly due to differences in the biomarkers of study (e.g., DNA, glutathione, lipid), the assay techniques for the specific markers used (e.g., MDA, TBARS) (Bloomer and Goldfarb, 2004).

RONS cause cellular damage, an important part of which is the oxidation of amino acid residues on proteins, forming protein carbonyls (PC), which is the most widely used marker of oxidative modification of proteins (Chevion et al., 2000). In relation to aerobic exercise, Bloomer et al. (2007a) examined the effect of aerobic exercise (30, 60 or 120 min at 70% VO\textsubscript{2max}) on plasma PC concentrations, a marker of protein oxidation, in aerobically trained men and women. These data suggest that PC concentration is elevated by cycling exercise performed at 70% VO\textsubscript{2max}, being greater in longer duration rides and begins to recover within one hour after the exercise. Similarly, in another study, PC derivatives increased in plasma, erythrocytes and lymphocytes, while neutrophils maintained basal levels after the exhaustive exercise (cycling stage, 171.8 Km) in professional cyclists (Sureda et al., 2005). In addition, Radak et al. (2003) indicated that PC level in the serum of runners increased significantly after the first day of running and remained elevated throughout the super-marathon race. As regards anaerobic exercise, blood PC were elevated following both squat (one set of 15 repetitions of barbell squats using 70% of one repetition maximum) and sprint exercise (30-s Wingate test) in resistance trained men (Bloomer et al., 2007b) and eccentric exercise (five sets of 15 eccentric maximal voluntary contractions) (Paschalis et al., 2007). Moreover, Saxton et al. (1994) found that after concentric leg exercise, skeletal muscle PC concentration was significantly higher than that observed after eccentric work. In this study, we found that PC levels were not affected by either aerobic or anaerobic exercise. Some studies have compared aerobic and anaerobic exercise bouts with oxidative tissue damage. Bloomer et al. (2005) reported that protein oxidation seems to be more greatly affected by anaerobic exercise (squatting at 70% of 1 RM for 30 min) and the magnitude of protein oxidation is greater following anaerobic exercise compared with submaximal aerobic exercise (cycling at 70% of VO\textsubscript{2max} for 30 min on a cycle ergometer) in young cross-trained men. The authors also indicated that the transient rise in PC following aerobic exercise is likely due to increased oxygen flux through the mitochondrial electron transport chain because oxygen uptake during exercise may increase approximately 10-fold but returns rapidly to baseline levels postexercise. On the other hand, Alessio et al. (2000) observed a significant increase in plasma PC immediately post exhaustive aerobic, but not nonaerobic maximal isometric exercise in young adult subjects. They noted that mechanisms for isometric exercise-induced oxidative stress may also include mechanical stress and it contributes to exercise induced damage of muscle fibers, even in the absence of increased VO\textsubscript{2}. These disparate results might be due to differences in exercise test protocols or training status of individuals.

Cellular DNA damage can be caused by ROS generated under different conditions, and several techniques have been developed to measure the oxidatively modified nucleobases in DNA. Usually 8-hydroxy-2-deoxyguanosine (8-OHdG) is measured as an index of oxidative DNA damage (Dalle-Donne et al., 2006). Tsai et al. (2001) found that DNA damage increased following marathon race in male runners in peripheral blood mononuclear cells. However, other studies showed that no increase in DNA damage was observed after submaximal or maximal aerobic exercise (Demirbağ et al., 2006; Umegaki et al., 1998). Moreover, several authors reported that serum DNA oxidation was minimally affected by aerobic or anaerobic exercise in trained men (Bloomer et al., 2005, 2007b). Experimental studies in rats demonstrated that acute strenuous exercise resulted in significant DNA damage (Pożzi et al., 2010; Sakai et al., 1999; Wierzba et al., 2006). Paradoxically, the results from the present study indicate that plasma 8-OHdG levels were significantly less than 30 min post-exercise than pre-exercise for anaerobic exercise, with no significantly detected reduction observed for aerobic exercise. Asami et al. (1998) investigated the effect of physical exercise on the level of 8-hydroxyguanine (8-OH-Gua), a form of oxidative DNA damage, and its repair activity in human peripheral leukocytes. They found that although the repair activity of untrained subjects significantly increased after exercise, 8-OH-Gua level and its repair activity did not change before and after the exercise in the trained athletes. They also observed inter-individual differences in 8-OH-Gua levels and its repair activities. Also, some investigators observed that DNA migration levels in white blood cells increased significantly 24 h post-exercise in trained and untrained men. After 72 h, DNA migration levels decreased to about control level (Hartmann et al., 1994; Niess et al., 1996). In our study, reductions in the level of 8-OHdG after anaerobic exercise may be explained by the increased DNA repair enzymes or timing of blood sampling or differences in the type of the sample analyzed (plasma, blood cells, serum, tissue etc.).

One of the limitations of the study is that because the blood samples were collected only three times, the results are limited as well, so if blood samples were increased in number, oxidative stress markers might be
affected differently. Another is that the subjects did not engage in any standard diet program throughout the experimental research.

**Conclusion**

The results of the present study show that high-intensity aerobic or anaerobic exercise does not have detrimental effect on oxidative damage to lipids, proteins and DNA.

**ACKNOWLEDGEMENTS**

This research was supported by the Selçuk University, Scientific Research Projects Coordinatorship (S.U.-BAP, Konya, Turkey), (Project no: 09401077). The author thanks Firuze Kurtoğlu, PhD (Department of Biochemistry, Faculty of Veterinary Medicine, Selçuk University, Turkey) for the biochemical analyses. Also, he thanks Sükri Serdar Balci, PhD (Department of Trainer Education, School of Physical Education and Sports, Selçuk University, Turkey) for the statistical analysis.

**REFERENCES**


