Effects of Anaerobic Exercise and Aerobic Exercise on Biomarkers of Oxidative Stress

Minyi SHI1, Xin WANG2, Takao YAMANAKA3, Futoshi OGITA3, Koji NAKATANI3 and Toru TAKEUCHI1

1Department of Environmental Medicine, Graduate School of Medical and Dental Sciences, Kagoshima University, Kagoshima, Japan
2Division of Antiviral Chemotherapy, Center for Chronic Viral Diseases at Graduate School of Medical and Dental Sciences, Kagoshima University, Kagoshima, Japan
3Department of Physiological Sciences, National Institute of Fitness and Sports, Kanoya, Japan

Abstract

Objectives: In addition to having health-promoting effects, exercise is considered to induce oxidative stress. To clarify whether increased oxygen consumption during exercise induces oxidative stress, we investigated the effects of aerobic exercise and anaerobic exercise on a series of oxidative damage markers.

Methods: One group of subjects performed aerobic exercise and another group performed anaerobic exercise with similar workloads, but with different levels of oxygen consumption. Blood and urine samples were collected before, immediately after, and 3, 9, and 24 h after exercise. Serum uric acid (UA) and creatine phosphokinase were evaluated. As markers of oxidative damage to lipids, proteins and DNA, we evaluated serum 4-hydroxy-2-nonenal, urinary F₂-isoprostanes, serum protein carbonyls, and leukocyte 8-hydroxydeoxyguanosine.

Results: Oxygen consumption was significantly greater during aerobic exercise. Although UA level increased immediately after aerobic exercise and decreased thereafter, UA level did not change after anaerobic exercise. The two types of exercise had significantly different effects on the change in UA level. After anaerobic exercise, the levels of 8-hydroxydeoxyguanosine and 4-hydroxy-2-nonenal significantly increased at 24 h and 3 h, respectively. The levels of creatine phosphokinase and F₂-isoprostanes decreased after exercise. The two types of exercise caused no apparent significant differences in the levels of these biomarkers.

Conclusion: The findings suggest that similar workloads of anaerobic exercise and aerobic exercise induce reactive oxygen species (ROS) differently: aerobic exercise seems to initially generate more ROS, whereas anaerobic exercise may induce prolonged ROS generation. Although more oxygen was consumed during aerobic exercise, the generated ROS did not induce significant oxidative damage. Oxygen consumption per se may not be the major cause of exercise-induced oxidative damage.

Key words: aerobic exercise, anaerobic exercise, oxidative stress, uric acid, human

Introduction

As a result of oxygen metabolic processes, cells continuously produce free radicals and reactive oxygen species (ROS). These free radicals are generally neutralized by the antioxidant defense system comprising enzymes including catalase, superoxide dismutase, glutathione peroxidase and low-molecular-weight antioxidants such as vitamins A, E and C (1). Oxidative stress is defined as a situation in which an increased level of ROS generation overwhelms the antioxidative defense capacity, resulting in oxidative damage to lipids, proteins and DNA (2). Regular physical exercise is recommended for reducing the risk of cancer and cardiovascular disease, and for its other beneficial effects (3, 4). However, strenuous physical exercise also considerably increases oxygen consumption level by as much as 10-fold the resting level (5). This may, in
turn, increase ROS production (6). Because an increased oxygen consumption level and electron transport disturbance can cause an enhanced leakage of superoxide radicals, the likelihood that aerobic exercise results in ROS production is well accepted (7). Moreover, other studies have suggested that even anaerobic exercise, which involves less oxygen circulation throughout the body than aerobic exercise, is associated with an increased ROS generation level through other pathways; among those implicated are the followings: activation of xanthine oxidase (8) and NADPH oxidase (9), ischemia-reperfusion (10), enhanced purine oxidation, damage to iron-containing proteins, disruption of Ca\(^{2+}\) homeostasis (11), and catecholamine autooxidation (12).

Such findings have made "oxidative stress" an interesting topic for physical exercise scientists, athletic trainers, and other specialists in sports and exercise. Although indications that increased damage to lipids, proteins, and DNA is associated with physical exercise have been well documented (13–16), some investigators have been unable to find signs of exercise-induced oxidative stress (17, 18). These discrepant results motivated us to investigate oxidative stress in humans after physical exercise. It seems reasonable to assume that the probable principal factor contributing to oxidative stress is elevated oxygen consumption. Only a few single-study reports have so far compared the effects of anaerobic exercise and aerobic exercise on oxidative stress (19–21). To contribute to this literature, we designed a protocol. We investigated the effects on the levels of multiple biomarkers of oxidative stress, of completing similar workloads using anaerobic exercise and aerobic exercise, which involve different levels of oxygen consumption. We speculated that these different types of exercise would have different effects on oxidative stress owing to differences in oxygen consumption and exercise intensity. We found, however, that both types of exercise induced similar patterns of oxidative stress, suggesting that oxygen consumption per se is not the major cause of exercise-induced oxidative damage.

**Materials and Methods**

**Chemicals**

Except where otherwise noted, all chemical reagents were obtained from Nacalai Tesque, Inc. (Kyoto, Japan). Tween 20 and human serum albumin (HSA) were supplied by Sigma Chemical Co. (St. Louis, MO), Block-Ace came from Dainihon-Seiyaku (Osaka, Japan), and 4-hydroxy-2-nonenal (4-HNE) was obtained from Cayman Chemical (Ann Arbor, MI). The Japanese Aging Control Institute (Shizuoka, Japan) supplied the mouse monoclonal antibody against 4-HNE-histidine adducts (HNEJ-2) and Binding Site (Birmingham, UK) supplied the peroxidase-conjugated antibody against HSA. The ABTS\(^{\ast}\) peroxidase substrate were from KPL (Maryland, USA), and ELISA microtiter plates (Maxisorp) were from Nalge Nunc International (Tokyo, Japan). Creatine phosphokinase test kits (CPKII-Test WAKO), creatinine test kits and uric acid assay kits (Uric Acid C-Test WAKO) were obtained from Wako Pure Chemicals, Inc. (Osaka, Japan). Oxford Biomedical Research, Inc. (Oxford, MI) provided urinary isoprostane ELISA kits.

**Subjects**

Ten healthy male volunteers aged 22–38 years were enrolled in this study. The study was fully explained to, and written informed consent was obtained from, each participant. Each volunteer regularly trained on 5 or 6 days per week in his preferred sporting activity: soccer, basketball, swimming, track running or rowing. Each had qualified for national and/or Kyushu prefectural championships. During the experiment, the subjects were housed in a dormitory and provided with the same diet without any extra antioxidants or other nutritional supplements. They did not do any other strenuous physical activity during the experiment. All the subjects were non-smokers and had no known medical problems.

**Exercise protocol**

Before enrollment in the study, all the subjects completed a maximal oxygen consumption (VO\(_{2\text{max}}\)) test and their VO\(_{2\text{max}}\) scores were determined. Controlled physical activity was carried out on a cycle ergometer. The total workload was set to 40 J because this intensity was judged endurable for anaerobic exercise. The subjects were randomly assigned to one of two exercise groups. Five subjects carried out anaerobic exercise, with the intensity set at 107% to 136% of VO\(_{2\text{max}}\) and the duration set at 2.5 min to generate a 40 J workload. The other group performed aerobic exercise, with the intensity set at 50% of VO\(_{2\text{max}}\) and the duration set at 8.5–15.5 min to generate a 40 J workload. Oxygen consumption during exercise was measured using the Douglas bag method. The exercise data are summarized in Table 1. The exercise protocol was approved by the Institutional Ethics Committee.

**Urine and blood samples**

Subjects were asked to collect urine samples prior to, immediately after, and 3, 9, and 24 h after exercise. A 24 h urine sample was also collected with URINEMATE® P (Sumitomo Bakelite, Tokyo, Japan). From both groups, the 24 h urine volumes were similar. Urine samples were stored at −80°C until analysis.

Blood samples were also drawn before exercise, immediately after exercise (0 h) and 3, 9, and 24 h after exercise. Except at 9 h, when 5 ml of blood was drawn, 10 ml blood samples were obtained each time. Serum was separated immediately and kept at −80°C until evaluation of creatine phosphokinase (CPK), uric acid (UA), 4-HNE, and protein carbonyls. Leukocytes used for 8-hydroxydeoxyguanosine (8OhDg) determination were isolated from whole blood using the dextran extraction method (22) and stored at −80°C.

**Detection of CPK and UA**

Serum CPK level was measured using the CPKII-Test WAKO. Serum and urinary UA levels were measured using the Uric Acid C-Test WAKO.

**Determination of 8OhDg in leukocytes**

Owing to the quantity of blood required to collect leukocytes, 8OhDg was only evaluated in pre-exercise, and in 0, 3, and 24 h post-exercise samples. DNA in leukocytes was extracted and digested under anaerobic conditions (23).
quantities of 80HdG and deoxyguanosine (dG) were determined, as previously described, by HPLC with electrochemical detection and UV absorption (24). The levels of 80HdG were expressed as the molar ratio of 80HdG per 10^4 dG.

Analysis of urinary F₂-isoprostanes (F₂-IP)

Urinary F₂-IP was measured using urinary isoprostane ELISA kits (EA 85). Calibration, curve fitting, and data analysis were carried out according to the manufacturer’s instructions. F₂-IP level was adjusted with creatinine level.

Analysis of urinary creatinine

Urinary creatinine was evaluated using creatinine test kits.

Detection of 4-HNE protein adducts

As described previously (25), with minor modification, 4-HNE-modified albumin in serum was measured by ELISA. Microtiter plate wells were precoated with 10 μg/ml monoclonal antibody HNEJ-2 for 16 h at 4°C, then washed four times with Dulbecco’s phosphate buffered saline (DPBS) before being blocked with diluted Block-Ace for 2 h at room temperature. After discarding the blocking solution, serum samples diluted to 1:16 with Block-Ace were placed in the wells and incubated for 2 h at room temperature and then washed four times with DPBS. Thereafter, each well was incubated with peroxidase-conjugated antibodies against HSA for 2 h at room temperature. After washing with 0.05% Tween 20 in DPBS, a peroxidase substrate was added for color presentation. The plate was measured at 405 nm with a microplate reader (Benchmark Plus, BioRad, Hercules, CA). HNE-HSA was prepared according to a previously described standard method (26).

Determination of protein carbonyl contents

The carbonyl contents of total serum protein were determined spectrophotometrically with 2,4-dinitrophenylhydrazine as described previously (27). Absorbance was measured with a microplate reader. The protein carbonyl contents were calculated using a molar extinction coefficient of 22.0 mM⁻¹·cm⁻¹. Protein was determined according to the Bio-Rad method using bovine serum albumin as a standard.

Statistical analyses

Data were analyzed using SPSS 12.0 J (SPSS Japan, Tokyo, Japan) with advanced modules. Because of the large inter-individual variations in the measured data, we calculated the ratios of post-exercise to pre-exercise values. Values are reported as mean±SE (standard error of the mean). Two-way ANOVA was used to detect significant differences between the two types of exercise. Comparisons between pre-exercise and post-exercise within one type of exercise were performed by one way ANOVA with the LSD test. The exercise data listed in Table 1 were analyzed with the t-test; P<0.05 was considered to be statistically significant. For biomarker results from pre-exercise samples, t-testing revealed no significant differences between the two groups.

Results

Exercise data are summarized in Table 1. While there was no significant difference between the two groups in terms of the workload imposed by the exercise, the exercise intensity was significantly different: 121%±13% of VO₂max for anaerobic exercise compared with 50% of VO₂max for aerobic exercise. In addition, more oxygen was consumed during aerobic exercise than during anaerobic exercise.

Figure 1 shows the changes in CPK levels following aerobic exercise and anaerobic exercise: no significant differences between the two types of exercise were detected. After either form of exercise, CPK level changed significantly during the sampling period. Slight but non-significant increases over pre-exercise levels were observed immediately after both types of exercise, but thereafter these decreased to below pre-exercise levels (P<0.05).

Figure 2 shows the effects of exercise on serum UA levels. Immediately after aerobic exercise, serum UA level increased significantly (P<0.05) and decreased to below pre-exercise level thereafter. In contrast, following anaerobic exercise, serum UA level did not change. We found a significant difference (P<0.05) between anaerobic exercise and aerobic exercise in terms of the change in serum UA levels over time. The anaerobic group had a higher serum UA level than the aerobic group. On the other hand, during the 24 h after exercise, we found no significant differences in the total urinary UA excretion between the two groups (981.0±81.9 mg/day for aerobic group, and 939.8±45.4 mg/day for aerobic group).

The levels of oxidative DNA damage following the two types of exercise are shown in Figure 3. After aerobic exercise, no significant change in leukocyte 80HdG level was apparent. In contrast, a significant increase was detected in samples taken 24 h after anaerobic exercise. All the subjects in the anaerobic exercise group had an increased 80HdG at 24 h, but only 3 subjects in the aerobic exercise group showed a similar increase. Even so, the overall differences between the two types of exercise were not significant.

Urinary F₂-IP levels were lower in samples taken 3 and 24 h after aerobic exercise (Fig. 4). After anaerobic exercise, although the general trend for F₂-IP level was similar to that after aerobic exercise, a significant difference was observed at 3 h. The effects of the two types of exercise on urinary F₂-IP level did not show significant differences. No significant difference was found between the types of exercise in terms of total F₂-IP excretion level during the 24 h after anaerobic or aerobic exercise (1116.4±355.7 ng/day for anaerobic exercise, and 1022.8±173.3 ng/day for aerobic exercise).

Table 1 Summary of exercise data

<table>
<thead>
<tr>
<th></th>
<th>Anaerobic exercise (N=5)</th>
<th>Aerobic exercise (N=5)</th>
</tr>
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<tbody>
<tr>
<td>Exercise time (min)</td>
<td>2.5*</td>
<td>10.5±1.3</td>
</tr>
<tr>
<td>Exercise workload (J)</td>
<td>37.6±3.6</td>
<td>39.7±2.5</td>
</tr>
<tr>
<td>% of VO₂max</td>
<td>121.4%±5.7%*</td>
<td>50%</td>
</tr>
<tr>
<td>Total oxygen consumption (L)</td>
<td>6.5±0.4*</td>
<td>17.5±2.1</td>
</tr>
</tbody>
</table>

Data are given as values±SE. * Significantly different compared to aerobic exercise (P<0.01).
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Exercise and Oxidative Stress

Fig. 1 Serum CPK levels before (before), immediately after (0 h), and 3, 9, and 24 h after aerobic (AE) exercise and anaerobic (AN) exercise. Data are expressed as ratios to pre-exercise levels and are shown as means±SE. The average level in samples before anaerobic exercise was 113.1±11.2 mU/ml and that in samples before aerobic exercise was 241.0±79.4 mU/ml. * P<0.05 compared with before aerobic exercise; ** P<0.05 compared with before anaerobic exercise.

Fig. 2 Serum UA levels before (before), immediately after (0 h), and 3, 9, and 24 h after aerobic (AE) exercise and anaerobic (AN) exercise. Data are expressed as ratios to pre-exercise levels and are shown as means±SE. The average level in samples before anaerobic exercise was 5.72±0.36 mg/dl and that in samples before aerobic exercise was 6.01±0.27 mg/dl. * P<0.05 compared with before aerobic exercise.

Fig. 3 Leukocyte 8OHdG levels before (before), immediately after (0 h), and 3 and 24 h after aerobic (AE) exercise and anaerobic (AN) exercise. Data are expressed as ratios to pre-exercise levels and are given as means±SE. The average level in samples before anaerobic exercise was 0.20±0.03 8OHdG/10^6dG and that in samples before aerobic exercise was 0.19±0.04 8OHdG/10^6dG. ** P<0.05 compared with before anaerobic exercise.

Fig. 4 Urinary F2-IP concentrations before (before), immediately after (0 h), and 3, 9, and 24 h after aerobic (AE) exercise and anaerobic (AN) exercise. Data are expressed as ratios to pre-exercise levels and are shown as means±SE. The average level in samples before anaerobic exercise was 1.60±0.47 ng/mg creatinine and that in samples before aerobic exercise was 0.78±0.09 ng/mg creatinine. * P<0.05 compared with before aerobic exercise; ** P<0.05 compared with before anaerobic exercise.

Fig. 5 Serum 4-HNE-modified albumin contents before (before), immediately after (0 h), and 3, 9, and 24 h after aerobic (AE) exercise and anaerobic (AN) exercise. Data from duplicate experiments are expressed as ratios to pre-exercise levels and are shown as means±SE. The average level in samples before anaerobic exercise was 3.97±0.70 21 nmol/ml and that in samples before aerobic exercise was 4.09±0.21 nmol/ml. ** P<0.05 compared with before anaerobic exercise.

Fig. 6 Protein carbonyl contents in serum before (before), immediately after (0 h), and 3, 9, and 24 h after aerobic (AE) exercise and anaerobic (AN) exercise. Data from duplicate experiments are expressed as ratios to pre-exercise levels and are shown as means±SE. The average level in samples before anaerobic exercise was 9.96±1.00 nmol/mg protein and that in samples before aerobic exercise was 10.35±1.38 nmol/mg protein.
Figure 5 shows the effects of anaerobic exercise and aerobic exercise on serum 4-HNE-modified albumin levels. After anaerobic exercise, the 4-HNE level significantly increased 3 h. A similar increase in 4-HNE level was also observed in samples taken 3 h after aerobic exercise (P=0.06). No significant difference was observed between the two types of exercise.

Serum protein carbonyl levels did not change after either anaerobic or aerobic exercise and no significant differences were observed between the two types of exercise (Fig. 6).

Discussion

Advised by physicians and convinced of the beneficial effects on health, more and more individuals are participating in regular aerobic or anaerobic exercise. Considerable uncertainty still remains, however, about the relationship between exercise and the oxidative stress that may occur owing to increased oxygen circulation during exercise. Few studies have yet compared aerobic exercise and anaerobic exercise in terms of the oxidative stress that they may induce (19–21). Because factors such as the level of fitness, age, and gender could all influence the oxidative stress caused by exercise, for a more effective comparison we selected highly fit, of similar age, single-gender subjects. All the exercise was done on an ergocycle to achieve similar workloads aerobically and anaerobically. Moreover, sample collection was carried out at the same times relative to the exercise protocol and under the same conditions. From the samples, we sought evidence of oxidative stress associated with exercise by utilizing multi-biomarker sets for several types of macromolecular damage.

For all the subjects, the total workload was 40. J. We considered this to be an endurable limit for members of the anaerobic exercise group. Each man who performed the anaerobic exercise afterwards complained of severe muscle pain. Despite this, no significant CPK increase was found, indicating that neither type of exercise induced muscle damage. Song has already reported no increase in CPK level after an intensive treadmill run to exhaustion (28) thus, CPK level does not necessarily increase after strenuous exercise. CPK decreased after 3 h of exercise: factors that may account for this include resting after exercise and circadian variation (29). Our results are in line with those of a previous study in which it was found that trained subjects, in particular, are unlikely to show increases in CPK levels after exercise and that their CPK levels continuously decrease reaching 60% the following morning (29). Although the workload imposed in this study might not have been sufficient to induce oxidative damage in the case of aerobic exercise, if oxygen consumption at rest is considered to be 3.5 ml/kg/min (30), the participants consumed seven times more oxygen during the aerobic exercise.

During exercise, it is considered that energy-rich purine phosphates are used and catabolized, resulting in the accumulation of UA and, at the same time, the formation of ROS (7). The increase we found in serum UA level immediately after aerobic exercise seems to support this proposed mechanism involving the enhanced catabolism of purine nucleotides. Accordingly, this should be followed by the production of ROS. Even so, 3 or more hours after aerobic exercise, UA levels declined to below pre-exercise levels. This result may have been due to circadian variation in UA levels, which are reported to peak in the morning (31). The lower levels obtained the next morning may have been influenced by resting after exercise. On the other hand, UA level did increase, but not significantly, 0 and 3 h after anaerobic exercise, and did not subsequently decrease. The result may be evidence of the catabolism of purines and an associated delayed and prolonged generation of ROS after anaerobic exercise. Such a situation may have similarities with ischemia-reperfusion (10). Meanwhile, the amounts of UA excretion and the levels of CPK were similar after both anaerobic exercise and aerobic exercise. These findings also support the notion that there is a delayed and increased level of purine catabolism after anaerobic exercise, and suggest, therefore, that ROS generation is affected differently depending on the type of exercise.

The level of typical oxidative DNA damage, marked by 80HdG, significantly increased 24 h after anaerobic exercise. However, we detected no anaerobic and aerobic exercise differences in the change in 80HdG levels over time. Moreover we found no significant differences in the levels of oxidative lipid damage (F2-IP nor 4HNE), although 3 h after anaerobic exercise, the level of 4HNE increased significantly. Anaerobic exercise and aerobic exercise were not associated with differences in F2-IP excretion. In addition we found no difference attributable to anaerobic or aerobic exercise in oxidative protein damage, marked by protein carbonyls. These results indicate that both types of exercise at this workload had similar effects on oxidative damage markers, and that these effects were slight. The results also suggest that the amount of oxygen consumed was not the determining factor for oxidative damage.

Although both types of exercise had similar effects on oxidative damage, after anaerobic exercise, the levels of biomarkers of oxidative DNA damage and lipid damage showed only a slight increase: leukocyte 80HdG was significantly elevated at 24 h and the serum 4-HNE level was significantly high only at 3 h. The time course for UA level was also different following the two types of exercise. Exercise intensity has been reported to be a critical factor mediating increases in blood UA level (32). Our findings may support the contention that more ROS are generated in response to high-intensity exercise, although less oxygen was consumed during anaerobic exercise. Similar results have also been reported by Cuevas et al. (33).

The significant increases we detected in 80HdG and 4-HNE levels after anaerobic exercise are in line with the results of other reports those indicated that aerobic exercise of moderate intensity does not induce oxidative stress (18, 34, 35). Discrepancies among the different types of macromolecular oxidation could be attributed to the differences in vulnerability to ROS of different molecules and the differences in turnover time for damage to occur. Previous reports have presented evidence of mixed results for different types of damage. Alessio et al. (13), for example, reported an increase in LOOH level immediately and 1 h after isometric handgrip exercise, whereas little increase was observed for the levels of protein carbonyls and malondialdehyde immediately after such exercise. Chevion et al. studied plasma antioxidant status and cell injury after 50
and 80 km marches and noted elevated plasma UA and CPK levels, but a decreased protein carbonyl level (36).

This study was subject to a number of limitations. Our study design required a homogenous population and, consequently, we could recruit only a limited number of volunteers. Furthermore, the workload was restricted by the need to keep the muscle pain within acceptable limits and to avoid the risk of fatigue accidents during anaerobic exercise.

In summary, similar workloads imposed by ergocycle exercise might induce ROS generation differently, depending on whether the exercise is anaerobic or aerobic, although aerobic exercise might initially induce more ROS owing to higher oxygen consumption, the time course of UA level suggested a prolonged generation of ROS after anaerobic exercise. Anaerobic exercise increased the levels of certain biomarkers of oxidative stress in the blood. Although more oxygen was consumed during aerobic exercise, no significant increase in oxidative stress was observed. With higher workloads, differences between anaerobic exercise and aerobic exercise may become more evident. To investigate this, it is necessary to study repeated sessions of anaerobic exercise and aerobic exercise.

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