

# Cigarette Smoking does not Induce Plasma or Pulmonary Oxidative Stress after Moderate-intensity Exercise

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**Abstract.** [Purpose] Cigarette smoking increases oxidative stress, which is a risk factor for several diseases. Moreover, strenuous exercise has been shown to induce plasma and pulmonary oxidative stress in young cigarette smokers. However, no previous reports have demonstrated whether plasma and pulmonary oxidative stress occur after moderate-intensity exercise. Therefore, the aim of this study was to clarify whether moderate-intensity exercise induces pulmonary and plasma oxidative stress in smokers. [Subjects] Ten young male smokers and 10 young male nonsmokers participated in this study. [Methods] Plasma hydroperoxide concentrations were measured at baseline and then immediately and 15 min after moderate-intensity exercise. Hydrogen peroxide concentrations in exhaled breath condensate were measured at baseline and after exercise. [Results] No significant interactions were found between smokers and nonsmokers in terms of hydroperoxide or hydrogen peroxide concentrations following moderate-intensity exercise at any time point. [Conclusion] These findings suggested that moderate-intensity exercise did not induce plasma or pulmonary oxidative stress in young cigarette smokers.

**Key words:** Oxidative stress, Cigarette smoker, Moderate-intensity exercise

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## INTRODUCTION

Oxidative stress is produced by reactive oxygen species (ROS) and is regulated by the expression and activity of antioxidants. Cigarette smoke is a complex mixture of thousands of chemical compounds, ROS, and other oxidants<sup>1)</sup> and is associated with increased oxidative stress in the blood and lungs<sup>2, 3)</sup>. Oxidative stress is one of the pathogenic mechanisms of several pulmonary and cardiovascular diseases<sup>4–6)</sup>.

Exercise also induces oxidative stress. Strenuous exercise has been shown to induce plasma ROS generation in nonsmokers<sup>7–10)</sup>. Moreover, cigarette smoking increases the levels of plasma and pulmonary oxidative stress in concert with exercise. Interestingly, strenuous exercise produces higher amounts of ROS generation in the plasma and lungs of cigarette smokers than in those of nonsmokers<sup>11–13)</sup>.

Moderate-intensity exercise promotes health, rehabilitation, and improvement of physical fitness<sup>14)</sup>. Therefore, it

is important to clarify whether plasma and pulmonary oxidative stress are induced by moderate-intensity exercise in smokers. However, no reports have investigated this topic. Thus, the aim of this study was to clarify whether smokers have increased plasma and pulmonary oxidative stress responses to moderate-intensity exercise compared with nonsmokers.

## SUBJECTS AND METHODS

### Subjects

The participants consisted of 10 male smokers and 10 male nonsmokers. All participants were untrained, did not participate in a regular exercise program, and were not regular cyclists. Participants were excluded from enrollment in the study if they had a history of metabolic, cardiovascular, or pulmonary disease or if they had orthopedic limitations. None of the participants were treated with antioxidant compounds (including vitamins) or anti-inflammatory medications. This protocol was approved by the Ethics Committee of the Hiroshima University Graduate School of Health Sciences (#1047). Written informed consent was obtained from all subjects.

### Methods

Subjects were instructed to abstain from strenuous phys-

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ical activity on the test day. Smokers refrained from smoking cigarettes for 12 h before the study to avoid the acute effects of cigarette smoking on hydroperoxide concentrations in plasma and hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) concentrations in exhaled breath condensate (EBC)<sup>3, 12, 13</sup>.

Heart rate (HR) and oxygen consumption during cardiopulmonary exercise testing (CPX) using a cycle ergometer were measured by an electrocardiogram monitor with a telemetry system (Dynascope DS-3140, Fukuda Denshi, Tokyo, Japan) and a gas analysis system (AE-300; Minato Medical Science, Tokyo, Japan). The predicted  $\text{VO}_2$  max was calculated by extrapolation of the heart rate and oxygen consumption at several submaximal workloads. The target HR during moderate-intensity exercise corresponded to that of 60% of the  $\text{VO}_2$  max. A week or more after CPX, subjects performed moderate-intensity exercise, which required pedaling at their target HR.

Blood samples were collected from subjects' fingertips using a single-use lancing device (ACCU-Chek Safe-T-Pro Plus; Roche, Mannheim, Germany) at baseline and then immediately and 15 min after exercising. Blood samples were centrifuged at 6,000 rpm for 2 min using a centrifuge (2420; Kubota, Tokyo, Japan) and then frozen at  $-30^\circ\text{C}$  until analysis.

EBC samples were collected for 15 min at baseline and after moderate-intensity exercise using a dedicated breath refrigeration circuit (ECoScreen; Jaeger, Wurzburg, Germany)<sup>12</sup>. Participants wore a nose clip and a mask, and the mask was connected to a dedicated breath refrigeration circuit cooled to  $-20^\circ\text{C}$ . The collected EBC samples were immediately used for measurement of  $\text{H}_2\text{O}_2$  concentrations.

Plasma hydroperoxide concentrations were determined with a Diacron derivatives of reactive oxygen metabolites (d-ROMs) test kit (Grosseto, Italy) using a spectrophotometer (Free Radical Elective Evaluator; Diacron)<sup>13</sup>.  $\text{H}_2\text{O}_2$  concentrations were measured in EBC samples with a d-ROMs Exhalation Test Kit (Diacron) using a spectrophotometer<sup>12</sup>.

Results are expressed as means  $\pm$  standard deviations (SDs). Predicted  $\text{VO}_2$  max values were analyzed using unpaired t-tests for comparison between smokers and nonsmokers. Baseline levels of oxidative stress markers in plasma and EBC samples were analyzed using unpaired t-tests for comparison between smokers and nonsmokers. Oxidative stress markers in plasma and EBC samples were compared using 2-way analysis of variance with repeated measures. The relationship between cumulative cigarette consumption and levels of oxidative stress markers in plasma and EBC samples were assessed using Pearson's correlation coefficients. p-values of less than 0.05 were considered statistically significant. All statistical analyses were performed using statistics software (IBM SPSS Statistics 21, IBM Japan, Tokyo, Japan).

## RESULTS

There were no significant differences in mean age, height, weight, or body mass index between smokers and nonsmokers (mean age,  $27.4 \pm 4.4$  vs.  $26.7 \pm 3.1$  years; height,  $1.73 \pm 0.06$  vs.  $1.73 \pm 0.07$  m; weight,  $70.2 \pm 8.9$

vs.  $66.4 \pm 4.8$  kg; body mass index,  $23.4 \pm 3.2$  vs.  $22.2 \pm 1.7$   $\text{kg}/\text{m}^2$ ). Cumulative cigarette consumption of smokers was  $6.8 \pm 4.5$  pack-years. Spirometric data of smokers were within normal limits. There were no significant differences in spirometric parameters between smokers and nonsmokers (percent of predicted forced vital capacity [FVC],  $93.0 \pm 10.8\%$  vs.  $89.5 \pm 5.3\%$ ; forced expiratory volume in 1 s (FEV1.0)/FVC,  $84.1 \pm 3.9\%$  vs.  $88.4 \pm 6.9\%$ ; percent of predicted FEV1.0,  $94.4 \pm 4.8\%$  vs.  $99.3 \pm 7.1\%$ ). There were no significant differences in predicted  $\text{VO}_2$  max values in the CPX between smokers and nonsmokers (smokers,  $43.5 \pm 3.2$   $\text{mL}/\text{kg}/\text{min}$  vs. nonsmokers,  $45.1 \pm 6.5$   $\text{mL}/\text{kg}/\text{min}$ ).

Plasma hydroperoxide concentrations in smokers were  $307.8 \pm 33.6$  U. Carr at baseline,  $312.5 \pm 33.2$  U. Carr immediately after exercise, and  $312.4 \pm 52.4$  U. Carr at 15 min after exercise. Plasma hydroperoxide concentrations in nonsmokers were  $283.8 \pm 42.9$  U. Carr at baseline,  $310.6 \pm 47.1$  U. Carr immediately after exercise, and  $297.8 \pm 37.7$  U. Carr at 15 min after exercise. No significant interactions between groups were observed at any time point. Additionally, while no main effect of group was observed, a main effect of time was observed ( $p < 0.05$ ). The  $\text{H}_2\text{O}_2$  concentrations in EBC samples from smokers were  $3.2 \pm 1.3$   $\mu\text{mol}/\text{L}$  at baseline and  $1.3 \pm 0.8$   $\mu\text{mol}/\text{L}$  after exercise, while those for nonsmokers were  $2.2 \pm 1.0$   $\mu\text{mol}/\text{L}$  at baseline and  $1.4 \pm 0.8$   $\mu\text{mol}/\text{L}$  after exercise. There were no significant differences in baseline EBC  $\text{H}_2\text{O}_2$  concentrations between smokers and nonsmokers. No significant interactions were observed between groups by time. Again, while no main effect of group was observed, a main effect of time was observed ( $p < 0.05$ ).

There was no significant correlation between cumulative cigarette consumption (pack-years) and levels of oxidative stress markers at baseline (plasma hydroperoxide concentration,  $r = -0.050$ ,  $p = 0.890$ ; EBC  $\text{H}_2\text{O}_2$  concentration,  $r = -0.152$ ,  $p = 0.675$ ).

## DISCUSSION

The present study examined plasma and pulmonary oxidative stress in response to moderate-intensity exercise in smokers and nonsmokers. We found that there were no significant interactions between groups in terms of plasma and pulmonary oxidative stress markers following moderate-intensity exercise at any time point. In addition, there were no relationships between cumulative cigarette consumption and levels of oxidative stress markers in plasma or EBC samples.

Production of ROS has been reported to be dependent on the intensity<sup>15</sup> and duration of exercise<sup>16</sup> because ROS generation results from the increase in oxygen consumption observed during exercise. In particular, many reports have found that high-intensity exercise and endurance training induce oxidative damage<sup>10, 17, 18</sup>. Exercise-induced oxidative stress is also increased by cigarette smoking. Indeed, multiple studies have found that plasma oxidative stress after strenuous exercise is higher in smokers than in nonsmokers<sup>11, 13</sup>. However, these effects have not been examined following moderate-intensity exercise, which is opti-

mal for promoting health, improvement of physical fitness, and rehabilitation. The results of this study suggested that cigarette smoking did not increase plasma oxidative stress following moderate-intensity exercise in young cigarette smokers, as evidenced by the fact that no significant interaction in plasma oxidative stress markers was observed between groups at any time point.

Smoking has been reported to increase oxidative stress in the lungs<sup>3)</sup>. Although the EBC H<sub>2</sub>O<sub>2</sub> concentrations in nonsmokers were not increased by 30 s of anaerobic exercise<sup>19)</sup>, the EBC H<sub>2</sub>O<sub>2</sub> concentrations in smokers were significantly increased by this exercise<sup>12)</sup>. In our current study, no significant interaction in EBC H<sub>2</sub>O<sub>2</sub> concentrations was observed between groups at any time. Thus, these data suggested that cigarette smoking did not increase pulmonary oxidative stress after moderate-intensity exercise.

There were no significant differences between smokers and nonsmokers in terms of plasma hydroperoxide concentrations and EBC H<sub>2</sub>O<sub>2</sub> concentrations at baseline, and there were no significant relationships between levels of oxidative stress markers at baseline and cumulative cigarette consumption in the present study. Nowak et al.<sup>3)</sup> reported that smokers with a long smoking history have high EBC H<sub>2</sub>O<sub>2</sub> concentrations and that there is a positive correlation between H<sub>2</sub>O<sub>2</sub> levels in the EBC and cumulative cigarette consumption. Cumulative cigarette consumption may be related to pulmonary oxidative stress. However, we may have observed no relationship between cumulative cigarette consumption and pulmonary oxidative stress in this study because the cumulative cigarette consumption of the participants in our study was much lower than that in some previous studies (17.8<sup>3)</sup> and 22.0 pack-years<sup>20)</sup>).

A limitation of this study was that the young cigarette smokers enrolled in the study had relatively low cumulative cigarette consumptions. It was reported that the amount of systemic or airway inflammation in elderly smokers who had long smoking histories was larger than in young smokers. If elderly smokers stopped smoking, systemic and airway inflammation persists for long periods<sup>21)</sup>. Therefore, further studies of elderly smokers who undergo exercise therapy under the guidance of physical therapists are needed to address changes in oxidative stress markers in response to moderate-intensity exercise.

In conclusion, our data demonstrated that moderate-intensity exercise may not increase the risk of systemic and pulmonary oxidative damage in young cigarette smokers.

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