The Effects of Aerobic Exercise at Hypoxic Condition during 6 Weeks on Body Composition, Blood Pressure, Arterial Stiffness, and Blood Lipid Level in Obese Women -

Hun-Young Park and Kiwon Lim

1Physical Activity and Performance Institute (PAPI), Konkuk University, Seoul, Republic of Korea
2Department of Physical Education, Konkuk University, Seoul, Republic of Korea

*Address for Correspondence: Kiwon Lim, Department of Physical Education, Konkuk University, Seoul, Republic of Korea, Tel: +82-2-450-3827; E-mail: exercise@konkuk.ac.kr

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ABSTRACT
The purpose of this study was to determine the effectiveness of aerobic exercise at hypoxic condition during 6 weeks on body composition, blood pressure, arterial stiffness, and blood lipid level in 30 to 60 years old women. Thirty-five obese women were volunteered to participate in the study as participants and were divided into three groups such as 20.9% O₂ normoxic training group (n = 12), 16.5% O₂ hypoxic training group (n = 11), and 14.5% O₂ training group (n = 12). The six weeks of training consisted 30 min of treadmill and cycle ergometer exercise, respectively (total 1 hour). Exercise intensity was maximal heart rate of 75% that developed Miyashita in 1985. Exercise frequency was 1 hour a day, 5 days a week, during 6 weeks. As a result, there was a significant decrease (p<0.05) in body fat mass and percentage of body fat in all groups. The reduction rate of body fat mass and percent of body fat were larger in the both hypoxic training group compared to the normoxic training group. Therefore, body weight was significantly decreased (p<0.05) only in 14.5% O₂ hypoxic training group. Both hypoxic training groups showed a greater decrease (p<0.05) in blood pressure and pulse wave velocity than normoxic training group. In addition, total cholesterol and low-density lipoprotein cholesterol showed a greater decrease (p<0.05) in both hypoxic training group compared with normoxic training group. Based on these results, our study demonstrated that 16.5% O₂ and 14.5% O₂ hypoxic training had a positive effect and possibility of efficiency on body composition, blood pressure, arterial stiffness and blood lipid level in obese women compared with normoxic training.

INTRODUCTION
Obesity is characterized by an increase in adipose tissue due to a positive energy balance of dietary intake and energy consumption and is now recognized as a very important social problem in increasing morbidity and mortality in the public health field [1]. In particular, the increase in visceral fat cells by obesity reduces the vasodilation of vascular endothelial cells and increases the risk of arteriosclerosis [2]. Many obese people often exhibit chronic inflammatory response to adipose tissue, which induces the onset and development of a variety of obesity-related diseases and increases oxidative stress in adipose tissue. In particular, obesity has been reported to cause metabolic dysfunction such as insulin resistance, type 2 diabetes and non-alcoholic fatty liver, and causes systemic inflammation, oxidative stress, and cardiovascular related complications [3].

In general, dietary and exercise combined treatment is the most commonly used to prevent obesity and various diseases [4]. However, this method has been reported to require long-term treatment for more than 12 weeks and does not inhibit the increased appetite with exercise, resulting in no consistent results via a relatively large number of positive and negative results [5]. In addition, pharmacotherapy and surgery can be used in the treatment of obesity, but these methods are not recommended except for severe obesity patients because of the various adverse effects that can adversely affect health [6].

In recent years, it has been recommended to hypoxic therapy (e.g. aerobic exercise in hypoxic condition) as a way to treat and prevent obesity and various lifestyle diseases by suppressing appetite and minimizing adverse effects [7]. In general, exercise in a high altitude environment or artificial hypoxic condition is well known to improve athletic performance [8]. In addition, exercise at hypoxic condition basically improves carbohydrate and lipid metabolism efficiently, it regulate oxygen transport capacity, process, carbohydrate metabolism, and satiety by increase the production of transcription factor hypoxia-inducible factor (HIF) -1 [9]. In particular, the hypoxic condition strongly induces weight loss through regulation of ghrelin, leptin, insulin, neuropeptide A, which increases fat metabolism and satiety [10-12]. Therefore, it is reported that exercise in the hypoxic condition is more effective in a shorter period of weight loss than exercise in the normoxic condition [7]. Exposure and exercise in a hypoxic condition leads to a decrease in arterial stiffness and an increase in blood flow to the skeletal muscle via vasodilation of the arteriole and production of nitric oxide (NO) [13]. These mechanisms are thought to play an important role in the prevention and treatment of various cardiovascular diseases induced by obesity through normalizing blood pressure and blood vessel elasticity.

Therefore, using a randomized and controlled design, this study aimed to investigate the effects of 6 weeks of aerobic exercise at hypoxic condition corresponding to 16.5% and 14.5% O₂ compared with exercise in normoxic condition. We measured the effects on body composition, blood pressure, arterial stiffness, and blood lipid level in obese women.

MATERIALS AND METHODS
Participants
To determine the sample size, an a priori power analysis was performed with G-power for the body composition parameter (percent of body fat) based on previous research [14], indicating that a sample size of total 30 participants (10 participants per group) would be required to provide 80% power at an α-level of 0.05. We anticipated a more than 10% dropout rate and aimed for a starting population of 36.

Thirty-six middle-aged obese women (30 to 55 year), who were not taking any medication and with >30 BMI (by Broca’s index) and >30% in percent body weight were selected as participants of this study. These women were housewives with low levels of activity who had not performed any kind of exercise over the last 6 months. Applicants’ menopausal status was not assessed. The participants consented by signature, after sufficient explanation of the experiment and an understanding of the possible adverse effects, and were randomly assigned into a normoxic training group (n = 12), 16.5% O₂ hypoxic training group (n = 11), and 14.5% O₂ hypoxic training group (n = 12). Thirty-five of the participants completed the study (>95% compliance), thus, only their data were used in the analyses. Data from the remaining one subject was discarded due to medication (n=1). There were no significant differences in physical characteristics among groups before training (Table 1). All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration.

Study Design
Thirty-five middle-aged obese women in a normoxic training group (n = 12), 16.5% O₂ hypoxic training group (n = 11), and 14.5% O₂ hypoxic training group (n = 12) performed training...
The arterial stiffness was evaluated by pulse wave velocity (PWV) and it was measured using PWC 5.0 (Korea Medical Technology, Korea), a noninvasive device. All subjects were placed in a sitting position, two electrodes and one electrode were attached to the left arm and the right arm, respectively, and then the PWV of the upper limb was measured after inserting the sensor into two thumbs. Then, the sensor attached to the thumb was replaced with a big toe, and the PWV of the lower extremity was measured again. The result is recorded the mean value by selecting stable interval for 20 seconds from the data measured for 30 seconds.

For the analysis of blood lipid level, about 4 mL of blood was collected in the median antebibrachial vein after 10 hours of fasting before and after 6 weeks of training at each environmental condition. After that, it was put into a SST (serum separating tube), centrifuged at 3,500 rpm for 10 minutes, and serum was collected and stored at -70°C for quick freezing. Then, the Green Cross Medical Foundation (Certified organization in The Korea Society for Laboratory Medicine) analyzed blood samples; a modular analytic PE (Roche, Germany) will be used to evaluate blood lipid levels. The enzymatic colorimetric assay method was used by FFA kit (Roche, Germany), CHOL kit (Roche, Germany), HDL-C plus 3rd generation kit (Roche, Germany), and LDL-C plus 2nd generation kit (Roche, Germany).

### Statistical Analysis

All statistical analyses were conducted using SPSS 23.0 (IBM Corp., Armonk, USA) for windows. Data are presented as the mean ± SD. The Kolmogorov-Smirnov test was used to ensure that all data had a normal distribution. All data were presented as means±SD. Paired t tests and analysis of variance (ANOVA) were used to evaluate within-participant differences between environmental conditions. The level of significance was set a priori at 0.05.

### RESULTS

Body composition data (body weight, free fat mass, body fat mass, and percent of body fat), measured before and after training, is reported in Table 2. There was a significant decrease ($p < 0.05$) in body fat mass and percentage of body fat in all groups. The reduction rate of body fat mass and percent of body fat were larger in the both hypoxic training group compared to the normoxic training group. Therefore, body weight was significantly decreased ($p < 0.05$) only in 14.5% $O_2$ hypoxic training group.

Blood pressure (systolic and diastolic) data before and after training is shown in table 2. Systolic blood pressure was significantly decrease ($p < .05$) in the 16.5% and 14.5% $O_2$ hypoxic training group by 6 weeks of training. Diastolic blood pressure decreased significantly ($p < .05$) in all groups, but both hypoxic training group showed a larger decrease tendency than the normoxic training group.

As shown in table 2, arterial stiffness was evaluated by measuring the PWV at left hand, right hand, left foot, and right foot. The PWV showed no change by 6 weeks of normoxic training. However, the 16.5% $O_2$ hypoxic training group showed a significant decrease ($p < 0.05$) in PWV at left hand, right hand, and left foot. The 14.5% $O_2$ hypoxic training group showed improved arterial stiffness through a significant reduction ($p < 0.05$) in PWV at all sites.

Table 2 depicts before and after training data for FFA, TC, HDL-C, and LDL-C in all training groups. There was no significant difference in FFA and HDL-C by 6 weeks of training in all groups. TC significantly decreased ($p < 0.05$) in all groups. However, LDL-C showed a significant decrease ($p < 0.05$) only in the hypoxic group.

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### Table 1: Selected Participants Characteristics (Mean ± S.D.)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Normoxic training group</th>
<th>16.5% $O_2$ hypoxic training group</th>
<th>14.5% $O_2$ hypoxic training group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Environmental condition</td>
<td>Sea-level</td>
<td>2,000m simulated altitude</td>
<td>3,000m simulated altitude</td>
</tr>
<tr>
<td>Number</td>
<td>12</td>
<td>11</td>
<td>12</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>47.2 ± 6.3</td>
<td>42.0 ± 4.4</td>
<td>46.6 ± 5.7</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>157.7 ± 3.9</td>
<td>157.6 ± 6.3</td>
<td>158.3 ± 5.3</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>65.6 ± 7.4</td>
<td>62.8 ± 8.9</td>
<td>64.6 ± 9.7</td>
</tr>
<tr>
<td>Body fat mass(kg)</td>
<td>34.3 ± 3.3</td>
<td>34.8 ± 4.6</td>
<td>32.8 ± 2.2</td>
</tr>
</tbody>
</table>

Note: S.D: Standard Deviation
leptin secretion in adiposities, thereby increasing fat metabolism. Reported that exercise training in a hypoxic condition increases basal metabolic rate [20-22]. In addition, Netzer, et al. [23] conducted a single blind study to test the hypothesis that training under hypoxia (HG, n = 24, FiO2 = 15%) results in similar or even greater improvement in body weight and metabolic risk markers compared with exercise under normoxia (NG, n = 21, FiO2 = 21%).

**DISCUSSION**

Many studies related to exercise training in high altitude or hypoxic conditions have been conducted primarily to improve aerobic exercise capacity of athletes [15]. Changes in substrate utilization and increased maximal oxygen consumption associated with improved aerobic exercise capacity are closely related to oxidative capacity of mitochondria in skeletal muscle [16]. In particular, improved aerobic exercise capacity is manifested by qualitative and quantitative adaptation of mitochondria [17]. Thus, aerobic exercise training in a hypoxic condition can be described as an effective treatment modality that can improve oxidative capacity and mitochondrial dysfunction in obese and type 2 diabetic patients [18,19]. However, based on these physiological mechanisms, studies on the application of aerobic exercise training at hypoxic condition for improving obesity and metabolic diseases are insufficient.

Therefore, Thirty-five middle-aged obese women in a normoxic training group (n = 12), 16.5% O2 hypoxic training group (n = 11), and 14.5% O2 hypoxic training group (n = 12) performed training session (treadmill and bicycle exercise at a total duration of 1 hour, 5 days per week, for 6 weeks) equivalent to 75% of maximal heart rate (HRmax) at each environment. As results, we found that 6 weeks of aerobic exercise training in the hypoxic conditions was more effective in improvement of body weight, systolic blood pressure, arterial stiffness, and LDL-C compared with the normoxic training group, which was consistent with previous studies, and these results are valid.

Regarding blood lipid levels, Roberts, et al. [27] reported that exposure to the hypoxic condition increased the utilization of fat as a substrate, positively affecting blood lipid level. Tinkov & Aksenov [28]. Reported that 46 patients with ischemic heart disease and 45 men with coronary artery disease showed decrease of TC, LDL-C, and triglyceride after exposure and exercise in a hypoxic condition. Thus, they insisted that hypoxic condition is an important stimulus condition to improve blood lipid level and health of cardiovascular disease patients. Wiesner, et al. [19]. conducted a single blind study in overweight to obese subjects to test the hypothesis that training under hypoxia (HG, n = 24, FiO2 = 15%) results in similar or even greater improvement in body weight and metabolic risk markers compared with exercise under normoxia (NG, n = 21, FiO2 = 21%).

**Table 2: Before- and after-training data for all outcomes by 6 weeks of training at each environmental condition (Mean ± S.D.)**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Normoxic training group</th>
<th>16.5% hypoxic training group</th>
<th>14.5% hypoxic training group</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body composition</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>65.6 ± 7.4</td>
<td>65.1 ± 7.7</td>
<td>62.8 ± 8.9</td>
</tr>
<tr>
<td>Free fat mass (kg)</td>
<td>40.5 ± 3.7</td>
<td>40.6 ± 3.8</td>
<td>38.4 ± 3.9</td>
</tr>
<tr>
<td>Body fat mass (kg)</td>
<td>22.6 ± 4.4</td>
<td>21.9 ± 4.9*</td>
<td>22.1 ± 5.8</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>34.3 ± 3.4</td>
<td>33.5 ± 3.6*</td>
<td>34.8 ± 4.6</td>
</tr>
<tr>
<td><strong>Blood pressure</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>116.2 ± 10.5</td>
<td>113.1 ± 5.2</td>
<td>123.3 ± 11.6</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>82.7 ± 9.1</td>
<td>79.0 ± 8.7*</td>
<td>76.7 ± 9.4</td>
</tr>
<tr>
<td><strong>Arterial stiffness(PWV)</strong></td>
<td></td>
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<tr>
<td>Left hand (ms)</td>
<td>216.2 ± 14.7</td>
<td>208.2 ± 19.3</td>
<td>203.5 ± 13.7</td>
</tr>
<tr>
<td>Right hand (ms)</td>
<td>221.2 ± 23.8</td>
<td>222.3 ± 42.8</td>
<td>206.1 ± 11.3</td>
</tr>
<tr>
<td>Left foot (ms)</td>
<td>306.4 ± 25.1</td>
<td>291.5 ± 25.9</td>
<td>295.8 ± 17.9</td>
</tr>
<tr>
<td>Right foot (ms)</td>
<td>305.1 ± 20.3</td>
<td>291.1 ± 24.0</td>
<td>302.1 ± 18.2</td>
</tr>
<tr>
<td><strong>Blood lipid levels</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>198.3 ± 33.2</td>
<td>175.7 ± 36.6*</td>
<td>186.6 ± 33.4</td>
</tr>
<tr>
<td>High density lipoprotein cholesterol (mg/dL)</td>
<td>50.7 ± 8.6</td>
<td>46.3 ± 9.6</td>
<td>47.4 ± 8.9</td>
</tr>
<tr>
<td>Low density lipoprotein cholesterol (mg/dL)</td>
<td>122.2 ± 41.4</td>
<td>102.4 ± 37.4</td>
<td>129.1 ± 33.2</td>
</tr>
</tbody>
</table>

Note: S.D: Standard Deviation; PWV: Pulse Wave Velocity;* p < .05 vs. before in the same training group.

Regarding blood lipid levels, Roberts, et al. [27] reported that exposure to the hypoxic condition increased the utilization of fat as a substrate, positively affecting blood lipid level. Tinkov & Aksenov [28]. Reported that 46 patients with ischemic heart disease and 45 men with coronary artery disease showed decrease of TC, LDL-C, and triglyceride after exposure and exercise in a hypoxic condition. Thus, they insisted that hypoxic condition is an important stimulus condition to improve blood lipid level and health of cardiovascular disease patients. Wiesner, et al. [19]. conducted a single blind study in overweight to obese subjects to test the hypothesis that training under hypoxia (HG, n = 24, FiO2 = 15%) results in similar or even greater improvement in body weight and metabolic risk markers compared with exercise under normoxia (NG, n = 21, FiO2 = 21%).
They conclude that in obese subjects, training in hypoxia elicits a similar or even better response in terms of physical fitness, metabolic risk markers, and body composition at a lower workload. The fact that workload and, therefore, mechanic strain can be reduced in hypoxia could be particularly beneficial in obese patients with orthopedic co morbidities. As with previous studies of this positive outcome, in this study, we also confirmed that exercise training in hypoxic condition is more effective in improving blood lipids such as LDL-C. The reduction of blood lipid levels in this study seems to be a complicated phenomenon appear to be caused by changes in dietary control mechanisms and improvement of body composition and cardiovascular function by exposure and training in hypoxic conditions. Therefore, various studies are needed to find out the precise mechanism and proper hypoxic stimulation condition.

CONCLUSION

The present study demonstrates that exercise training at 16.5% and 14.5% O2 hypoxic conditions had a positive effect and possibility of efficiency on body composition, blood pressure, arterial stiffness and blood lipid level in obese women compared with normoxic training. It can be explained that there is a need to confirm the effect of hypoxic training on the basis of a review of various variables related to obesity and health during a longer treatment period in the future.

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