Effects of normoxic and hypoxic exercise regimens on cardiac, muscular, and cerebral hemodynamics suppressed by severe hypoxia in humans

Jong-Shyan Wang,1,2 Min-Huan Wu,1 Tso-Yen Mao,3 Tieh-cheng Fu,2 and Chih-Chin Hsu2
1Graduate Institute of Rehabilitation Science, Chang Gung University, Tao-Yuan; 2Heart Failure Center, Department of Physical Medicine and Rehabilitation, Chang Gung Memorial Hospital, Keelung; and 3Department of Athletic Training & Health Science, National Taiwan Sport University, Tao-Yuan, Taiwan

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Wang J, Wu M, Mao T, Fu T, Hsu C. Effects of normoxic and hypoxic exercise regimens on cardiac, muscular, and cerebral hemodynamics suppressed by severe hypoxia in humans. J Appl Physiol 109: 219–229, 2010. First published April 29, 2010; doi:10.1152/japplphysiol.00138.2010.—Hypoxic preconditioning prevents cerebrovascular/cardiovascular disorders by increasing resistance to acute ischemic stress, but severe hypoxic exposure disturbs vascular hemodynamics. This study compared various exercise regimens with/without hypoxia to affect hemodynamics and oxygenation in cardiac, muscle, and cerebral tissues during severe hypoxic exposure. Sixty sedentary males were randomly divided into five groups. Each group (n = 12) received one of five interventions: 1) normoxic (21% O2) resting control, 2) hypoxic (15% O2) resting control, 3) normoxic exercise (50% maximum work rate under 21% O2; N-E group), 4) hypoxic-relative exercise (50% maximal heart rate reserve under 15% O2; H-RE group), or 5) hypoxic absolute exercise (50% maximum work rate under 15% O2; H-AE group) for 30 min/day, 5 days/wk, for 4 wk. A recently developed noninvasive bioreactance device was used to measure cardiac hemodynamics, and near-infrared spectroscopy was used to assess perfusion and oxygenation in the vastus lateralis (VL)/gastrocnemius (GN) muscles and frontal cerebral lobe (FC). Our results demonstrated that the H-AE group had a larger improvement in aerobic capacity compared with the N-E group. Both H-RE and H-AE ameliorated the suppression of cardiac stroke volume and the GN hyperemic response (Δtotal Hb/min) and reoxygenation rate by acute 12% O2 exposure. Simultaneously, the two hypoxic interventions enhanced perfusion (Δtotal Hb) and O2 extraction [Δdeoxygenated Hb] of the VL muscle during the 12% O2 exercise. Although acute 12% O2 exercise decreased oxygenation (ΔO2-Hb) of the FC, none of the 4-wk interventions influenced the cerebral perfusion and oxygenation during normoxic/hypoxic exercise tests. Therefore, we conclude that moderate hypoxic exercise training improves cardiopulmonary fitness and increases resistance to disturbance of cardiac hemodynamics by severe hypoxia, concurrence with enhancing O2 delivery/utilization in skeletal muscles but not cerebral tissues.

oxygen; physical activity; circulation

CHRONIC INTERMITTENT HYPOXIA is known to enhance aerobic capacity by increasing pulmonary ventilation, adaptation of the hematopoietic system, and tissue utilization of O2 in sedentary males (43, 44), endurance athletes (10, 13), and elderly subjects with or without vascular diseases (8). Animal studies have also indicated that hypoxic preconditioning reduced the volume of cerebral infarction and edema (41) and attenuated the suppression of cardiac stroke volume and the GN hyperemic response after 4 wk of intermittent exposure to 12% O2; when the concentration of O2 was set to 15%, the risk of vascular complications became negligible. Hence, whether the effect of a hypoxic intervention on circulation systems is beneficial or adverse may depend on the exposing concentration of O2 in the air. To our knowledge, a “safe and effective” exercise strategy combined with hypoxia that promotes aerobic fitness but minimizes hemodynamic limitations of the heart, skeletal muscles, and cerebral tissues caused by severe hypoxia has not yet been established. We hypothesized that moderate hypoxic (15% O2) exercise training effectively improves physical fitness in a severe hypoxic (12% O2) environment by simultaneously ameliorating hemodynamic disturbances in the heart and enhancing O2 delivery/utilization in skeletal muscles or cerebral tissues.

The objective of the study was to clarify how different exercise regimes with/without hypoxia influence cardiac hemodynamics and skeletal muscular/cerebral perfusion and oxygenation during normoxic and hypoxic exercise tests. The invasiveness of present methods of assessing tissue hemodynamics precluded direct testing for this study. Therefore, this work specifically used a new, noninvasive, bioreactance device [i.e., a noninvasive continuous cardiac output (CO) monitoring system (NICOM)] to assess cardiac hemodynamics (22, 25).
and simultaneously used near-infrared (NIR) spectroscopy (NIRS) to monitor changes in skeletal muscular/cerebral perfusion and oxygenation (35).

**METHODS**

**Subjects and Interventions**

The Ethics Committee of Chang Gung Memorial Hospital approved the study protocol, which followed institutional guidelines (No. 98-1374B). Sixty healthy subjects who were nonsmokers, nonmedication/vitamin users, infection and cardiopulmonary disease risk free, and nondiabetic were recruited from Chang Gung University. No subject had engaged in any regular physical activity (i.e., exercise frequency ≤ 1 time/wk, duration < 20 min) or exposure to high altitude (altitude of ≥3,000 m) for at least 1 yr before the experiment. All subjects gave informed consent after the experimental procedures were explained. Subjects were randomly divided into the following five groups: normoxic control (N-C; n = 12), hypoxic control (H-C; n = 12), normoxic exercise (N-E; n = 12), hypoxic-relative exercise (H-RE; n = 12), and hypoxic-absolute exercise (H-AE; n = 12). Anthropometric data for the five groups did not differ significantly. Subjects were instructed to fast for at least 8 h and to refrain from exercise for at least 24 h before the testing sessions. All subjects arrived at the testing center at 9:00 AM to eliminate any possible diurnal effect.

Subjects in the five groups were exposed to 21% Po2 (N-C; 159 Torr, N-C group) or 15% O2 (P02; 114 Torr, H-C group) at rest or were trained on a bicycle ergometer (Corvial 400, Lode, Groningen, The Netherlands) at 50% of the maximal work rate (50% Wmax) under 21% O2 in air (N-E group), 50% of the maximal heart rate (HR) reserve (50% HRmax) under 15% O2 in air (H-RE group), or 50% Wmax under 15% O2 in air (H-AE group) for 30 min/day, 5 days/wk, 4 days before and 4 days after the intervention, 2) normoxic (and hypoxic) continuous exercise tests (CXTs) on the third (and second) day before and on the second (and third) day after the intervention, and 3) normoxic and hypoxic ischemia-reperfusion tests (IRTs) 1 day before and 1 day after the intervention.

<p>| Table 1. Intervention conditions in the various normoxic and hypoxic regimens |
|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|</p>
<table>
<thead>
<tr>
<th></th>
<th>N-C Group</th>
<th>H-C Group</th>
<th>N-E Group</th>
<th>H-RE Group</th>
<th>H-AE Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air O2, %</td>
<td>20.9 ± 0.0</td>
<td>15.0 ± 0.2</td>
<td>20.9 ± 0.0</td>
<td>15.0 ± 0.3†</td>
<td>15.0 ± 0.3‡</td>
</tr>
<tr>
<td>SaO2, %</td>
<td>97.4 ± 0.3</td>
<td>90.2 ± 0.6</td>
<td>97.2 ± 0.5</td>
<td>87.5 ± 1.5*</td>
<td>84.3 ± 1.2‡</td>
</tr>
<tr>
<td>Work rate, W</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>95 ± 3</td>
<td>82 ± 3*</td>
<td>99 ± 4‡</td>
</tr>
<tr>
<td>Maximal work rate, %</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>50.0 ± 0.0</td>
<td>44.1 ± 1.5*</td>
<td>50.0 ± 0.0‡</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>73 ± 2</td>
<td>77 ± 3</td>
<td>135 ± 4</td>
<td>147 ± 5†‡</td>
<td>147 ± 5†‡</td>
</tr>
<tr>
<td>Maximal HR reserve, %</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>50.1 ± 1.2</td>
<td>50.2 ± 1.1</td>
<td>58.8 ± 1.5†‡</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>86.5 ± 2.1</td>
<td>87.4 ± 2.0</td>
<td>97.3 ± 3.1</td>
<td>93.3 ± 2.3*</td>
<td>98.2 ± 3.2*</td>
</tr>
<tr>
<td>VE, l/min</td>
<td>6.5 ± 1.2</td>
<td>7.5 ± 2.0</td>
<td>45.2 ± 3.8</td>
<td>46.5 ± 3.5</td>
<td>59.2 ± 3.8‡</td>
</tr>
<tr>
<td>V̇O2, ml·min⁻¹·kg⁻¹</td>
<td>3.6 ± 0.5</td>
<td>3.5 ± 0.5</td>
<td>21.8 ± 1.5</td>
<td>17.5 ± 1.2*</td>
<td>21.8 ± 1.8</td>
</tr>
</tbody>
</table>

Values are means ± SE. Subjects were divided into the following five groups: normoxic control (N-C), hypoxic control (H-C), normoxic exercise (N-E), hypoxic-relative exercise (H-RE), and hypoxic-absolute exercise (H-AE). SaO2 arterial O2 saturation; HR, heart rate; MAP, mean arterial pressure; VE, minute ventilation; V̇O2, O2 consumption. *P < 0.05, N-E group vs. H-RE group; †P < 0.05, N-E group vs. H-AE group; ‡P < 0.05, H-RE group vs. H-AE group.
for 4 wk in an air-conditioned normobaric hypoxia chamber (Colorado Mountain Room, Boulder, CO). The O2 concentrations of 15% and 21% correspond to altitudes of ~2,733 m and sea level, respectively. The hypoxia chamber was maintained at a temperature of 22 ± 0.5°C with a relative humidity of 60 ± 5%; a CO2 scrubber eliminated CO2 in the air (≤3,500 ppm) (43–45). Interventionsal conditions in various normoxic and hypoxic regimens are shown in Table 1 and Fig. 1. In the design of exercise intensity, both N-E and H-AE were done to match to the normoxic work rate (50% Wmax), whereas H-RE was performed according to the normoxic exercise HR (50% HRRmax). Both N-C and H-C groups were exposed to 21% or 15% O2 in a sitting position at rest. All subjects recorded their daily activity using a physical activity questionnaire, which was collected weekly until the end of the study. During the experiment, subjects were instructed to refrain from other physical activity (i.e., exercise frequency ≤ 1 time/wk, duration ≤ 20 min). The rate of compliance with the five interventions was 100%.

Graded Exercise Tests

Each subject performed graded exercise tests (GXTs) 4 days before and 4 days after the intervention. GXTs were performed using a bicycle ergometer. The test consisted of 2 min of unloaded pedaling; the loading increased by 20–30 W every 3 min until exhaustion [i.e., progressive exercise up to maximal O2 consumption (V˙O2max)]. HR, minute ventilation (VE), O2 consumption (V˙O2), and CO2 production (V˙CO2) were measured using an automated system (System 2000, Medical Graphics, St. Paul, MN) (42). V˙O2max was the value at which the level of V˙O2 increased < 2 ml·kg–1·min–1 after at least 2 min. HR exceeded its predicted maximum, the respiratory exchange ratio exceeded 1.2, or the venous lactate concentration exceeded 8 mM. Additionally, the ventilation threshold was determined when V˙e/V˙CO2 increased without a corresponding increase in the V˙e-to-V˙CO2 ratio, end-tidal P02 increased without a decrease in end-tidal PCO2, or there was a departure from linearity for Ve (42, 43). Blood pressure (BP) was monitored using an automatic BP system (model 412, Quinton, Bothell, WA), arterial O2 saturation (Sao2) was monitored by finger pulse oximetry (model 9500, Nonin Onyx, Plymouth, MN), blood lactate concentrations were determined using an i-STAT clinical analyzer (Sysmex SF-3000, Kobe, Japan) (43).

Normoxic and Hypoxic Continuous Exercise Tests

Each subject performed normoxic continuous exercise tests (NCXTs) [and hypoxic continuous exercise tests (HCXTs)] on the third (and second) day before the intervention and on the second (and third) day after the intervention. NCXTs (and HCXTs) on the bicycle ergometer required 50 W of warm-up for 3 min, an increase of work rate to 100 W of continuous exercise for 20 min, and then recovery to 50 W of cool-down for 3 min. During the test, the O2 concentration was set to 21% (or 12%). We chose 100 W as the work rate for the exercise tests for this study because it is considered to be moderate-intensity exercise (~50% Wmax) and could fit the activities required for normal daily life in these tested subjects (1). Additionally, exercise for 20 min is considered to the minimal duration for reaching the exercise effect on improvement of physical fitness according to recommendations from the American College of Sports Medicine (1). The O2 concentrations of 12% and 21% correspond to altitudes of ~4,460 m and sea level, respectively. Cardiac, skeletal muscular, or cerebral hemodynamic parameters at the 20-min continuous exercise stage were averaged using as an index of the tissue hemodynamic response to NCXT (or HCXT).

Cardiac hemodynamic measurements. The NICOM bioreactance-based system (Cheetah Medical, Wilmington, DE) enables the analysis of relative phase shifts (Δª) of oscillating currents that occur when traversing the thoracic cavity (22, 25). The system included a
radiofrequency generator to apply a high-frequency current across the thorax, four dual-surface electrodes were established electrical contact with the body, a receiving amplifier to record the transesophageal voltage in response to the injected current, and circuitry for determining $\Delta \Phi$ between the injected current and recorded voltage. One of the dual electrodes was used by the high-frequency current generator, whereas the other was used by the input voltage amplifier. Signals were applied to and recorded from the left and right sides of the thorax; these signals were processed separately and averaged after digital processing. During NCXTs or HCXTs, electrodes were placed on the subject’s back so that the cables would not interfere with upper body motion. The signal processing unit of the system determined $\Delta \Phi$ between the input signal relative to the output signal. $\Delta \Phi$ was then recorded as an indicator of the change in aortic blood flow. Stroke volume (SV) was estimated using the following equation: $SV = C \times VET \times \Delta \Phi / \text{dmax}$, where $C$ is a constant of proportionality that is preset by the manufacturer (Cheetah Medical). VET is the ventricular ejection time indicated by NICOM and ECG signals, and $t$ is time. Both CO and total peripheral resistance (TPR) were then calculated using the following equations: $CO = SV \times HR$ and $TPR = MAP / CO$, where $MAP$ is mean arterial pressure.

Skeletal muscular and cerebral hemodynamic measurements. During NCXTs or HCXTs, subjects were instrumented with two pairs of NIR probes to monitor the absorption of light across cerebral and muscle tissues (Oxymon, Artinis, The Netherlands) (35). Subjects were specially designed headsets that positioned one NIR emitter and detector pair over the left frontal cortex (FC) region of the forehead. The spacing between optodes was adjusted to ensure proper placement (range: 2.5–3.5 cm) and signal strength (10–30%) on each subject. A second emitter and detector pair was affixed over the lateral border of the patella and 3–5 cm lateral to the midline of the thigh. A skinfold measurement was made in the sagittal plane midway between optodes to account for skin and adipose thickness. Probes were held in place by a plastic spacer with an optode distance of 3.5–4.5 cm and secured to the skin using double-sided tape. Elastic bandages were used to shield the optodes from ambient light. The Beer-Lambert law was used to calculate micromolar changes in tissue oxygenation ($[\Delta O_2 \text{Hb}]$ and $[\Delta \text{HHb}]$) over time using received optical densities from two continuous wavelengths of NIR light (780 and 850 nm) and published differential path lengths of 4.95 and 5.93 (11, 40) cm for muscle and cerebral tissue, respectively. The total Hb concentration ($[\Delta \text{THb}]$) was calculated as the sum of $[\Delta O_2 \text{Hb}]$ and $[\Delta \text{HHb}]$ and used as an index of change in regional blood volume (39). Data were recorded at 10 Hz and filtered with a Savitzky-Golay smoothing algorithm before analysis. Because $[\Delta \text{THb}]$ is closely associated with changes in venous $O_2$ content and is less sensitive to $[\Delta \text{THb}]$ than $[\Delta O_2 \text{Hb}]$, $[\Delta \text{HHb}]$ provides a highly sensitive measure of relative tissue deoxygenation due to $O_2$ extraction (19).

Ischemia-Reperfusion Tests

All groups performed normoxic and hypoxic ischemia-reperfusion tests (IRTs) 1 day before and 1 day after the intervention. In the first IRT, hemodynamic parameters of the gastrocnemius (GN) muscle were measured by NIRS after 1 h of supine rest under the 21% $O_2$ condition. Immediately after this normoxic IRT, subjects were exposed to 12% $O_2$ for 1 h, during which period they were asked to remain in a supine position, and then performed the second IRT to evaluate the GN hemodynamic response to hypoxic stress. An emitter and detector pair was affixed over the lateral border of the GN muscle on the left lower leg. Probes were attached to a plastic spacer with an optode distance of 3.5–4.5 cm and secured to the skin using double-sided tape, and the lower extremities were elevated to a 30° angle from the examination couch. The subdiastolic occlusion pressure in the left lower leg was set at 60 mmHg to measure basal arterial blood flow (21). This occlusion procedure was conducted three times with

<table>
<thead>
<tr>
<th>Table 3. Effects of the various normoxic and hypoxic continuous exercise tests</th>
<th>N-C Group</th>
<th>H-C Group</th>
<th>N-E Group</th>
<th>H-RE Group</th>
<th>H-AE Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Preintervention</td>
<td>Postintervention</td>
<td>Preintervention</td>
<td>Postintervention</td>
<td>Preintervention</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>21% $O_2$</td>
<td>142</td>
<td>147</td>
<td>150</td>
<td>147</td>
</tr>
<tr>
<td></td>
<td>12% $O_2$</td>
<td>174</td>
<td>155</td>
<td>160</td>
<td>159</td>
</tr>
<tr>
<td>Stroke volume, ml</td>
<td>21% $O_2$</td>
<td>97.4</td>
<td>97.8</td>
<td>98.0</td>
<td>97.9</td>
</tr>
<tr>
<td></td>
<td>12% $O_2$</td>
<td>106.9</td>
<td>107.4</td>
<td>107.6</td>
<td>108.0</td>
</tr>
<tr>
<td>Cardiac output, ml/min</td>
<td>21% $O_2$</td>
<td>7.1</td>
<td>7.3</td>
<td>7.4</td>
<td>7.5</td>
</tr>
<tr>
<td></td>
<td>12% $O_2$</td>
<td>8.0</td>
<td>8.2</td>
<td>8.3</td>
<td>8.4</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>21% $O_2$</td>
<td>106.9</td>
<td>107.4</td>
<td>107.6</td>
<td>108.0</td>
</tr>
<tr>
<td></td>
<td>12% $O_2$</td>
<td>107.7</td>
<td>108.2</td>
<td>108.4</td>
<td>108.7</td>
</tr>
<tr>
<td>Total peripheral resistance, mmHg · l/min</td>
<td>21% $O_2$</td>
<td>7.1</td>
<td>7.3</td>
<td>7.4</td>
<td>7.5</td>
</tr>
<tr>
<td></td>
<td>12% $O_2$</td>
<td>8.0</td>
<td>8.2</td>
<td>8.3</td>
<td>8.4</td>
</tr>
</tbody>
</table>

Values are means ± SE. *$P < 0.05$; preintervention vs. postintervention; †$P < 0.01$; N-E group vs. H-RE or H-AE groups; ‡$P < 0.05$; N-C group vs. H-RE or H-AE groups.
each occlusion lasting 10 s. Basal arterial blood flow described the maximal velocity of the blood volume change, indicated by $\Delta [THb]/min$, in the measured GN muscle during venous occlusion.

Arterial inflow and reoxygenation in the case of reactive hyperemia were measured after 4 min of arterial occlusion (21). At the beginning of the occlusion phase, the cuff pressure was set to suprasystolic pressure (systolic BP plus 50 mmHg) to produce complete arterial occlusion. Muscle $V_{\text{O}_2}$ ($\Delta [O_2Hb]/min$) was calculated from the rate of linear decrease in $O_2Hb$ over the initial 5 s after the suprasystolic occlusion. The hyperemic arterial blood flow ($\Delta [THb]/min$) and reoxygenation rate ($\Delta [O_2Hb]/min$) were calculated by measuring the rates of increases in THb and $O_2Hb$, respectively, during the initial 5 s after the cessation of suprasystolic occlusions. The slopes of changes in the NIRS parameters were determined by standard computer algorithms (Oxymon, Artinis, The Netherlands).

Statistical Analysis

Data are expressed as means ± SE. The statistical software package StatView (SAS Institute) was used for data analysis. Experimental results were analyzed by 5 (groups) × 4 (time sample points; i.e., hypoxic and normoxic tests before and after the interventions) repeated-measures ANOVA and Bonferroni’s post hoc test to compare cardiac/skeletal muscular/cerebral hemodynamic parameters during NCXTs/HCXTs and normoxic/hypoxic IRTs at the beginning of this study and after 4 wk in the various groups. Additionally, the comparison of cardiopulmonary fitness at ventilation threshold or maximal exercise performance during GXTs at the beginning of this study and 4 wk later in the various groups were analyzed by 5 (groups) × 2 (time sample points; i.e., GXTs before and after the interventions) repeated-measures ANOVA and Bonferroni’s post hoc test. The criterion for significance was $P < 0.05$.

RESULTS

Exercise Performance and Hematological Parameters

The five experimental groups did not differ significantly in anthropometric parameters or in basic hematological (i.e., red blood cells, Hb, and hematocrit) parameters at the beginning of the study (Table 2). After 4 wk of the interventions, N-E, H-RE, and H-AE subjects revealed an increased work rate, VE, and $V_{\text{O}_2}$ at ventilation threshold and maximal exercise performance (Table 2). The H-AE group revealed a greater improvement in aerobic capacity than the N-E group (Table 2). However, no significant changes in exercise performance occurred after either the N-C or H-C interventions (Table 2). Additionally, all interventions for 4 wk did not influence the levels of red blood cells, Hb, and hematocrit at rest in the tested subjects (Table 2).

Cardiovascular Hemodynamics

Although NCXTs and HCXTs had similar CO levels (Table 3), acute exposure to 12% O$_2$ during exercise resulted in a lower SV (Fig. 2, A–E) and higher HR (Fig. 2, F–J) than the 21% O$_2$.

To assess the reliabilities of cardiac/skeletal muscular/cerebral hemodynamic responses to exercise and ischemia-reperfusion, subjects ($n = 6$) in a previous study were tested twice at 1-day intervals. The results of cardiac, VL, and FC hemodynamic responses to NCXTs and HCXTs were highly reproducible from day to day, and single-measure intraclass corrections were from 0.831 to 0.922 for test-retest reliability. Additionally, intraclass corrections of GN hemodynamic responses to normoxic and hypoxic IRTs were from 0.842 to 0.918 in this study.

Fig. 2. Effects of various normoxic and hypoxic regimens on stroke volume (SV; A–E) and HR (F–J) during normoxic CXTs (NCXTs) and hypoxic CXTs (HCXTs). A and F: N-C group; B and G: H-C group; C and H: N-E group; D and I: H-RE group; E and J: H-AE group. Pre-N, NCXT before the intervention; Pre-H, HCXT before the intervention; Post-N, NCXT after the intervention; Post-H, HCXT after the intervention. Levels of SV (A–E) and HR (F–J) at the 20-min continuous exercise stage were averaged and used as an index of the cardiac hemodynamic response to NCXT (or HCXT). Experimental results were analyzed by repeated-measures ANOVA and Bonferroni’s post hoc test. Values are means ± SE.
condition did. N-E only lowered exercise HR under the 21% O₂ condition (Fig. 2H) but did not affect exercise SV (Fig. 2C) under either the 12% or 21% O₂ condition. Both H-RE (Fig. 2I) and H-AE (Fig. 2J) decreased exercise HR under both 12% and 21% O₂ conditions. They simultaneously attenuated the suppression of exercise SV by the 12% O₂ exposure (Fig. 2, D and E). However, values of SaO₂, CO, MAP, or TPR during NCXTs or HCXTs remained unchanged after 4-wk interventions with N-C, H-C, H-E, H-RE, or H-AE (Table 3).

Perfusion and Oxygenation in Skeletal Muscles and Cerebral Tissues

The 12% O₂ exercise resulted in lower oxygenation (ΔO₂Hb) and higher deoxygenation (ΔHHb) of the VL muscle and FC compared with the 21% O₂ exercise, whereas perfusion (ΔTHb) of the VL muscle and FC did not significantly differ between NCXTs and HCXTs (Table 4). After 4 wk of interventions, H-RE and H-AE groups exhibited increased perfusion (Fig. 3, D and E) and deoxygenation (Fig. 3, I and J) of the VL muscle during both NCXTs and HCXTs (Table 4). However, the N-E group only exhibited enhanced 21% O₂ exercise-induced perfusion (Fig. 3C) and deoxygenation (Fig. 3H) of the VL muscle (Table 4). None of the normoxic or hypoxic interventions affected perfusion (Fig. 4, A–E) or deoxygenation (Fig. 4, F–J) of the FC during either NCXTs or HCXTs (Table 4).

The Hyperemic Response, Reoxygenation Rate, and Muscle VO₂ in Skeletal Muscles

Figure 5 showed a NIRS analysis of GN hemodynamics during the IRTs. Although acute exposure to 12% O₂ did not change basal arterial blood flow in the GN muscle, both hyperemic arterial blood flow and the reoxygenation rate at the reperfusion phase after arterial occlusion were suppressed while subjects were exposed to this hypoxic environment (Table 5). Moreover, the 12% O₂ exposure also decreased muscle VO₂ in the GN muscle at the period of arterial occlusion (Table 5). Four-week interventions of H-RE and H-AE ameliorated the suppressions of the arterial hyperemic response, reoxygenation, and muscle VO₂ in the GN muscle by acute 12% O₂ exposure and actually enhanced these GN hemodynamic functions in this hypoxic environment (Table 5). However, no significant changes in GN vascular and metabolic responses to normoxic and hypoxic IRTs occurred after 4-wk interventions with N-C, H-C, and N-E (Table 5).

DISCUSSION

This investigation demonstrates clearly that exercise training combined with or without hypoxic exposure significantly improves the aerobic fitness of sedentary males by increasing their pulmonary ventilation and tissue O₂ delivery/utilization at ventilation threshold and maximal performance. Moreover, for enhancing aerobic fitness, H-AE training is more effective than N-E training. Notably, both H-RE and H-AE training regimens simultaneously 1) ameliorated the suppression of exercise cardiac SV caused by severe hypoxia, 2) increased skeletal muscle blood flow (i.e., increased ΔTHb) and O₂ extraction (i.e., increased ΔHHb) during both NCXTs and HCXTs, and 3) enhanced the skeletal muscle hyperemic response (i.e., increased ΔTHb/min) and reoxygenation rate (i.e., increased ΔO₂Hb/min) induced by hypoxic IRTs. Although the N-E...
training increased skeletal muscular perfusion and O₂ utilization during NCXTs, this training regimen does not result in adaptations of hemodynamic responses to HCXTs in the heart and skeletal muscles. None of the normoxic and hypoxic regimens affected the extent of cerebral perfusion and oxygenation during NCXTs or HCXTs.

Cardiac Hemodynamics

Noninvasive methods used to assess cardiac hemodynamic adaptations to hypoxic exercise training in humans have not been established until now. Recently, preclinical and clinical data have demonstrated the feasibility of using blood flow-related phase shifts of transthoracic electric signals for continuous noninvasive CO monitoring (22, 25, 34). Moreover, the accuracy, precision, and responsiveness of this bioreactance-based device have also been validated compared with the thermodilution technique, which is the gold standard for CO determination in the clinical setting (22). By applying a bioreactance-based measurement, this investigation is the first to demonstrate convincingly that, compared with the normoxic condition, acute exposure to 12% O₂ enhances the elevation of HR and diminishes the elevation of SV during exercise despite a similar change in the exercise CO level. However, 4 wk of the 15% O₂ exercise training regimens such as H-RE and H-AE can effectively ameliorate the suppression of exercise SV by 12% O₂ exposure. These results imply that the two hypoxic exercise regimens may induce hypoxic preconditioning effects on the heart, namely, daily episodes of moderate hypoxic exercise (i.e., 50% W_max or HRR_max under 15% O₂), which increases the resistance of the heart to severe hypoxic (12% O₂) stress. However, when exercise is performed under the same workload during normoxia and hypoxia, a relative higher intensity, indicated by elevated exercise HR, occurs during exposure to hypoxia (Table 1). Alternatively, if the exercise HR level is the same as in N-E, H-RE requires a less intensive training workload than H-AE to improve aerobic capacity and simultaneously increase resistance to depression of exercise SV caused by severe hypoxia. However, neither the N-E nor H-C interventions affected the depression of exercise SV by the 12% O₂ exposure, which suggests that either moderate exercise (50% W_max) alone or hypoxic (15% O₂) intervention alone is insufficient to trigger myocardial preconditioning against cardiac dysfunction when performing exercise in an extremely hypoxic environment.

Animal studies (2, 6) have indicated that acute intermittent hypoxia improves myocardial tolerance to ischemia by activating nitric oxide synthase (NOS) and mitochondrial ATP-sensitive K⁺ (K_{ATP}) channels (2, 6). Moreover, the severity of intervened hypoxia is apparently a required condition for cardioprotection (2, 3). Hypoxia and physical exercise are independent and highly potent metabolic stressors (26). Acute hypoxic exposure reduces the S_aO₂ level, whereas physical exercise elevates V\dot{O}_₂ by working organs (e.g., the heart and skeletal muscles). Therefore, hypoxic exercise markedly lowers O₂ concentration or saturation of working organs by simultaneously decreasing the O₂ supply and increasing the O₂ demand. Because of this augmented hypoxemia by hypoxic exercise, the effect of myocardial preconditioning by enhanced
Fig. 4. Effects of various normoxic and hypoxic regimens on perfusion (A–E) and deoxygenation (F–J) in the frontal cerebral lobe (FC) during NCXTs and HCXTs. A and F: N-C group; B and G: H-C group; C and H: N-E group; D and I: H-RE group; E and J: H-AE group. Changes in the levels of perfusion (A–E) and deoxygenation (F–J) at the 20-min continuous exercise stage were averaged and used as an index of the FC hemodynamic response to NCXTs (or HCXTs). Values are means ± SE.

Fig. 5. Near-infrared spectroscopic analysis on gastrocnemius (GN) hemodynamics during IRTs. The basal arterial inflow describes the maximal velocity of the blood volume change (THb, green line) in the measured GN muscle during venous occlusion (subdiastolic occlusion pressure: 60 mmHg). Muscle O₂ consumption was calculated from the rate of the linear decrease in O₂Hb over the initial 5 s after arterial occlusion (systolic blood pressure plus 50 mmHg). Hyperemic arterial inflow and reoxygenation were calculated by measuring the rates of increases in THb (green line) and O₂Hb (red line), respectively, during the initial 5 s after the cessation of arterial occlusion.

NOS or/and K<sub>ATP</sub> channel activation(s) may be larger than that obtained by hypoxia or exercise alone. Additionally, the protective effects of hypoxic exercise training on cardiovascular systems are also likely associated with increasing coronary blood flow and cardiomyoglobin content as well as upregulated expression of antioxidant enzymes and stress-related proteins (12, 15). Recently, Wang et al. (43) also demonstrated that intermittent 12–15% O₂ exposures for 4–8 wk simultaneously hindered lipid peroxidation and proinflammatory cytokine IL-1β production generated by severe exercise. Moreover, chronic intermittent 12% O₂ exposure further increased circulatory anti-inflammatory cytokines IL-6 and IL-10, which inhibit IL-1β production during intense exercise (43). Therefore, these adaptations of hypoxic interventions may protect against oxidative stress and inflammation associated with cardiac dysfunctional processes.

Some investigators (5) have suggested that chronic continuous hypoxia, such as acclimatization to high altitude, induces erythropoiesis by increased erythropoietin production, which contributes to enhance O₂ delivery to tissues. However, the present study indicated that 15% O₂ exercise training in a normobaric hypoxia chamber did not influence the levels of red blood cells, Hb, and hematocrit at rest in tested subjects. Therefore, the restoration of the increased SV response to exercise in both the H-RE and H-AE groups may not result from increased myocardial O₂ delivery by accelerated erythropoiesis. A previous investigation (18) has also demonstrated that intermittent hypobaric hypoxia exposure (3 h/day, 5 days/wk, at 4,000–5,500 m) did not accelerate erythropoiesis despite the increase in serum erythropoietin. Hence, a possible explanation for the different findings in hematological parameters from chronic continuous hypoxia is that the relatively short episode (30 min/day for 4 wk) of moderate hypoxia (15% O₂) with exercise may fail to cause sustained accelerated erythropoiesis.
Table 5. Effects of the various normoxic and hypoxic regimens on hemodynamics in the gastrocnemius muscle during ischemia-reperfusion tests

<table>
<thead>
<tr>
<th>N-C Group</th>
<th>H-C Group</th>
<th>N-E Group</th>
<th>H-RE Group</th>
<th>H-AE Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal arterial inflow, (M)/min</td>
<td>2.2</td>
<td>21.5</td>
<td>3.1</td>
<td>20.6</td>
</tr>
<tr>
<td>[THb] (M)/min</td>
<td>2.1</td>
<td>20.8</td>
<td>2.1</td>
<td>20.1</td>
</tr>
<tr>
<td>Hyperemic arterial inflow, (M)/min</td>
<td>2.7</td>
<td>21.5</td>
<td>2.2</td>
<td>20.1</td>
</tr>
<tr>
<td>[THb] (M)/min</td>
<td>2.1</td>
<td>20.8</td>
<td>2.1</td>
<td>20.1</td>
</tr>
<tr>
<td>VO2, (M)/min</td>
<td>2.15</td>
<td>2.10</td>
<td>2.15</td>
<td>2.1</td>
</tr>
<tr>
<td>[Hb] (M)/min</td>
<td>2.1</td>
<td>2.1</td>
<td>2.1</td>
<td>2.1</td>
</tr>
<tr>
<td>Reoxygenation, (M)/min</td>
<td>4.5</td>
<td>4.6</td>
<td>4.5</td>
<td>4.6</td>
</tr>
</tbody>
</table>

Values are means ± SE. *P < 0.05, preintervention vs. postintervention; †P < 0.05, N-E group vs. H-RE or H-AE groups; ‡P < 0.05, H-C group vs. N-E group; •P < 0.05, 12% O2 vs. 21% O2.

One the other hand, our recent study (21) indicated that both exposures to 12–15% O2 decreased total power and high-frequency power and increased low-frequency power and the ratio of low-frequency to high-frequency power, indicating a change in cardiac autonomic activity of the sinus node in response to acute hypoxic stimulation. However, only exposure to 12% O2, but not 15% O2, downregulates autonomic responses to physical stress. Long-term exercise training is known to improve HR variability in healthy adults and patients with cardiovascular diseases (32). Moreover, Miura et al. (23) demonstrated that cardiac sympathetic nerve injury during coronary occlusion was attenuated by ischemic preconditioning via the activation of KATP channels. However, no clear and comprehensive picture of hypoxic exercise training-mediated HR variability has emerged in the literature. Whether 15% O2 exercise training ameliorated the impairment of cardiac autonomic function, as with the SV response to exercise, by the 12% O2 exposure needs to be further investigated.

Skeletal Muscle Hemodynamics

The NIRS signal reflects the dynamic balance between muscle capillary blood flow and muscle VO2 in the microenvironment. These factors may induce an adaptive response to hypoxic exercise training mediated by hypoxia-inducible factor-1 (33). Hypoxia-inducible factor-1 is activated in hypoxic conditions and results in transcriptional initiation response mediated by hypoxia-inducible factor-1 (33). Hypoxia-inducible factor-1 is activated by hypoxia through a well-defined mechanism and results in upregulated expression of numerous gene-encoding proteins, such as erythropoietin, VEGF, and inducible NOS (33). Additionally, hypoxia-induced elevation of plasma IL-6 has been suggested to have a costimulatory effect on VEGF (9) production, which further promotes angiogenesis to acclimatize to a hypoxic environment. These factors may induce an adaptive response to hypoxia that increases tissue perfusion and oxygenation, thereby overcoming severe hypoxic insult.

Cerebral Hemodynamics

Under hypoxic environmental conditions such as high altitude, reductions in global cerebral oxygenation are associated with impairments in central motor drive (29, 37). Although Gonzalez-Alonso et al. (17) argued that reduced cerebral oxygenation does not represent a limit to exercise performance, Nielsen et al. (27) reported that the administration of supplemental O2 maintains cerebral oxygenation and increases time...
trial performance without affecting muscle oxygenation, which suggests a central limit to exercise performance. However, Møller et al. (24) demonstrated that cerebral blood flow and oxidative metabolism are unchanged after acclimatization to high altitude, despite marked adaptation in systemic respiratory functions. In the present investigation, HCXTs before the normoxic or hypoxic interventions elicited more deoxygenation in the FC (indicated by increased ΔHHb) than NCXTs did, even when FC perfusion was comparable (indicated by similar ΔTHb) in NCXTs and HCXTs. However, 4 wk of either normoxic or hypoxic exercise training did not influence the extent of cerebral perfusion and oxygenation during NCXTs and HCXTs. These findings are in agreement with a previous report (24) showing that acclimatization to a hypoxic environment may not involve adaptations of cerebral hemodynamics.

Limitations of the Study and Technical Considerations

As in numerous other investigations, a limitation of this study is that the subjects tended to be young and healthy. Thus, further clinical evidence is needed to extrapolate the present results to elderly patients or to those with abnormal cerebrovascular/cardiovascular systems such as patients with stroke or myocardial ischemia. Additionally, the small group size ($n = 12$) is also a major limitation of this study. However, the results obtained from this investigation have high values of statistical power, from 0.886 to 1.000. Moreover, the subjects in this study had no the hemodynamic adaptation status to exercise or hypoxic stimulations before the intervention because none of the subjects had engaged in any regular physical activity or exposure to high altitude. In fact, subjects who failed to demonstrate cardiac/skeletal muscular/cerebral hemodynamic responses to the $12\%$ O$_2$ exercise had only $\leq 1$ person/group before the intervention.

NIRS measurements have been well correlated with intracellular O$_2$ tension in muscle (38) and venous O$_2$ saturation in both muscle (14) and cerebral tissue (20). However, the relative contribution of skin blood flow to NIRS tissue signals has been questioned because NIRS probes are typically placed on the skin surface. The influence of skin blood flow has been estimated to account for $\approx 15–23\%$ of NIR light attenuation; thus, underlying tissues are the primary determinants of the resulting NIRS measurements (7). This study used skinfold measurements to calculate the thickness of the skin and adipose, and no significant changes in the thicknesses of the tested surface areas were found after any intervention (data not shown). Therefore, the skin blood flow detected by NIRS measurements is not likely to affect the conclusions of this investigation. In this study, acclimatization of cerebral hemodynamics to the hypoxic environment was not observed after either H-RE or H-AE interventions. Since the cranium makes up a significant portion of the head, it is possible that the reason that no hemodynamic changes were seen in the cerebral measurements resulted from the fact that little cerebral tissue was investigated. Additionally, the possibility that hemodynamics in other cerebral lobes were altered by hypoxic preconditioning cannot be ruled out because the cerebral hemodynamic measurements in this study were limited to the FC lobe. Therefore, the assessment of various cerebral lobe hemodynamic responses to hypoxic exercise using NIRS with multiple probes needs further investigation. On the other hand, we did not have direct measures of CO with which to compare the NICOM device despite the high reproducibility of the bioreactance-based measurements. Direct evaluation of CO responses to normoxic or hypoxic interventions that uses a “gold standard” measurement, such as thermodilution, needs further work.

Conclusions

As with intermittent hypoxia, as previously investigated, the hypoxic exercise regimens designed in this study can enhance pulmonary ventilation and aerobic capacity in a normoxic environment. This investigation further demonstrated that both H-AE and H-RE improved exercise performance under the $12\%$ O$_2$ condition, apparently by ameliorating suppression of myocardial contractility and increasing perfusion and O$_2$ utilization of exercising skeletal muscles rather than by regulating hemodynamics of cerebral tissues. Moreover, the H-RE intervention conferred similar cardiac and muscular hemodynamic adaptations in a hypoxic environment as the H-AE intervention. Since the H-RE intervention requires a lighter training workload than the H-AE intervention, H-RE training can be considered an “effective and modest workload” strategy for improving individual aerobic capacity while minimizing the risk of hemodynamic dysfunction caused by severe hypoxia.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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