Endurance training attenuates the increase in peripheral chemoreflex sensitivity with intermittent hypoxia

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Miller AJ, Sauder CL, Cauffman AE, Blaha CA, Leuenberger UA. Endurance training attenuates the increase in peripheral chemoreflex sensitivity with intermittent hypoxia. Am J Physiol Regul Integr Comp Physiol 312: R223–R228, 2017. First published December 30, 2016; doi:10.1152/ajpregu.00105.2016.—Patients with heart failure and sleep apnea have greater chemoreflex sensitivity, presumably due to intermittent hypoxia (IH), and this is predictive of mortality. We hypothesized that endurance training would attenuate the effect of IH on peripheral chemoreflex sensitivity in healthy humans. Fifteen young healthy subjects (9 female, 26 ± 1 yr) participated. Between visits, 11 subjects underwent 8 wk of endurance training that included running four times/wk at 80% predicted maximum heart rate and interval training, and four control subjects did not change activity. Chemoreflex sensitivity (the slope of ventilation responses to serial oxygen desaturations), blood pressure, heart rate, and muscle sympathetic nerve activity (MSNA) were assessed before and after 30 min of IH. Endurance training decreased resting systolic blood pressure (119 ± 3 to 113 ± 3 mmHg; P = 0.027) and heart rate (67 ± 3 to 61 ± 2 beats/min; P = 0.004) but did not alter respiratory parameters at rest (P > 0.2). Endurance training attenuated the IH-induced increase in chemoreflex sensitivity (pretraining: Δ 0.045 ± 0.026 vs. posttraining: Δ −0.028 ± 0.040 l·min⁻¹·% O₂ desaturation⁻¹; P = 0.045). Furthermore, IH increased mean blood pressure and MSNA burst rate before training (P < 0.05), but IH did not alter these measures after training (P > 0.2). All measurements were similar in the control subjects at both visits (P > 0.05). Endurance training attenuates chemoreflex sensitization to IH, which may partially explain the beneficial effects of exercise training in patients with cardiovascular disease.

intermittent hypoxia; peripheral chemoreflex; exercise training; blood pressure; muscle sympathetic nerve activity

EXERCISE TRAINING has profound beneficial effects on cardiovascular function and improves outcomes in patients with a range of cardiovascular diseases. Although the mechanisms remain incompletely understood, it is known that exercise training evokes vascular adaptations (6, 15), alters autonomic reflexes (19, 28), and increases antioxidants (8, 20).

The peripheral chemoreflex is an autonomic reflex mediated by O₂-sensing cells in the carotid body that signal increases in ventilation and muscle sympathetic nerve activity (MSNA) to avert hypoxia (25). Patients with congestive heart failure, hypertension, and sleep apnea have enhanced peripheral chemoreflex sensitivity (30). In heart failure, peripheral chemoreflex sensitivity increases in proportion to disease severity and predicts prognosis (23). In these disease states, it is hypothesized that repetitive hypoxic stress alters carotid body function and autonomic reflexes (5, 30). Furthermore, intermittent exposure to hypoxia can increase the sensitivity of this reflex in healthy humans. Our group and others found that exposure to short-term intermittent hypoxia (IH) increases MSNA, blood pressure (BP), and chemoreflex sensitivity to hypoxic stimuli in healthy humans (3, 10, 11, 13). Thus, in healthy humans, exposure to IH appears to induce a state of sympathetic overactivity and altered chemoreflex function that characterizes disease states. Therefore, studying ways to ameliorate the effects of IH on peripheral chemoreflex potentiation is imperative.

Animal studies reveal that chronic intermittent hypoxia increases reactive oxygen species (ROS) activity in the carotid body, which increases peripheral chemoreflex sensitivity (24). In animal models of heart failure, exercise training decreases chemoreflex sensitivity (12, 14) and improves autonomic function by increasing antioxidant activity (7). Whether exercise training alters peripheral chemoreflex sensitivity in humans is unknown. Therefore, the purpose of this study was to investigate the effects of 8 wk of endurance training on peripheral chemoreflex sensitization to short-term IH. We hypothesized that endurance training would attenuate the IH-induced increase in peripheral chemoreflex sensitivity in healthy humans.

METHODS

Design and subjects. The study utilized a within-subjects, repeated-measures design, in which physiological parameters were measured at two sets of laboratory visits (two visits in each set) separated by two months. Visits 1 and 2 (pretraining baseline), and visits 3 and 4 (posttraining or time control), respectively, were performed during the same week. In laboratory visits 2 and 4, data were acquired continuously at baseline and after 30 min of consecutive hypoxic breath holds (intermittent hypoxia, IH). This IH protocol has previously been shown to lead to a sustained increase in muscle sympathetic nerve activity (10, 11) and an increase in the sympathetic responses to sustained hypoxia (11). All study protocols were approved in advance by the Institutional Review Board of the Penn State Milton S. Hershey Medical Center and conformed to the Declaration of Helsinki. All volunteers provided written informed consent.

Fifteen young healthy subjects (9 female, 26 ± 1 yr) participated in this study. Eleven subjects were enrolled as training subjects and underwent 8 wk of endurance training between visits 2 and 3. A sample size calculation conducted after the first four subjects had completed chemoreflex testing before and after training revealed that if the true difference in the change in chemoreflex sensitivity to intermittent hypoxia with endurance training was 0.15 l·min⁻¹·% O₂ desaturation⁻¹, with a standard deviation of
0.13, we would need to enroll 10 subjects to have 0.9 power and an α of 0.05. We enrolled 11 subjects in the training protocol to account for attrition and potential missing data. Four subjects (three female, 30 ± 2 yr) were enrolled as control subjects and did not change their activity between visits. All subjects had supine resting blood pressures below 120/80 mmHg and were nonasthmatic, nonobese, nonsmokers, not taking any prescription or vasoactive medication, and were in good health, as determined by a history and physical examination. All subjects were moderately active but not competitive athletes. All subjects refrained from caffeine, alcohol, and exercise for 24 h before each laboratory visit.

Protocol. All subjects completed four laboratory visits on separate days. Visits 1 and 2 (pretraining baseline), as well as visits 3 and 4 (posttraining or time control), respectively, were performed a few days apart, whereas the pretraining baseline and posttraining or time control studies were separated by 2 mo. All study protocols were performed in a thermoneutral laboratory (20–21°C) in the morning or afternoon hours.

Endurance testing. At visits 1 and 3, maximal exercise capacity (V\textsubscript{O2peak}) was determined to assess individual fitness levels before and after training (or time control). At both visits, V\textsubscript{O2peak} (ml·kg\textsuperscript{-1}·min\textsuperscript{-1}) was determined during graded treadmill exercise and inspiratory/expiratory gas analysis, as performed previously by our group (2, 27). After a 5-min warm-up to determine speed, subjects began exercising at a 0% incline. The incline increased by 2.5% every 2 min up to a 10% incline (8 min) or fatigue. Oxygen consumption plateaued in all subjects, indicating maximal exertion. V\textsubscript{O2peak} represented the average value of V\textsubscript{O2} over the last 30 s of the test.

Chemoreflex testing. At visits 2 and 4, chemoreflex sensitivity was assessed before and after IH. All testing at visits 2 and 4 was performed in the supine position. Chemoreflex testing was performed before and after IH using the Dejous technique that has been described previously (4, 10, 23). Briefly, subjects were exposed to a series of 10 brief trials of pure N\textsubscript{2} inhalation (2–8 breaths) to produce a range of transient O\textsubscript{2} desaturations [70–90% arterial O\textsubscript{2} saturation (SpO\textsubscript{2})]. In each trial, the maximal ventilatory response averaged over two breaths was plotted against the coinciding SpO\textsubscript{2} nadir. Chemoreflex slopes (1·min\textsuperscript{-1}·% O\textsubscript{2} desaturation\textsuperscript{-1}) were calculated.

Intermittent hypoxia. After baseline measurements of chemoreflex sensitivity, subjects performed 30 consecutive 20-s hypoxic breath holds (1 per minute for 30 min), as described previously (10, 11). To enhance hypoxia, subjects were given hypoxic gas (F\textsubscript{IO\textsubscript{2}} 0.1) for 20 s before each breath hold. This produced SpO\textsubscript{2} nadirs with each breath hold of ~80%. After IH, hemodynamic and ventilatory parameters were measured over 5 min (post-IH recovery) before chemoreflex testing was repeated.

Handgrip exercise. Five of the subjects performed isometric handgrip to see whether IH and/or training altered other autonomic reflexes, such as the exercise pressor reflex. The handgrip trial was skipped if subjects were unable to comply with the protocol for the additional required time. For the first handgrip trial (pre-IH at visit 2) subjects contracted their hand and forearm muscles at 30% of their maximal voluntary contraction until fatigue using a handgrip dynamometer. For subsequent handgrip trials, subjects gripped for the same amount of time and workload as their first trial. Ten seconds before relaxation, a pneumatic cuff (Hokanson, Bellevue, WA) on the arm was inflated to 250 mmHg to trap the metabolites in the exercising muscle and isolate the metabolic component of the exercise pressor reflex (16). There was a 15-min rest period after handgrip and before intermittent hypoxia to allow physiological parameters to return to baseline.

Training protocol. Between visits 2 and 3, training subjects (n = 11) underwent endurance exercise training, which consisted of four supervised running sessions per week for 8 wk. Subjects wore a heart rate (HR) monitor (Polar S160; Polar Electro, New York, NY) during training sessions and ran at a pace sufficient to maintain a HR of at least 80% of their age-predicted maximum HR. The training schedule began with 20 min of running each session and increased by 5 min each week up to 60 min. After the first 2 wk of training, twice weekly interval exercises (hills and sprints) were included in training sessions to maximize the training effect. Interval training increased in difficulty each week, as previously described (2, 27). Control subjects (n = 4) did not change their activity between visits.

Measurements. At visits 2 and 4, HR and BP were acquired continuously with a three-lead ECG (CardioCap5; GE Healthcare, New York, NY) and a finger BP pressure cuff (Finometer, FMS, Amsterdam, The Netherlands). Resting BPs were obtained in triplicate by automated oscillometry of the left brachial artery (SureSigns V3S; Philips Healthcare, Baltimore, MD) after 15 min of quiet rest and were used to verify the Finometer values, as previously described (29). To measure ventilatory parameters, subjects were instrumented with a pneumotrace to monitor respiratory movement and a face mask with separate inlet and outlet valves. The inlet port was open to ambient room air or connected to nitrogen (N\textsubscript{2}, F\textsubscript{IO\textsubscript{2}}, 1.0) or hypoxic gas (F\textsubscript{IO\textsubscript{2}}, 0.1). The expiratory port was connected to a respiratory gas monitor (Ohmeda RGM 5200; GE Healthcare) to monitor minute ventilation (Ve), breathing rate, and end-tidal CO\textsubscript{2}. SpO\textsubscript{2} was measured with an ear oximeter.

Multifiber recordings of MSNA were obtained via peroneal nerve microneurography, as previously described (10, 11, 17, 31). Briefly, a tungsten microelectrode was inserted into the peroneal nerve below the fibular head, and a reference electrode was placed subcutaneously 2–3 cm away from the recording electrode. The recording electrode was adjusted until a site with clear sympathetic nerve traffic to skeletal muscle was identified using established criteria (36). The nerve signal was amplified, band-pass filtered with a bandwidth of 500–5,000 Hz, and integrated with a time constant of 0.1 s (662C-3 Nerve Traffic Analysis System, University of Iowa Bioengineering, Iowa City, IA). The nerve signal was also routed to a loudspeaker and a computer for monitoring throughout the study. The integrated MSNA signal was evaluated offline using custom software to identify bursts based on fixed criteria of shape, latency following the R-wave of the ECG, and 3:1 signal-to-noise ratio compared with the baseline.

Data analysis and statistics. All data were sampled at 500 Hz via PowerLab (PowerLab 16/35, ADInstruments) and analyzed offline. Data were analyzed in 1-min bins and then were averaged across the baseline, stressor, or recovery period. To assess the effects of IH, data were acquired for 5 min before and after IH. The effects of time on physiological parameters were analyzed with paired Student’s t-tests. In training subjects, repeated-measures ANOVA was used to determine significant interactions between time (pretraining, posttraining) and treatment (pre-IH, post-IH). For significant interactions, post hoc Tukey’s tests were employed to determine the effects of IH at each time point. In control subjects, data were compared between visits with Student’s t-tests. Between-group comparisons were not made due to variable sample sizes. All data are shown as means ± SE. Significance was set at P < 0.05 for all tests.

RESULTS

Anthropometric and resting hemodynamic data before and after endurance training are shown in Table 1. Eight weeks of endurance training increased V\textsubscript{O2peak} in all subjects on average by 3.7 ± 0.2 ml·kg\textsuperscript{-1}·m\textsuperscript{-1}. Endurance training also decreased resting systolic BP and HR but did not change resting diastolic BP, weight, or body mass index (BMI). Training did not alter mean BP (P = 0.256) or any respiratory parameters (P > 0.2; Table 2). Resting hemodynamics and V\textsubscript{O2peak} did not change in control subjects between visits (Table 1).
The effects of endurance training and IH on physiological measures are shown in Table 2. IH increased mean BP pretraining (P = 0.031) but not posttraining (P = 0.107). IH decreased HR pretraining (P = 0.018) but did not alter HR posttraining (P = 0.340). IH decreased Sp O2 minimally pretraining (P = 0.019) but not posttraining (P = 0.334). IH decreased end-tidal CO 2 both slightly before (P = 0.003) and after training (P = 0.005). IH did not affect V E pretraining (P = 0.100) or posttraining (P = 0.361). Pretraining, IH increased ventilatory chemoreflex sensitivity to hypoxia (P = 0.050). Yet, posttraining the chemoreflex slope was not changed (P = 0.246). Endurance training attenuated the IH-induced increase in chemoreflex sensitivity (pretraining: Δ0.045 ± 0.026 vs. posttraining: Δ−0.028 ± 0.040 l·min −1·% O 2 desaturation −1; P = 0.045, Fig. 1). Individual chemoreflex sensitivity data from the 11 subjects (i.e., chemoreflex slopes) are shown in Table 3.

Successful MSNA recordings were acquired at both study visits (pretraining and posttraining) in six training subjects. Training did not alter baseline MSNA (P = 0.406). Pretraining, IH increased MSNA burst rate (P = 0.044). However, posttraining, IH did not change MSNA burst rate (P = 0.259). A subset of five training subjects performed isometric handgrip exercise at 30% maximum voluntary contraction to fatigue at both study visits before and after IH. Successful MSNA recordings were obtained during handgrip exercise at both visits in three subjects. Physiological responses to handgrip exercise were similar before and after training. Training did not alter the mean BP (P = 0.242), HR (P = 0.169), or MSNA (P = 0.286) responses to handgrip exercise. The mean BP response to handgrip exercise was not affected by IH pretraining (pre-IH: Δ17 ± 4 vs. post-IH: Δ12 ± 6 mmHg; P = 0.124) or posttraining (pre-IH: Δ20 ± 5 vs. post-IH: Δ15 ± 5 mmHg; P = 0.171). The HR response to handgrip was similar before and after IH pretraining (Δ22 ± 4 vs. Δ15 ± 6 beats/min, P = 0.190) and posttraining (Δ18 ± 4 vs. Δ26 ± 6 beats/min, P = 0.322). MSNA responses to handgrip were not different before and after IH pretraining (Δ10 ± 2 vs. Δ11 ± 4 bursts/min; P = 0.814) and posttraining (Δ7 ± 2 vs. Δ5 ± 2 bursts/min; P = 0.467).

Control subjects were moderately active and were phenotypically similar to subjects who subsequently underwent training (BMI: 23.5 ± 1.8 kg/m 2, V O2peak: 35.2 ± 0.8 ml·kg −1·min −1). Resting minute ventilation and hemodynamics were also similar to the training group (mean BP: 86 ± 4 mmHg, HR: 65 ± 6 beats/min, V E: 8.14 ± 0.48 l/min, CO 2: 39.4 ± 0.71 mmHg, Sp O2: 97 ± 1%). In control subjects, IH increased chemoreflex sensitivity from 0.345 ± 0.022 to 0.408 ± 0.083 l·min −1·% O 2 desaturation −1 at initial testing and from 0.362 ± 0.098 to 0.413 ± 0.078 l·min −1·% O 2 desaturation −1 8 wk later. The effect of IH on chemoreflex sensitivity was not statistically different between visits in control subjects (P = 0.896). BMI, V O2peak, and resting BP and HR also did not vary between visits in control subjects studied 8 wk apart (Table 1).

Table 2. Effects of endurance training and intermittent hypoxia on hemodynamic, respiratory, and neural parameters

<table>
<thead>
<tr>
<th></th>
<th>Pretraining (n = 11)</th>
<th>Post-IH</th>
<th>Pretraining (n = 11)</th>
<th>Post-IH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean BP, mmHg</td>
<td>85 ± 2</td>
<td>86 ± 2*</td>
<td>83 ± 2†</td>
<td>84 ± 2</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>67 ± 3</td>
<td>63 ± 3*</td>
<td>61 ± 2</td>
<td>60 ± 2</td>
</tr>
<tr>
<td>Minute ventilation, l/min</td>
<td>7.66 ± 0.36</td>
<td>7.59 ± 0.41</td>
<td>7.28 ± 0.39</td>
<td>7.98 ± 0.55</td>
</tr>
<tr>
<td>Respiratory frequency, breaths/min</td>
<td>11 ± 1</td>
<td>13 ± 1</td>
<td>12 ± 1</td>
<td>14 ± 1</td>
</tr>
<tr>
<td>Tidal volume, liters</td>
<td>0.69 ± 0.06</td>
<td>0.61 ± 0.06</td>
<td>0.70 ± 0.12</td>
<td>0.60 ± 0.05</td>
</tr>
<tr>
<td>End-tidal CO 2, mmHg</td>
<td>40.7 ± 0.8</td>
<td>37.9 ± 0.6*</td>
<td>40.1 ± 0.5</td>
<td>37.5 ± 0.7*</td>
</tr>
<tr>
<td>Sp O2, %</td>
<td>98 ± 0.2</td>
<td>97 ± 0.3*</td>
<td>97 ± 0.2</td>
<td>97 ± 0.2</td>
</tr>
<tr>
<td>Chemoreflex slope, l·min −1·% O 2 desaturation −1</td>
<td>0.261 ± 0.032</td>
<td>0.305 ± 0.032*</td>
<td>0.290 ± 0.044</td>
<td>0.262 ± 0.036</td>
</tr>
<tr>
<td>MSNA burst rate, bursts/min (n = 6)</td>
<td>16 ± 3</td>
<td>23 ± 5*</td>
<td>15 ± 3</td>
<td>20 ± 2</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SE; n, number of subjects. IH, intermittent hypoxia; Sp O2, arterial O 2 saturation; MSNA, muscle sympathetic nerve activity. *P < 0.05 pre-IH vs. post-IH. †P < 0.05 Pretraining vs. Posttraining.
ROS. Given that both ROS and chemoreflex sensitivity are chemoreflex sensitization to IH is partially attributed to pretreatment with ROS scavengers prevents the chronic that may alter autonomic reflexes (22, 26). Furthermore, increases oxidative stress in the carotid body and brain stem, similar to what occurs in ischemia-continuous hypoxia, IH includes periodic reoxygenation current and in previous studies (10, 11). Compared with peripheral chemoreflex in healthy humans, as shown in the \( n \)-fold increase in MSNA, but not HR and BP, responses to handgrip exercise (18, 31, 32). However, our training protocol concluded that running did not specifically train the forearm muscles, and our sample size for MSNA during handgrip was small \( (n = 3) \). Our data support that the effect of endurance training specifically prevents the rise in peripheral chemoreflex sensitivity, BP, and MSNA but does not alter other autonomic reflexes, such as the exercise pressor reflex.

While our previous work supports that IH increases chemoreflex sensitivity (11), all data from each subject were collected on the same day, and the test-retest reliability of chemoreflex sensitivity measurements was previously unknown. Although our study was not powered to test this hypothesis with equivalence testing \( (n = 4) \), the data from our control subjects support that measures of hypoxic ventilatory chemoreflex responses before and following IH are repeatable.

Limitations. We recognize that our study sample is small, and markers of intracellular oxidative stress and antioxidant capacity are lacking. Therefore, we cannot determine whether the mechanism responsible for the apparent protective effect of exercise training on the peripheral chemoreflex is due to an attenuation of oxidative stress. While investigations in animal models support the role of antioxidants in chemoreflex protection (7, 12), future studies are needed to investigate potential mechanisms in humans. Whereas all studies were performed during the daytime hours, we did not account for potential diurnal influences on chemoreflex function since some repeat studies in some subjects took place at different times during the day. In addition, for reasons of subject preference, we did not...
randomize the subjects to the training or control groups. Furthermore, in the women studied, we made no concerted effort to account for potential effects of the menstrual cycle phase on chemoreflex function. However, while menstrual cycle may affect cardiovascular control, all subjects served as their own control, and the pretraining and posttraining studies took place 8 wk (approximately two menstrual cycles) apart, making it likely that the pretraining and posttraining chemoreflex studies took place in the same endocrine environment. Finally, although our goal was to examine chemoreflex sensitivity to hypoxia, the repetitive breath holds in the IH paradigm also resulted in intermittent elevations of CO2 that may have affected chemoreflex function. Therefore, whereas intermittent rises of CO2 could have contributed to the effects of intermittent hypoxia, the prevailing end-tidal CO2 during chemoreflex testing before and after IH, and before and after training was comparable, making it unlikely that differences in CO2 would affect the interpretation of the study.

Perspectives and Significance

We found that endurance training attenuates the IH-induced peripheral chemoreflex potentiation. These data may also help explain the beneficial effects of exercise training on the cardiovascular system and in patients with heart failure. In addition, this study may inform future investigations on mechanisms that underlie the effects of exercise on carotid body chemoreceptor activity.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


REFERENCES


