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RESEARCH ARTICLE

Hypoxia

Short exposure to intermittent hypoxia increases erythropoietin levels in healthy individuals

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Abstract

Few minutes of hypoxic exposure stabilizes hypoxia-inducible factor-1α, resulting in erythropoietin (EPO) gene transcription and production. The objective of this study was to identify the shortest intermittent hypoxia protocol necessary to increase serum EPO levels in healthy individuals. In a first experiment, spontaneous EPO changes under normoxia (NORM) and the EPO response to five 4-min cycles of intermittent hypoxia (IH5) were determined in six individuals. In a second experiment, the EPO response to eight 4-min cycles of intermittent hypoxia (IH8) and 120 min of continuous hypoxia (CONT) was determined in six individuals. All hypoxic protocols were performed at a targeted arterial oxygen saturation of 80%. There was no significant change in EPO levels in response to normoxia or in response to five cycles of intermittent hypoxia (NORM: 9.5 ± 1.8 to 10.5 ± 1.8, IH5: 11.4 ± 2.3 to 13.4 ± 2.1 mU/mL, main effect for time $P = 0.35$). There was an increase in EPO levels in response to eight cycles of intermittent hypoxia and 120 min of continuous hypoxia, with peak levels observed 4.5 h after the onset of hypoxia (IH8: 11.2 ± 2.0 to 16.7 ± 2.2, CONT: 11.1 ± 3.8 to 19.4 ± 3.8 mU/mL, main effect for time $P < 0.01$). Eight cycles of intermittent hypoxia increased EPO levels to a similar extent as 120 min of continuous hypoxia (main effect for condition $P = 0.36$). Eight 4-min cycles of intermittent hypoxia represent the shortest protocol to increase serum EPO levels in healthy individuals.

NEW & NOTEWORTHY The objective of this study was to identify the shortest intermittent hypoxia protocol necessary to increase serum erythropoietin levels in healthy individuals. Eight 4-min bouts of intermittent hypoxia, representing a hypoxic duration of 32 min at an arterial oxygen saturation of 80%, significantly increased erythropoietin levels in healthy individuals. These findings suggest that a short session of intermittent hypoxia has the potential to increase oxygen-carrying capacity.

INTRODUCTION

Hypoxia, or low partial pressure of oxygen, triggers the release of erythropoietin (EPO), a glycoprotein that stimulates red blood cell production in order to increase oxygen-carrying capacity (1, 2). Exposure to hypoxia stabilizes hypoxia-inducible factor-1α (HIF-1α) within few minutes, which results in EPO gene transcription and production (2). Continuous exposure to hypoxia lasting between 84 to 120 min consistently increases serum EPO levels (3–9). However, exposure to several short successive bouts of hypoxia also stabilizes HIF-1α and triggers similar increases in EPO levels. Indeed, a 4-h intermittent hypoxia protocol consisting of 2.5 min of hypoxia alternating with 1.5 min normoxia significantly increased EPO levels (5). Moreover, several sessions of intermittent hypoxia, consisting of 3–5 hypoxic cycles lasting 3–5 min interspersed with 3-min normoxic cycles at an arterial oxygen saturation of ~80% increased red blood cell count in elderly men with and without coronary artery disease, suggesting that each session of intermittent hypoxia triggers the release of EPO (10). These findings suggest that intermittent hypoxia represents a time-efficient approach to induce increases in EPO levels. The objective of the present study was to identify the shortest intermittent hypoxia protocol necessary to increase EPO levels. The EPO response to the following four protocols was determined: *1*) spontaneous change under normoxia; *2*) five 4-min cycles of intermittent hypoxia; *3*) eight 4-min cycles of intermittent hypoxia; and *4*) 120 min of continuous hypoxia. All hypoxic protocols were performed at a targeted arterial oxygen saturation of 80%. It was hypothesized that both intermittent hypoxia protocols would significantly increase EPO levels in healthy individuals.

METHODS

A total of seven healthy, recreationally active adults participated in the study. Participants were excluded from the study if they had uncontrolled hypertension, were smokers, pregnant, had a history of cardiovascular disease, diabetes or lung disease, or were taking medication affecting the cardiovascular system. All participants provided informed written consent to participate in the study, which was approved by the Institutional Review Board of the University of Texas at

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Austin. The study consisted of two separate experiments. In *experiment 1*, the EPO response to five 4-min cycles of intermittent hypoxia (IH5) was compared with the spontaneous change in EPO levels under normoxic conditions (NORM) to account for diurnal variations. In *experiment 2*, the EPO response to eight 4-min cycles of intermittent hypoxia (IH8) was compared with the EPO response to 120 min of continuous hypoxia (CONT), a protocol that consistently increases EPO levels (4–6, 9). All protocols within one experiment were separated by at least 1 day. *Experiments 1* and *2* were performed approximately 3 mo apart. Data analysis for *experiment 1* was conducted before performing *experiment 2*, which resulted in slight modifications to the study protocol for *experiment 2*.

Hypoxic Protocols

Participants inhaled hypoxic air through a mask connected to a two-way rebreathing valve, which was connected to a 5-L nondiffusing gas bag (Hans Rudolph, Inc, Shawnee, KS). The nondiffusing bag was itself connected to a gas tank of compressed air. Air was made hypoxic by titrating nitrogen into the breathing circuit. The flow of nitrogen was controlled to achieve an arterial oxygen saturation of 80%. Due to the high individual variability in hypoxic ventilatory responses, a fixed fraction of inspired oxygen can result in a wide range of arterial oxygen saturation across individuals. In addition, arterial oxygen saturation increases over the course of five cycles of intermittent hypoxia at a fixed oxygen level (11). Thus, exposure to hypoxia was not performed at a fixed oxygen level but at a targeted arterial oxygen saturation to induce the same level of hypoxemia for each cycle of intermittent hypoxia and throughout the continuous hypoxia protocol in all participants. An arterial oxygen saturation of 80% was chosen based on our previous observation that an arterial oxygen saturation of 90% corresponding to a fraction of inspired oxygen of 0.12 ± 0.01 was not sufficient to significantly increase EPO levels in young healthy individuals (12). Each 4-min hypoxic bout started once the participant reached an arterial oxygen saturation of 83%. For the IH5 protocol, each 4-min hypoxic bout was followed by 4 min of normoxia. To shorten the total duration of the protocol, normoxic bouts of the IH8 protocol ended once arterial oxygen saturation reached baseline levels, which took approximately 2 min.

Erythropoietin Levels

In previous studies, EPO levels consistently peaked 4 to 4.5 h following the onset of a continuous hypoxic exposure (4, 5, 7–9). In *experiment 1*, venous blood samples were therefore collected before, 2.5 and 4.5 h after the beginning of the IH5 and NORM protocols. However, a delayed EPO response was observed following intermittent hypoxia, with EPO levels peaking 6 h following the start of the exposure (5). In *experiment 2*, venous blood samples were therefore collected before, 4.5 and 6 h after the beginning of the IH8 and CONT protocols. Blood was centrifuged, serum aliquoted, and stored at −80°C for subsequent analyses. Erythropoietin levels were determined using an enzyme-linked immunosorbent assay (Abcam, Cambridge, UK). The coefficient of variations for the erythropoietin assay were 6.8% and 11.0% for *experiments 1* and *2*, respectively.

Pulmonary Gas Exchange and Hemodynamics

Breath-by-breath measures of pulmonary gas exchange were collected and analyzed every 10 s throughout all hypoxic protocols using a metabolic cart calibrated with room air and standardized gas (Ultima Cardio2, MGC Diagnostics, St. Paul, MN). The pneumotachometer was mounted between the mask and the nonrebreathing valve of the breathing circuit. An arterial waveform obtained by finger plethysmography from the middle finger of the left hand was continuously recorded during all hypoxic protocols (NOVA, Finapres Medical Systems, Amsterdam, the Netherlands). Brachial arterial blood pressure, heart rate, stroke volume, and cardiac output were derived from the arterial waveform, a method validated against invasive measures (13). Arterial oxygen saturation was monitored by pulse oximetry throughout all hypoxic protocols. All data were recorded in LabChart for later analysis (Powerlab, ADI Instruments Inc., Colorado Springs, CO).

Data and Statistical Analyses

Participants' characteristics from both experiments were compared using a Student's *t* test. In *experiment 1*, a two-way repeated-measures analysis of variance was used to evaluate the effect of condition (NORM and IH5) and time (baseline, 2.5 h, and 4.5 h) on EPO levels. In *experiment 2*, a two-way repeated-measures analysis of variance was used to evaluate the effect of condition (IH8 and CONT) and time (baseline, 4.5 h, and 6 h) on EPO levels.

For the IH5 and IH8 protocols, 4-min average values for pulmonary gas exchange and hemodynamic variables were calculated for each hypoxic bout. For the CONT protocol, 5-min average values for pulmonary gas exchange and hemodynamic variables were calculated every 30 min (min 26–30, 56–60, 86–90, and 116–120). Baseline values for each physiological variable consisted of the 1-min average preceding the start of the hypoxic protocols. A one-way repeated-measured analysis of variance was used to evaluate the effect of each hypoxic bouts on all physiological variables. When main effects were significant, post hoc analyses were performed using Tukey's test. Unless specified, all values are presented as means ± standard deviations. Significance was set at $P \leq 0.05$.

RESULTS

Six individuals (3 women) participated in *experiment 1*, and six individuals (3 women) participated in *experiment 2*. Five of these six individuals participated in both studies. Age, weight, height, systolic and diastolic blood pressure, heart rate, and physical activity levels were similar between experiments (Table 1).

Erythropoietin Levels

There was no significant spontaneous change in EPO levels under normoxic conditions, and there was no significant increase in EPO levels in response to five cycles of intermittent hypoxia (Figs. 1 and 2). Greater EPO levels were observed

Table 1. *Participants' characteristics*

Variables	*Experiment 1*	*Experiment 2*
Age, yr	28 ± 7	28 ± 7
Weight, kg	78.1 ± 19.3	81.3 ± 17.4
Height, cm	176 ± 11	178 ± 7
Systolic blood pressure, mmHg	121 ± 12	122 ± 15
Diastolic blood pressure, mmHg	77 ± 11	77 ± 10
Heart rate, beats/min	62 ± 19	70 ± 12
Physical activity levels, h/wk	4.9 ± 4.2	6.7 ± 3.7

during IH5 in comparison with NORM (main effect for condition, P = 0.046) (Fig. 1). There was a significant increase in EPO levels in response to eight cycles of intermittent hypoxia and 120 min of continuous hypoxia, with peak levels observed 4.5 h following the start of the hypoxic exposure for both protocols (main effect for time, $P <$ 0.01) (Figs. 1 and 2). Serum EPO levels increased to a similar extent in response to both protocols, with observed increases of 65 ± 65% and 85 ± 76% in response to eight cycles of intermittent hypoxia and 120 min of continuous hypoxia, respectively.

Pulmonary Gas Exchange and Hemodynamics

Experiment 1.

By design, intermittent hypoxia induced an average arterial oxygen saturation of 79 ± 1% (Fig. 3), which translated to a fraction of inspired oxygen of 10.3 ± 0.7% (Fig. 4) (main effect for hypoxia, $P <$ 0.001 for both variables). Intermittent hypoxia did not significantly affect minute ventilation (Fig. 4). Exposure to intermittent hypoxia did not affect blood pressure but triggered an increase in heart rate (main effect for hypoxia, P = 0.01), which resulted in an increased cardiac output (main effect for hypoxia, $P <$ 0.01) (Fig. 3). Specifically, heart rate during the first hypoxic cycle was greater than at baseline and during the last hypoxic cycle, and cardiac output was greater than baseline during the first two hypoxic cycles.

Experiment 2.

Similar to *experiment 1*, the arterial oxygen saturation of 80 ± 1% (Fig. 3) achieved during intermittent hypoxia was equivalent to a fraction of inspired oxygen of 10.4 ± 0.2% (Fig. 4) (main effect for hypoxia, $P <$ 0.001 for both variables). Intermittent hypoxia did not significantly affect minute ventilation (Fig. 4). Exposure to eight cycles of intermittent hypoxia did not affect blood pressure or cardiac output (Fig. 3) but increased heart rate (main effect for hypoxia, P = 0.02), with a greater heart rate observed during the first two hypoxic cycles in comparison with baseline.

Two hours of continuous hypoxia at an arterial oxygen saturation of 81 ± 2% (Fig. 3) resulted in a fraction of inspired oxygen of 11.9 ± 0.5% (Fig. 4) (main effect for hypoxia, $P <$ 0.001 for both variables). Exposure to continuous hypoxia increased systolic and diastolic blood pressures (main effect for hypoxia of P = 0.03 and P = 0.04, respectively) but did not affect heart rate or cardiac output (Fig. 3). In comparison to baseline, minute ventilation was reduced during min 56–60 and min 86–90 of continuous hypoxia (main effect for hypoxia, P = 0.02) (Fig. 4). The reduced ventilation was caused by a significantly reduced respiratory rate at min 86–90 when compared with baseline (main effect for hypoxia, P = 0.03; baseline: 12.4 ± 3.0 vs. min 86–90: 11.7 ± 2.5 breaths/min).

DISCUSSION

The objective of the present study was to identify the shortest intermittent hypoxia protocol needed to stimulate an increase in serum EPO levels in healthy individuals. The primary finding was that eight cycles of intermittent hypoxia increased EPO levels to a similar extent as 120 min of continuous hypoxia. Conversely, five cycles of intermittent hypoxia were not sufficient to increase EPO levels in healthy individuals. The longer total hypoxic duration or the additional hypoxic bouts may be responsible for the increase in EPO levels observed following eight, but not five, cycles of intermittent hypoxia.

Two hours of continuous hypoxia at a fraction of inspired oxygen ranging from 10% to 12.5% consistently increase EPO levels by 28–52%, with peak values observed 4 h after the onset of the hypoxic exposure (4–6, 9). Accordingly, the present 2 h of continuous hypoxia at a fraction of inspired oxygen of 11.9 ± 0.5% triggered an average 85% increase in EPO levels observed 4.5 h after the onset of hypoxia. Only one study previously determined whether intermittent hypoxia leads to a similar increase in EPO levels. A 4-h intermittent hypoxia protocol consisting of 2.5 min of hypoxia at a fraction of inspired oxygen of 10.5% alternating with 1.5 min of normoxia, representing a total hypoxic duration of ~108 min, resulted in a 52% increase in EPO levels (5). In the present study, the shorter intermittent hypoxia protocol consisting of eight 4-min bouts of hypoxia at a fraction of inspired oxygen of 10.4 ± 0.2%, translating to a total of 32 min under an arterial oxygen saturation of 83%, resulted in a similar 65% average increase in serum EPO levels. These findings suggest that brief successive bouts of hypoxia can stabilize HIF-1α and lead to EPO gene transcription and production to the same extent as 2 h of continuous hypoxia. These results are also

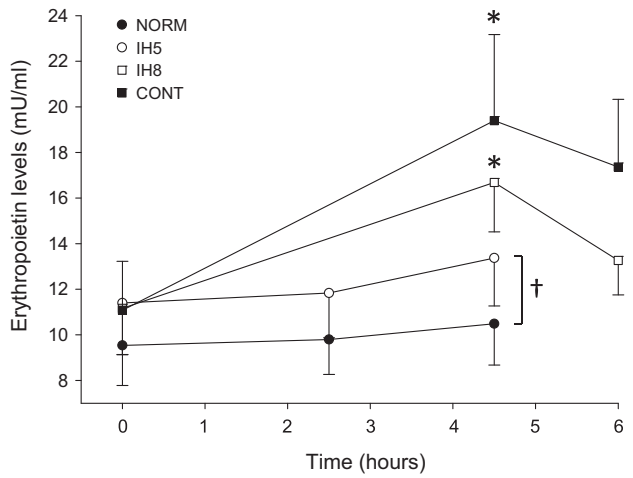

Figure 1. Erythropoietin response to spontaneous change under normoxia (NORM), five cycles of intermittent hypoxia (IH5), eight cycles of intermittent hypoxia (IH8), and 120 min of continuous hypoxia (CONT). Values are presented as means ± standard error of the mean. *$P <$ 0.05 between baseline and 4.5 h; †Main effect for condition.

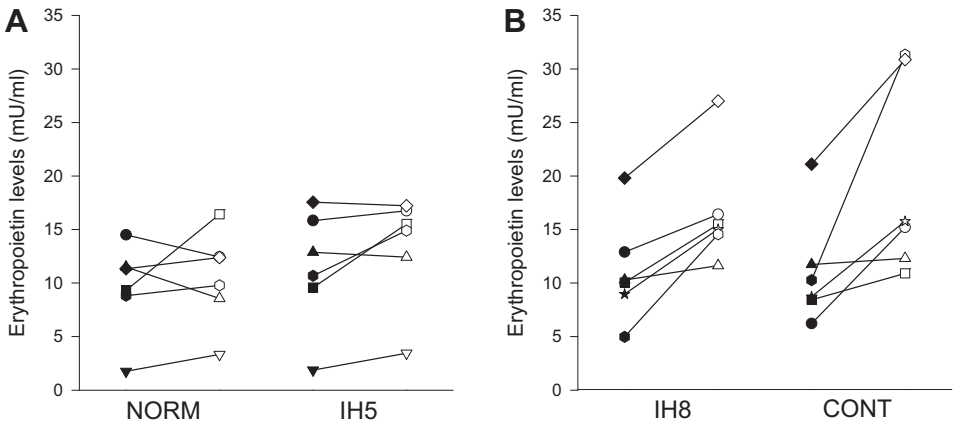

Figure 2. Individual EPO responses from baseline (closed symbols) to 4.5 h (open symbols) to spontaneous change under normoxia (NORM) and five cycles of intermittent hypoxia (IH5) (*A*) and eight cycles of intermittent hypoxia (IH8) and 120 min of continuous hypoxia (CONT) (*B*).

consistent with the observation that an intermittent hypoxia protocol, consisting of six 5-min hypoxic bouts interspersed with 5-min normoxic bouts, and 2 h of continuous hypoxia induced comparable stabilization of HIF-1α in mice (14). Interestingly, it has been reported that 1 h of continuous hypoxia at a fraction of inspired oxygen of 10.5% does not induce an increase in EPO levels (5). Thus, the intermittent stimulus appears to have an important effect on the release of EPO. However, five cycles of intermittent hypoxia at a fraction of inspired oxygen of 10.3 ± 0.7%, representing a total duration of 20 min under an arterial oxygen saturation of 83%, was not sufficient to trigger a significant increase in EPO levels. Therefore, at a fraction of inspired oxygen of 10.5%, a total hypoxic duration above 20 min or more than five hypoxic bouts are needed to induce increases in serum EPO levels.

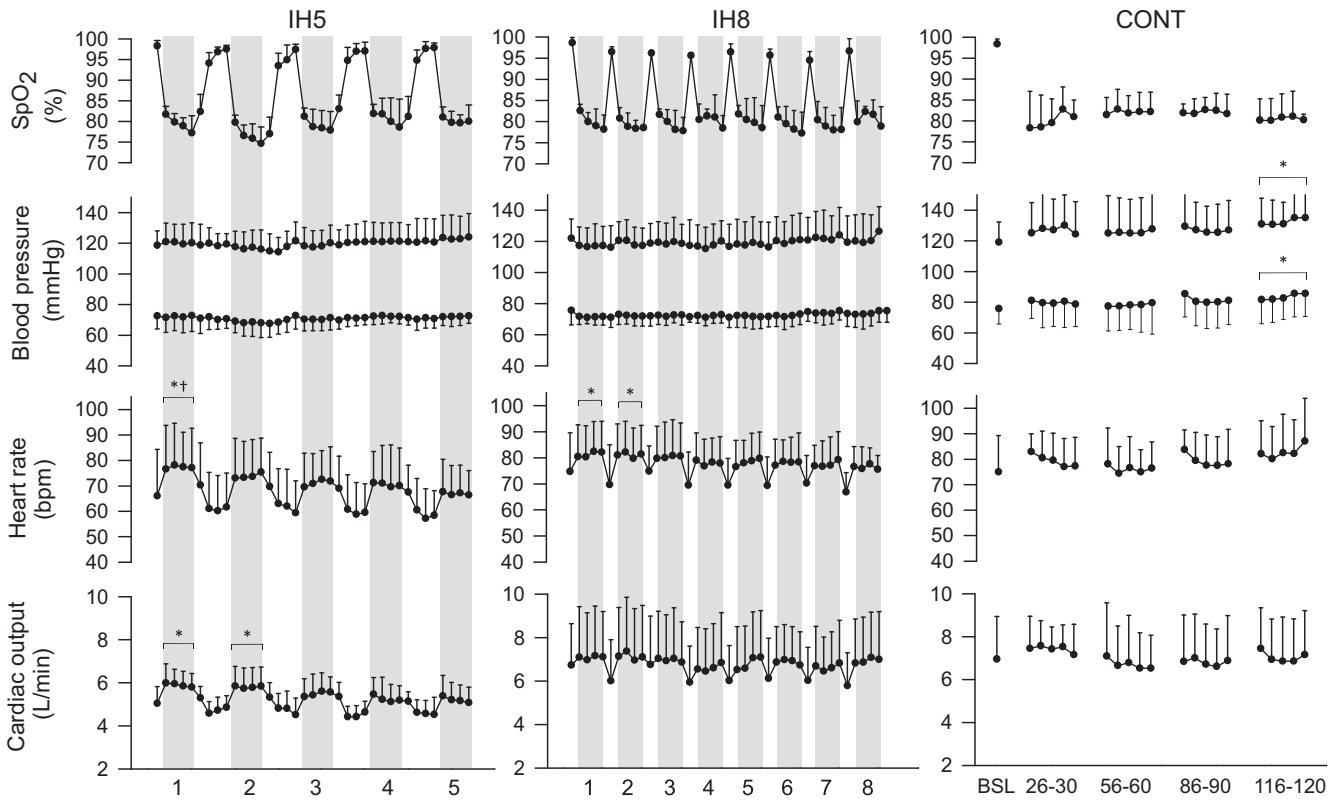

Figure 3. Five cycles of intermittent hypoxia (IH5): 1-min averages for baseline and each hypoxic and normoxic bouts. Eight cycles of intermittent hypoxia (IH8): 1-min averages for baseline, each hypoxic bouts and the highest 10-s average based on arterial oxygen saturation during each normoxic bout. Two hours of continuous hypoxia (CONT): 1-min averages for baseline and min 26–30, 56–60, 86–90, and 116–120. Main effect for hypoxia ($P < 0.001$) for arterial oxygen saturation (SpO_2) all conditions. Main effect for hypoxia for heart rate for IH5 ($P = 0.01$) and IH8 ($P = 0.02$). Main effect for hypoxia for cardiac output ($P < 0.01$) for IH5. Main effect for hypoxia for systolic and diastolic blood pressures for CONT ($P = 0.03$ and $P = 0.04$, respectively). *$P <$ 0.05 different from baseline.

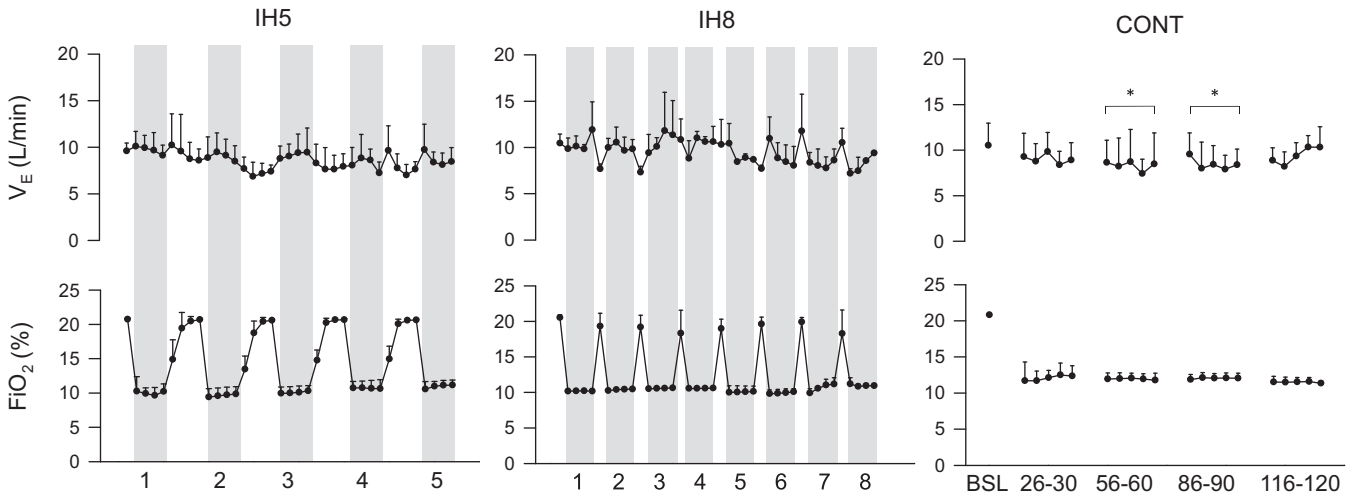

Figure 4. Five cycles of intermittent hypoxia (IH5): 1-min averages for baseline and each hypoxic and normoxic bouts. Eight cycles of intermittent hypoxia (IH8): 1-min averages for baseline, each hypoxic bouts, and the highest 10-s average based on arterial oxygen saturation during each normoxic bout. Two hours of continuous hypoxia (CONT): 1-min averages for baseline and min 26–30, 56–60, 86–90, and 116–120. Main effect for hypoxia ($P < 0.001$) for fraction of inspired oxygen (FiO_2) for all conditions. Main effect for hypoxia ($P = 0.02$) for ventilation ($\dot{V}E$) for CONT. $*P < 0.05$ different from baseline.

We previously reported that a single session of intermittent hypoxia consisting of five 4-min hypoxic bouts at a targeted arterial oxygen saturation of 90% was not sufficient to increase EPO levels in young healthy individuals (12). An arterial oxygen saturation of ~80% was administered during repeated sessions of intermittent hypoxia that increased red blood cell count in elderly men with and without coronary artery disease (10). Thus, protocols of five and eight cycles of intermittent hypoxia at a targeted oxygen saturation of 80% were performed to determine the contribution of hypoxic duration on the release of EPO. Eight, but not five, cycles of intermittent hypoxia at an arterial oxygen saturation of 80% induced the release of EPO, establishing the minimal hypoxic duration needed to trigger a release of EPO in healthy individuals. Future studies are required to determine whether a more severe hypoxic exposure would trigger an increase in EPO levels following five cycles of intermittent hypoxia.

Intermittent hypoxia at an arterial oxygen saturation of 80% did not affect minute ventilation. These findings are in accordance with our previous observations that intermittent hypoxia protocols of three or five 4-min bouts of poikilocapnic hypoxia at an average arterial oxygen saturation of 87% and 89%, respectively, did not affect pulmonary gas exchange in young healthy individuals (12, 15). However, the observed lack of change in ventilation contradicts previous findings that intermittent hypoxia protocols of five 6-min hypoxic bouts at oxygen levels of 10% or at a targeted arterial oxygen saturation of 80% as well as an intermittent hypoxia protocol of seven 5-min hypoxic bouts at an arterial oxygen saturation of 70%–80% increased ventilation (11, 16, 17). The lack of significant effect on ventilation observed in the present study may result from the shorter duration of the hypoxic cycles. While others (16) reported a lack of increase in minute ventilation during 1 h of continuous hypoxia at an arterial oxygen saturation of 70%–80%, the observed reduction in minute ventilation during min 56–60 and 86–90 of the continuous hypoxia protocol was unexpected. However, this statistically significant reduction in minute ventilation represents a small 2 L/min difference caused by a slightly greater respiratory rate at baseline. The lack of increase in minute ventilation during continuous hypoxia was possibly due to a progressive reduction in the fraction of inspired oxygen when titrating nitrogen in the breathing circuit.

Exposure to continuous hypoxia increased systolic and diastolic blood pressure when compared with baseline values. These findings are in accordance with previous observations that 1 h of continuous hypoxia at an arterial oxygen saturation of 70%–80% increased systolic and diastolic blood pressure (16), and that 20 min of continuous hypoxia at an arterial oxygen saturation of 80% increased sympathetic activity, systolic blood pressure, and mean arterial pressure (18, 19). On the other hand, both intermittent hypoxia protocols did not significantly affect blood pressure, which is in agreement with our previous findings that intermittent hypoxia consisting of three or five 4-min hypoxic cycles at a targeted arterial oxygen saturation of 90% did not have any effect on blood pressure (12, 15). Moreover, repetitive bouts of normobaric, poikilocapnic hypoxia consisting of 5–6 min at a fraction of inspired oxygen of 0.10 or at an arterial oxygen saturation of 80% did not affect blood pressure in young healthy individuals (11, 17, 20). Hypercapnia, but not hypocapnia, induces a sympathoexcitation that persists following a 20-min continuous hypoxic exposure (21). Therefore, the isocapnia or hypocapnia observed during short hypoxic bouts likely contributes to the lack of increase in blood pressure observed with intermittent hypoxia.

A reduced partial pressure of oxygen stimulates erythropoiesis. In the present study, exposure to hypoxia was performed at a targeted arterial oxygen saturation to induce the same level of hypoxemia across cycles and across participants. However, this targeted saturation potentially corresponds to varying partial pressures of oxygen across individuals. Measures of partial pressures of oxygen through arterial blood gases would allow true uniformity of the stimulus across individuals. Three of the four women participating

in the study were using hormonal contraceptives, and there was no control for menstrual cycle phase when performing experiments. However, the menstrual cycle phase does not seem to affect the erythropoietic response to hypoxia as similar increases in EPO levels were reported upon ascent to altitude in women in the luteal or follicular phase of the menstrual cycle (22). In conclusion, eight 4-min bouts of intermittent hypoxia, or 32 min under an arterial oxygen saturation of 83%, represent the shortest hypoxic exposure to significantly increase EPO levels in healthy individuals. A rise in EPO levels eventually leads to the creation of reticulocytes that mature into red blood cells within 5–6 days (23). A single 90-min session of continuous hypoxia increased EPO levels by 39% and led to an increased number of immature red blood cells in untrained young healthy men (8). Thus, future studies should determine whether a single session of intermittent hypoxia can significantly increase red blood cells volume and hemoglobin mass in different populations ranging from endurance-trained athletes to clinical populations with impaired oxygen-carrying capacity such as patients with type 2 diabetes (24, 25).

DISCLOSURES

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

AUTHOR CONTRIBUTIONS

F.W. and S.L. conceived and designed research; F.W., S.S.-G., and M.J.N. performed experiments; S.L. analyzed data; F.W. and S.L. interpreted results of experiments; S.L. prepared figures; F.W. and S.L. drafted manuscript; S.S.-G. and M.J.N. edited and revised manuscript; F.W., S.S.-G., M.J.N. and S.L. approved final version of manuscript.

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