J Appl Physiol 135: 88–93, 2023.
First published June 1, 2023; doi:10.1152/japplphysiol.00172.2023

american physiological society

JOURNAL OF APPLIED PHYSIOLOGY

RESEARCH ARTICLE

Brief exposure to intermittent hypoxia increases erythropoietin levels in older adults

Frank Wojan, Sten Stray-Gundersen, Sahar D. Massoudian, and Sophie Lalande

Department of Kinesiology and Health Education, The University of Texas at Austin, Texas, United States

Abstract

Eight 4-min cycles of intermittent hypoxia represent the shortest hypoxic exposure to increase erythropoietin (EPO) levels in young adults. The impact of aging on the EPO response to a hypoxic stimulus remains equivocal. Thus, the objective of this study was to determine the effect of the same intermittent hypoxia protocol on EPO levels in older adults. Twenty-two participants (12 women, age: 53 ± 7 yr) were randomly assigned to an intermittent hypoxia group (IH, n = 11) or an intermittent normoxia group (IN, n = 11). Intermittent hypoxia consisted of eight 4-min cycles at a targeted oxygen saturation of 80% interspersed with normoxic cycles to resaturation. Air was made hypoxic by titrating nitrogen into a breathing circuit. Intermittent normoxia consisted of the same protocol, but nitrogen was not added to the breathing circuit. EPO levels were measured before and 4.5 h after the beginning of each protocol. Intermittent hypoxia lowered oxygen saturation to 82 ± 3%, which corresponded to a fraction of inspired oxygen of 10.9 ± 1.0%. There was a greater increase in EPO levels following intermittent hypoxia than intermittent normoxia (IH: 3.2 ± 2.2 vs. IN: 0.7 ± 0.8 mU/mL, $P < 0.01$). A single session of eight 4-min cycles of hypoxia increased EPO levels, the glycoprotein stimulating red blood cell production, in older adults. Exposure to intermittent hypoxia has therefore the potential to increase oxygen-carrying capacity in a population with reduced red blood cell volume.

NEW & NOTEWORTHY We previously identified the shortest intermittent hypoxia protocol necessary to increase erythropoietin levels in young adults. The objective of this study was to determine whether the same intermittent hypoxia protocol increases erythropoietin levels in older adults. Eight 4-min bouts of hypoxia, representing a hypoxic duration of 32 min at a targeted oxygen saturation of 80%, increased erythropoietin levels in older adults, suggesting that exposure to intermittent hypoxia has the potential to increase oxygen-carrying capacity in an aging population.

aging; erythropoietin; intermittent hypoxia

INTRODUCTION

Maximal oxygen consumption, the ability of the cardiovascular system to transport and use oxygen during maximal exercise, predicts mortality to the same or potentially greater extent as traditional risk factors such as smoking, hypertension, high cholesterol, and type 2 diabetes (1). Maximal oxygen consumption progressively decreases with advancing age (2). Oxygen transport from the lungs to the exercising muscles occurs through the binding of oxygen to hemoglobin contained in red blood cells, therefore, both hemoglobin mass and red blood cell volume strongly correlate with maximal oxygen consumption (3). A reduced red blood cell volume, mainly due to a lower fat-free mass and decreased physical activity levels, has been reported in older men and women (4, 5). In these studies, red blood cell volume was calculated from plasma volume and hematocrit levels, which are greatly influenced by hydration status (6). When directly assessed, red blood cell count was also reported to decrease with aging in men (7). Therefore, an intervention that increases hemoglobin mass and red blood cell volume could ultimately improve maximal oxygen consumption in an older population characterized by reduced fat-free mass and physical activity levels.

Erythropoietin (EPO) stimulates red blood cell production in response to hypoxia (8). We previously reported that eight 4-min bouts of intermittent hypoxia represent the shortest hypoxic exposure to increase EPO levels in young adults (9). With aging, circulating EPO levels have been reported to be either higher (10–14), lower (15), or not different (16, 17) from the EPO levels observed in young individuals. However, the impact of aging on the EPO response to a hypoxic stimulus remains equivocal. Thus, the objective of this study was to determine whether eight 4-min bouts of intermittent hypoxia also trigger an increase in EPO levels in older adults. A rise in EPO levels results in the creation of reticulocytes that mature into red blood cells within 7 days (18). Indeed, a transient increase in EPO levels induced by a single 90-min session of continuous hypoxia resulted in an increased number of reticulocytes 2 days following the hypoxic exposure (19). Thus, a secondary objective of this study was to determine whether a single session of intermittent hypoxia leads to an

Correspondence: S. Lalande (sophie.lalande@austin.utexas.edu).
Submitted 20 March 2023 / Revised 26 May 2023 / Accepted 26 May 2023

increase in hemoglobin mass in older adults. It was hypothesized that a single exposure to intermittent hypoxia would increase EPO levels, which would lead to an increased hemoglobin mass in this population.

METHODS

Participants and Study Design

Both men and women were recruited to participate in the study. Participants provided written informed consent for participating in the study, which was approved by the Institutional Review Board of the University of Texas at Austin (IRB study number 2017090015). Participants were excluded from the study if they had uncontrolled hypertension or were taking more than one antihypertensive medication, were smokers, were pregnant, or had a history of cardiovascular disease, diabetes, or lung disease. Twenty-two participants were randomly assigned to an intermittent hypoxia group (IH, n = 11, 6 women) or a placebo intermittent normoxia group (IN, n = 11, 6 women). The study consisted of three visits over a period of 8 days. Measurements of hemoglobin mass were performed on *visits 1* and *3*. The EPO response to intermittent hypoxia or intermittent normoxia was assessed on *visit 2*, which always took place in the morning (start time ranging between 7:00 and 10:45 am). *Visit 3* took place 7 days after *visit 2*. Since menstrual blood loss has no impact on hemoglobin mass (20), visits were scheduled during any phase of the menstrual cycle in the three premenopausal women participating in the study. All participants were asked to avoid alcohol and intense physical activity on the day preceding all visits.

Intermittent Hypoxia and Intermittent Normoxia

The intermittent hypoxia protocol consisted of eight 4-min hypoxic cycles at a targeted oxygen saturation of 80% interspersed with normoxic cycles to resaturation (9). Participants inhaled hypoxic air through a mask connected to a two-way rebreathing valve that was itself connected to a five-liter nondiffusing bag (Hans Rudolph, Inc, Shawnee, KS). The nondiffusing bag was connected to a gas tank of compressed air and a gas tank of nitrogen. Air was made hypoxic by titrating nitrogen into the breathing circuit to achieve an oxygen saturation of 80%. Each 4-min hypoxic cycle began once the participant reached an oxygen saturation of 83%. In the present study, it took an average of 3 min 19 s to achieve the targeted oxygen saturation, which was similar to the average desaturation time of 3 min 10 s previously observed in young adults (9). Resaturation duration was on average 1 min 49 in older adults, which was longer than the average resaturation duration of 1 min 20 s observed in young adults (9). Intermittent normoxia consisted of the same protocol, but nitrogen was not introduced in the breathing circuit.

Erythropoietin Levels

EPO levels consistently peak 4 to 4.5 h following the onset of a continuous hypoxic exposure or intermittent hypoxia exposure (9, 19, 21–24). Thus, venous blood samples were collected before and 4.5 h after the beginning of intermittent hypoxia and intermittent normoxia. 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 average coefficient of variation for the erythropoietin assays was 7.2%.

Hematological Variables

Hemoglobin mass was determined using a modified version of the optimized carbon monoxide rebreathing technique (25, 26). A venous blood draw was obtained to determine baseline carboxyhemoglobin, hematocrit, and hemoglobin levels (ABL 80 FLEX OSM, Radiometer, Copenhagen, Denmark). Participants rebreathed a bolus of carbon monoxide from a low-volume closed-circuit system containing air over a period of 2 min. Carboxyhemoglobin levels were measured again 10 min after the start of the carbon monoxide rebreathing. Hemoglobin mass, red blood cell volume, plasma volume, and total blood volume were calculated from the change in carboxyhemoglobin levels induced by carbon monoxide rebreathing (27). In our laboratory, the coefficient of variation for hemoglobin mass, based on duplicate measures performed on consecutive days in five individuals, is 2.6%.

Pulmonary Gas Exchange and Hemodynamics

On *visit 1*, average heart rate and blood pressure were calculated from two measures obtained following 5 min of supine rest (Omron Healthcare, Inc., Lake Forest, IL). Breath-by-breath measures of pulmonary gas exchange were collected using a metabolic cart calibrated with standardized gas (Ultima Cardio2, MGC Diagnostics, St. Paul, MN) and averaged every 10 s throughout intermittent hypoxia and intermittent normoxia. The pneumotachometer was mounted between the mask and the nonrebreathing valve of the breathing circuit. An arterial waveform obtained via finger plethysmography and oxygen saturation obtained via pulse oximetry were continuously recorded throughout both protocols (NOVA, Finapres Medical Systems, Amsterdam, the Netherlands). Brachial arterial blood pressure, heart rate, stroke volume, cardiac output, and total peripheral resistance were derived from the arterial waveform. All data were recorded in LabChart for later analysis (PowerLab, ADInstruments Inc., Colorado Springs, CO).

Data and Statistical Analyses

Participants' characteristics were compared using a Student's t test. A two-way analysis of variance was used to evaluate the effect of condition (intermittent hypoxia vs. intermittent normoxia) and time (pre- vs. postintervention) on EPO levels and hematological variables. When main effects or interactions were significant, post hoc analyses were performed using Tukey's test. Average values for each hemodynamic and pulmonary gas exchange were calculated for each 4-min hypoxic cycle of the intermittent hypoxia protocol and each 4-min normoxic cycle of the intermittent normoxia protocol. Baseline values for each physiological variable consisted of the 1-min average preceding the start of the hypoxic and normoxic protocols. A two-way analysis of variance was used to evaluate the effect of time (baseline and 8 cycles) and condition (intermittent hypoxia and intermittent normoxia) on hemodynamic and ventilatory variables. When the main effects

were significant, post hoc analyses were performed using Tukey's test. Pearson's correlation was used to determine the relation between oxygen saturation, fraction of inspired oxygen, and changes in EPO levels. A post hoc power analysis performed using EPO levels before and after intermittent hypoxia and intermittent normoxia in our 22 participants resulted in a power of 0.61. Significance was set at $P \leq 0.05$. Unless stated otherwise, all values are presented as means ± standard deviations.

RESULTS

Age, weight, height, body mass index, hematocrit levels, hemoglobin concentration, blood pressure, heart rate, and physical activity levels were not different between groups (Table 1). None of the participants had anemia according to the World Health Organization's criteria (hemoglobin levels <12.0 g/dL in women and <13.0 g/dL in men). *Visit 3* took place 7 days following *visit 2* for all but one participant for whom *visit 3* took place 8 days following *visit 2*. EPO levels were greater during intermittent hypoxia than intermittent normoxia (main effect for condition, $P = 0.02$), with greater postintervention levels observed with intermittent hypoxia than intermittent normoxia (Fig. 1). EPO levels tended to increase following exposure to intermittent hypoxia and intermittent normoxia (main effect for time, $P = 0.08$). The change in EPO levels was greater following intermittent hypoxia than intermittent normoxia (IH: 3.2 ± 2.2 vs. IN: 0.7 ± 0.8 mU/mL, $P < 0.01$). There was no sex difference for the change in EPO levels in response to intermittent hypoxia (women: 3.5 ± 2.1 vs. men: 2.8 ± 2.5 mU/mL, $P = 0.59$). There was a correlation between changes in EPO levels and oxygen saturation ($r = -0.63$, $P < 0.01$) and between changes in EPO levels and fraction of inspired oxygen ($r = -0.75$, $P < 0.01$). There was no change in any of the hematological variables in response to either intermittent hypoxia or intermittent normoxia (Table 2).

Intermittent hypoxia resulted in a lower oxygen saturation compared with intermittent normoxia (Fig. 2). A greater heart rate and a lower diastolic blood pressure were observed in the intermittent hypoxia group in comparison with the intermittent normoxia group (Fig. 2). Intermittent hypoxia induced a lower fraction of inspired oxygen in comparison with intermittent normoxia but did not affect any other ventilatory variables (Fig. 3). A lower respiratory rate, a greater tidal volume, and a greater minute ventilation were observed in the intermittent hypoxia group in comparison to the intermittent normoxia group (Fig. 3).

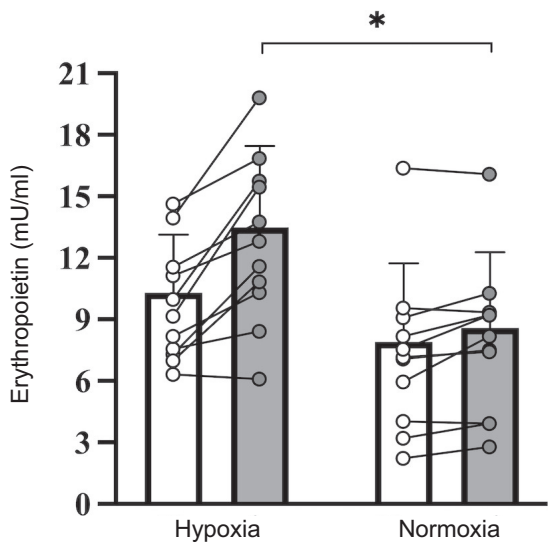

Figure 1. Average and individual erythropoietin levels before (white circles and bars) and after (gray circles and bars) eight cycles of intermittent hypoxia ($n = 11$, 6 women) and intermittent normoxia ($n = 11$, 6 women). Values are presented as means ± standard deviations. Main effect for condition, *$P < 0.05$ different from intermittent normoxia.

Table 1. *Participants' characteristics*

Variables	IH	IN
Age, yr	53 ± 8	54 ± 7
Height, cm	176 ± 10	172 ± 11
Weight, kg	73.2 ± 10.5	73.4 ± 20.0
Body mass index, kg/m^2	23.7 ± 2.7	24.6 ± 5.6
Systolic blood pressure, mmHg	116 ± 13	120 ± 10
Diastolic blood pressure, mmHg	75 ± 9	77 ± 6
Heart rate, beats/min	60 ± 10	59 ± 11
Hemoglobin, g/dL	13.9 ± 1.3	14.0 ± 1.0
Hematocrit, %	43 ± 4	43 ± 3
Physical activity, h/wk	7.6 ± 9.0	5.1 ± 4.0
Medication use, *n*		
ACE inhibitor	1	0
Angiotensin II blocker	1	0
Beta-blockers	0	1
Statin	2	2
Levothyroxine	0	3
Estrogen/progesterone	0	3

IH, Intermittent hypoxia; IN, Intermittent normoxia; ACE, angiotensin-converting enzyme.

DISCUSSION

The purpose of the present study was to determine whether a single session of intermittent hypoxia increases EPO levels in older adults. Eight 4-min hypoxic cycles at an oxygen saturation of 82 ± 3% corresponding to a fraction of inspired oxygen of 10.9 ± 1.0% induced a 31% increase in EPO levels in older adults. In contrast, intermittent normoxia induced a 9% increase in EPO levels, consistent with the reported 15% diurnal variation in EPO levels from midmorning to late afternoon (21). A secondary objective of the present study was to determine whether this single session of intermittent hypoxia leads to an increase in hemoglobin

Table 2. *Hematological variables before and after intermittent hypoxia and intermittent normoxia*

Variables	IH		IN	
	Pre	Post	Pre	Post
Hemoglobin mass, g	752 ± 189	754 ± 189	800 ± 179	801 ± 186
Hemoglobin mass, g/kg	10.3 ± 2.2	10.2 ± 2.1	10.9 ± 1.5	10.9 ± 1.8
Red blood cell volume, L	2.31 ± 0.58	2.31 ± 0.57	2.45 ± 0.55	2.45 ± 0.57
Plasma volume, L	3.56 ± 0.51	3.68 ± 0.46	3.81 ± 0.77	3.69 ± 0.72
Blood volume, L	5.87 ± 1.04	5.99 ± 1.00	6.26 ± 1.29	6.15 ± 1.24
Blood volume, mL/kg	80.7 ± 13.3	81.5 ± 12.1	86.5 ± 14.5	84.8 ± 15.4

IH, Intermittent hypoxia; IN, Intermittent normoxia.

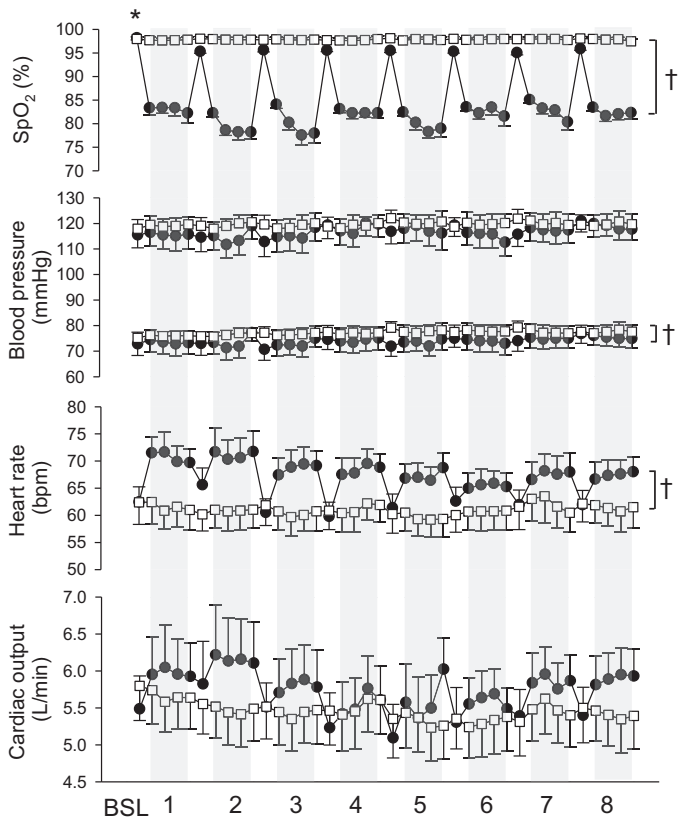

Figure 2. Oxygen saturation (SpO_2), blood pressure, heart rate, and cardiac output at baseline (BSL) and in response to eight cycles of intermittent hypoxia at a targeted oxygen saturation of 80% (black circles, n = 11, 6 women) and eight cycles of intermittent normoxia (white squares, n = 11, 6 women). Values are presented as means ± standard error. †Main effect for condition, *main effect for time: $P < 0.05$ different from cycles 1 to 8.

mass. Contrary to our hypothesis, one session of intermittent hypoxia did not induce a rise in hemoglobin mass.

Exposure to hypoxia stabilizes hypoxia-inducible factors (HIFs) within few minutes, which results in EPO gene transcription and production (8). Aging seems to negatively impact the EPO response to hypoxia. Indeed, exposure to 3 h of continuous hypoxia at a targeted oxygen saturation of 80% increased EPO levels in both young and older adults, however, EPO levels were approximately three times greater in young versus older adults (28). It was therefore suggested that an age-dependent defect in HIF-1 action reduced EPO gene expression in response to hypoxia (29). In the present study, eight 4-min hypoxic cycles, corresponding to a total hypoxic duration of 32 min at a targeted oxygen saturation of 80%, increased EPO levels in older adults. Similarly, this rise in EPO levels was half the rise in EPO levels previously observed in response to the same intermittent hypoxia protocol in young adults (65% vs. 31%) (9). Nonetheless, the present findings further confirm that a short, intermittent hypoxic stimulus triggers EPO production, challenging the long-standing belief that continuous hypoxic exposures ranging between 84 and 120 min are necessary to trigger an increase in EPO levels (19, 21–24, 30). The present findings are supported by the observation that exposure to 30 min of intermittent hypoxia or 2 h of continuous hypoxia induced comparable activation of the HIF pathway as defined by stabilization of HIF-1α protein (31). Thus, intermittent hypoxia represents an efficient approach to elicit EPO production.

A rise in EPO levels leads to the creation of reticulocytes that eventually mature into red blood cells (18). An increase in EPO levels induced by a single 90-min session of continuous hypoxia previously resulted in an increased number of reticulocytes 2 days following the hypoxic exposure in untrained young men (age: 28.7 ± 4.3 yr) (19). Despite the observed increase in EPO levels in the present study, eight 4-min hypoxic cycles did not result in an increased hemoglobin mass. It is therefore hypothesized that additional sessions of intermittent hypoxia are necessary to induce an increase in red blood cell volume and, thereby, hemoglobin mass. Although EPO levels were not assessed, five consecutive days of a similar intermittent hypoxia protocol, consisting of 4–6 min hypoxic bouts at a mean oxygen saturation of 85% for a total hypoxic duration of ∼70 min, increased red blood cell count in young (32) and older adults (33). Moreover, 15 sessions of intermittent hypoxia increased red blood cell count and hemoglobin mass in elderly men with and without coronary artery disease and in individuals at risk for or with mild chronic obstructive pulmonary disease (34, 35).

The lower average fraction of inspired oxygen of 10.9% accompanying intermittent hypoxia did not acutely affect the respiratory rate, tidal volume, or minute ventilation. These results are in agreement with our previous findings

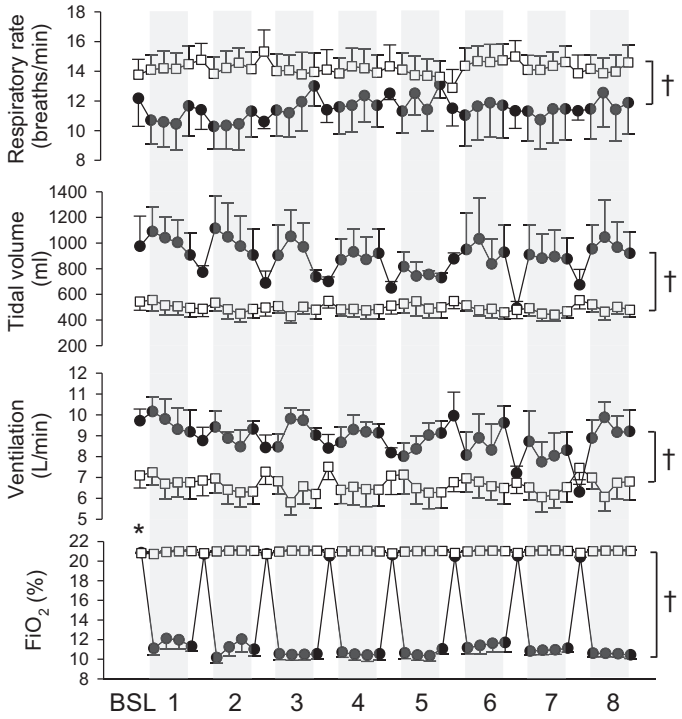

Figure 3. Respiratory rate, tidal volume, ventilation, and fraction of inspired oxygen (FiO_2) at baseline (BSL) and in response to eight cycles of intermittent hypoxia at a targeted oxygen saturation of 80% (black circles, n = 6, 2 women) and eight cycles of intermittent normoxia (white squares, n = 9, 5 women). Values are presented as means ± standard error. †Main effect for condition, *main effect for time: $P < 0.05$ different from cycles 1 to 8.

that three 4-min hypoxic cycles at a fraction of inspired oxygen of 11.4% and eight 4-min hypoxic cycles at a fraction of inspired oxygen of 10.4% did not affect minute ventilation (9, 36), and in accordance with others who showed that seven 5-min hypoxic cycles at a targeted oxygen saturation of 75% did not affect ventilation (37). However, the present findings are in contrast with previous findings that five 6-min hypoxic cycles at a fraction of inspired oxygen of 10% or at a targeted oxygen saturation of 80% and seven 5-min hypoxic cycles at an oxygen saturation of 70–80% increase ventilation (38–40) through an increase in tidal volume (39). The present intermittent hypoxia protocol did not induce change in blood pressure. These results are consistent with previous findings that repetitive bouts of normobaric, poikilocapnic hypoxia, consisting of 4–6 min at a fraction of inspired oxygen of 10% or at an oxygen saturation of 80%, did not affect arterial blood pressure in young and older individuals (9, 36, 38, 39, 41). Heart rate increased during intermittent hypoxia in comparison with intermittent normoxia, which is consistent with previous findings that seven 5-min hypoxic cycles at an oxygen saturation of 75% increased heart rate in older adults (37). Thus, intermittent hypoxia consisting of eight 4-min hypoxic cycles at a targeted oxygen saturation of 80% has minimal impact on hemodynamics and ventilation in older adults.

In conclusion, a single session of intermittent hypoxia elicited a rise in EPO levels in older men and women. Future studies are needed to determine the minimum number of intermittent hypoxia sessions necessary to increase hemoglobin mass and maximal oxygen consumption in this population. Thus, intermittent hypoxia potentially represents a novel intervention to mitigate the decline in oxygen-carrying capacity associated with the reduced maximal oxygen consumption observed with aging.

DATA AVAILABILITY

Data will be made available upon reasonable request.

GRANTS

This work was supported by the Small Grants Program from the College of Education of the University of Texas at Austin.

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

REFERENCES

1. **Ross R**, **Blair SN**, **Arena R**, **Church TS**, **Després JP**, **Franklin BA**, **Haskell WL**, **Kaminsky LA**, **Levine BD**, **Lavie CJ**, **Myers J**, **Niebauer J**, **Sallis R**, **Sawada SS**, **Sui X**, **Wisløff U**; **American Heart Association Physical Activity Committee of the Council on Lifestyle and Cardiometabolic Health, Council on Clinical Cardiology, Council on Epidemiology and Prevention, Council on Cardiovascular and Stroke Nursing, Council on Functional Genomics and Translational Biology, Stroke Council.** Importance of assessing cardiorespiratory fitness in clinical practice: a case for fitness as a clinical vital sign: a scientific statement from the American Heart Association. *Circulation* 134: e653–e699, 2016. doi:10.1161/CIR.0000000000000461.
2. **Robinson S.** Experimental studies of physical fitness in relation to age. *Arbeitsphysiologie* 10: 251–323, 1938. doi:10.1007/BF02011412.
3. **Schmidt W**, **Prommer N.** Impact of alterations in total hemoglobin mass on VO_{2max}. *Exerc Sport Sci Rev* 38: 68–75, 2010. doi:10.1097/JES.0b013e3181d4957a.
4. **Davy KP**, **Seals DR.** Total blood volume in healthy young and older men. *J Appl Physiol (1985)* 76: 2059–2062, 1994. doi:10.1152/jappl.1994.76.5.2059.
5. **Jones PP**, **Davy KP**, **DeSouza CA**, **van Pelt RE**, **Seals DR.** Absence of age-related decline in total blood volume in physically active females. *Am J Physiol Heart Circ Physiol* 272: H2534–H2540, 1997. doi:10.1152/ajpheart.1997.272.6.H2534.
6. **Chapman AB**, **Zamudio S**, **Woodmansee W**, **Merouani A**, **Osorio F**, **Johnson A**, **Moore LG**, **Dahms T**, **Coffin C**, **Abraham WT**, **Schrier RW.** Systemic and renal hemodynamic changes in the luteal phase of the menstrual cycle mimic early pregnancy. *Am J Physiol Renal Physiol* 273: F777–F782, 1997. doi:10.1152/ajprenal.1997.273.5.F777.
7. **Zierk J**, **Krebs A**, **Rauh M**, **Metzler M**, **Löscher A**, **Strasser E**, **Krause SW.** Blood counts in adult and elderly individuals: defining the norms over eight decades of life. *Br J Haematol* 189: 777–789, 2020. [Erratum in *Br J Haematol* 190: 294, 2020]. doi:10.1111/bjh.16430.
8. **Semenza GL.** O_2-regulated gene expression: transcriptional control of cardiorespiratory physiology by HIF-1. *J Appl Physiol (1985)* 96: 1173–1177, 2004. doi:10.1152/japplphysiol.00770.2003.
9. **Wojan F**, **Stray-Gundersen S**, **Nagel MJ**, **Lalande S.** Short exposure to intermittent hypoxia increases erythropoietin levels in healthy individuals. *J Appl Physiol (1985)* 130: 1955–1960, 2021. doi:10.1152/japplphysiol.00941.2020.
10. **Ershler WB**, **Sheng S**, **McKelvey J**, **Artz AS**, **Denduluri N**, **Tecson J**, **Taub DD**, **Brant LJ**, **Ferrucci L**, **Longo DL.** Serum erythropoietin and aging: a longitudinal analysis. *J Am Geriatr Soc* 53: 1360–1365, 2005. doi:10.1111/j.1532-5415.2005.53416.x.
11. **Goodnough LT**, **Price TH**, **Parvin CA.** The endogenous erythropoietin response and the erythropoietic response to blood loss anemia: the effects of age and gender. *J Lab Clin Med* 126: 57–64, 1995.
12. **Kario K**, **Matsuo T**, **Kodama K**, **Nakao K**, **Asada R.** Reduced erythropoietin secretion in senile anemia. *Am J Hematol* 41: 252–257, 1992. doi:10.1002/ajh.2830410406.
13. **Kario K**, **Matsuo T**, **Nakao K.** Serum erythropoietin levels in the elderly. *Gerontology* 37: 345–348, 1991. doi:10.1159/000213283.
14. **Malaguarnera M**, **Bentivegna P**, **Giugno I**, **Romano M**, **Di Fazio I**, **Motta M**, **Trovato BA.** Erythropoietin in healthy elderly subjects. *Arch Gerontol Geriatr* 22: 131–135, 1996. doi:10.1016/0167-4943(95)00687-7.
15. **Joosten E**, **Van Hove L**, **Lesaffre E**, **Goossens W**, **Dereymaeker L**, **Van Goethem G**, **Pelemans W.** Serum erythropoietin levels in elderly inpatients with anemia of chronic disorders and iron deficiency anemia. *J Am Geriatr Soc* 41: 1301–1304, 1993. doi:10.1111/j.1532-5415.1993.tb06479.x.
16. **Mori M**, **Murai Y**, **Hirai M**, **Kawakami M**, **Saito T**, **Takanashi N**, **Urabe A**, **Takaku F.** Serum erythropoietin titers in the aged. *Mech Ageing Dev* 46: 105–109, 1988. doi:10.1016/0047-6374(88)90118-2.
17. **Powers JS**, **Krantz SB**, **Collins JC**, **Meurer K**, **Failinger A**, **Buchholz T**, **Blank M**, **Spivak JL**, **Hochberg M**, **Baer A**, **Cotes PM**, **Goldwasser E.** Erythropoietin response to anemia as a function of age. *J Am Geriatr Soc* 39: 30–32, 1991. doi:10.1111/j.1532-5415.1991.tb05902.x.
18. **Jelkmann W.** Erythropoietin. *Front Horm Res* 47: 115–127, 2016. doi:10.1159/000445174.
19. **Schmidt W**, **Eckardt KU**, **Hilgendorf A**, **Strauch S**, **Bauer C.** Effects of maximal and submaximal exercise under normoxic and hypoxic conditions on serum erythropoietin level. *Int J Sports Med* 12: 457–461, 1991. doi:10.1055/s-2007-1024713.
20. **Keller MF**, **Harrison ML**, **Lalande S.** Impact of menstrual blood loss and oral contraceptive use on oxygen-carrying capacity. *Med Sci Sports Exerc* 52: 1414–1419, 2020. doi:10.1249/MSS.0000000000002252.

21. **Klausen T**, **Christensen H**, **Hansen JM**, **Nielsen OJ**, **Fogh-Andersen N**, **Olsen NV.** Human erythropoietin response to hypocapnic hypoxia, normocapnic hypoxia, and hypocapnic normoxia. *Eur J Appl Physiol Occup Physiol* 74: 475–480, 1996. doi:10.1007/BF02337729.
22. **Knaupp W**, **Khilnani S**, **Sherwood J**, **Scharf S**, **Steinberg H.** Erythropoietin response to acute normobaric hypoxia in humans. *J Appl Physiol (1985)* 73: 837–840, 1992. doi:10.1152/jappl.1992.73.3.837.
23. **Rodríguez FA**, **Ventura JL**, **Casas M**, **Casas H**, **Pagés T**, **Rama R**, **Ricart A**, **Palacios L**, **Viscor G.** Erythropoietin acute reaction and haematological adaptations to short, intermittent hypobaric hypoxia. *Eur J Appl Physiol* 82: 170–177, 2000. doi:10.1007/s004210050669.
24. **Turner G**, **Gibson OR**, **Watt PW**, **Pringle JSM**, **Richardson AJ**, **Maxwell NS.** The time course of endogenous erythropoietin, IL-6, and TNFα in response to acute hypoxic exposures. *Scand J Med Sci Sports* 27: 714–723, 2017. doi:10.1111/sms.12700.
25. **Lalande S**, **Kelsey JW**, **Joyner MJ**, **Johnson BD.** Determination of blood volume by pulse CO-oximetry. *Physiol Meas* 33: 19–27, 2012. doi:10.1088/0967-3334/33/1/19.
26. **Schmidt W**, **Prommer N.** The optimised CO-rebreathing method: a new tool to determine total haemoglobin mass routinely. *Eur J Appl Physiol* 95: 486–495, 2005. doi:10.1007/s00421-005-0050-3.
27. **Burge CM**, **Skinner SL.** Determination of hemoglobin mass and blood volume with CO: evaluation and application of a method. *J Appl Physiol (1985)* 79: 623–631, 1995. doi:10.1152/jappl.1995.79.2.623.
28. **Törpel A**, **Peter B**, **Hamacher D**, **Schega L.** Dose-response relationship of intermittent normobaric hypoxia to stimulate erythropoietin in the context of health promotion in young and old people. *Eur J Appl Physiol* 119: 1065–1074, 2019. doi:10.1007/s00421-019-04096-8.
29. **Rivard A**, **Berthou-Soulie L**, **Principe N**, **Kearney M**, **Curry C**, **Branellec D**, **Semenza GL**, **Isner JM.** Age-dependent defect in vascular endothelial growth factor expression is associated with reduced hypoxia-inducible factor 1 activity. *J Biol Chem* 275: 29643–29647, 2000. doi:10.1074/jbc.M001029200.
30. **Eckardt KU**, **Boutellier U**, **Kurtz A**, **Schopen M**, **Koller EA**, **Bauer C.** Rate of erythropoietin formation in humans in response to acute hypobaric hypoxia. *J Appl Physiol (1985)* 66: 1785–1788, 1989. doi:10.1152/jappl.1989.66.4.1785.
31. **Scortegagna M**, **Ding K**, **Zhang Q**, **Oktay Y**, **Bennett MJ**, **Bennett M**, **Shelton JM**, **Richardson JA**, **Moe O**, **Garcia JA.** HIF-2α regulates murine hematopoietic development in an erythropoietin-dependent manner. *Blood* 105: 3133–3140, 2005. doi:10.1182/blood-2004-05-1695.
32. **Tobin B**, **Costalat G**, **Renshaw GMC.** Pre-acclimation to altitude in young adults: choosing a hypoxic pattern at sea level which provokes significant haematological adaptations. *Eur J Appl Physiol* 122: 395–407, 2022. doi:10.1007/s00421-021-04837-8.
33. **Tobin B**, **Costalat G**, **Renshaw GMC.** Intermittent not continuous hypoxia provoked haematological adaptations in healthy seniors: hypoxic pattern may hold the key. *Eur J Appl Physiol* 120: 707–718, 2020. doi:10.1007/s00421-020-04310-y.
34. **Burtscher M**, **Haider T**, **Domej W**, **Linser T**, **Gatterer H**, **Faulhaber M**, **Pocecco E**, **Ehrenburg I**, **Tkatchuk E**, **Koch R**, **Bernardi L.** Intermittent hypoxia increases exercise tolerance in patients at risk for or with mild COPD. *Respir Physiol Neurobiol* 165: 97–103, 2009. doi:10.1016/j.resp.2008.10.012.
35. **Burtscher M**, **Pachinger O**, **Ehrenbourg I**, **Mitterbauer G**, **Faulhaber M**, **Pühringer R**, **Tkatchouk E.** Intermittent hypoxia increases exercise tolerance in elderly men with and without coronary artery disease. *Int J Cardiol* 96: 247–254, 2004. doi:10.1016/j.ijcard.2003.07.021.
36. **Stray-Gundersen S**, **Massoudian SD**, **Wojan F**, **Tanaka H**, **Lalande S.** Hypoxic preconditioning reduces endothelial ischemia-reperfusion injury in older adults. *Am J Physiol Regul Integr Comp Physiol* 323: R832–R838, 2022. doi:10.1152/ajpregu.00200.2022.
37. **Chacaroun S**, **Borowik A**, **Doutreleau S**, **Belaidi E**, **Wuyam B**, **Tamisier R**, **Pépin JL**, **Flore P**, **Verges S.** Cardiovascular and metabolic responses to passive hypoxic conditioning in overweight and mildly obese individuals. *Am J Physiol Regul Integr Comp Physiol* 319: R211–R222, 2020. doi:10.1152/ajpregu.00311.2019.
38. **Iwamoto E**, **Hanson BE**, **Bock JM**, **Casey DP.** Intermittent hypoxia enhances shear-mediated dilation of the internal carotid artery in young adults. *J Appl Physiol (1985)* 129: 603–611, 2020. doi:10.1152/japplphysiol.00274.2020.
39. **Liu X**, **Xu D**, **Hall JR**, **Ross S**, **Chen S**, **Liu H**, **Mallet RT**, **Shi X.** Enhanced cerebral perfusion during brief exposures to cyclic intermittent hypoxemia. *J Appl Physiol (1985)* 123: 1689–1697, 2017. doi:10.1152/japplphysiol.00647.2017.
40. **Chacaroun S**, **Borowik A**, **Morrison SA**, **Baillieul S**, **Flore P**, **Doutreleau S**, **Verges S.** Physiological responses to two hypoxic conditioning strategies in healthy subjects. *Front Physiol* 7: 675, 2016. doi:10.3389/fphys.2016.00675.
41. **Zhang P**, **Downey HF**, **Chen S**, **Shi X.** Two-week normobaric intermittent hypoxia exposures enhance oxyhemoglobin equilibrium and cardiac responses during hypoxemia. *Am J Physiol Regul Integr Comp Physiol* 307: R721–R730, 2014. doi:10.1152/ajpregu.00191.2014.