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ORIGINAL ARTICLE

Dose–response relationship of intermittent normobaric hypoxia to stimulate erythropoietin in the context of health promotion in young and old people

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Abstract

Purpose Erythropoietin (EPO) has multifactorial positive effects on health and can be increased by intermittent normobaric hypoxia (IH). Recommendations about the intensity and duration of IH to increase EPO exist, but only for young people. Therefore, the aim of the study was to investigate the dose–response relationship regarding the duration of hypoxia until an EPO expression and the amount of EPO expression in old vs. young cohorts.

Methods 56 young and 67 old people were assigned to two separate investigations with identical study designs (3-h hypoxic exposure) but with different approaches to adjust the intensity of hypoxia: (i) the fraction of inspired oxygen (FiO_2) was 13.5%; (ii) the FiO_2 was individually adjusted to an oxygen saturation of the blood of 80%. Age groups were randomly assigned to a hypoxia or control group (normoxic exposure). EPO was assessed before, during (90 and 180 min), and 30 min after the hypoxia.

Results EPO increased significantly after 180 min in both cohorts and in both investigations [old: (i) + 16%, $p = 0.007$ and (ii) + 14%, $p < 0.001$; young: (i) + 27%, $p < 0.001$ and (ii) + 45%, $p = 0.007$]. In investigation (i), EPO expression was significantly higher in young than in old people after 180 min of hypoxic exposure ($p = 0.024$) and 30 min afterwards ($p = 0.001$).

Conclusion The results indicate that after a normobaric hypoxia of 180 min, EPO increases significantly in both age cohorts. The amount of EPO expression is significantly higher in young people during the same internal intensity of hypoxia than in old people.

Keywords EPO · Aging · Hypoxic intensity · Hypoxic duration · Altitude training

Abbreviations

CaO_2	Arterial oxygen content
CG	Control group
EPO	Erythropoietin
FiO_2	Fraction of inspired oxygen
Hb	Hemoglobin
Hct	Hematocrit
HG	Hypoxia group
HIF	Hypoxia-inducible factor
H-ext	Investigation where the intensity of hypoxia was applied to external parameters
H-int	Investigation where the intensity of hypoxia was applied to internal parameters
IH	Intermittent hypoxia
RBC	Red blood cells
SpO_2	Oxygen saturation of the blood
TrkB	Receptor tyrosine kinase B

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Introduction

Erythropoietin (EPO) is a 165-amino acid (~ 30 kDa) glycoprotein hormone which belongs to the cytokine type I superfamily. EPO is primarily associated with erythropoiesis because it stimulates erythroid cell production in the bone

marrow. Furthermore, it is also a cytoprotective agent for the brain and the cardiovascular system (Jelkmann 2005; Brines and Cerami 2006; Santhanam et al. 2010; Westenbrink et al. 2007). Due to some signaling pathways as in hematopoiesis, EPO has a neuroprotective effect (e.g., against stroke), promotes myocardial survival, and acts as vascular growth factor through a combination of several mechanisms (Genc et al. 2004; Bartesaghi et al. 2005; Fliser and Haller 2007). Additionally, EPO may be involved in neurogenic processes in the brain causing cerebral adaptions. It has been assumed that the long latencies of those adaptions are associated with an EPO-dependent mRNA expression and a production of the brain-derived neurotrophic factor (BDNF). BDNF, in turn, leads to a long-term activation of the specific receptor tyrosine kinase B (TrkB) (Viviani et al. 2005). Because of the above-mentioned adaptations, EPO has been discussed to be a meaningful preventive and therapeutic endogenous hormone to prevent neurocognitive disorders as well as cardiovascular diseases (Westenbrink et al. 2007; Vogiatzi et al. 2010; Liu et al. 2008). It especially exhibits a particular value for old people due to the intersection between age and those disorders/diseases (Borson 2010; North and Sinclair 2012).

One of the main stimuli for increasing EPO is tissue hypoxia. Tissue hypoxia can be reached by breathing oxygen-reduced air (normobaric hypoxia). Therefore, the controlled exposure to normobaric hypoxia is a valuable non-pharmacological treatment strategy to enhance EPO above the basal level (blood serum concentration range from 6 to 32 U/l; Jelkmann 1992) to initiate the above-mentioned adaptations (Verges et al. 2015). In response to a hypoxia, the hypoxia-inducible factor (HIF), particularly the HIF-2 (Haase 2013), will ubiquitously be stabilized as key transcription factor for EPO gene expression in the renal cortex (predominant peripheral production), liver, and brain (predominant central production in temporal cortex, hippocampus and amygdala) (Jelkmann 2005). While the increase in EPO can be up to 200-fold in the renal cortex, a 3- to 20-fold increase is possible in the brain. Although the increase in central EPO is lower, the kinetics of EPO expression occurs in a very similar way (Marti 2004; Fandrey 2004). To increase the EPO level, normobaric hypoxia does not need to be applied continuously (e.g., the whole day), but temporarily. The temporary exposure to hypoxia, interspersed with periods of normoxia, is a special hypoxic protocol called intermittent hypoxia (IH). The efficacy of IH is mainly depending on five variables: (1) the intensity of hypoxia, (2) the duration of hypoxia, (3) the number of cycles, (4) the pattern of IH presentation (e.g., consecutive days vs. alternating days), and (5) the total protocol duration (see Navarrete-Opazo and Mitchell 2014). The intensity and the duration of hypoxia are especially crucial for increasing EPO in IH-sessions. Here, the intensity of hypoxia should be differentiated between external and internal hypoxia. With regard to the discussion by Millet et al. (2016) about the characteristics of the hypoxic dose concerning the intensity of hypoxia, this distinction is crucial to elucidate physiological adaptation processes and a dose–response relationship. While the external intensity of hypoxia determines the type of the environmental condition (e.g., the fraction of inspired oxygen: FiO_2), the internal intensity of hypoxia is characterized by the amount of oxygen deficit in the organism (e.g., the oxygen saturation of the blood: SpO_2, measured by a pulse oximeter). Here, the intensity of internal hypoxia depends among others on the intensity of external hypoxia, some physiological processes (e.g., lung diffusion capacity), the chemoreceptor sensitivity (García-Río et al. 2007) and the compensatory response of the organism (e.g., the cardiorespiratory system; Bärtsch and Gibbs 2007). With regard to the latter three factors, inter-individual differences and age-specific changes can be determined (García-Río et al. 2007; Lhuissier et al. 2012; Stam et al. 1994). Consequently, there are inter-individual differences of the internal intensity of hypoxia during the administration of an external hypoxia which may result in a different severity of the hypoxic stimulus for the organism (variations by up to 10% or more of the SpO_2, see Chacaroun et al. 2017; Burtscher et al. 2004; Harshman et al. 2015).

Regarding the dose–response relationship of these two hypoxic variables (intensity and duration of hypoxia) and the EPO expression, there is a good body of evidence for people in the age from 20 to 40 years. We know that in this cohort, EPO expression depends on the intensity of hypoxia (e.g., Savourey et al. 2004; Ge et al. 2002; Mackenzie et al. 2008) and that EPO increases after a duration of approximately 2 h of hypoxic exposure (e.g., Knaupp et al. 1992; Eckardt et al. 1989; Turner et al. 2017; Montero and Lundby 2018). To date, however, there is no evidence with respect to an effective dose–response relationship in older cohorts. Thus, we aimed to investigate the acute EPO expression during and after an exposure to normobaric hypoxia in old vs. young individuals. The cohorts were compared regarding (1) the duration of hypoxic exposure until a significant increase of EPO was observed as well as (2) the amount of EPO expression during and after the hypoxic exposure.

Materials and methods

External hypoxia vs. internal hypoxia (H-ext vs. H-int)

To elucidate the dose–response relationships of hypoxic exposure and EPO expression as a function of age, we conducted two investigations with a comparable study design

but different approaches to adjust the intensity of hypoxia (external vs. internal hypoxia, see "Introduction").

In the first investigation, we adjusted the intensity of hypoxia regarding parameters of the external intensity of hypoxia. Here, we have used a mean external intensity of normobaric hypoxia like in IH studies in therapeutic settings: recommendation for the FiO_2 ranged between 17% and 10% (see Navarrete-Opazo and Mitchell 2014; Mateika et al. 2015; Verges et al. 2015). We chose a FiO_2 of 13.5% as external intensity of hypoxia for the first investigation (acronym for this investigation: H-ext).

In the second investigation, we adjusted the intensity of hypoxia regarding parameters of the internal intensity of hypoxia. Here, the FiO_2 was individually adjusted to a SpO_2 of 80% (acronym for this investigation: H-int). The SpO_2 of 80% corresponds to the average internal intensity of hypoxia during an external hypoxia with a FiO_2 of 17–10% (see Burtscher et al. 2004; Chacaroun et al. 2017).

Participants

For the investigations H-ext and H-int, we recruited a total of 56 young (age: 18 to 35 years; H-ext: $n=27$; H-int: $n=29$) and 67 old (age 60–75 years; H-ext: $n=33$; H-int: $n=34$) volunteers (see Table 1). The hematological parameters red blood cells (RBC), hemoglobin (Hb), and hematocrit (Hct) were in the range of the standard values for each participant (see Table 1). Therefore, we ruled out that the participants had restrictions of the erythropoietic system.

The participants had neither cardiovascular or respiratory diseases nor hematological or renal disorders. The involved subjects successfully passed a medical anamnesis by a medical doctor and were briefed about the aims and the experimental protocol of this study before signing a written informed consent (according to the Declaration of Helsinki). This study was officially approved and authorized by the Ethical Committee of the Otto von Guericke University Magdeburg, Germany (Nr. 74/15).

Study design

The H-ext and H-int were single-blind (subject) simple randomized (permuted block randomization, proportion 1:1, using the software RITA—Randomization In Treatment Arms, Evidat®, Germany) controlled trials in a repeated measures design. In both investigations, the participants breathed normobaric hypoxic air in a sitting position over 3 h. The intensity of hypoxia was administered following the above-mentioned approaches. In the H-ext, the participants of the hypoxia groups (HG-Young, HG-Old) received the normobaric hypoxic air in the altitude room of the Institute of Sports Science at the Otto von Guericke University Magdeburg (altitude system by Hypoxico®; the FiO_2 was continuously controlled by an oxygen measuring device: GOX100, Greisinger electronic GmbH, Germany). If the SpO_2 of participants critically decreases (< 75%), they were asked to cancel the investigation. In the H-int, the normobaric hypoxic air was administered through a facemask (CPAP double-port mask, hand-held or fixed with a bandeau) by an altitude generator (Everest Summit II, Hypoxico®, USA). The use of an altitude generator ensured a short-term regulation of the FiO_2 to the target SpO_2 of 80%. The FiO_2 can be gradually adjusted (gradually in 0.3% steps). We determined the FiO_2 for each setting in advance which helped us to know the applied FiO_2 needed to get a SpO_2 of 80%.

The SpO_2 was measured with a finger-pulse oximeter (MD300C2, Beijing Choice Electronic Tech. Co, Ltd®, China) at each 15 min to control the intensity of internal hypoxia in the H-ext and to adjust the intensity of external hypoxia in the H-int. The same investigation procedure was performed within the control groups (CG-Young, CG-Old) in the H-ext and H-int, whereas those received a placebo–air mixture (ambient air, FiO_2 20.9%).

Table 1 Values (mean ± standard deviation) of personal parameters and values of hematological parameters of the young and old people in the interventions H-ext and H-int

Groups	*N*	Age (years)	Gender	RBC (Mio./µl)	Hb (mmol/l)	Hct (%)
H-ext						
HG-young	13	23.5 ± 2.8	♀3/♂10	5.0 ± 0.3	9.2 ± 0.6	43.6 ± 2.4
CG-young	14	23.3 ± 2.6	♀2/♂12	5.1 ± 0.5	9.4 ± 1.0	44.5 ± 4.1
HG-old	15	69.1 ± 2.6	♀6/♂9	4.8 ± 0.4	9.1 ± 0.5	42.9 ± 2.1
CG-old	18	66.9 ± 4.2	♀8/♂10	4.9 ± 0.3	9.2 ± 0.6	44.0 ± 2.6
H-int						
HG-young	14	23.5 ± 2.8	♀4/♂10	5.1 ± 0.4	9.5 ± 0.7	44.6 ± 2.6
CG-young	15	23.3 ± 2.6	♀3/♂12	5.0 ± 0.4	9.1 ± 0.8	43.2 ± 3.3
HG-old	17	69.1 ± 2.6	♀5/♂12	4.9 ± 0.4	9.2 ± 0.7	43.4 ± 2.7
CG-old	17	66.9 ± 4.2	♀9/♂8	4.9 ± 0.4	9.2 ± 0.6	43.3 ± 2.6

Red blood cells (RBC), hemoglobin (Hb), and hematocrit (Hct)

Blood analyses

To determine the peripheral EPO levels, we draw blood samples of approximately 5 ml immediately prior to the investigations (baseline: BL), at 90 and 180 min of the hypoxic exposure (90 min, 180 min), and 30 min after the exposure (30 min post). Apart from that, the baseline blood samples were used to determine red blood cells (RBC), hemoglobin (Hb), and hematocrit (Hct) to be able to rule out that the participants had any diseases of the erythropoietic system. EPO was measured in serum of the blood samples by the fully automated random access two-side sandwich enzyme-enhanced chemiluminescent immunoassay method (CLIA) with the IMMULITE® 2000 immunoassay system (Siemens Healthcare Diagnostics®, Germany) (Owen and Roberts 2011). The medical doctor who drew the blood samples was blinded regarding the group allocation of the participants.

Statistical analyses

For statistical analyses, all EPO values were reported as percentage changes (relative changes with respect to the baseline measurement) to be able to compare those among cohorts. Then the data were imported and analyzed with the statistical computer software SPSS 24 (IBM®, Germany). The data were checked for normal distribution using the Kolmogorov–Smirnov test. Differences in EPO between the hypoxia and control group within the age cohorts (HG-young vs. CG-young, HG-old vs. CG-old) or between the age cohorts within the hypoxia or control group (HG-young vs. HG-old, CG-young vs. CG-old) over the time points (baseline, 90-min, 180-min, 30-min) were analyzed with a two-way ANOVA with repeated measures (interaction effect). We used the post hoc Bonferroni correction of a one-way ANOVA with repeated measures, to determine differences between time points for EPO (time effect). Values of SpO_2 and FiO_2 were averaged for the 3 h of exposure to hypoxia or normoxia. Differences between groups were determined with the post hoc Bonferroni correction of a one-way ANOVA without repeated measures for the EPO, SpO_2 and FiO_2 (group effects). In conjunction with the interaction effects, main time effects, and main group effects, the effect size partial eta squared (η_p^2) was reported (Lakens 2013).

p values < 0.05 were considered significant. The analyst conducting statistical procedures was blinded to the group allocation.

Results

SpO_2 and FiO_2 in the H-ext and H-int

Regarding the H-ext and H-int, the mean values (± standard deviation) of the SpO_2 and FiO_2 are shown in Fig. 1.

Main group effects were observed for the SpO_2 in the H-ext ($F_{3,60} = 178.4$; $p < 0.001$; $\eta_p^2 = 0.905$) as well as in the H-int ($F_{3,63} = 1277.5$; $p < 0.001$; $\eta_p^2 = 0.985$). Also for the H-int, a main group effect was found for the FiO_2 ($F_{3,63} = 546.5$; $p < 0.001$; $\eta_p^2 = 0.965$). We were not able to

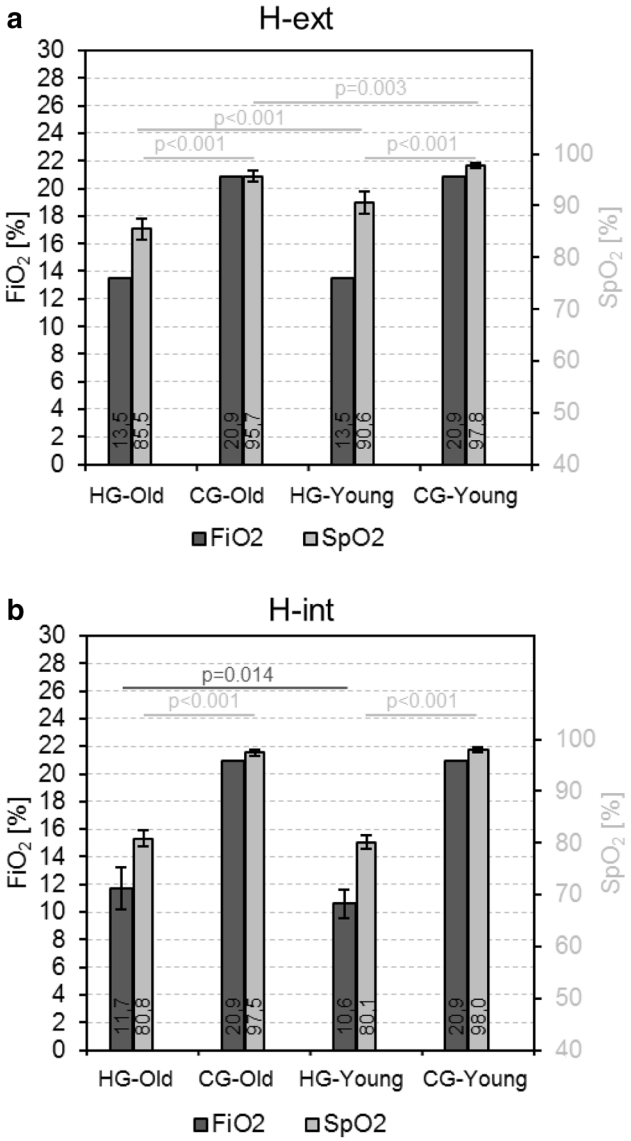

Fig. 1 Mean values of the fraction of inspired oxygen (FiO_2) and the oxygen saturation of the blood (SpO_2) during an exposure to a normobaric hypoxia over 3 h. The intensity of hypoxia was either adjusted to a fraction of inspired oxygen (FiO_2) of 13.5% (H-ext; 1a) or to an oxygen saturation of the blood (SpO_2) of 80% (H-int; 1b)

calculate main group effects of the FiO_2 in the H-ext due to the lack of variance.

Regarding the post hoc calculated group effects, there were two main findings: first, significant lower values of SpO_2 were found in the HG-old as compared to the HG-young when the FiO_2 was fixed in the H-ext (see Fig. 1a). Second, the FiO_2 must be significantly lower adjusted in the HG-young to reach the predefined SpO_2 of 80% as compared to the HG-old in the H-int (see Fig. 1b). In the H-ext and H-int, the SpO_2 values were significantly lower in both age cohorts in the HG as compared to CG (see Fig. 1a, b).

Acute response of EPO to a normobaric hypoxia in young and old people

The mean (± standard deviation) percentage changes of EPO during the H-ext and H-int are shown in Fig. 2 as well as Table 2.

There was a significant interaction effect between the groups (HG, CG) within the age cohorts on the EPO in the H-ext and H-int (interaction effects, see Table 2).

The main time effect over all measurement time points was significant in both investigations for the hypoxia groups but not for the control groups (main time effects, see Table 2). In the post hoc analysis with Bonferroni correction, we observed a significant increase in EPO after

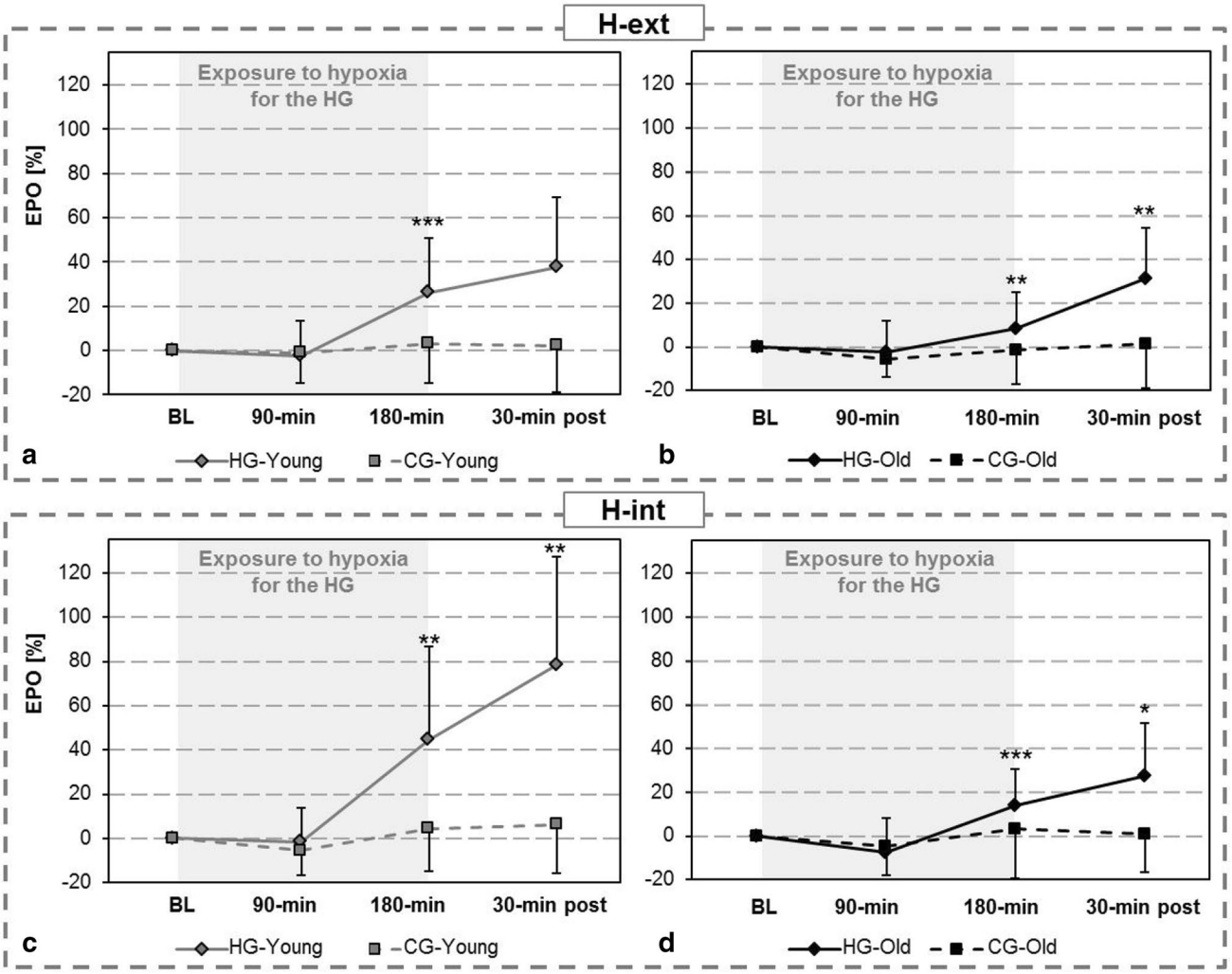

Fig. 2 Percentage change of serum EPO related to the baseline during a single normobaric hypoxic exposure over 3 h as well as 30 min after administration of hypoxia in young and old people. The intensity of hypoxia was either adjusted to a fraction of inspired oxygen (FiO_2) of 13.5% (H-ext) for **a** young and **b** old people or to an oxygen saturation of the blood (SpO_2) of 80% (H-int) for **c** young and **d** old people (time points for measurement EPO: baseline: BL; after 90 and 180 min of hypoxic exposure: 90 min, 180 min; 30 min after the exposure to hypoxia: 30-min post; *HG* hypoxia group, *CG* control group; time effects in relation to the previous time point: $*p < 0.050$, $**p < 0.010$, $***p < 0.001$)

Table 2 Percentage change of serum EPO related to the baseline during a single normobaric hypoxic exposure over 3 h as well as 30 min after administration to hypoxia in young and old people

Groups	90 min (mean ± SD, time effect[a])	180 min (mean ± SD, time effect[a])	30 min post (mean ± SD, time effect[a])	Main time effect	Interaction effect[b]
H-ext					
HG-young	−2.4 ± 15.9, $p = 1.000$	26.5 ± 24.1, $p < 0.001$	37.9 ± 31.2, $p = 0.419$	$F_{3,36} = 19.9$; $p < 0.001$; $\eta_p^2 = 0.623$	$F_{3,66} = 9.3$; $p = 0.001$; $\eta_p^2 = 0.297$
CG-young	−0.7 ± 14.0, $p = 1.000$	3.1 ± 17.8, $p = 1.000$	2.5 ± 21.3, $p = 1.000$	$F_{3,30} = 0.6$; $p = 0.535$; $\eta_p^2 = 0.058$	
HG-old	−2.3 ± 13.8, $p = 1.000$	15.7 ± 24.2, $p = 0.007$	31.2 ± 23.2, $p = 0.002$	$F_{3,30} = 21.7$; $p < 0.001$; $\eta_p^2 = 0.685$	$F_{3,66} = 8.6$; $p = 0.001$; $\eta_p^2 = 0.282$
CG-old	−5.6 ± 8.3, $p = 0.093$	−1.3 ± 15.8, $p = 0.961$	1.5 ± 20.5, $p = 1.000$	$F_{3,36} = 1.3$; $p = 0.282$; $\eta_p^2 = 0.100$	
Main group effect	$F_{3,56} = 0.342$ $p = 0.795$ $\eta_p^2 = 0.019$	$F_{3,56} = 5.1$ $p = 0.004$ $\eta_p^2 = 0.228$	$F_{3,49} = 7.5$ $p < 0.001$ $\eta_p^2 = 0.333$		
H-int					
HG-young	−1.5 ± 15.2, $p = 1.000$	44.6 ± 41.9, $p = 0.007$	78.5 ± 48.7, $p = 0.004$	$F_{3,30} = 23.7$; $p < 0.001$; $\eta_p^2 = 0.703$	$F_{3,75} = 17.2$; $p < 0.001$; $\eta_p^2 = 0.474$
CG-young	−5.5 ± 11.6, $p = 1.000$	4.5 ± 19.5, $p = 1.000$	6.4 ± 22.4, $p = 1.000$	$F_{3,27} = 0.6$; $p = 0.565$; $\eta_p^2 = 0.060$	
HG-old	−7.6 ± 15.4, $p = 0.055$	14.1 ± 16.5, $p < 0.001$	27.5 ± 23.7, $p = 0.026$	$F_{3,36} = 21.2$; $p < 0.001$; $\eta_p^2 = 0.638$	$F_{3,75} = 7.5$; $p = 0.001$; $\eta_p^2 = 0.232$
CG-old	−4.6 ± 13.6, $p = 1.000$	3.5 ± 23.0, $p = 1.000$	1.2 ± 18.1, $p = 1.000$	$F_{3,39} = 1.0$; $p = 0.386$; $\eta_p^2 = 0.070$	
Main group effect	$F_{3,59} = 0.5$ $p = 0.691$ $\eta_p^2 = 0.026$	H-int $F_{3,56} = 6.9$ $p = 0.001$ $\eta_p^2 = 0.283$	$F_{3,51} = 17.1$ $p < 0.001$ $\eta_p^2 = 0.522$		

The intensity of hypoxia was either adjusted to a fraction of inspired oxygen (FiO_2) of 13.5% (H-ext) for young and old people or to an oxygen saturation of the blood (SpO_2) of 80% (H-int) for young and old people; time points for measurement EPO: after 90 and 180 min of hypoxic exposure: 90 min, 180 min; 30 min after the exposure to hypoxia: 30 min post; HG: hypoxia group, CG: control group

[a]Time effect at 90 min (from baseline to 90 min), at 180 min (from 90 min to 180 min) and at 30 min post (from 180 min to 30 min post)

[b]Interaction effect between the HG and CG over all measurement time points (90 min, 180 min, 30 min post) within the age cohorts

180 min of hypoxic exposure in the H-ext and H-int for the hypoxia groups of the young and old people (time effects, see Table 2; Fig. 2). 30 min after the exposure, EPO continued to increase significantly in the hypoxia groups, except in the HG-young of the H-ext (time effects, see Table 2; Fig. 2).

Significant main group effects were identified for the time point 180 min as well as 30 min post, but not for 90 min in the H-ext and H-int (main group effects, see Table 2). The post hoc analysis with Bonferroni correction showed that in the H-int, the EPO level was significantly greater (approximately three times) at 180 min ($p = 0.024$) and 30 min post ($p = 0.001$) in HG-young vs. HG-old. No group effects were in the H-ext between HG-young and HG-old.

In addition to the variance shown in Fig. 2, Fig. 3 shows the inter-individual percentage change of EPO of every single participant due to the exposure to hypoxia and afterwards.

Discussion

The acute response of EPO to a normobaric hypoxia as a function of age

With respect to the aim of this study, there are two primary findings:

First, an exposure to a normobaric hypoxia between 90 and 180 min is needed to yield a significant acute increase in EPO in young and old people, see Table 2 and Fig. 2.

Second, the amount of acute EPO expression in young people is significantly higher than in old people if internal intensity of hypoxia will be similarly administered (no significant difference if normobaric hypoxia is administered with a similar external intensity of hypoxia), see Table 2.

In addition to our two primary findings, we want to highlight also one observation of the current study: there was a high dispersion of EPO expression in response to a hypoxic exposure in both age cohorts, see Fig. 3.

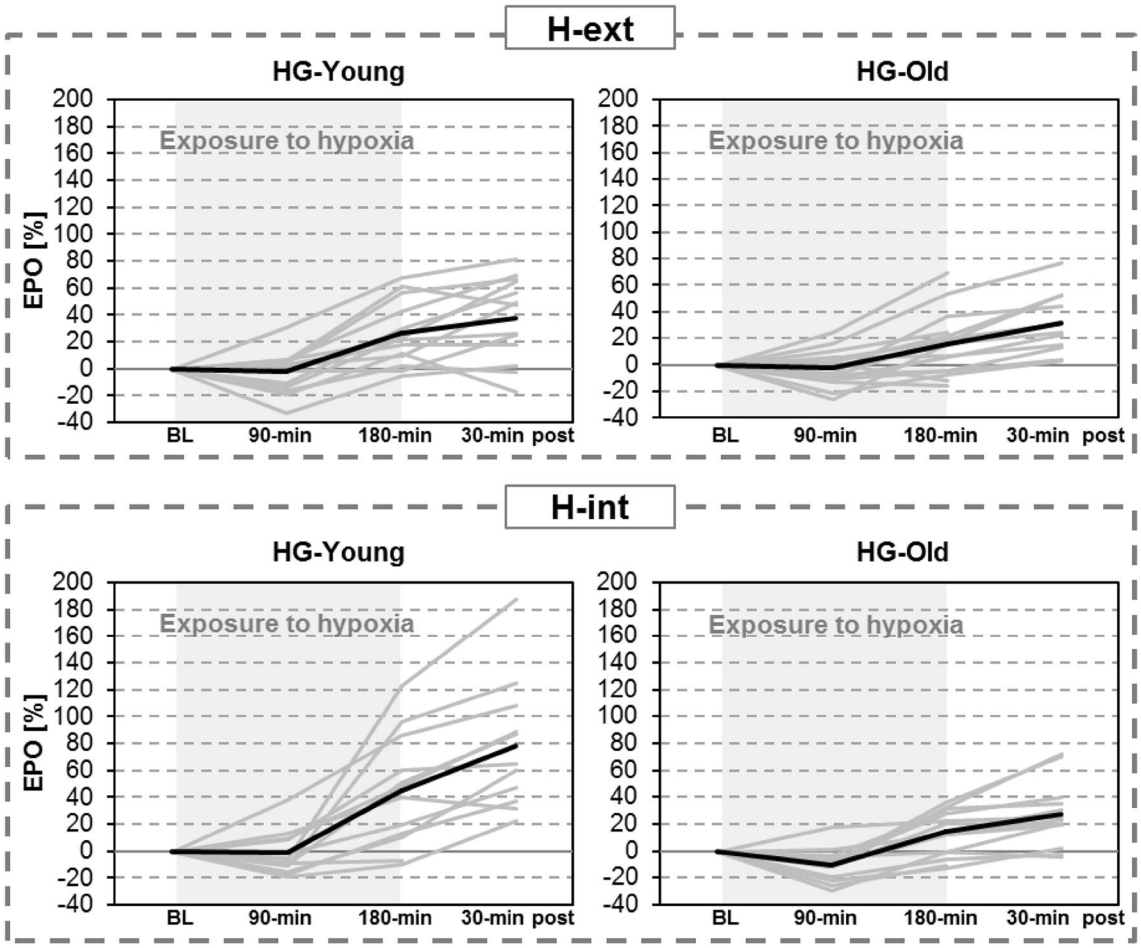

Fig. 3 Inter-individual percentage change of serum EPO related to the baseline measurement during a single normobaric hypoxic exposure over 3 h as well as 30 min after administration of hypoxia in young and old people (the gray lines denote single participants, the black lines denote the mean value of the group). The severity of hypoxia was either adjusted to a fraction of inspired oxygen (FiO_2) of 13.5% (H-ext) for young and old people or to an oxygen saturation of the blood (SpO_2) of 80% (H-int) for young and old people (time points for measurement EPO: baseline: BL; after 90 and 180 min of hypoxic exposure: 90 min, 180 min; 30 min after the exposure to hypoxia: 30 min post; *HG* hypoxia group, *CG* control group)

The first primary finding is in line with the results of Eckardt et al. (1989), Knaupp et al. (1992), Wahl et al. (2013), Turner et al. (2017), as well as Montero and Lundby (2018) who identified that approximately 2–3 h of hypoxic exposure are required for a significant increase in EPO. So far, however, this has only been investigated for young people (20–40 years). Although we were not able to identify the exact time point for an initial EPO expression as a function of age, we can at least ensure that the duration of hypoxia exposure for an EPO expression is between 90 and 180 min for old people, too.

Regarding our second primary finding, this is the first study, to our knowledge, that examines the amount of EPO expression in response to a normobaric hypoxia in old people (vs. young people). The lower hypoxic-induced EPO expression in old people as compared to young people could be explained by various age-dependent morphological and functional alterations of the organism. Concerning this, we want to highlight the following two aspects which might be crucial factors:

(i) It has been observed that the stabilization of HIF is impaired with aging which, in turn, could restrict the production of EPO (Rivard et al. 2000).
(ii) Besides this, the renal function could be restricted by inflammatory processes and sclerosing modifications of the kidney tissue in the age (Costa et al. 2013; Zhou et al. 2008).

To clarify the exact underlying mechanisms of the above-mentioned age-dependent phenomenon, further investigations should be carried out. Moreover, we observed for the first time that there is a further increase in EPO after a hypoxic exposure also for old people. This was, so far,

only known among young people (e.g., Eckardt et al. 1989; Knaupp et al. 1992; Turner et al. 2017; Ge et al. 2002). Thus, the release of EPO is not limited to the hypoxic period in old people, too.

The phenomenon of an inter-individual variability of the EPO expression in response to hypoxia is for the first time also observed for old people (so far, just known for young people; see Friedmann et al. 2005, as well as; Chapman et al. 1998, 2010, for review, see Płoszczyca et al. 2018). However, the reason for this dispersion is still unclear. Even though it is known that the level of the arterial oxygen content (CaO_2) is crucial for EPO expression (Montero and Lundby 2018), it is controversially discussed which superordinate governor determines the amount of EPO expression in response to hypoxia. Relating to this, Witkowski et al. (2002), Jedlickova et al. (2003), and Hennis et al. (2010) discussed several genetic determinants, but they could not provide a final explanation. Perhaps, inter-individual genetic differences (genotype) are the reason for the mentioned phenomenon which might explain, on the one hand, the occurrence of responders and non-responders (see Friedmann et al. 2005; Chapman et al. 1998) and, on the other hand, the contradicting results regarding the relationship between the intensity of normobaric hypoxia and the amount of EPO expression (mean relationship: Savourey et al. 2004; Eckardt et al. 1989, 1990; Ge et al. 2002; Mackenzie et al. 2008, vs. no relationship: Turner et al. 2017).

External vs. internal intensity of hypoxia as a function of age

We found age-specific significant differences between the external and internal intensity of hypoxia if one of these parameters will be kept constant. This was shown in two respects: (i) the internal intensity of hypoxia is significantly lower in old people as compared to young people during the same external intensity of hypoxia; (ii) for a comparable internal intensity of hypoxia, the external intensity of hypoxia must be significantly higher for young people compared to old people, see Fig. 1. These results are in line with the results of García-Río et al. (2007), Korkushko et al. (2009), and Lhuissier et al. (2012). The observed phenomenon could be reasoned by a changed acute compensatory response to hypoxia (such as the increase of the cardiorespiratory system; Bärtsch and Gibbs 2007) whereby very different responses have been detected for old people in comparison to young people (comparable response of the cardiorespiratory system: Pokorski and Marczak 2003; Ahmed et al. 1991; Vovk et al. 2004; decreased ventilatory response: Kronenberg and Drage 1973; Serebrovskaya et al. 2000; increased ventilatory response with lower cardiac activity: Lhuissier et al. 2012). Different acute hypoxic responses of the cardiorespiratory system could be reasoned by various degenerative physiological and/or morphological changes such as decreased sensitivity of chemoreceptors (Pokorski et al. 2004; García-Río et al. 2007), defect of the HIF-1α action (Rivard et al. 2000; Prabhakar 2013), degenerative changes of the lung morphology (Janssens et al. 1999; Sharma and Goodwin 2006), and reduced response of the heart rate evoked by reduced β-adrenergic responsiveness (Christou and Seals 2008).

Consequently, we recommend adjusting the external intensity of hypoxia individually to reach a homogeneous and inter-individual comparable internal intensity of hypoxia. Here, the SpO_2 can be used as a sensitive target parameter. With this approach, it can be assured that the internal hypoxic stimulus is provided in a comparable and safe manner for each person in IH treatments, independent of age.

Conclusion

All in all, the current study provides the following insights:

- An exposure to a normobaric hypoxia between 90 and 180 min is needed to yield a significant acute increase in EPO in both young and old people (the exact initial time point of an acute increase has not been identified).
- As already known in young people, a further EPO expression can be observed after a hypoxic exposure also in old people.
- The amount of EPO expression after a hypoxic exposure of 3 h (same internal intensity of hypoxia) as well as 30 min after the hypoxic exposure is significantly higher in young people as compared to old people (approximately three times).
- The internal intensity of hypoxia (SpO_2) is significantly lower in old people as compared to young people during the same external intensity of hypoxia (FiO_2). Accordingly, for a comparable internal intensity of hypoxia (SpO_2), the external intensity of hypoxia (FiO_2) must be significantly higher for young people compared to old people.

Future IH-studies focusing on investigating health effects of EPO should consider these insights regarding the dose–response relationship between IH and EPO expression as well as the application of the hypoxic intensity.

Compliance with ethical standards

Conflict of interest None of the authors have any conflicts of interests.

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