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Research Paper

Effect of ageing on hypoxic exercise cardiorespiratory, muscle and cerebral oxygenation responses in healthy humans

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New Findings

- **What is the central question of this study?**
  This study aimed to determine the effect of ageing on cardiorespiratory and tissue oxygenation responses to hypoxia during maximal incremental exercise.
- **What is the main finding and its importance?**
  Older healthy subjects had preserved hypoxic cardiorespiratory and tissue oxygenation responses at rest and during moderate exercise. At maximal exercise, they had a reduced hypoxic ventilatory response but similar maximal power output reduction compared with young individuals. This study suggests that until moderate exercise, hypoxic responses are preserved until the age of 70 years and therefore that ageing is not a contraindication for high-altitude sojourn.

This study assessed the effects of ageing on cardiorespiratory and tissue oxygenation responses to hypoxia both at rest and during incremental maximal exercise. Sixteen young (20–30 years old) and 15 older healthy subjects (60–70 years old) performed two maximal incremental cycling tests in normoxia and hypoxia (inspiratory oxygen fraction 12%). Cardiorespiratory responses, prefrontal cortex and quadriceps tissue oxygenation (near-infrared spectroscopy) were measured during exercise as well as during hypercapnia at rest. The hypoxic ventilatory response was similar in young compared with older individuals at rest and during moderate-intensity exercise (50% maximal power output: young 0.9 ± 0.2 *versus* older 1.1 ± 0.8 l min^{-1} %$^{-1}$; $P > 0.05$) but larger in young subjects during high-intensity exercise (maximal power output: 2.2 ± 0.8 *versus* 1.8 ± 1.1 l min^{-1} %$^{-1}$; $P < 0.05$). The hypoxic cardiac response did not differ between groups both at rest and during exercise. During exercise in hypoxia, young subjects showed greater deoxygenation than older subjects, at both the prefrontal cortex and quadriceps levels. The hypoxia-induced reduction in maximal power output (young −32 ± 5% *versus* older −30 ± 6%; $P > 0.05$) and the hypercapnic responses did not differ between groups. Older healthy and active individuals below the age of 70 years have cardiorespiratory and tissue oxygenation responses to hypoxia similar to young individuals both at rest and during moderate-intensity exercise.

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Despite a lower hypoxic ventilatory response at maximal exercise, older individuals have similar oxygen desaturation and maximal power output reduction compared with young subjects.

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Introduction

The physiological responses to hypoxia involve a series of cardiorespiratory and tissue mechanisms that act to mitigate the reduction in tissue oxygen availability. Ageing can be associated with an alteration in several of these mechanisms, e.g. lung function, chemosensitivity and vasoreactivity (Janssens *et al.* 1999; Marín & Rodríguez-Martínez, 1999), raising the issue of a potential reduction in hypoxic tolerance in older individuals. Hypoxaemia can occur in older individuals, either because of the effect of ageing on the respiratory system (Janssens *et al.* 1999) or because of the occurrence of pathologies, such as chronic obstructive pulmonary disease or sleep apnoea. Older people can also be exposed to hypoxia during travel at high altitude, where altitude mountain sickness can develop. The effect of ageing on the physiological response and tolerance to hypoxia is therefore of particular relevance both in high-altitude activities and in clinical conditions that cause local or systemic hypoxia.

The hypoxic ventilatory response is thought to be an important mechanism during hypoxic exposure. The evaluation of the effect of ageing on the ventilatory response has led to contrasting results. Similar isocapnic hypoxic ventilatory response at rest has been observed in young and older men and women (Smith *et al.* 2001; Pokorski *et al.* 2004; Vovk *et al.* 2004), whereas others have reported reduced isocapnic hypoxic ventilatory responses in older men and women (Kronenberg & Drage, 1973; Serebrovskaya *et al.* 2000; García-Río *et al.* 2007; Hartmann *et al.* 2015). Two studies have also reported an increased isocapnic hypoxic ventilatory response in older men and women at rest (Chapman & Cherniack, 1987) and larger poikilocapnic hypoxic ventilatory response at rest and during mild-intensity exercise in older men only (Lhuissier *et al.* 2012) compared with young control subjects. Fewer data are available regarding the effect of ageing on the hypoxic cardiac response. A blunted cardiac response has been reported to isocapnic hypoxia at rest in elderly men (Chapman & Cherniack, 1987) and to poikilocapnic hypoxia at rest and during mild-intensity exercise in older men and women (Lhuissier *et al.* 2012).

In addition to the cardiorespiratory responses, the brain and muscle haemodynamic responses play a major role in cognitive function and physical performance in hypoxia (Verges *et al.* 2012). Ageing is known to reduce cerebral blood flow and oxygenation at rest and during exercise (Murrell *et al.* 2012; Fisher *et al.* 2013). The cerebrovascular response to hypercapnia in older individuals was reported to be similar (Davis *et al.* 1983; Kastrup *et al.* 1998; Galvin *et al.* 2010; Murrell *et al.* 2012) or reduced (Yamamoto *et al.* 1980; Reich & Rusinek, 1989) compared with younger individuals. Only one study recently evaluated the hypoxic cerebrovascular response in older individuals at rest and reported blunted cerebral blood velocity sensitivity to hypoxia compared with young control subjects (Hartmann *et al.* 2015). At the muscle level, reduced oxygenation has been reported in older individuals at rest and during exercise in normoxia (Costes *et al.* 1999; Ferri *et al.* 2007). Some results suggest that the muscle vasodilator response to hypoxia may be reduced at rest in the elderly (Kirby *et al.* 2012) and either reduced or similar during exercise (Casey *et al.* 2011; Limberg *et al.* 2012) in older individuals compared with young control subjects. Therefore, the effects of ageing on the brain and muscle haemodynamic responses to hypoxia remain to be clarified both at rest and during exercise in order to determine whether older individuals might have reduced ability to prevent cerebral and muscle deoxygenation when arterial oxygen delivery is reduced.

The aim of this study was to compare the cardiorespiratory, cerebral and muscular oxygenation responses to hypoxia and hypercapnia in young and older men and women. Hypoxic responses were assessed during an incremental maximal exercise to account for the effect of exercise intensity and to assess, for the first time, the effect of ageing on hypoxic responses at maximal exercise. We hypothesized that ageing might be associated with reduced cardiorespiratory responses and greater tissue deoxygenation in hypoxia both at rest and during maximal exercise.

Methods

Subjects

Sixteen young healthy subjects (eight females) between 20 and 30 years old and 15 older healthy subjects (seven females) between 60 and 70 years old were included. Young and older subjects were paired for sex, body mass index and habitual physical activity. All subjects were physically active and engaged one to three times a week in low- to moderate-intensity endurance activities,

Table 1. Subjects' characteristics

Characteristic	Older ($n = 15$)	Young ($n = 16$)
Number of females/ males	7/8	8/8
Age (years)	65 (3)	25 (2)*
Height (cm)	167 (10)	172 (10)
Weight (kg)	66 (10)	68 (9)
Quadriceps skinfold thickness (mm)	9 (3)	10 (2)
Systolic blood pressure (mmHg)	124 (14)	123 (14)
Diastolic blood pressure (mmHg)	85 (13)	84 (7)
$\dot{V}_{O_2peak}$ (ml min^{-1} kg^{-1})	34.1 (7.2)	43.7 (7.0)*
Endurance activities (sessions week^{-1})	1.5 (0.9)	1.3 (1.1)
Residential altitude (m)	329 (210)	244 (40)

Data are means (SD). Abbreviation: $\dot{V}_{O_2peak}$, normoxic peak oxygen consumption. *Significant difference between groups ($P < 0.05$).

such as walking, jogging or cycling (based on individual interview). None was sedentary or highly trained. All subjects lived at low altitude (<1000 m) and were unacclimatized to high altitude at the time of the tests (no sojourn above 2000 m over the past 2 months). Subjects' characteristics are shown in Table 1. Subjects refrained from physical exercise on the 2 days before the tests, abstained from drinking caffeinated beverages on the test day, and had their last meal at least 2 h before the tests. All subjects were healthy, without cardiovascular, respiratory and neurological diseases. Four young females were taking a low-dose combined oral contraceptive, and all older females were postmenopausal. The study was approved by the local ethics committee and performed according to the *Declaration of Helsinki*. Subjects were fully informed of the procedure and risks involved and gave their written consent.

Experimental design

During the first visit, a maximal incremental cycling test was performed in normoxia (inspiratory oxygen fraction = 0.21; altitude 212 m). Subjects exercised on a computer-controlled electrically braked cycle ergometer (Ergometrics 800; Ergoline, Bitz, Germany), with breath-by-breath gas analysis and ECG (Medisoft, Dinant, Belgium). After a 2 min resting period, subjects started to cycle at 80 W (young males), 60 W (young females and older males) or 40 W (older females) for 3 min, followed by 20 W (young males), 15 W (young females and older males) or 10 W (older females) increments every 2 min until volitional exhaustion.

During the second visit, at least 48 h after the first visit, hypercapnic cardiorespiratory and tissue oxygenation responses were measured at rest. Subjects sat on a deckchair and initially inhaled ambient air for 5 min, followed by a normoxic hypercapnic gas mixture (inspiratory oxygen fraction = 0.21, inspiratory carbon dioxide fraction = 0.05) for 5 min. After these resting measurements, a maximal incremental test was performed in hypoxia. Subjects sat on the cycle ergometer and inhaled a hypoxic gas mixture (inspiratory oxygen fraction = 0.12) at rest for 10 min, i.e. a wash-in period of sufficient duration to allow muscle and cerebral tissue deoxygenation (Rupp *et al.* 2013). They started cycling at 60 W (young males), 40 W (young females and older males) or 20 W (older females) for 3 min, followed by 15 W (young males) or 10 W (females and older females and males) increments every 2 min until volitional exhaustion. Owing to the expected large difference in maximal power outputs between subpopulations (young and older individuals, females and males) in the present study, using identical initial power output for all individuals would have led to very low or very high initial power output for some subpopulations and to highly different increments in order to obtain similar exercise duration for all individuals and conditions (normoxia and hypoxia). To address this aspect, initial power outputs and subsequent increments were determined in order to obtain similar exercise duration in young and older males and females, both in normoxia and hypoxia. Gas mixtures were delivered by an IsoCap-Altitrainer 200® (SMTEC, Nyon, Switzerland) via a face mask. The order of the normoxic and hypoxic maximal incremental tests was not randomized for ethical reasons, i.e. to allow the detection of any cardiorespiratory abnormalities in normoxia that would prevent the subject from performing maximal hypoxic exercise.

Measurements

Cardiorespiratory measurements. Minute ventilation ($\dot{V}_E$), gas exchanges and heart rate (HR) were measured continuously breath by breath by an automated ergospirometer (Ergocard; Medisoft, Dinant, Belgium). Arterial oxygen saturation (S_{pO_2}) was measured continuously by earlobe pulse oximetry (Masimo Radical 7; Masimo Corp., Irvine, CA, USA). Blood lactate concentration at exhaustion (Lactate Plus; Nova Biomedical Corporation, Waltham, MA, USA) was determined during each exercise test. Resting arterial blood pressure was measured with an automatic sphygmomanometer on the dominant arm (Medisoft). In all exercise tests, at least three of the following four criteria were achieved: (i) a plateau in oxygen consumption ($\dot{V}_{O_2}$) despite increasing power output; (ii) a respiratory exchange ratio >1.1; (iii) HR >95% of age-predicted maximal HR (220 minus age); and (iv) blood lactate concentration >8 mmol l^{-1}. The peak $\dot{V}_{O_2}$ measured at

the end of each incremental cycling test is referred to as $\dot{V}_{O_2peak}$.

Near-infrared spectroscopy (NIRS). Oxyhaemoglobin (HbO_2) and deoxyhaemoglobin (HHb) concentration changes and tissue oxygenation index (TSI) were measured throughout testing sessions over multiple sites using a two-wavelength (780 and 850 nm) multichannel, continuous wave NIRS system (Oxymon MkIII; Artinis Medical Systems, Elst, The Netherlands). Total haemoglobin concentration ([HbTot]) was calculated as the sum of [HbO_2] and [HHb] and reflects the changes in tissue blood volume within the illuminated area (Hoshi *et al.* 2001; Van Beekvelt *et al.* 2001). Theoretical and performance details of NIRS have been described previously (Rolfe, 2000; Ferrari *et al.* 2004). Near-infrared spectroscopy parameters reflect the dynamic balance between O_2 demand and O_2 supply in the tissue microcirculation. The [HbO_2] and [HbTot] are mostly sensitive to blood flow and O_2 delivery, whereas [HHb] is closely associated with changes in venous O_2 content and therefore tissue oxygen extraction (Rolfe, 2000; Ferrari *et al.* 2004). Left quadriceps vastus lateralis muscle NIRS signal was assessed using an interoptode distance of 4 cm. The vastus lateralis probe holder was secured to the skin using double-sided tape and covered with a black sweatband to shield the optodes from ambient light. The vastus lateralis probe was positioned on the lower third of the muscle belly. Skinfold thickness was measured at the site of application of the NIRS probe using Harpenden skinfold callipers (Baty International, Burgess Hill, UK). The left prefrontal cortex NIRS signal was assessed between Fp1 and F3 locations according to the international 10–20 EEG system with an interoptode distance of 3.5 cm. The cortical probe holder was secured to the skin with double-sided tape and maintained with black Velcro headbands. Optode positioning was identical during both sessions for each subject. Data were recorded continuously at 10 Hz and filtered with a 3 s width moving Gaussian smoothing algorithm before analysis.

The HbO_2 and HHb concentrations are measured as differences from the previous normocapnic period during the hypercapnic test and as differences from the initial rest period during the exercise tests.

Data analysis

Cardiorespiratory and NIRS data were averaged over the last 30 s of each step during exercise and over the last 30 s of the normoxic and hypercapnic periods during the hypercapnic test. To compare normoxic and hypoxic exercise tests at iso-power output and at exhaustion, values corresponding to rest, 25 and 50% of the maximal normoxic power output (25%N and 50%N), 100% of the maximal hypoxic power output (Exh H) and 100% of the maximal normoxic power output (Exh N) were analysed. Comparisons at identical submaximal workload (90 W) are also provided. This absolute workload was chosen because it was the highest workload reached by all subjects in both normoxia and hypoxia. When the power output increment did not provide data at 90 W, data were linearly interpolated between the exercise intensities immediately below and above 90 W.

The hypoxic ventilatory (absolute or relative to body weight) and cardiac responses during the exercise test (at rest, 25%N, 50%N, Exh H and at 90 W) were calculated as follows (Richalet *et al.* 2012; Puthon *et al.* 2016):

$$(\dot{V}_{E,hypoxia} - \dot{V}_{E,normoxia})/(S_{pO_2,\,normoxia} - S_{pO_2,\,hypoxia}) \quad (\text{in } 1\,\text{min}^{-1}\%^{-1}),$$

$$(\dot{V}_{E,hypoxia} - \dot{V}_{E,normoxia})/(S_{pO_2,\,normoxia} - S_{pO_2,\,hypoxia})/\text{Body weight} \quad (\text{in } 1\,\text{min}^{-1}\%^{-1}\,\text{kg}^{-1}),$$

$$(HR_{hypoxia} - HR_{normoxia})/(S_{pO_2,\,normoxia} - S_{pO_2,\,hypoxia})/ \quad (\text{in beats min}^{-1}\%^{-1}).$$

The hypercapnic ventilatory and cardiac responses during the resting hypercapnic response test were calculated as follows (Richalet *et al.* 2012; Puthon *et al.* 2016):

$$(\dot{V}_{E,hypercapnia} - \dot{V}_{E,normocapnia})/(P_{ET,CO_2,hypercapnia} - P_{ET,CO_2,normocapnia}) \quad (\text{in } 1\,\text{min}^{-1}\text{mmHg}^{-1}),$$

$$(HR_{hypercapnia} - HR_{normocapnia})/(P_{ET,CO_2,hypercapnia} - P_{ET,CO_2,normocapnia}) \quad (\text{in beats min}^{-1}\text{mmHg}^{-1}),$$

where P_{ET,CO_2} is the end-tidal partial CO_2 pressure.

Statistical analysis

Normality of distribution and homogeneity of variances of the main variables were confirmed using a skewness–kurtosis normality test and Levene's test, respectively. Two-way ANOVAs (age × condition) with repeated measures were performed for maximal power output and maximal cardiorespiratory parameters during exercise. Three-way ANOVAs (age × condition × exercise intensity) with repeated measures were performed for cardiorespiratory parameters and NIRS data measured during exercise. Tukey's *post hoc* tests were applied to determine a difference between two mean values if the ANOVA revealed a significant main effect or interaction effect. Student's unpaired *t* tests were used to compare cardiorespiratory and NIRS hypercapnic responses between groups. Statistical analysis was performed using Statistica version 10 (Statsoft, Tulsa, OK, USA). For all statistical analyses, a two-tailed α level of

Table 2. Cardiorespiratory responses during exercise in young and older individuals

Parameter	Age	Conditions	Rest	25%N	50%N	Exh H	Exh N
Power output (W)	O		0	51 (10)	86 (24)	117 (29)	170 (45)
	Y		0	70 (11)*	118 (31)*	160 (45)*	234 (59)*
$\dot{V}_{O_2}$ (ml min^{-1} kg^{-1})	O	N	4.5 (0.8)	15.5 (2.3)	20.7 (3.6)	20.6 (4.7)	34.1 (7.1)
		H	2.9 (1.2)	12.2 (2.6)	18.5 (4.3)	25.4 (5.3)	n.a.
	Y	N	5.1 (0.9)	18.7 (1.3)*	25.5 (3.3)*	33.6 (6.3)*	43.7 (7.0)*
		H	3.2 (1.1)	11.5 (2.5)	20.0 (4.0)*	31.9 (4.0)*	NA
HR (beats min^{-1})	O	N	72 (10)	103 (14)	123 (13)	141 (14)	168 (11)
		H	73 (10)	119 (18)	143 (15)	159 (11)	NA
	Y	N	75 (9)	118 (13)*	142 (13)*	165 (10)*	192 (9)*
		H	83 (15)*	138 (16)*	165 (13)*	181 (10)*	n.a.
HVR_{BW} (ml min^{-1} %$^{-1}$ kg^{-1})	O		2.9 (8.3)	9.1 (4.8)	16.2 (9.7)	26.6 (13.7)	n.a.
	Y		0.9 (3.8)	5.7 (4.7)	13.5 (2.8)	32.2 (10.2)*	n.a.
HCR (beats min^{-1} %$^{-1}$)	O		0.2 (1.3)	0.9 (0.3)	0.9 (0.3)	0.8 (0.4)	n.a.
	Y		0.5 (0.4)	0.8 (0.5)	0.9 (0.4)	0.6 (0.2)	n.a.

Data are means (SD). Abbreviations: Exh H, variables at maximal hypoxic power output; Exh N, variables at maximal normoxic power output; H, hypoxia; HCR, hypoxic cardiac response; HR, heart rate; HVR_{BW}, hypoxic ventilatory response relative to body weight; N, normoxia; n.a., variables not available at this power output in hypoxia; 25%N and 50%N, variables at 25 and 50% of the maximal normoxic power output, respectively; O, older subjects; $\dot{V}_{O_2}$, oxygen consumption; and Y, young subjects. *Significant difference between groups ($P < 0.05$).

0.05 was used as the cut-off for significance. All data are presented as mean values ± SD.

Results

Young and older subjects had similar anthropometric characteristics, lived at similar low-altitude levels and performed a similar amount of endurance activities (Table 1). Exercise duration was 22 ± 4 and 21 ± 3 min in young individuals in normoxia and hypoxia, respectively, and 21 ± 4 and 20 ± 4 min in older individuals in normoxia and hypoxia, respectively ($P > 0.05$). The $\dot{V}_{O_2peak}$ values were lower in older compared with young subjects ($P < 0.001$) and indicated that subjects had good average fitness levels.

Cardiorespiratory responses

Table 2 and Figs 1 and 2 show normoxic and hypoxic cardiorespiratory responses at rest and during exercise in young and older subjects. Maximal power output was reduced from normoxia to hypoxia to a similar extent in both groups (young −32 ± 5% *versus* older −30 ± 6%; $P = 0.385$), as was $\dot{V}_{O_2peak}$ (young −26 ± 11% *versus* older −24 ± 14%; $P = 0.419$).

At rest, older individuals had lower P_{ET,CO_2} and HR compared with young individuals. The S_{pO_2}, $\dot{V}_E$, ventilatory and cardiac hypoxic responses at rest did not differ between groups. No difference between groups was observed in resting arterial blood pressure in normoxia (mean arterial pressure 101 ± 8 and 103 ± 11 mmHg in young and older individuals, respectively) and hypoxia (97 ± 8 and 100 ± 14 mmHg, respectively; $P > 0.05$).

Despite slightly lower average values during exercise in hypoxia in young subjects, differences in S_{pO_2} (Fig. 1*A*) between young and older subjects were not significant (age × condition interaction: $F = 4.4$, $P = 0.055$). The P_{ET,CO_2} was higher in young compared with older subjects in both normoxia and hypoxia except at Exh H in hypoxia and at Exh N (age × condition × intensity interaction: $F = 10.9$, $P < 0.001$; Fig. 1*B*).

The hypoxia-induced increase in $\dot{V}_E$ during exercise was significantly larger in young compared with older subjects at Exh H only (age × condition × intensity interaction: $F = 6.6$, $P = 0.001$; Fig. 2*A*). The maximal $\dot{V}_E$ expressed as a percentage of the theoretical maximal ventilation (35 × forced expiratory volume in 1 s) was significantly smaller in young compared with older subjects in both normoxia (91 ± 12 *versus* 112 ± 25%, $P = 0.007$) and hypoxia (82 ± 15 *versus* 99 ± 21%, $P = 0.012$). Absolute hypoxic ventilatory responses (age × intensity interaction: $F = 4.5$, $P = 0.014$; Fig. 2*B*) and hypoxic ventilatory responses relative to body weight (age × intensity interaction: $F = 3.3$, $P = 0.029$; Table 2) were larger at Exh H ($P = 0.036$) in young compared with older subjects. The hypercapnic ventilatory response was similar in young and older subjects (young 1.3 ± 0.9 *versus* older 1.5 ± 1.0 l min^{-1} mmHg^{-1}; $P = 0.634$).

Heart rate during exercise was significantly higher in young compared with older subjects (main effect of age: $F = 18.9$, $P = 0.001$) in both normoxia and hypoxia (age × condition interaction: $F = 2.9$,

$P = 0.112$; Table 2). The hypoxic cardiac response during exercise did not differ between young and older subjects (age × intensity interaction: $F = 1.4$, $P = 0.265$; Table 2). The hypercapnic cardiac response was similar in young and older subjects (young 0.5 ± 0.6 *versus* older 0.2 ± 0.3 beats min^{-1} mmHg^{-1}; $P = 0.092$).

At 90 W, $\dot{V}_E$ and P_{ET,CO_2} were higher and lower, respectively, in older individuals compared with young individuals ($\dot{V}_E$ normoxia 46.6 ± 10.5 *versus* 36.6 ± 3.7 l min^{-1}, hypoxia 66.5 ± 11.9 *versus* 51.8 ± 7.7 l min^{-1}; P_{ET,CO_2} normoxia 38 ± 4 *versus* 43 ± 3 mmHg, hypoxia 30 ± 4 *versus* 36 ± 4 mmHg, respectively). No difference in hypoxic ventilatory or cardiac responses was observed between groups at 90 W.

Tissue oxygenation response

The NIRS data are shown in Figs 3 and 4.

Prefrontal cortex HbO_2 increased during exercise to a greater extent in young compared with older subjects

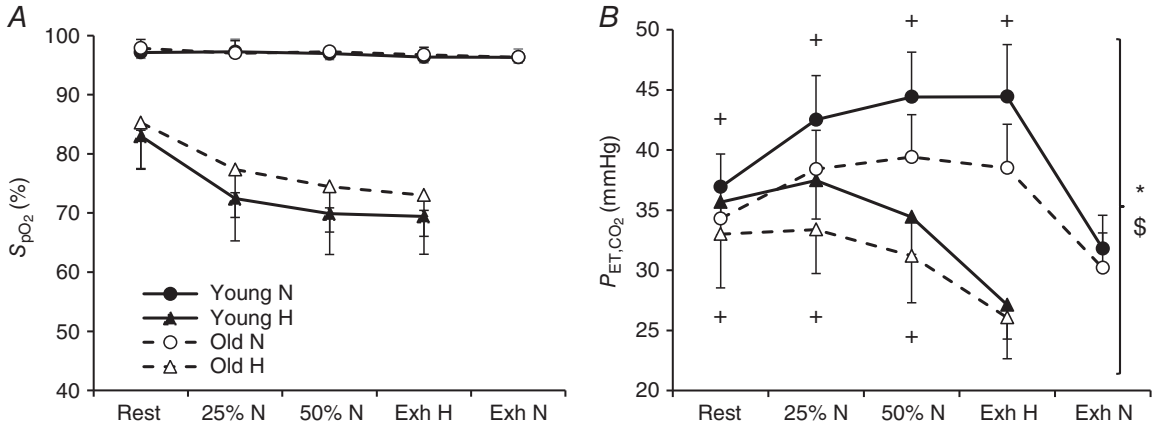

Figure 1. Arterial oxygen saturation (*A*) and end-tidal partial CO_2 pressure (*B*) during exercise in normoxia and hypoxia
Abbreviations: Exh H, values at maximal hypoxic power output; Exh N, values at maximal normoxic power output; H, hypoxia; N, normoxia; 25% N and 50% N, values at 25% and 50% of the maximal normoxic power output; Old, older subjects; P_{ET,CO_2}, end-tidal partial CO_2 pressure; S_{pO_2}, arterial oxygen saturation; and Young, young subjects. $^{+}$Significant difference between groups ($P < 0.05$). *Significant age × condition × intensity interaction ($P < 0.05$). $^{\$}$Significant age main effect ($P < 0.05$).

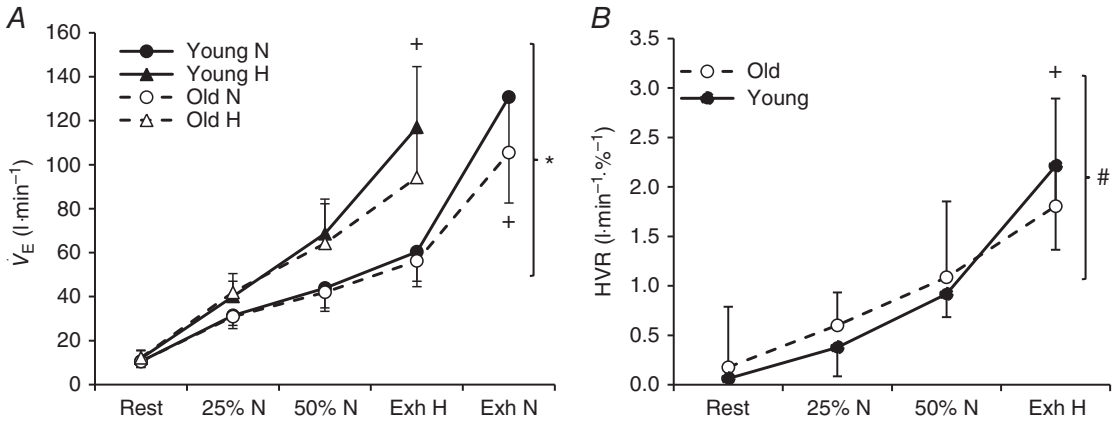

Figure 2. Minute ventilation (*A*) and hypoxic ventilatory response (*B*) during exercise in normoxia and hypoxia
Abbreviations: HVR, hypoxic ventilatory response; $\dot{V}_E$, minute ventilation; other abbreviations as in Fig. 1. $^{+}$Significant difference between groups ($P < 0.05$). *Significant age × condition × intensity interaction ($P < 0.05$). $^{\#}$Significant age × intensity interaction ($P < 0.05$).

(age × intensity interaction: $F = 12.43$, $P < 0.001$), especially in normoxia (age × condition interaction: $F = 5.47$, $P = 0.050$; Fig. 3*A*).

The hypoxia-induced increase in prefrontal cortex HHb during exercise was larger in young compared with older subjects (age × condition interaction: $F = 17.6$, $P = 0.001$; Fig. 3*B*). Prefrontal cortex HbTot increased during exercise to a greater extent in young compared with older subjects, in both normoxia and hypoxia (age × intensity interaction: $F = 11.1$, $P < 0.001$; Fig. 3*C*). Prefrontal cortex TSI did not differ between young and older subjects in both normoxia and hypoxia (Fig. 5).

Vastus lateralis HbO_2 did not differ between young and older subjects in both normoxia and hypoxia (age × condition interaction: $F = 2.2$, $P = 0.162$). The hypoxia-induced increase in vastus lateralis HHb during exercise was larger in young compared with older subjects (age × condition × intensity interaction: $F = 3.8$, $P = 0.034$; Fig. 4*B*). Vastus lateralis HbTot increased during exercise in normoxia and hypoxia in both groups except in young subjects at Exh H in hypoxia and at Exh N (age × condition × intensity interaction: $F = 6.4$, $P = 0.005$; Fig. 4*C*). Vastus lateralis TSI did not differ between young and older subjects in both normoxia and hypoxia (Fig. 5).

No difference in tissue oxygenation responses at 90 W in normoxia and hypoxia was observed between groups (results not shown, all $P > 0.05$).

No age × sex interaction was observed for all cardiorespiratory and tissue oxygenation responses (all $P > 0.05$).

Discussion

This study is the first to compare hypoxic responses during an incremental maximal exercise test in young and older healthy subjects. The results indicate that the hypoxic ventilatory response was similar in young compared with

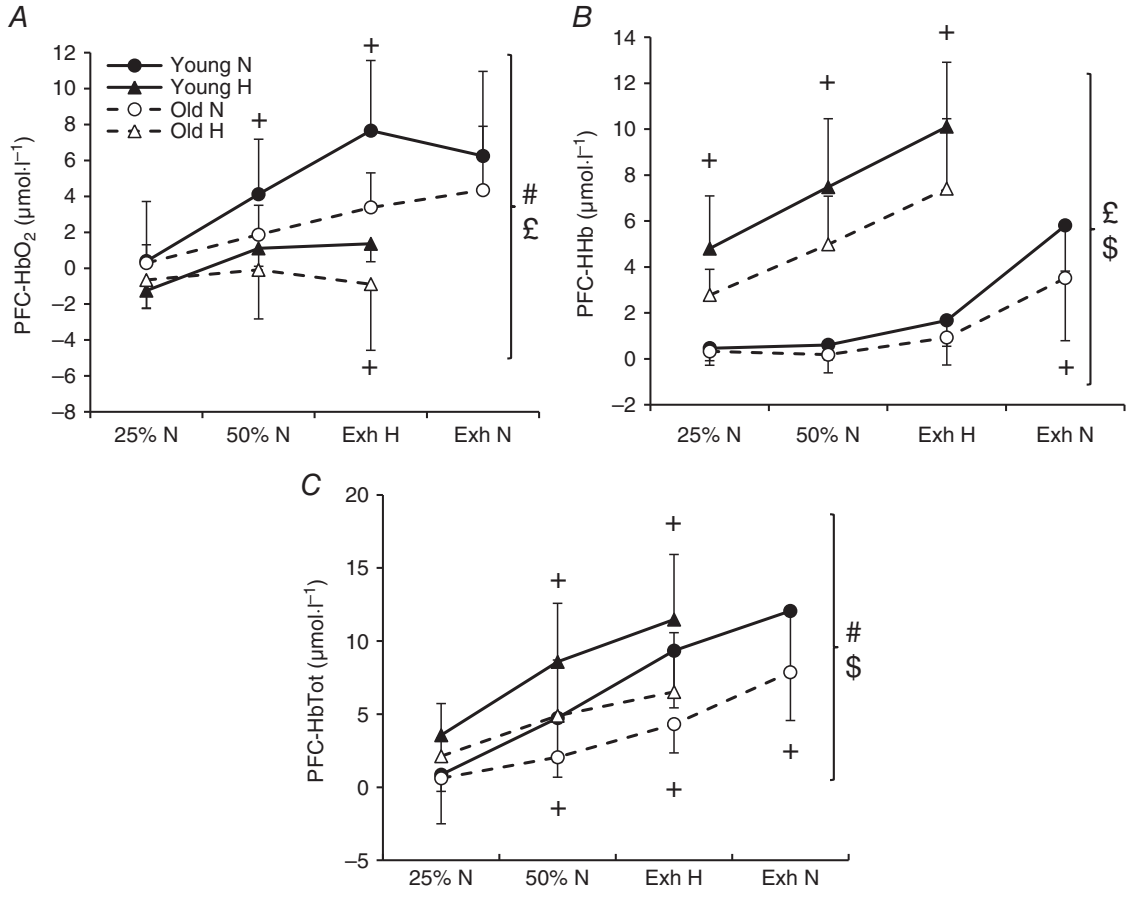

Figure 3. Changes in prefrontal cortex oxygenation during exercise in normoxia and in hypoxia
Abbreviations: HbO_2, oxyhaemoglobin; HHb, deoxyhaemoglobin; HbTot, total haemoglobin; PFC, prefrontal cortex; other abbreviations as in Fig. 1. Data are changes from resting values. $^{+}$Significant difference between groups ($P < 0.05$). $^{\#}$Significant age × intensity interaction ($P < 0.05$). $^{£}$Significant age × condition interaction ($P \leq 0.05$). $^{\$}$Significant age main effect ($P < 0.05$).

older individuals at rest and during moderate-intensity exercise, but larger in young subjects during high-intensity exercise. The hypoxic cardiac response did not differ between young and older subjects both at rest and during exercise. These cardiorespiratory responses were associated with similar hypoxia-induced reductions in maximal power output in young and older subjects. Young subjects also showed larger hypoxia-induced prefrontal cortex and vastus lateralis deoxygenation during exercise. Altogether, older healthy and active individuals appeared to have preserved cardiorespiratory and tissue oxygenation responses to hypoxia both at rest and during moderate-intensity exercise.

Cardiorespiratory responses

The hypoxic ventilatory response (estimated by using pulse oxygen saturation as a surrogate for arterial blood oxygenation level) was larger in young compared with older subjects during high-intensity exercise only. This difference was significant both when the hypoxic ventilatory response was expressed in absolute values and relative to body weight, as previously suggested (Lhuissier *et al.* 2012). Similar hypoxic and hypercapnic (estimated by using P_{ET,CO_2} as a surrogate for arterial blood CO_2 partial pressure) ventilatory responses at rest suggest that chemosensitivity was similar in young and older subjects. The similar hypoxic response is in accordance with animal studies reporting unchanged ventilatory responsiveness despite some alterations in carotid body morphology in older animals (Pokorski *et al.* 2004; Pokorski & Antosiewicz, 2010).

Older subjects were able to increase their minute ventilation in response to hypoxia at rest and during moderate-intensity exercise to a similar extent to young subjects (Fig. 2). Likewise, the hypoxic ventilatory response at identical submaximal workload (90 W) did not differ between groups. However, near maximal exercise, mechanical constraints may limit the ability of older subjects to increase ventilation as much as young subjects. Older subjects exhibited exhausted ventilatory reserve at maximal exercise in both normoxia and hypoxia (as shown by minute ventilation near 100% of theoretical maximal ventilation and according to previous reports in the

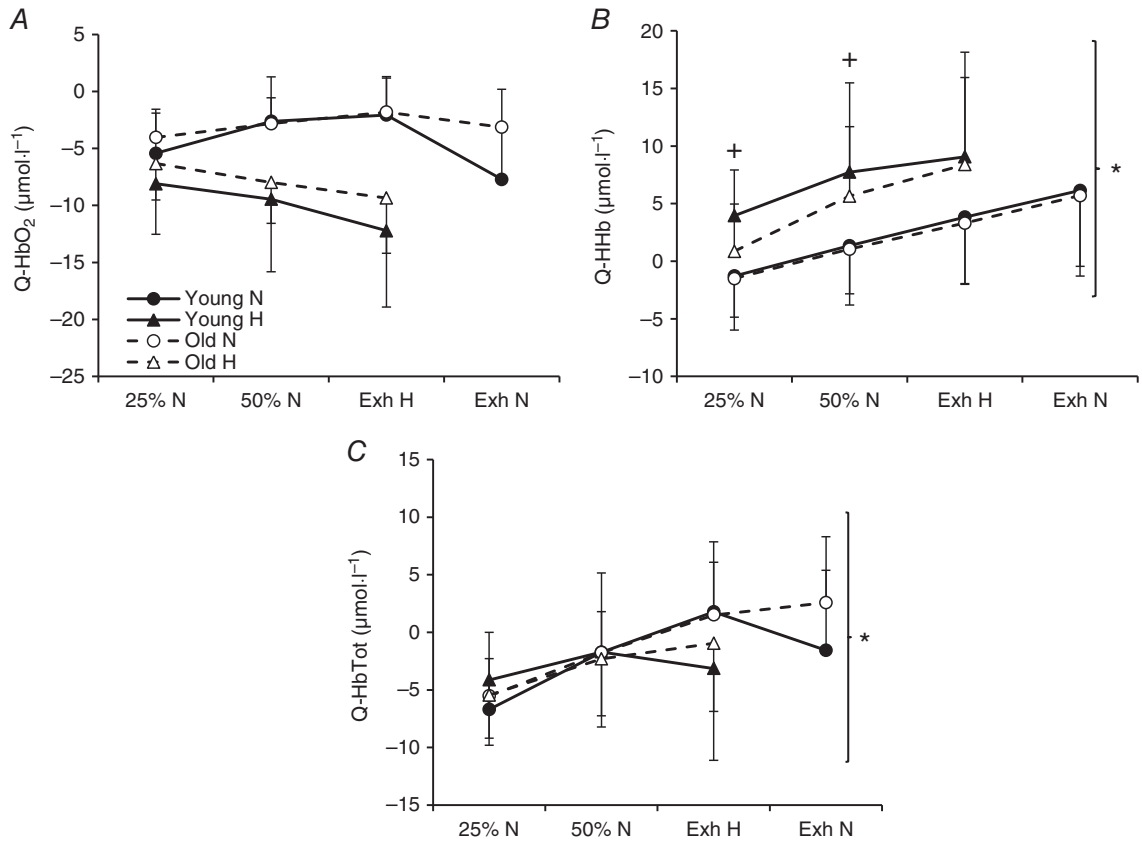

Figure 4. Changes in muscle oxygenation during exercise in normoxia and in hypoxia
Abbreviations: HbO_2, oxyhaemoglobin; HHb, deoxyhaemoglobin; HbTot, total haemoglobin; Q, quadriceps; other abbreviations as in Fig. 1. Data are changes from resting values. $^{+}$Significant difference between groups ($P < 0.05$). *Significant age × condition × intensity interaction ($P < 0.05$).

literature, e.g. Johnson & Dempsey, 1991) and therefore could not reach minute ventilation levels at exhaustion as high as young subjects. Impaired lung elastic recoil, chest compliance and respiratory muscle strength as well as exaggerated work of breathing are potential mechanisms reducing the maximal response of the respiratory system to hypoxia during intensive exercise in older individuals (Janssens *et al.* 1999).

Despite lower hypoxic ventilatory response during intense exercise in older subjects, S_{pO_2} did not differ between young and older subjects. Young subjects even tended to have slightly lower S_{pO_2} during exercise in hypoxia, as previously observed during mild-intensity exercise (Lhuissier *et al.* 2012). Therefore, the lower hypoxic ventilatory response during intense exercise in older subjects did not seem to accentuate their arterial deoxygenation or their loss of maximal power output compared with young subjects. It should be acknowledged that S_{pO_2} measurement at maximal exercise can be affected by metabolic acidosis and that slightly lower arterial pH in young subjects (e.g. Fisher *et al.* 2013) might have lowered S_{pO_2} and therefore artificially reduced their hypoxic ventilatory response (i.e. $\Delta \dot{V}_E / \Delta S_{pO_2}$) at maximal exercise. Conversely, greater acidosis might also have stimulated their peripheral chemoreceptors to a greater extent, therefore enhancing the hypoxic ventilatory response. Future studies are therefore needed to clarify the role of differences in lung mechanics (e.g. presence of flow limitation, work of breathing) and arterial blood gases (e.g. arterial pH) regarding the hypoxic ventilatory response at maximal exercise in young and older individuals.

Given that β-adrenergic sensitivity decreases with ageing (Lakatta, 1986), previous studies suggested that a reduced β-adrenergic sensitivity may be responsible for an impaired hypoxic cardiac response in older individuals (Lhuissier *et al.* 2012; Limberg *et al.* 2012). The present results show similar a hypoxic cardiac response in young and older subjects both at rest and during exercise. As exercise training has been shown to improve β-adrenergic sensitivity in older individuals (Spina *et al.* 1998), the fact that our older subjects were physically active might account for their preserved hypoxic cardiac response.

Previous studies reported heterogeneous results regarding the effect of sex on hypoxic cardiorespiratory responses (Lhuissier *et al.* 2012; MacNutt *et al.* 2012). In a retrospective study with a large sample, Lhuissier *et al.* (2012) reported that ageing was associated with reduced hypoxia-induced arterial deoxygenation and increased ventilatory hypoxic response in males only and with reduced cardiac hypoxic response in both males and females. In the present study, no interaction between age and sex was obtained for cardiorespiratory hypoxic responses, suggesting that the effects of age were similar in active males and females below the age of 70 years.

Cerebral oxygenation

The lower prefrontal cortex HbO_2 and HbTot increases during exercise observed in older subjects are in accordance with the impaired cerebral response to exercise with ageing previously reported in the literature by using transcranial Doppler ultrasonography (Murrell *et al.* 2012; Fisher *et al.* 2013). Lucas *et al.* (2012) also showed that ageing affects the cerebral response to exercise assessed by transcranial Doppler ultrasonography (middle cerebral artery) and NIRS measurement (prefrontal cortex), with the latter showing specific impairments at the prefrontal microvascular levels in older individuals. In contrast to our hypothesis, this reduced cerebral haemodynamic response to exercise in older individuals was not accentuated in hypoxia. Young subjects even showed a larger hypoxia-induced reduction in prefrontal cortex HbO_2 and increase in HHb compared with older individuals. Prefrontal cortex HbTot showed similar

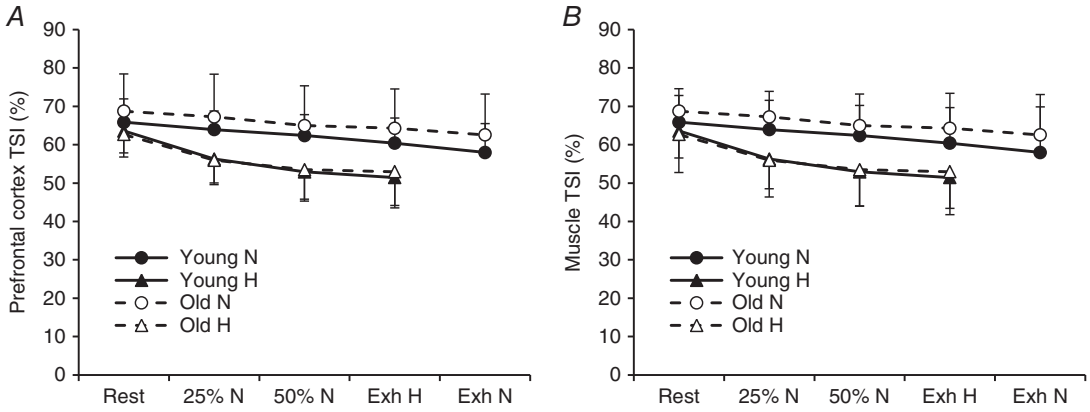

Figure 5. Changes in prefrontal cortex and muscle tissue saturation index (TSI) during exercise in normoxia and in hypoxia
Abbreviations are as in Fig. 1.

changes from normoxia to hypoxia in both groups. This can be interpreted as similar hypoxia-induced changes in cerebral blood volume in both groups. Larger hypoxia-induced changes in HHb and HbO_2 in young subjects might therefore reflect a greater imbalance between prefrontal cortex oxygen delivery and consumption in young compared with older individuals during exercise in hypoxia (Hoshi *et al.* 2001). The effect of hypoxia on cerebral metabolism both at rest and during exercise in older individuals remains to be investigated in order to gain a better understanding of the interaction between ageing, hypoxaemia and brain function.

In young healthy subjects in normoxic conditions, cerebral blood flow is known to increase when performing at mild-to-moderate exercise intensities, whereas at higher intensities near maximal exercise, the hyperventilation-induced hypocapnia can promote cerebral vasoconstriction and reduce cerebral blood flow (Ogoh & Ainslie, 2009). In accordance with previous studies, older subjects had reduced P_{ET,CO_2} compared with young individuals both at rest and during submaximal exercise (Vovk *et al.* 2004; Marsden *et al.* 2012; Fisher *et al.* 2013). Owing to the cerebral vasoconstrictive effect of hypocapnia, lower P_{ET,CO_2} during exercise in older subjects might explain, at least in part, the lower prefrontal cortex HbO_2 and HbTot during both normoxic and hypoxic exercise. At maximal exercise, however, P_{ET,CO_2} decreased in young subjects to a similar extent as in older subjects. This may explain the similar prefrontal cortex HbO_2 at Exh N in young and older subjects because of a larger hypocapnia-induced vasoconstrictive effect in young subjects, as previously suggested (Marsden *et al.* 2012).

Muscle oxygenation

Normal ageing is associated with a number of changes, such as structural vascular alterations, increased muscle sympathetic neural outflow and/or impaired local vascular control mechanisms (Marín & Rodríguez-Martínez, 1999; Proctor & Parker, 2006), which could compromise muscle blood flow and oxygenation during hypoxic exercise. During moderate-intensity normoxic leg exercise, active muscle blood flow is generally attenuated in older individuals, probably because of a heightened state of vasoconstriction (Proctor & Parker, 2006). In the present study, normoxic exercise induced similar changes in vastus lateralis NIRS parameters in young and older individuals; however, the hypoxia-induced larger increase in vastus lateralis HHb during exercise in young subjects suggests significantly greater deoxygenation and oxygen extraction in response to hypoxia compared with older subjects. This greater extraction may be attributable to larger absolute workload at identical submaximal relative intensities in young compared with older individuals or to other mechanisms, as recently observed in young athletes *versus* active subjects (Van Thienen & Hespel, 2016). This greater hypoxia-induced increase in oxygen extraction may be needed because, despite the larger absolute power output and therefore oxygen requirement during exercise in young individuals, a similar increase in vastus lateralis muscle blood flow (as suggested by similar HbO_2 and HbTot during exercise in both groups) was observed in young compared with older subjects. Hence, although some previous data suggested impaired muscle hypoxic vasodilator responses at rest with ageing (Ferri *et al.* 2007; Kirby *et al.* 2012), the present study does not suggest an impairment in muscle hypoxic response during exercise in older compared with young subjects.

Limitations

Investigation of both sexes is an important strength in physiological studies, given the predominance of male-only reports in the literature. The present study, however, was not designed and insufficiently powered to address the effect of sex on hypoxic responses. Half of the young females were taking oral contraceptives, and the phase of the menstrual cycle was not the same for all test sessions. Although recent results indicate that the menstrual cycle may not affect hypoxic and hypercapnic ventilatory responses at rest and during exercise (MacNutt *et al.* 2012), future studies on the effect of age and sex on hypoxic responses should be controlled for contraceptive treatment and menstrual cycle.

The present data apply to older individuals in their seventh decade, but one could wonder whether individuals older than those in the present study may show larger impairments of hypoxic responses. Previous studies reported changes in cardiorespiratory hypoxic responses as early as in the fifth decade (Lhuissier *et al.* 2012), and García-Río *et al.* (2007) showed that hypoxic sensitivity gradually decreased as age increased to 70–74 years and remained unchanged from 75 years of age onward. Hence, these results suggest that our group of older individuals between 60 and 70 years old should have been sufficiently old to show potential effects of ageing on hypoxic responses. Further studies are nonetheless needed to investigate older individuals.

Exercise responses of young and older individuals can be compared for identical absolute or relative power outputs. In the present study, young and older individuals were compared as follows: (i) at identical relative power output (25 and 50% of individual maximal normoxic power output); (ii) at identical absolute power output (at 90 W only because this was the highest power output reached by all individuals); and (iii) at maximal exercise. Although comparing young and older individuals at identical absolute power output presents obvious limitations (90 W corresponded to low-intensity exercise

for young males in normoxia and to near-maximal exercise for older females in hypoxia), group comparisons at identical relative and absolute submaximal power outputs and at maximal exercise provide an exhaustive analysis of the present data. Also, while the effect of ageing on hypoxic exercise responses was assessed by using a short-duration (<30 min) maximal incremental test, whether more prolonged exercise duration at moderate intensity might reveal additional differences between young and older individuals remains to be investigated.

Near-infrared spectroscopy signals are known potentially to be influenced by blood flow in superficial layers (Sørensen *et al.* 2015), but given that the young and older individuals had similar vastus lateralis skinfold thickness and because relative changes from baseline were compared for each individual in normoxia and hypoxia, the present results regarding differences between young and older individuals are unlikely to be affected by superficial layers. Finally, one should acknowledge that the inability to randomize the normoxic and hypoxic conditions cannot entirely rule out a potential ordering effect.

Conclusion

In conclusion, cardiorespiratory and tissue oxygenation responses to hypoxia both at rest and during moderate-intensity exercise appear to be similar in healthy young and older active subjects at least until the age of 70 years. Only the hypoxic ventilatory response during high-intensity exercise was slightly lower in older compared with young individuals, but this was not associated with larger arterial oxygen desaturation or greater maximal power output decrement. Although further studies considering both longer hypoxic exposure and prolonged exercise duration are needed, these results emphasize that ageing is not a contraindication for high altitude sojourn, providing no pathological condition interferes and physical fitness is compatible with the expected physical demand at high altitude.

References

Casey DP, Walker BG, Curry TB & Joyner MJ (2011). Ageing reduces the compensatory vasodilatation during hypoxic exercise: the role of nitric oxide. *J Physiol* **589**, 1477–1488.

Chapman KR & Cherniack NS (1987). Aging effects on the interaction of hypercapnia and hypoxia as ventilatory stimuli. *J Gerontol* **42**, 202–209.

Costes F, Denis C, Roche F, Prieur F, Enjolras F & Barthélémy JC (1999). Age-associated alteration of muscle oxygenation measured by near infrared spectroscopy during exercise. *Arch Physiol Biochem* **107**, 159–167.

Davis SM, Ackerman RH, Correia JA, Alpert NM, Chang J, Buonanno F, Kelley RE, Rosner B & Taveras JM (1983). Cerebral blood flow and cerebrovascular CO_2 reactivity in stroke-age normal controls. *Neurology* **33**, 391–399.

Ferrari M, Mottola L & Quaresima V (2004). Principles, techniques, and limitations of near infrared spectroscopy. *Can J Appl Physiol* **29**, 463–487.

Ferri A, Adamo S, Longaretti M, Marzorati M, Lanfranconi F, Marchi A & Grassi B (2007). Insights into central and peripheral factors affecting the "oxidative performance" of skeletal muscle in aging. *Eur J Appl Physiol* **100**, 571–579.

Fisher JP, Hartwich D, Seifert T, Olesen ND, McNulty CL, Nielsen HB, van Lieshout JJ & Secher NH (2013). Cerebral perfusion, oxygenation and metabolism during exercise in young and elderly individuals. *J Physiol* **591**, 1859–1870.

Galvin SD, Celi LA, Thomas KN, Clendon TR, Galvin IF, Bunton RW & Ainslie PN (2010). Effects of age and coronary artery disease on cerebrovascular reactivity to carbon dioxide in humans. *Anaesth Intensive Care* **38**, 710–717.

García-Río F, Villamor A, Gómez-Mendieta A, Lores V, Rojo B, Ramírez T & Villamor J (2007). The progressive effects of ageing on chemosensitivity in healthy subjects. *Respir Med* **101**, 2192–2198.

Hartmann SE, Waltz X, Kissel CK, Szabo L, Walker BL, Leigh R, Anderson TJ & Poulin MJ (2015). Cerebrovascular and ventilatory responses to acute isocapnic hypoxia in healthy aging and lung disease: effect of vitamin C. *J Appl Physiol* **15**, 363–373.

Hoshi Y, Kobayashi N & Tamura M (2001). Interpretation of near-infrared spectroscopy signals: a study with a newly developed perfused rat brain model. *J Appl Physiol* **90**, 1657–1662.

Janssens JP, Pache JC & Nicod LP (1999). Physiological changes in respiratory function associated with ageing. *Eur Respir J* **13**, 197–205.

Johnson BD & Dempsey JA (1991). Demand vs. capacity in the aging pulmonary system. *Exerc Sport Sci Rev* **19**, 171–210.

Kastrup A, Dichgans J, Niemeier M & Schabet M (1998). Changes of cerebrovascular CO_2 reactivity during normal aging. *Stroke* **29**, 1311–1314.

Kirby BS, Crecelius AR, Voyles WF & Dinenno FA (2012). Impaired skeletal muscle blood flow control with advancing age in humans: attenuated ATP release and local vasodilation during erythrocyte deoxygenation. *Circ Res* **111**, 220–230.

Kronenberg RS & Drage CW (1973). Attenuation of the ventilatory and heart rate responses to hypoxia and hypercapnia with aging in normal men. *J Clin Invest* **52**, 1812–1819.

Lakatta EG (1986). Diminished beta-adrenergic modulation of cardiovascular function in advanced age. *Cardiol Clin* **4**, 185–200.

Lhuissier FJ, Canouï-Poitrine F & Richalet JP (2012). Ageing and cardiorespiratory response to hypoxia. *J Physiol* **590**, 5461–5474.

Limberg JK, Evans TD, Pegelow DF, Eldridge MW, Sebranek JJ, Proctor LT & Schrage WG (2012). Heterogeneous vascular responses to hypoxic forearm exercise in young and older adults. *Eur J Appl Physiol* **112**, 3087–3095.

Lucas SJ, Ainslie PN, Murrell CJ, Thomas KN, Franz EA & Cotter JD (2012). Effect of age on exercise-induced alterations in cognitive executive function: relationship to cerebral perfusion. *Exp Gerontol* **47**, 541–551.

MacNutt MJ, De Souza MJ, Tomczak SE, Homer JL & Sheel AW (2012). Resting and exercise ventilatory chemosensitivity across the menstrual cycle. *J Appl Physiol* **112**, 737–747.
Marín J & Rodríguez-Martínez MA (1999). Age-related changes in vascular responses. *Exp Gerontol* **34**, 503–512.
Marsden KR, Haykowsky MJ, Smirl JD, Jones H, Nelson MD, Altamirano-Diaz LA, Gelinas JC, Tzeng YC, Smith KJ, Willie CK, Bailey DM & Ainslie PN (2012). Aging blunts hyperventilation-induced hypocapnia and reduction in cerebral blood flow velocity during maximal exercise. *Age (Dordr)* **34**, 725–735.
Murrell CJ, Cotter JD, Thomas KN, Lucas SJ, Williams MJ & Ainslie PN (2012). Cerebral blood flow and cerebrovascular reactivity at rest and during sub-maximal exercise: effect of age and 12-week exercise training. *Age (Dordr)* **35**, 905–920.
Ogoh S & Ainslie PN (2009). Cerebral blood flow during exercise: mechanisms of regulation. *J Appl Physiol* **107**, 1370–1380.
Pokorski M & Antosiewicz J (2010). Alterations in the hypoxic ventilatory response with advancing age in awake rats. *J Physiol Pharmacol* **61**, 227–232.
Pokorski M, Walski M, Dymecka A & Marczak M (2004). The aging carotid body. *J Physiol Pharmacol* **55** Suppl 3, 107–113.
Proctor DN & Parker BA (2006). Vasodilation and vascular control in contracting muscle of the aging human. *Microcirculation* **13**, 315–327.
Puthon L, Bouzat P, Rupp T, Robach P, Favre-Juvin A & Verges S (2016). Physiological characteristics of elite high-altitude climbers. *Scand J Med Sci Sports* **26**, 1052–1059.
Reich T & Rusinek H (1989). Cerebral cortical and white matter reactivity to carbon dioxide. *Stroke* **20**, 453–457.
Richalet JP, Larmignat P, Poitrine E, Letournel M & Canouï-Poitrine F (2012). Physiological risk factors for severe high-altitude illness: a prospective cohort study. *Am J Respir Crit Care Med* **185**, 192–198.
Rolfe P (2000). In vivo near-infrared spectroscopy. *Annu Rev Biomed Eng* **2**, 715–754.
Rupp T, Leti T, Jubeau M, Millet GY, Bricout VA, Levy P, Wuyam B, Perrey S & Verges S (2013). Tissue deoxygenation kinetics induced by prolonged hypoxic exposure in healthy humans at rest. *J Biomed Opt* **18**, 095002.
Serebrovskaya TV, Karaban IN, Kolesnikova EE, Mishunina TM, Swanson RJ, Beloshitsky PV, Ilyin VN, Krasuk AN, Safronova OS & Kuzminskaya LA (2000). Geriatric men at altitude: hypoxic ventilatory sensitivity and blood dopamine changes. *Respiration* **67**, 253–260.
Smith WD, Poulin MJ, Paterson DH & Cunningham DA (2001). Dynamic ventilatory response to acute isocapnic hypoxia in septuagenarians. *Exp Physiol* **86**, 117–126.
Sørensen H, Rasmussen P, Siebenmann C, Zaar M, Hvidtfeldt M, Ogoh S, Sato K, Kohl-Bareis M, Secher NH & Lundby C (2015). Extra-cerebral oxygenation influence on near-infrared-spectroscopy-determined frontal lobe oxygenation in healthy volunteers: a comparison between INVOS-4100 and NIRO-200NX. *Clin Physiol Funct Imaging* **35**, 177–184.
Spina RJ, Turner MJ & Ehsani AA (1998). β-Adrenergic-mediated improvement in left ventricular function by exercise training in older men. *Am J Physiol Heart Circ Physiol* **274**, H397–H404.
Van Beekvelt MC, Colier WN, Wevers RA & Van Engelen BG (2001). Performance of near-infrared spectroscopy in measuring local O_2 consumption and blood flow in skeletal muscle. *J Appl Physiol* **90**, 511–519.
Van Thienen R & Hespel P (2016). Enhanced muscular oxygen extraction in athletes exaggerates hypoxemia during exercise in hypoxia. *J Appl Physiol* **120**, 351–361.
Verges S, Rupp T, Jubeau M, Wuyam B, Esteve F, Levy P, Perrey S & Millet GY (2012). Cerebral perturbations during exercise in hypoxia. *Am J Physiol Regul Integr Comp Physiol* **302**, R903–R916.
Vovk A, Smith WD, Paterson ND, Cunningham DA & Paterson DH (2004). Peripheral chemoreceptor control of ventilation following sustained hypoxia in young and older adult humans. *Exp Physiol* **89**, 647–656.
Yamamoto M, Meyer JS, Sakai F & Yamaguchi F (1980). Aging and cerebral vasodilator responses to hypercarbia: responses in normal aging and in persons with risk factors for stroke. *Arch Neurol* **37**, 489–496.

Additional information

Competing interests

None declared.

Author contributions

L.P., P.B., P.R., A.F.-J., S.D. and S.V. conceived and designed the work. L.P., A.F.-J., S.D. and S.V. acquired and analysed the data. L.P., P.B., P.R., A.F.-J., S.D. and S.V. interpreted the results and drafted and revised the manuscript. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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