Supplemental Material

Methods

Experimental measures

Hemoglobin mass (Hb_{mass}) and blood volumes. Hemoglobin mass (Hb_{mass}) was determined using the classic carbon monoxide (CO) rebreathing technique integrated in a semi-automated system with a very low typical error of measurement (TE ≤ 1.2 %), as previously described (1-3). In brief, following 20 min of supine rest, 2 ml of blood (baseline) was sampled from the median cubital vein via a 20-G venflon (BD, USA) and analysed immediately in duplicate for percent carboxyhemoglobin (%HbCO), hemoglobin (Hb) concentration and hematocrit (Hct) (ABL80, Radiometer, Denmark). Then, individuals performed the orthostatic test with unaltered haematological variables. Following 20 min of supine recovery, they breathed 100 % O_2 for 4 min to flush the nitrogen from the airways. After closing the O_2 input, a bolus of 1.5 ml/kg of 99.5 % chemically pure CO (Air Liquide, Canada) was administrated into the breathing circuit. Individuals rebreathed this gas mixture for 10 min. Then, an additional 2 ml blood sample was obtained and analysed in duplicate as aforementioned. The change in %HbCO is used to calculate Hb_{mass}, taking into account the small amount of CO that remains in the rebreathing circuit at the end of the procedure. Total red blood cell volume (RBCV), plasma volume (PV) and blood volume (BV) were determined from Hb_{mass}, baseline Hb concentration and Hct (1-3).

Transthoracic echocardiography and central hemodynamics. Apical four-chamber and two-chamber cine-loops were recorded via high-resolution ultrasound (Mindray Medical M9, USA) and analyzed offline (TOMTEC Imaging Systems, Royal Philips, Netherlands) at rest and during predetermined levels of LBNP (-10, -20, -30, -40, and -50 mm Hg). Following the American Society of Echocardiography and the European Association of Cardiovascular Imaging recommendations, cardiac chamber quantification was performed using the modified Simpson method (biplane method of disks) by tracing the endocardial border in both apical four-chamber and two-chamber views at end-diastole and end-systole (4, 5). Systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean arterial pressure (MAP) at the heart level were continuously assessed non-invasively via Finometer PRO (Finapres Medical Systems, Netherlands) (6), with data exported into a pre-established acquisition software (Labchart 7, AD Instruments, UK). Stroke volume (SV) was determined as left ventricular end-diastolic volume

(LVEDV) minus left ventricular end-systolic volume (LVESV), while the product of SV and HR provided cardiac output (Q). Systemic vascular resistance (SVR) was calculated as the ratio of MAP and Q. Echochardiographic variables are commonly normalized by body surface area ($BSA = 0.007184 \cdot weight^{0.425} \cdot height^{0.725}$) (7). The reproducibility of key echocardiographic and hemodynamic measurements (within-subject coefficient of variation (CV)) during incremental LBNP in our laboratory is ≤ 5 % for LV volumes and ≤ 3 % for blood pressures.

Orthostatic test. The participants rested for 20 min in supine position with their lower body inside a LBNP chamber sealed via an elastic belt at the level of the iliac crest. The volume of tissue exposed to LBNP could vary according to potential individual anthropometrical differences in the proportion of tissue below versus above the iliac crest. The LBNP chamber was designed for accurate echocardiography, comprising an electric hydraulic jack that enabled left lateral tilting. A moderate left semilateral body position (17˚ relative to the horizontal) was implemented for high-quality and reproducible cardiac imaging throughout the test. The LBNP protocol comprised a ramp test including incremental LBNP levels to facilitate the detection of progressive cardiac and hemodynamic alterations (8). The negative pressure inside the chamber was increased every 10 min by -10 mm Hg, from 0 to -50 mm Hg. The test was terminated immediately after completion of the last 10 min LBNP (-50 mm Hg) level or in the presence of presyncope, which was defined according to any of the following prevalent criteria: decrease in SBP to < 70 mm Hg, abrupt fall in SBP (> 15 mm Hg) and/or common signs and symptoms such as pallor, lightheadedness, profuse sweating and nausea accompanied by request from the subject to discontinue the test.

Blood uniformization. BV and O_2 carrying capacity were reduced in men to the same level of women on an individual basis. To this end, a 20 G venflon (BD, USA) was placed in the median cubital vein and a specific amount of blood (generally around 8 % of BV) was withdrawn immediately before starting the measurements, resulting in the same BV per kg between men and women. O_2 carrying capacity was defined as the concentration in blood of Hb able to carry O_2 (i.e., effective Hb (g/l) = total Hb – (HbCO + methemoglobin)). Accordingly, a small quantity of CO, determined by the difference in effective Hb between men and women, was introduced in the rebreathing system in which men breathed for 10 min in order to reduce their O_2 carrying

capacity to women's level. The level of effective Hb was monitored prior to and after CO rebreathing in each men via venous blood sampling to precisely control and corroborate the reduction of blood O_2 carrying capacity to the desired levels. During the blood uniformization procedure, men rested in supine position with their lower body inside the LBNP chamber, ready to initiate the test.

Statistical analysis

Statistical analyses were performed using SPSS 22.0 (SPSS, Chicago, IL). Data were tested for normal distribution with the Kolmogorov-Smirnov test and for homogeneity of variances with the Levene's test. Two-way ANOVA with repeated measures was performed to assess echocardiographic and hemodynamic variables in women and men prior to and after blood uniformization, with group (women, men prior to / after blood uniformization) and time (rest, -10, -20, -30, -40, and -50 mm Hg) as between- and within-subject factors, respectively. In addition, due to the progressive dropout of individuals (presyncope) during the orthostatic test, comparisons between groups were carried out at each time point using the independent sample *t* test. The chi-square test was used to determine between-group differences between observed frequency distributions of individuals who completed each LBNP level. Finally, the potential associations of (i) age with OT time and (ii) BV per unit of body mass with age and body mass index were assessed with linear regression analyses in women and men. A two-tailed *P*-value less than 0.05 was considered significant. All data are reported as mean (± SD) unless otherwise stated.

References

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