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Sex differences in cardiorespiratory fitness are explained by blood volume and oxygen carrying capacity

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Aims Intrinsic sex differences in fundamental blood attributes have long been hypothesized to contribute to the gap in cardiorespiratory fitness between men and women. This study experimentally assessed the role of blood volume and oxygen (O_2) carrying capacity on sex differences in cardiac function and aerobic power.

Methods and results Healthy women and men ($n = 60$) throughout the mature adult lifespan (42–88 yr) were matched by age and physical activity levels. Transthoracic echocardiography, central blood pressure, and O_2 uptake were assessed throughout incremental exercise (cycle ergometry). Main outcomes such as left ventricular end-diastolic volume (LVEDV), stroke volume (SV), cardiac output (Q), and peak O_2 uptake (VO_{2peak}), as well as blood volume (BV) were determined with established methods. Measurements were repeated in men following blood withdrawal and O_2 carrying capacity reduction matching women's levels. Prior to blood normalization, BV and O_2 carrying capacity were markedly reduced in women compared with men ($P < 0.001$). Blood normalization resulted in a precise match of BV (82.36 ± 9.83 vs. 82.34 ± 7.70 ml·kg^{-1}, $P = 0.993$) and O_2 carrying capacity (12.0 ± 0.6 vs. 12.0 ± 0.7 g·dl^{-1}, $P = 0.562$) between women and men. Body size-adjusted cardiac filling and output (LVEDV, SV, Q) during exercise as well as VO_{2peak} (30.8 ± 7.5 vs. 35.6 ± 8.7 ml·min^{-1}·kg^{-1}, $P < 0.001$) were lower in women compared with men prior to blood normalization. VO_{2peak} did not differ between women and men after blood normalization (30.8 ± 7.5 vs. 29.7 ± 7.4 ml·min^{-1}·kg^{-1}, $P = 0.551$).

Conclusions Sex differences in cardiorespiratory fitness are abolished when blood attributes determining O_2 delivery are experimentally matched between adult women and men.

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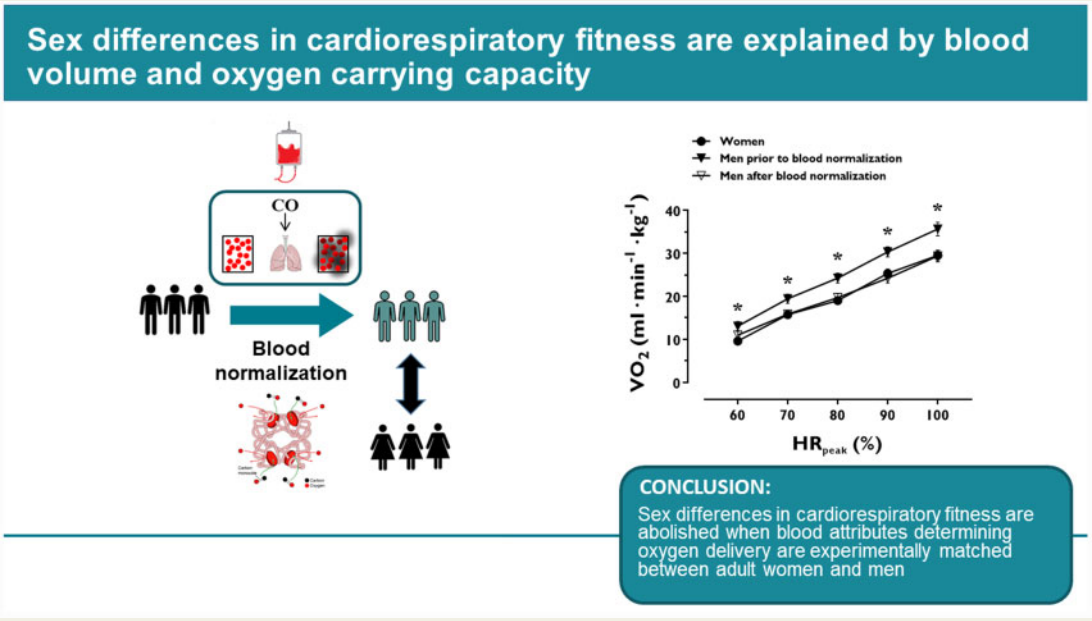
Graphical Abstract



Keywords Cardiac function • Blood volume • Haemoglobin mass • Aerobic power • Sex

1. Introduction

It is a truism that men and women are not equally endowed as far as certain physical capacities are concerned. Sex differences in body size and muscle mass definitely contribute to lower strength and power (anaerobic) performance in women.[1] Likewise, structural elements of the aerobic energy system are sex-specific.[2–4] Women commonly have a smaller heart compared with men, even when normalized by body size.[5] The fluid that fills the heart and the circulatory system, that is blood volume (BV), as well as blood oxygen (O_2) carrying capacity is also diminished in women in absolute and relative units.[6–10] Hence, according to current physiological principles,[8,11–17] women have a reduced cardiac filling and lower capacity to pump blood and deliver O_2, a fundamental determinant of peak O_2 uptake (VO_{2peak}) per kg of body weight,[15] considered a hallmark of cardiorespiratory fitness with strong prognostic value.[18,19] Indeed, women present on average a 15 to 25% lower VO_{2peak} than men for a given age and fitness status, which portrays a marked sex dimorphism in aerobic power.[20,21] Importantly, large population studies have found up to five-fold increases in all-cause and cardiovascular mortality in asymptomatic adults with low VO_{2peak} independently of traditional risk factors, relationships seemingly enhanced in middle-aged and older women.[22,23] Notwithstanding, women generally live longer than men, plausibly as a result of biological, behavioural, and environmental factors.[24] Yet, clinical evidence denotes that key aspects of cardiovascular and exercise physiology have yet to be understood in a sex-specific manner.[25,26] Specifically, whether the primary determinants of VO_{2peak} that were established in studies comprising men, that is BV and O_2 carrying capacity,[6,8,13,15,16,27,28] explain the gap in aerobic power between sexes remains uncertain. Namely, could the superior aerobic power of men be a consequence of increased O_2 delivery despite multiple skeletal muscle and metabolic attributes known to be sex-specific?[3,29] The answer to such question is far from irrelevant since blood attributes determining O_2 delivery are amenable to modification and thus potentially translated into effective targets to improve or preserve cardiovascular health in the general population.

The aim of the present study was to experimentally determine the role of fundamental sex differences in blood (BV and O_2 carrying capacity) in cardiac function, central haemodynamics, and O_2 uptake, from rest to peak incremental exercise, in healthy middle-aged and older men and women. We hypothesized that 'blood normalization' comprising the manipulation and matching of BV and O_2 carrying capacity between men and women would abolish sex differences in cardiorespiratory fitness.

2. Methods

2.1 Participants

Sixty moderately active adult women and men (42–88 yr) were recruited via electronic/printed advertisements on community notice boards in the city of Calgary. Moderate-to-vigorous physical activity (MVPA) levels were determined from established questionnaires as previously described.[30] Inclusion criteria comprised healthy status, absence of current medical symptoms or medication limiting incremental exercise testing, and no history of cardiac, pulmonary, or neuromuscular diseases. Individuals fulfilling the above criteria but having donated blood within 3 months prior to the study were excluded. The study was approved by the Conjoint Health Research Ethics Board (REB18-1654) of the University of Calgary and conducted in accordance with the Declaration of Helsinki. Prior to the start of the experiments, informed

oral and written consents were obtained from all participants following current guidelines (Supplementary Material).

2.2 Experimental design

Participants were required to report to our laboratory at least once, depending on sex and a voluntary familiarization visit. Each man was assessed twice, prior to and after blood normalization relative to a previously assessed woman with similar age and physical activity level (one-to-one matching). Time of day of testing sessions was kept consistent for each men and women–men matched pair with a minimum of 48 hr and a maximum of 7 days between the first (baseline) and second (blood normalization) sessions. All individuals were instructed to avoid strenuous exercise, alcohol, and caffeine from 24 hr prior to testing, as well as to maintain their usual baseline activity and daily dietary habits throughout the study. Body composition was assessed via dual-energy X-ray absorptiometry (DXA) (Hologic QDR 4500; Hologic, Inc, Bedford, USA). All measurements were performed after a 5 hr fasting period in a quiet room with controlled temperature between 22 and 23°C. Prior to testing, the participants completed demographic and clinical questionnaires and rested in supine position for 20 min in order to stabilize cardiovascular, hemodynamic, and haematological variables.

2.3 Experimental measures

2.3.1 Haemoglobin mass (Hb_{mass}) and blood volumes

Haemoglobin 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.[14,15,31] 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 per cent carboxyhaemoglobin (%HbCO), haemoglobin (Hb) concentration, and haematocrit (Hct) (ABL80, Radiometer, Denmark). Then, individuals performed the aerobic power 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 baseline Hct.[14,15,31]

2.3.2 Transthoracic echocardiography and central haemodynamics

Apical four-chamber and two-chamber cine-loops were recorded via high-resolution ultrasound (Mindray Medical M9, USA) and analysed off-line (TOMTEC Imaging Systems, Royal Philips, the Netherlands) at rest and during pre-determined levels of incremental exercise relative to peak heart rate (HR_{peak}) (60, 70, 80, 90, and 100% HR_{peak}) as well at a given submaximal workload (100 W). 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.[32,33] 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, the Netherlands),[34] 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. Total arterial compliance was determined by the pulse pressure method.[35] Echocardiographic variables were presented in common dimensional and flow units, as well as normalized by body surface area ($BSA = 0.007184 \cdot weight^{0.425} \cdot height^{0.725}$).[36] The reproducibility of key echocardiographic and hemodynamic measurements (within-subject coefficient of variation (CV)) during incremental exercise in our laboratory is ≤ 6% for LV volumes and ≤ 3% for blood pressures.

2.3.3 Aerobic power

An established incremental exercise protocol[14–16] was performed using an electromagnetic cycle ergometer (KICKR Core trainer, Wahoo, USA) integrated within a large lower body negative pressure chamber (LBNP) (165 × 82 × 108 cm) designed for exercise echocardiography. The LBNP comprises an electric hydraulic jack that enables to select any degree from 0 to 45° of left lateral tilting. The combination of left semi-lateral supine body position with lower body negative pressure allows for the simultaneous assessment of cardiac function, which requires a left lateral body position for high-quality and reproducible imaging, and aerobic power via the regulation of negative pressure inside the chamber (-50 mm Hg) to induce hemodynamic loads characteristic of the upright position, a physiological requirement to achieve VO_{2peak}.[37,38] The test started with a warm-up period of 3 min at 20–30 W workloads. Thereafter, the workload was increased by 15–30 W every 60 s until exhaustion was reached in a total duration of 7–10 min. O_2 uptake and CO_2 output were continuously measured (CardioCoach VO_2, KORR Medical, USA). Calibration of the gas analysers and the flowmeter was performed prior to each test. Breath-by-breath values were averaged over 15 s. The highest breath-by-breath average value was taken as VO_{2peak} provided that two of the following established criteria were fulfilled: plateau in O_2 uptake despite increased workload, age-predicted HR_{peak} +/- 10 bpm ($HR_{peak} = 211–0.64 \times age$),[39] respiratory exchange ratio ≥ 1.1.[15,40]

2.3.4 Blood normalization

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 certain amount of blood (generally around 7% of BV) was withdrawn immediately before starting the measurements, which resulted in identical 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 + methaemoglobin)). Accordingly, a small quantity of CO, determined by the difference in effective Hb between men and women, was introduced in the rebreathing system,[14,15,28] 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 as well right after exercise testing in each men via venous and earlobe blood sampling to precisely control and corroborate the reduction of blood O_2 carrying capacity to the desired levels.[41–43] During the blood normalization procedure, men rested

in supine position with their lower body inside the LBNP-exercise chamber, ready to initiate the incremental exercise test.

2.4 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, hemodynamic, and pulmonary variables in women and men prior to and after blood normalization, with group (women, men prior to/after blood normalization) and time (60, 70, 80, 90, and 100% HR_{peak}) as between- and within-subject factors, respectively. When F was significant in the ANOVA, pairwise specific comparisons were carried out via independent samples *t*-tests. Group differences at resting supine and submaximal absolute workload (100 W) were determined via independent samples *t*-tests. A two-tailed *P*-value less than 0.05 was considered significant. All data are reported as mean (± SD) unless otherwise stated.

3. Results

3.1 Baseline characteristics

Main general characteristics of the study participants are shown in *Table 1*. All individuals were non-smokers and non-obese (body mass index < 30 kg · m^{-2}). Age and physical activity levels were matched between women and men ($P \geq 0.551$). Women presented smaller anthropometric indices (height, weight, body surface area) compared with men ($P < 0.001$). Likewise, body composition variables were within normal age- and sex-related levels, with higher body fat percentage in women ($P < 0.001$). The vast majority of women (93%) had reached menopause. Hormone replacement therapy was used by a minority of participants (17%). Regarding resting supine cardiac and hemodynamic variables, HR was similar in women and men, whereas LVEDV and SV normalized by BSA were lower in women compared with men ($P \leq 0.022$). Central blood pressures and total arterial compliance did not differ between sexes, whereas SVR was higher in women ($P = 0.009$). Cardiorespiratory fitness, as represented by VO_{2peak} per kg of body weight, was reduced in women compared with men ($P < 0.001$), both groups falling within 50th and 99th percentiles of reference values according to age and sex in healthy populations.[40] Due to sex differences in body composition, women and men presented similar VO_{2peak} when expressed relative to lean mass ($P = 0.645$).

3.2 Blood O_2 carrying capacity and blood volume (BV)

Table 2 presents haematological variables in women and men prior to and after blood normalization. As expected, blood O_2 carrying capacity, as represented by effective Hb concentration, as well as BV was markedly diminished in women compared with men ($P < 0.001$). Blood normalization resulted in a precise match of effective Hb (12.0 ± 0.6 vs. 12.0 ± 0.7 g · dl^{-1}, $P = 0.562$) and BV (82.36 ± 9.83 vs. 82.34 ± 7.70 ml · kg^{-1}, $P = 0.993$) between women and men. The average absolute BV removed from men was 467 ± 178 ml, equivalent to a standard blood donation.[44]

3.3 Echocardiography and haemodynamics

Baseline sex differences in cardiac LVEDV (46.4 ± 9.2 vs. 50.8 ± 11.6 ml · m^{-2}, $P = 0.113$) and SV (32.7 ± 6.3 vs. 35.4 ± 8.0 ml · m^{-2}, $P = 0.153$) were no longer present at supine rest following blood normalization in men. Likewise, resting supine SVR did not differ in women compared with men after blood normalization (2613 ± 817 vs. 2161 ± 613 dyn· s· cm^{-5}, $P = 0.092$). Central blood pressures were similar between women and men following blood normalization at supine rest (MAP: 100.7 ± 17.8 vs. 101.5 ± 18.3 mm Hg, $P = 0.882$).

During incremental exercise (*Figure 1*), LVEDV and SV were lower in women compared with men prior to blood normalization ($P < 0.001$). Accordingly, women had less Q at any given HR ($P = 0.003$). Left ventricular filling and output variables (LVEDV, SV, and Q) remained mildly elevated in men after blood normalization ($P \leq 0.014$). Sex differences were

Table 1 Baseline characteristics of study subjects

	Women	Men
N	30	30
Age (years)	63.5 ± 8.6	63.8 ± 9.7
Height (cm)	162.8 ± 7.4	177.4 ± 7.3*
Weight (kg)	61.3 ± 10.2	79.0 ± 8.8*
BSA (m^2)	1.7 ± 0.15	2.0 ± 0.14*
MVPA (h·wk^{-1})	5.1 ± 3.0	5.6 ± 3.5
Smoking (%)	0	0
HRT (%)	17	0
Body composition		
Bone mineral content (kg)	2.0 ± 0.3	2.7 ± 0.4*
Lean mass (kg)	42.5 ± 5.2	60.8 ± 6.1*
Fat mass (kg)	17.4 ± 5.5	14.3 ± 4.7
Fat mass (%)	27.7 ± 5.4	18.1 ± 4.4*
Resting echocardiography		
HR (bpm)	57.4 ± 7.2	55.3 ± 5.4
RA (ml)	28.5 ± 8.7	41.8 ± 12.2*
RV EDA (cm^2)	17.1 ± 3.2	22.2 ± 4.4*
RV ESA (cm^2)	8.0 ± 2.9	10.3 ± 4.1*
LA (ml)	39.5 ± 18.9	45.3 ± 18.2
LVEDV (ml)	76.9 ± 17.4	105.1 ± 25.7*
LVESV (ml)	22.6 ± 8.7	31.7 ± 12.8*
LVEF (%)	70.9 ± 8.2	70.3 ± 7.4
SV (ml)	54.3 ± 12.8	73.4 ± 18.4*
Resting haemodynamics		
SBP (mm Hg)	145.6 ± 25.9	146.8 ± 18.4
DBP (mm Hg)	80.2 ± 16.9	77.9 ± 13.3
MAP (mm Hg)	100.7 ± 17.8	101.3 ± 12.4
SVR (dyn·s·cm^{-5})	2612.8 ± 817.5	2119.8 ± 578.1*
SV/PP (ml·mm Hg^{-1})	0.78 ± 0.39	1.12 ± 0.43
Aerobic power	-	-
VO_{2peak} (ml·min^{-1})	1868.7 ± 460.9	2782.8 ± 633.6*
VO_{2peak} (ml·min^{-1}·kg^{-1})	30.8 ± 7.5	35.6 ± 8.7*
VO_{2peak} (ml·min^{-1}·kg lean $mass^{-1}$)	44.7 ± 8.7	45.8 ± 9.7

Data are presented as mean ± SD.
*$P < 0.05$, women vs. men.
Statistical test: independent samples *t*-test.
BSA, body surface area; DBP, diastolic blood pressure; HR, heart rate; HRT, hormone replacement therapy; LA, left atria; LVEDV, left ventricular end-diastolic volume; LVEF, left ventricular ejection fraction; LVESV, left ventricular end-systolic volume; MAP, mean arterial pressure; MVPA, moderate-to-vigorous physical activity; PP, pulse pressure; RA, right atria; RV EDA, right ventricle end-diastolic area; RV ESA, right ventricle end-systolic area; SBP, systolic blood pressure; SV, stroke volume; SVR, systemic vascular resistance; VO_{2peak}, peak oxygen uptake (per kg of body weight).

not detected in LVESV irrespective of blood normalization ($P \geq 0.103$). Similar results were observed in the right side of the heart throughout incremental exercise, with end-diastolic area being smaller in women compared with men prior to ($P < 0.001$) and after blood normalization ($P = 0.002$). With respect to central blood pressures during incremental exercise (*Figure 2*), no differences were present between women and men prior to blood normalization ($P \geq 0.519$). After blood normalization, SBP was largely reduced in men compared with women ($P < 0.035$). SVR was higher in women compared with men prior to ($P = 0.007$) and after blood normalization ($P = 0.004$). At a given absolute submaximal exercise workload (100 W) (*Figure 4*), women presented lower LVEDV and SV ($P \leq 0.014$) but the concomitant increase in HR ($P < 0.001$) resulted in similar Q ($P = 0.986$) between sexes prior to as well as after blood normalization.

Table 2 Haematological variables in women and men prior to and after blood normalization

	Women	Men pre	Men post
Hb_{mass} (g)	604.3 ± 77.4	929.3 ± 116.6*	866.4 ± 117.7†
HbCO (%)	0.9 ± 0.3	0.9 ± 0.2	10.1 ± 4.5†
Effective Hb ($g \cdot dl^{-1}$)	12.0 ± 0.6	13.4 ± 0.9*	12.0 ± 0.7
Hct (%)	40.9 ± 1.8	45.5 ± 3.1*	45.2 ± 2.5†
RBCV ($ml \cdot kg^{-1}$)	33.74 ± 4.55	39.72 ± 4.02*	37.15 ± 4.16†
PV ($ml \cdot kg^{-1}$)	48.62 ± 5.73	47.67 ± 6.06	45.20 ± 4.67†
BV ($ml \cdot kg^{-1}$)	82.36 ± 9.83	87.39 ± 8.56*	82.34 ± 7.70

Data are presented as mean ± SD.
*$P < 0.05$, women vs. men pre.
†$P < 0.05$, women vs. men post.
Statistical test: independent samples *t*-test.
BV, blood volume; Effective Hb, blood concentration of haemoglobin able to carry oxygen; HbCO, carboxyhaemoglobin; Hb_{mass}, total circulating haemoglobin mass; Hct, haematocrit; PV, plasma volume; RBCV, red blood cell volume.

3.4 Aerobic power

O_2 uptake per kg of body weight during incremental exercise in women and men prior to and after blood normalization is presented in *Figure 3*. At any given HR, O_2 uptake was lower in women compared with men before blood normalization ($P < 0.001$). Following blood normalization, O_2 uptake during exercise is remarkably similar between sexes ($P = 0.885$). Specifically, women and men after blood normalization had similar VO_{2peak} (30.8 ± 7.5 vs. 29.7 ± 7.4 ml · min^{-1} · kg^{-1}, $P = 0.551$), unless expressed relative to lean mass whereby women presented higher values (44.7 ± 8.7 vs. 38.1 ± 8.0 ml · min^{-1} · kg lean $mass^{-1}$, $P = 0.004$). At a given absolute submaximal exercise workload (100 W) (*Figure 4*), O_2 uptake per kg of body weight was higher in women compared with men prior to ($P = 0.010$) and after blood normalization to ($P = 0.003$).

4. Discussion

This main purpose of the present study was to experimentally assess the role of major blood attributes determining O_2 delivery on sex differences in cardiorespiratory fitness. The major findings are: (i) blood normalization between men and women abolishes sex differences in VO_{2peak}; (ii) sex differences in cardiac filling and output remain after blood normalization; and (iii) the reduction of O_2 carrying capacity in men to women's level entails a decrease in cardiac afterload plausibly contributing to preserve cardiac filling and output in dissociation with O_2 uptake.

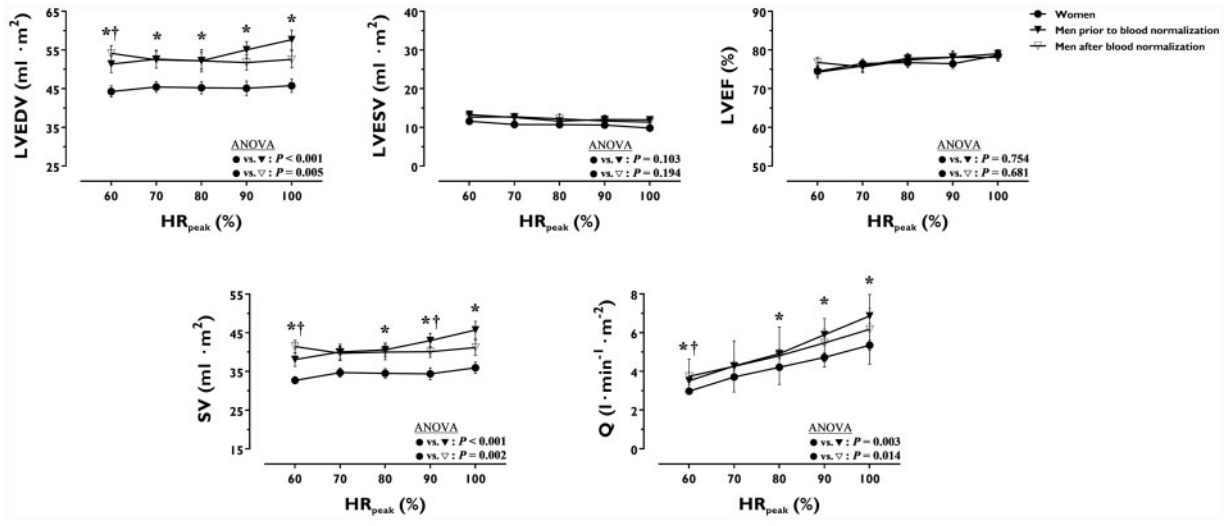


Figure 1 Left ventricular volumes and function during incremental exercise in women and men prior to and after blood normalization. *$P < 0.05$ (independent samples *t*-test) between women and men prior to blood normalization. †$P < 0.05$ (independent samples *t*-test) between women and men after blood normalization. Data are expressed as mean ± SEM. Number of biological observations for each graph: LVEDV ($n = 420$), LVESV ($n = 420$), LVEF ($n = 420$), SV ($n = 420$), Q ($n = 420$). Statistical tests: two-way ANOVA with repeated measures with group and time as between- and within-subject factors, respectively; independent samples *t*-test. HR_{peak}, peak heart rate; LVEDV, left ventricular end-diastolic volume; LVEF, left ventricular ejection fraction; LVESV, left ventricular end-systolic volume; Q, cardiac output; SV, stroke volume.

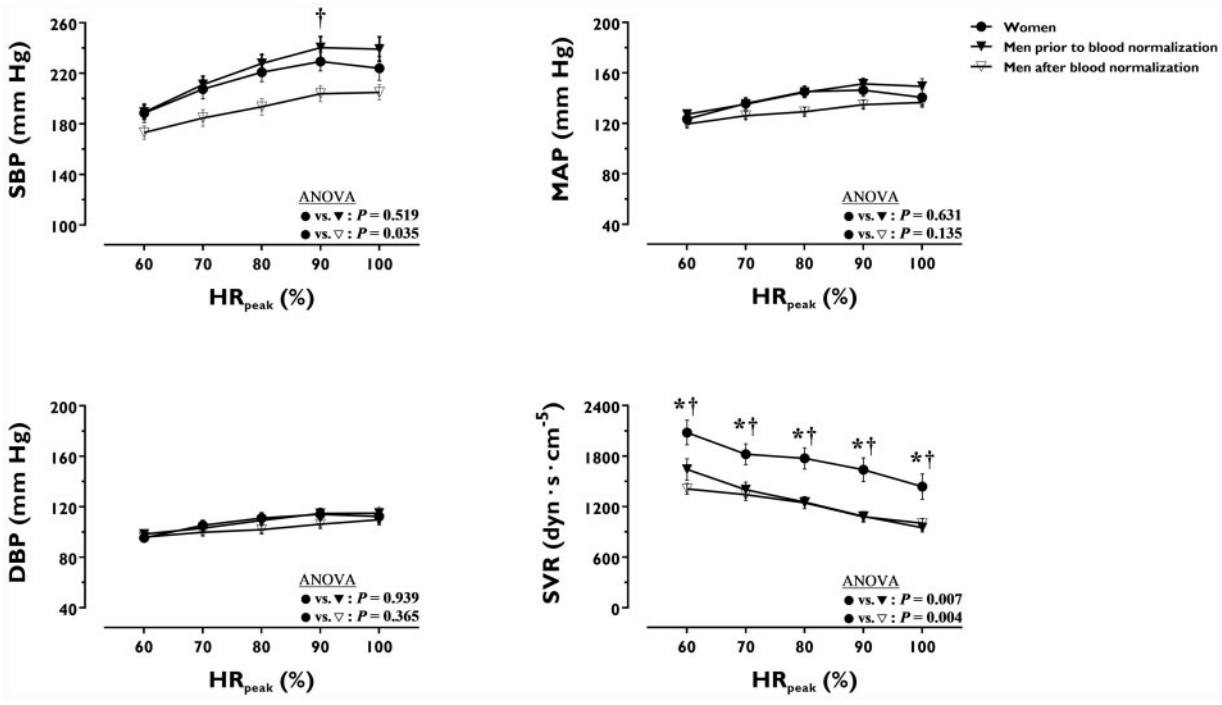


Figure 2 Central haemodynamics and peripheral resistance during incremental exercise in women and men prior to and after blood normalization. $^{*}P < 0.05$ (independent samples *t*-test) between women and men prior to blood normalization. $^{\dagger}P < 0.05$ (independent samples *t*-test) between women and men after blood normalization. Data are expressed as mean ± SEM. Number of biological observations for each graph: SBP ($n = 355$), DBP ($n = 355$), MAP ($n = 355$), SVR ($n = 335$). Statistical tests: two-way ANOVA with repeated measures with group and time as between- and within-subject factors, respectively; independent samples *t*-test. HR_{peak}, peak heart rate; DBP, diastolic blood pressure; MAP, mean arterial pressure; SBP, systolic blood pressure; Q, cardiac output; SVR, systemic vascular resistance.

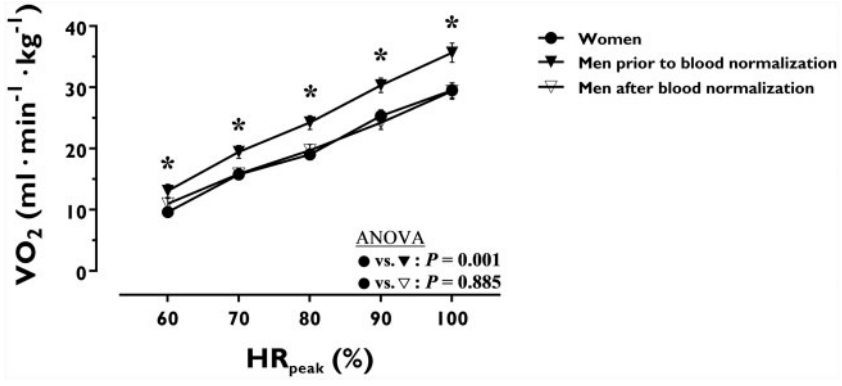


Figure 3 Oxygen uptake during incremental exercise in women and men prior to and after blood normalization. $^{*}P < 0.05$ (independent samples *t*-test) between women and men prior to blood normalization. $^{\dagger}P < 0.05$ (independent samples *t*-test) between women and men after blood normalization. Data are expressed as mean ± SEM. Number of biological observations in the graph: $n = 450$. Statistical tests: two-way ANOVA with repeated measures with group and time as between- and within-subject factors, respectively; independent samples *t*-test. HR_{peak}, peak heart rate; VO_2, oxygen uptake (per kg of body weight).

After a century of inquiries into the physiology of exercise, the pre-eminent albeit not exclusive role of the circulatory system in determining cardiorespiratory fitness, represented by VO_{2peak}, is firmly established.[12,13,45] The inherent constitution of the O_2 transport and utilization chain is asymmetrical, not all steps have the same potential to transport or consume O_2 in relation to VO_{2peak}.[12,46] Intracellular

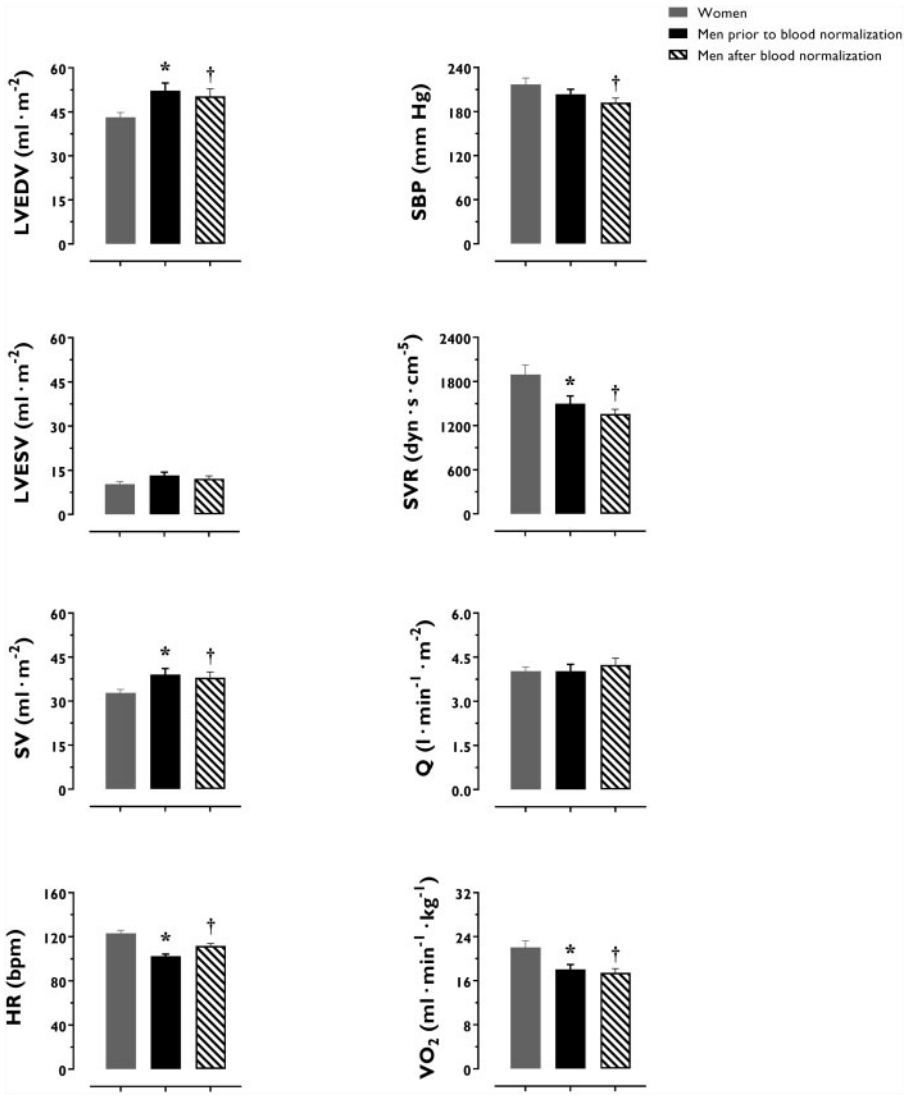


Figure 4 Cardiac, hemodynamic, and pulmonary variables at a given fixed submaximal exercise workload (100 W) in women and men prior to and after blood normalization. $^{*}P < 0.05$ (independent samples *t*-test) between women and men prior to blood normalization. $^{\dagger}P < 0.05$ (independent samples *t*-test) between women and men after blood normalization. Data are expressed as mean ± SEM. Number of biological observations for each graph: LVEDV ($n = 84$), LVESV ($n = 84$), SV ($n = 84$), HR ($n = 84$), SBP ($n = 71$), SVR ($n = 71$), Q ($n = 84$), VO_2 ($n = 88$). Statistical test: independent samples *t*-test. HR, heart rate; LVEDV, left ventricular end-diastolic volume; LVEF, left ventricular ejection fraction; Q, cardiac output; SBP, systolic blood pressure; SV, stroke volume; SVR, systemic vascular resistance; VO_2, oxygen uptake (per kg of body weight).

biochemical mechanisms that could in theory limit O_2 consumption are overbuilt in relation to the capacity to deliver O_2 through the circulatory system.[47–49] VO_{2peak}, as typically elicited by incremental exercise involving more than half of total muscle mass, is mainly determined by O_2 delivery even in the presence of compromised muscle oxidative capacity.[8,12,13,15,46] Systemic O_2 delivery is function of (i) cardiac pumping capacity (Q_{peak}) and (ii) arterial O_2 content, which in the presence of normal lung function reflects blood O_2 carrying capacity.[8,13,15] The heart's ability to pump blood is primarily determined by the filling level of the circulatory system, that is BV.[8,13,15] BV, along with venous tone, is the main contributing factor to the pressure gradient (venous reservoir-right atrium) that dictates venous return and cardiac filling.[24,25] Cardiac filling and output are intrinsically related by virtue of the Frank–Starling mechanism.[29,65] The longer the stretch of cardiomyocytes during filling (diastole), known as cardiac pre-load, the greater the tension developed at contraction (systole), until a plateau in SV is reached.[50–52] Given that HR_{peak} is essentially uniform for a given age irrespective of sex,[39] SV dictates Q_{peak}.[11,53] Hence, the generally smaller BV of women relative to men for a given fitness status results in reduced cardiac filling, SV, and Q_{peak} in women.[6] According to the above rationale, if only BV is normalized between men and women, systemic O_2 delivery would still be increased in men owing to their enhanced blood O_2 carrying capacity.[9,10] Both BV and effective Hb (i.e. Hb able to carry O_2) must be concurrently manipulated to match, in theory, the capacity to deliver O_2 between sexes. In agreement with the primary hypothesis, the matching of BV and O_2 carrying capacity between men and women precisely negated sex

differences in VO_{2peak}. The mechanistic basis and physiological implications of these findings are developed hereunder.

The matching of O_2 carrying capacity between men and women involves a 10% reduction of effective Hb concentration in the former. Thus, a relative state of anaemic hypoxia ensues. In this regard, moderate anaemic (or hypoxic) hypoxia is known to vasodilate arterial and venous beds via O_2-dependent mechanisms in proportion to the decrease in O_2 carrying capacity and thereby O_2 content in blood, thus termed compensatory vasodilation.[42,54] A key, yet not exclusive, vasodilator substance during hypoxic exercise is nitric oxide (NO).[55] Endothelium-dependent NO contributes to compensatory vasodilation, a mechanism that is attenuated with aging and partially restored via oral nitrate.[56,57] When arterial O_2 content falls from 200 to 180 ml· l^{-1} (10% reduction), compensatory vasodilation via NO, additional metabolites, as well as neural factors induces a similar or higher increment in peripheral vascular conductance so that O_2 delivery to active skeletal muscles is preserved.[11,55] Accordingly, we observed a consistent ≥ 10% reduction in exercise SBP, a marker of cardiac afterload,[54,55,58] concurring with the average reduction in O_2 carrying capacity in men after blood normalization. Such a systemic effect is expected to modulate the circulatory system in an integrative manner, including central and peripheral haemodynamics. In this regard, marked peripheral vasodilation via infusion of potent vasodilators in the femoral artery is known to increase cardiac pumping capacity during peak cycling exercise.[59] Specifically, peripheral arterial vasodilation contributes to increased SV via reduced cardiac afterload,[60,61] defined as the force (pressure) opposing myocardial contraction to eject blood into the arterial (high pressure) system. Therefore, when the blood of men is manipulated to have the O_2 carrying capacity of women, the concomitant decrease in cardiac afterload may contribute to enhance cardiac pumping capacity. The reduction of cardiac afterload must comprise the vasodilation of arterial beds in skeletal muscle, that is those regulating the bulk of blood flow and pressure during exercise; it should be noted that the infusion of potent vasodilators in peripheral veins alone does not alter central hemodynamics.[61] Moreover, the fact that cardiac filling was preserved in men after blood withdrawal implies that arterial vasodilation facilitates venous return during exercise, despite SVR is unaltered plausibly due to simultaneous venoconstriction associated with hypovolemia.[62] As a counterpart, arterial vasodilation in exercising limbs blunts O_2 extraction.[59] This could be due to changes in blood flow distribution underpinned by the vasodilation of arterioles perfusing inactive muscle fibres and non-muscular tissue, which under normoxic exercise are predominantly subjected to sympathetic-mediated vasoconstriction.[63] Collectively considered, men after blood normalization preserved cardiac filling and output, plausibly explained by *extra* arterial vasodilation, which in turn affected systemic O_2 extraction according to the consistent decrease of O_2 uptake at any relative exercise intensity. The current 'central' findings suggest that the circulatory system of men is adapted to a relatively high level of blood O_2 content in order to optimally regulate blood flow distribution and O_2 delivery.

Ultimately, two main factors may lead to reduced O_2 uptake in men after blood normalization: decreased systemic O_2 delivery resulting from lowered O_2 carrying capacity and suboptimal blood flow distribution to active muscles possibly attributed to extensive arterial vasodilation. It should be acknowledged, however, that the present study focuses on 'central' factors while strictly peripheral considerations remain to be ascertained. In this regard, the observation of higher VO_{2peak} per kg of lean mass in women compared with men after blood normalization implies a sex difference in muscle O_2 utilization. While skeletal muscle-related factors are not considered to limit VO_{2peak} in healthy individuals,[12,46–49] recent investigations have evidenced lower mitochondrial content and oxidative capacity in skeletal muscle of men compared with women matched by cardiorespiratory fitness.[3,64,65] Yet, the role of skeletal muscle factors and potential secondary effects of sex-specific substrate use during exercise on O_2 utilization needs to be experimentally addressed.

Consideration should be given to the administration of CO in the present study. The use of CO to manipulate arterial O_2 content for the assessment of cardiovascular regulation has a long history.[42,66–68] In healthy men, CO administration induces proportional reductions in O_2 carrying capacity and VO_{2peak}.[68,69] Importantly, moderate CO inhalation resulting in up to 18% decrements in O_2 carrying capacity in humans does not alter haematological (blood pH, bicarbonate, electrolytes, Hb concentration) and biophysical (temperature) characteristics of blood during cycling exercise.[42] Likewise, the CO administered essentially remains in the circulation during exercise, there is no diffusion into the tissue.[42] Yet, CO, which is produced endogenously with the degradation of the haem group, shares some of the biological properties of NO, including a vasodilatory effect.[70] Both CO- and NO-dependent vasodilation in skeletal muscle could be intertwined.[71] Thus, part of the peripheral vasodilation observed in men after blood normalization could be attributed to a direct effect of CO in the vasculature. In this respect, the additional increase of leg vascular conductance during cycling performed in hypoxic hypoxia is very similar to that observed with anaemic CO-mediated hypoxia, indicating no further vasodilatory effect of CO in exercise conditions.[42] In other respects, CO might alter O_2 extraction by increasing the affinity of Hb for O_2, which could contribute to lower O_2 extraction apart from the impact of extensive peripheral vasodilation during exercise. Nonetheless, O_2 extraction during exercise is similarly reduced (~10% decrements) by interventions inducing comparable increases peripheral vasodilation via the administration of either CO or vasodilator drugs not affecting Hb-O_2 affinity.[42,59] These observations point towards peripheral vasodilation as the main factor decreasing O_2 extraction with anaemic CO-mediated hypoxia. The fact that VO_{2peak} is ultimately reduced in proportion to the decrease of O_2 carrying capacity also suggests that any additional effect on O_2 uptake attributed to enhanced Hb-O_2 affinity should be minimal.[31,32] Notwithstanding, further research is needed to comprehensively ascertain the intrinsic effects of anaemic hypoxia, CO-mediated or not, in exercising humans.

There are some limitations in this study that require comment. First, we included middle-aged and older healthy individuals in order to limit the influence of disease-related confounding factors and assessed the acute effects of blood normalization. Whether the present findings can be extrapolated to young individuals or particular clinical (chronic) conditions will need to be eventually elucidated. Second, the investigators that performed the analyses, but not the study participants, were blinded to the experimental condition. Provided that a blinded intervention for phlebotomy and CO rebreathing could be successfully implemented, the main outcomes of the study are not thought to be altered by an hypothetical nocebo effect when standard physiological criteria are fulfilled.[15,72,73] Third, despite daily dietary habits were controlled throughout the study, further research may experimentally assess the potential role of body composition and dietary nitrates on sex differences in cardiorespiratory fitness. Finally, a low prevalence of individuals was taking hormone replacement therapy. We did not observe different results in these individuals, concurring with previous evidence indicating no effect of hormone replacement therapy on key measures of cardiovascular function during exercise and VO_{2peak} in our study population.[74]

Nonetheless, future studies may address the potential role of sex hormones and the associated NO axis in non-menopausal women.

In conclusion, the present study demonstrates that blood normalization between men and women eliminates differences in aerobic power. Moreover, the match of BV and O_2 carrying capacity between sexes reveals that the circulatory system of men is adjusted to a relatively high level of O_2 content in blood in order to optimally regulate blood pressure and flow distribution during exercise. The sex-specific signalling pathways underlying the coupled haematological–hemodynamic regulation during exercise remain speculative at present and will have to be characterized in future studies.

Supplementary material

Supplementary material is available at *Cardiovascular Research* online.

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Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

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Translational perspective

Low cardiorespiratory fitness is strongly associated with all-cause and cardiovascular mortality in asymptomatic adults independently of traditional risk factors, relationships seemingly enhanced in middle-aged and older women. Yet, whether the primary haematological determinants of cardiorespiratory fitness that were established in studies comprising men explain the difference between sexes remains uncertain. Importantly, blood attributes are amenable to modification and thus potentially translated into effective targets to improve or preserve cardiovascular health in the general population. The present experimental study demonstrates that blood normalization between men and women eliminate sex differences in cardiorespiratory fitness.