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Effects of 150 and 450 mL acute blood losses on maximal oxygen uptake and exercise capacity

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Abstract

Purpose: This study investigated whether maximal oxygen uptake ($\dot{V}O_{2max}$) and exercise capacity are affected by small acute blood loss (150 mL) and elucidated compensatory mechanisms. **Methods:** Thirteen male subjects ($\dot{V}O_{2max}$: 63±9 mL·kg^{-1}·min^{-1}; mean±SD) performed incremental exercise to exhaustion on a cycle ergometer in three experimental conditions: in euvolemia (control; blood volume, BV: 6.0±0.7 L) and immediately after acute BV reductions of 150 mL (BVR_{150mL}) and 450 mL (BVR_{450mL}). Changes in plasma volume (PV) and BV during exercise were calculated from hematocrit, hemoglobin concentration, and hemoglobin mass (carbon monoxide rebreathing). **Results:** The reduction in $\dot{V}O_{2max}$ per mL of BV reduction was 2.5-fold larger after BVR_{450mL} compared to BVR_{150mL} (-0.7 ± 0.3 vs -0.3 ± 0.6 mL · min^{-1} · mL^{-1}; P =.029). $\dot{V}O_{2max}$ was not significantly changed after BVR_{150mL} (-1±2%; P=.124), but reduced by 7±3% after BVR_{450mL} (P<.001) compared to control. Peak power output only decreased after BVR_{450mL} (P<.001). At maximal exercise, BV was restored after BVR_{150mL} compared to control (-50±185 mL; P=.375) attributed to PV-restoration, which was, however, insufficient in restoring BV after BVR_{450mL} (-281±184 mL; P<.001). The peak heart rate tended to increase (3±5 beats·min^{-1}; P=.062) whereas the O_2 pulse (-2±1 mL·beat^{-1}; P<.001) and Vastus Lateralis tissue oxygenation index (-4±8 %-points; P=.080) were reduced after BVR_{450mL}, suggesting decreased stroke volume and increased leg O_2 extraction. **Conclusion:** The deteriorations of $\dot{V}O_{2max}$ and of maximal exercise capacity accelerate with the magnitude of acute blood loss, likely because of a rapid PV restoration sufficient to establish euvolemia after a small but not after a moderate blood loss.

Keywords: blood volume; cardiac output; hemoglobin mass; maximal oxygen uptake; plasma volume, VO2max

Introduction

It is well established that the circulating blood volume (BV) and the total hemoglobin mass (Hb_{mass}) are of principal importance for maximal oxygen uptake ($\dot{V}O_{2max}$) and exercise capacity in humans (1, 2). A standard blood donation of 400-500 mL reduces $\dot{V}O_{2max}$ by ~5-10% (1, 3-5) and reinfusion of ~360-450 mL of freeze-preserved packed red blood cells (RBC) equaling ~800-900 mL of whole blood increases $\dot{V}O_{2max}$ by ~4-6% (6-8). The underlying mechanisms responsible are the accompanying changes in stroke volume (SV), maximal cardiac output ($\dot{Q}_{max}$) and the blood O_2 carrying capacity, thus, changing the systemic O_2 delivery (6, 8) that is the principal determinant of $\dot{V}O_{2max}$ during whole-body exercise, at sea level (9).

Endurance training increases BV (2). In a previous study (10), after 10 weeks of endurance training, we counteracted the individual training-induced BV expansion by phlebotomy. Surprisingly, the training-induced gain in $\dot{V}O_{2max}$ was preserved, which contrasts previous findings showing a clear deterioration of $\dot{V}O_{2max}$ and exercise capacity after acute blood loss (1, 3-5, 11, 12). One explanation for the discrepancies may originate from the magnitude of the BV reduction, as only a ~170 mL blood withdrawal was necessary to counteract the training-induced BV expansion (10), as compared to 360-500 mL acute blood losses carried out in previous investigations (1, 3-5, 11, 12). This may indicate that compensatory mechanisms exist that fully preserves $\dot{V}O_{2max}$ when the acute blood loss is below a threshold. However, no previous study has compared the effects of acute blood losses of different magnitudes within the same subjects.

To address this experimentally, we compared $\dot{V}O_{2max}$ and maximal exercise capacity immediately after BV removals of 150 mL (BVR_{150mL}) and 450 mL (BVR_{450mL}) to a euvolemic control situation. Changes in plasma volume (PV) and BV during submaximal and maximal exercise were calculated from measurements of hemoglobin concentration ([Hb]) and hematocrit (hct) obtained during rest and exercise and by knowing the Hb_{mass} (carbon monoxide rebreathing) (13). We hypothesized that $\dot{V}O_{2max}$ and peak power output ($\dot{W}_{peak}$) during incremental exercise to exhaustion are maintained after BVR_{150mL}, but reduced after BVR_{450mL}. Therefore, the reduction in $\dot{V}O_{2max}$ after BVR_{450mL} will be more pronounced even after standardizing to the BV removed.

Methods

Ethical approval

This study was approved by the Ethics Committee of the Norwegian School of Sport Sciences (ref. 100-290819) and The Norwegian Centre for Research Data (ref. 418763). Oral and written informed consents were obtained from all subjects before the start of this investigation, which was carried out in accordance with the Declaration of Helsinki.

Subjects

Thirteen moderately- to well-trained men that were accustomed to cycling were recruited and completed all tests (age: 27.3 ± 4.0 years; weight: 74.7 ± 6.5 kg; height: 1.79 ± 0.07 m; $\dot{V}O_{2max}$: 63.4 ± 8.7 mL · kg^{-1} · min^{-1}; Hb_{mass}: 891 ± 127 g; BV: 5991 ± 736 mL). An additional two subjects were recruited but dropped out due to factors unrelated to the study. All subjects were non-smokers and reported no contraindications to maximal exercise testing.

Experimental design

The subjects visited the laboratory on three occasions at the same time of day (08:00 or 14:00): on a familiarization day, a control day and an experimental day, each separated by 1 week. Exercise testing was repeated twice on each day, separated by 90 min rest, which is an adequate recovery duration for preserving $\dot{V}O_{2max}$ and $\dot{W}_{peak}$ when several maximal tests are conducted on the same day (14). Each trial included three 5-min submaximal power outputs followed by a maximal test with step-increments every minute until exhaustion for determination of $\dot{W}_{peak}$ and $\dot{V}O_{2max}$. The two trials on the control day were conducted in euvolemia (normal BV), whereas on the experimental day, the first and the second trial were preceded by the removal of 150 mL blood (referred to as BVR_{150mL}) and 450 mL blood (150 mL + 300 mL; BVR_{450mL}), respectively. In this way, the first and the second control trial served as controls for BVR_{150mL} and BVR_{450mL}, respectively. Between trials, recovery and food intake were standardized. *Ad libitum* water intake was recorded on the control day and was repeated on the experimental day. The subjects were instructed to abstain from strenuous exercise and caffeine consumption during the last 24 h and 12 h before lab visits, respectively. Hb_{mass} was assessed on the familiarization day for the calculation of intravascular volumes.

Measurements and procedures

Exercise trials

The trials started with a 3-min resting measurement while seated on the cycle ergometer (Excalibur Sport; Lode B.V., Groningen, The Netherlands). Afterward, the three 5-min submaximal power outputs (90 ± 32, 138 ± 36, and 185 ± 42 W, equalling 35 ± 4, 45 ± 5, and 56 ± 6 % of $\dot{V}O_{2max}$, respectively) were directly followed by a maximal test with step-increments of 25 W every minute until exhaustion. The starting level was the third submaximal power output +25 W. The submaximal power outputs were individually set to induce a capillary blood lactate concentration ≤ 2.5 mmol · L^{-1} (Biosen C-line; EKF Diagnostic, Cardiff, UK) on the third power output and a time to exhaustion of ~8 min on the maximal test. The power outputs were adjusted from the familiarization tests to meet these demands: if the subject completed the incremental test with a shorter/longer duration than 8 min, the submaximal power outputs were adjusted accordingly. This adjustment led to a capillary blood lactate concentration of 1.9 ± 0.5 mmol · L^{-1} on the third submaximal power output on the control day. The mean power output during the last 60 s was defined as $\dot{W}_{peak}$. After reaching exhaustion, the subjects cycled at 50-100 W for 10 min for warm-down.

Respiratory variables were measured using open-circuit indirect calorimetry with a mixing chamber (Oxycon Pro; Jaeger Instrument, Friedberg, Germany), as validated by Foss and Hallén (15). The subjects were connected to a two-way non-rebreathing valve (product number 2700; Hans Rudolph Inc., Shawnee, KS, USA), the expired gas was passed through a mixing chamber and a volume transducer (Triple V; Erick Jaeger GmbH, Höchberg, Germany), and the expired O_2 and CO_2 fractions were continuously monitored (rise time for gas analyzers, T_{10-90}: 80 ms after filtering). Before each trial, the gas analyzers were calibrated using a high-precision gas mixture (15.00% O_2 and 6.00% CO_2; AGA, Oslo, Norway) and ambient air. The volume transducer (resistance: 0.1 kPa · L^{-1} · s^{-1} at 15 L· s^{-1}) was calibrated using a 3.00-L high-precision syringe (5530 series; Hans Rudolph Inc.). $\dot{V}O_2$ was measured over the last 2.5 min at each submaximal power output and continuously during the resting measurements and incremental tests. On the submaximal power outputs, the average of the last 2 min served as the steady-state values. During the incremental tests to exhaustion, the highest 30-s average was taken as $\dot{V}O_{2max}$. Heart rate (HR) was measured continuously (electrocardiogram), and the highest 10-s average was defined as HR_{peak}. The average 30-s HR during $\dot{V}O_{2max}$ was used to calculate the O_2 pulse (O_2 pulse = $\dot{V}O_2$ / HR = SV · a-$\bar{v}O_2$ difference) as an indicator of SV. Blood samples were drawn at rest, during the

last 20 s of each submaximal power output and at exhaustion. Peak blood lactate concentration was measured 1 min after exhaustion.

As a substitute measure of arterial O_2 saturation, peripheral capillary O_2 saturation (SpO_2) was assessed using a pulse oximeter placed on the pre-heated right index finger (901-M; Masimo, Irvine, CA, USA). Arterial O_2 content (CaO_2) at maximal exercise was estimated as: (1.34 × [Hb] × SpO_2) + 3 mL O_2 dissolved in plasma per liter of blood. The tissue oxygenation index (TOI) was obtained from the Vastus Lateralis muscle as the ratio of oxygenated to total tissue Hb and myoglobin via continuous wavelength near-infrared spectroscopy (NIRS; PortaMon; Artinis Medical Systems, Elst, The Netherlands). NIRS primarily reflects capillary oxygenation but is also affected by the arteriolar and venular blood and muscle myoglobin. The Vastus Lateralis TOI correlates well with the femoral venous O_2 saturation and may serve as a substitute measure of leg O_2 extraction (16, 17). The oxygenation parameters were obtained using three light-emitting diodes (wavelengths: 760 and 850 nm) spaced 30, 35 and 40 mm from the detector. The NIRS optode was covered with Saran wrap, placed mid-way between the trochanter major and the lateral epicondyle and secured with elastic bandages around the thigh. Equivalent positioning across days was ensured by using skin and anatomical landmarks. The data was recorded at 10 Hz and analyzed using the Oxysoft software (v. 3.0.95; Aritnis). One minute after the termination of exercise, an 8.5 cm-wide cuff (Zimmer Biomet, Warsaw, IN, USA) was manually inflated (VBM Medizintechnik, Sulz am Neckar, Germany) to 300 mmHg to occlude the arterial blood flow for 6 min (prolonged up to 8 min if necessary) until the minimum TOI was reached. After cuff-release, the maximum TOI was recorded during hyperemia. The maximum and minimum TOI were presented with the data obtained during exercise to display the dynamic range of the device. The subjects had a skin- and subcutaneous tissue thickness of 5.3 ± 1.8 mm (range: 3.1–9.7) below the optode, assessed using an ultrasound device (Vivid E95; GE Vingmed Ultrasound AS, Horten, Norway).

The coefficient of variation for $\dot{W}_{peak}$ and $\dot{V}O_{2max}$, calculated across the four exercise trials in euvolemia (familiarization and control trials), was 1.7% and 1.9%, respectively.

Blood sampling and phlebotomy

During the control and the experimental day, blood samples (3 mL, EDTA; BD, Franklin Lakes, NJ, USA) were obtained via an 18 G catheter (BD) indwelling in an antecubital vein that was regularly flushed with normal saline (0.9% NaCl) to maintain patency. [Hb] was

measured in duplicate (triplicate if the measurements deviated by ≥ 0.4 g · dL^{-1}) on a hemoximeter (ABL80 CO-OX FLEX; Radiometer, Copenhagen, Denmark) and hct was measured in triplicate by the micro-centrifugation method (6 min at 12,800 rpm; Hettich, Tuttlingen, Germany) and adjusted for trapped plasma (3%). The intra-assay coefficient of variation was 1.6% and 0.5% for [Hb] and hct, respectively (n = 296-309). The same catheter was used for phlebotomy of 150 mL blood before BVR_{150mL}, and an additional 300 mL blood before BVR_{450mL} (450 mL in total). The blood was drawn slowly over 5 min (BVR_{150mL}) or 10 min (BVR_{450mL}) during which the blood pressure was monitored (ProBP 3400 series; Welch Allyn, Skaneateles, NY, USA). The subjects rested seated for 10 min after phlebotomy before moving to the cycle ergometer.

The RBCV, PV and BV at rest and during exercise were calculated assuming a constant Hb_{mass} over the experimental period (18, 19). The reductions in intravascular volumes induced by phlebotomy and blood sampling were included in these calculations, and it was assumed that all changes in [Hb] and hct could be accounted for by fluctuations in PV occurring due to factors such as sweating, transcapillary fluid shifts, swelling/shrinking of RBCs, intestinal fluid uptake and urine production (13, 20).

Hemoglobin mass

Hb_{mass} was measured using a carbon monoxide (CO) rebreathing method (21, 22). First, the subjects rested seated for 10 min, followed by capillary blood sampling in two 125-µL pre-heparinized tubes (Clinitubes; Radiometer, Copenhagen, Denmark) from a pre-heated fingertip. The subjects then inhaled a bolus of 1.2 mL per kg body weight of 99.97% chemically pure CO (AGA) administered via a 100-mL plastic syringe (Omnifix; Braun, Kronberg im Taunus, Germany) to a spirometer (Blood tec GmbH, Germany). In this closed circuit, the CO was rebreathed for 2 min together with 3 L of pure O_2 (AGA) while checking for leakages using a CO analyzer (Draeger, Lübeck, Germany). Two capillary blood samples were collected, 6 and 8 min after the administration of CO. All blood samples were immediately analyzed in duplicate for percent carboxyhemoglobin using an ABL80 CO-OX FLEX. After rebreathing, the CO not absorbed by the body was calculated by multiplying the CO concentration of the rebreathing bag by the bag volume and the subject's estimated residual lung volume (23). The CO exhaled between the time-point of disconnecting from the spirometer to the blood sampling was estimated by multiplying the difference in end-tidal CO concentration before and after rebreathing by the estimated alveolar ventilation (24). The Hb_{mass} was calculated by dilution of CO in the blood (21) with correction for loss of CO to

myoglobin (0.3% of the administered CO per minute) (22). The coefficient of variation of duplicate Hb_{mass} determinations conducted by Ø.S. in our lab is typically 1.1-1.6 % (10, 25).

Statistical analyses

Data in text and tables are presented as mean ± standard deviation (SD) and in graphs as mean ± 95% confidence limits. The data were initially assessed for normal distribution using the D'Agostino-Pearson test. Maximal responses and resting measurements were analyzed using a paired sample t-test and only the planned comparisons were conducted: control 1 vs BVR_{150mL} and control 2 vs BVR_{450mL}. Cohen's *d* effect size was calculated for the maximal responses by dividing the mean difference between a BVR trial and its respective control trial with the pooled SD for these two trials [(BVR_{mean} – $control_{mean}$) / pooled SD]. A paired sample t-test was used to analyze whether the change in $\dot{V}O_{2max}$ from control 1 to BVR_{150mL} was different from the change from control 2 to BVR_{450mL} (Δ value vs Δ value). The submaximal $\dot{V}O_2$, HR, TOI, intravascular volumes (BV, PV and RBCV) and hematological parameters ([Hb], hct and MCHC) were analyzed using a two-way repeated measures ANOVA (trial x power output) followed by Bonferroni's multiple comparisons test. Control 1 vs BVR_{150mL} and control 2 vs BVR_{450mL} were analyzed separately. The alpha-level was set to ≤ .05, and values between > .05 and ≤ .10 were considered to indicate trends. GraphPad Prism 8 (v.8.4.1; GraphPad Software, CA, USA) and Microsoft Office Excel 2016 (Microsoft Corporation, WA, USA) were used for statistical analysis.

Results

Maximal exercise tests

$\dot{V}O_{2max}$ was not significantly changed after BVR_{150mL} (-1.1 ± 2.3%; P = .124) but was reduced by 6.9 ± 3.1% after BVR_{450mL} (P < .001; Fig. 1) compared to the euvolemic control. $\dot{W}_{peak}$ only decreased after BVR_{450mL} (P < .001; Table 1). The reduction in $\dot{V}O_{2max}$ per milliliter blood removed was larger after BVR_{450mL} than after BVR_{150mL} (-0.7 ± 0.3 vs -0.3 ± 0.6 mL · min^{-1} · mL^{-1}, respectively; P = .029) and per gram Hb_{mass} removed (-4.9 ± 1.9 vs -2.0 ± 4.3 mL · min^{-1} · g^{-1}, respectively; P = .024). The estimated CaO_2 at maximal exercise (based on [Hb] and SpO_2) was reduced after BVR_{150mL} (-4 ± 7 mL · L^{-1}; P = .040) and BVR_{450mL} (-6 ± 6 mL · L^{-1}; P = .004), with no significant differences between BVR trials (P = .294). After BVR_{450mL}, HR_{peak} and Vastus Lateralis TOI displayed trends towards being increased (3 ± 5 beats · min^{-1}; P = .062; Fig. 2D) and decreased (P = .080; Fig. 2F),

respectively. After BVR_{450mL}, the O_2 pulse was decreased ($P < .001$; Fig. 2E), suggesting decreased SV. The peak ventilation, breathing frequency, respiratory exchange ratio, post-exercise blood lactate concentration and rating of perceived exertion were similar across trials (Table 1).

The body weight-indexed $\dot{V}O_{2max}$ ($mL \cdot kg^{-1} \cdot min^{-1}$) at control was not related to the reduction in $\dot{V}O_{2max}$ (in $mL \cdot min^{-1}$) after BVR_{150mL} ($R^2 = .01$; $P = .740$) nor after BVR_{450mL} ($R^2 = .01$; $P = .795$), indicating that the reduction in $\dot{V}O_{2max}$ induced by a fixed amount of blood withdrawal is independent of $\dot{V}O_{2max}$.

<<Table 1 and Fig. 1 here>>

Submaximal exercise

Blood withdrawal did not affect submaximal $\dot{V}O_2$ (main effect of trial: $P = .478$-$.621$). After BVR_{450mL}, the HR was increased at the second and third submaximal power output (Fig. 2D), whereas the O_2 pulse was decreased (Fig. 2E). The Vastus Lateralis TOI was reduced at rest ($P = .022$) and displayed a trend towards being reduced during submaximal exercise after BVR_{450mL} (main effect of trial: $P = .080$; Fig. 2F).

<<Fig. 2 here>>

Hematological fluctuations during exercise

The 150 mL blood removed before BVR_{150mL} consisted of 63 ± 4 mL RBCs and 87 ± 4 mL plasma. Before BVR_{450mL}, 186 ± 12 mL RBCs and 264 ± 12 mL plasma were removed in total. This represented 2.5 ± 0.3% (range: 2.1-3.2) and 7.4 ± 0.9% (range: 6.3-9.5) of the Hb_{mass} and 2.5 ± 0.3% (2.1-3.2) and 7.6 ± 1.0% (range: 6.4-9.6) of the subjects' total BV.

The changes in [Hb], hct and mean corpuscular hemoglobin concentration (MCHC) during the trials are presented in Fig 3. Despite removing 150 mL blood, the calculated BV at rest was unchanged after BVR_{150mL} compared to control 1 (mean difference: -71 ± 234 mL; $P = .296$; Fig. 3F). This was caused by hemodilution (Fig. 3A-B) that likely re-established the PV to the pre-phlebotomy level (mean difference: -12 ± 202 mL; $P = .835$; Fig. 3E). From rest to exhaustion, the calculated PV reduced similarly in control 1 and after BVR_{150mL} (-553 ± 173 vs -528 ± 110 mL, respectively) due to a similar hemoconcentration (Δ[Hb]; 10.3 ± 3.2% vs 10.0 ± 2.1%, respectively; $P = .730$). The BV at $\dot{W}_{peak}$ was similar (mean difference:

-50 ± 185 mL; P = .375) despite a significantly lower RBCV after BVR_{150mL} (mean difference: -63 ± 59 mL; P = .002; Fig. 3D).

Despite similar body weight during all trials (Table 1; P ≥ .30) and identical fluid intake between the two control tests and between BVR_{150mL} and BVR_{450mL} (6.9 ± 4.0 dL water), the PV showed a trend towards being elevated before the second phlebotomy compared to before control 2 (86 ± 164 mL; P = .083; comparison indicated with an arrow in Fig. 3E). After the second phlebotomy, the PV was lower than in control 2 at rest (-158 ± 173 mL; P = .006) and during submaximal exercise (mean differences: -169 to -193 mL; all P < .01; Fig. 3E), but showed only a trend towards being different at $\dot{W}_{peak}$ (-90 ± 165 mL; P = .073). The hemoconcentration was 10.5 ± 2.5% and 9.7 ± 2.5% during control 2 and BVR_{450mL}, respectively (P = .400). The RBCV and BV were lower at $\dot{W}_{peak}$ after BVR_{450mL} compared to control 2 (-191 ± 46; P < .001 and -281 ± 184 mL; P < .001, respectively), indicating that 38 ± 41 % of the total BV withdrawal (450 mL) was restored due to PV restoration.

<<Fig. 3 here>>

Discussion

To the best of our knowledge, this is the first study that has investigated the acute effects of a small (150 mL) vs a moderate (450 mL) blood withdrawal on $\dot{V}O_{2max}$. The novel finding is that the reduction $\dot{V}O_{2max}$ per mL of acute blood loss was 2.5-fold larger after a moderate compared to a small blood loss. The 150 mL blood loss was associated with re-established PV and BV, unchanged O_2 pulse (an indicator of stroke volume), and unchanged Vastus Lateralis TOI at maximal exercise compared with the euvolemic control trial. The PV and BV were not fully restored after the removal of 450 mL blood. As a result, $\dot{V}O_{2max}$ was reduced by ~7%. Together, these findings demonstrate that compensatory mechanisms partly enable the circulation to cope with small BV reductions to preserve $\dot{V}O_{2max}$ and maximal exercise capacity, and that the adverse effects of blood loss accelerate with the volume of blood removed.

The sensitivity of $\dot{V}O_{2max}$ to BV reductions

The findings of the present study comply with previous work, showing a negligible effect of a small BV reduction on $\dot{V}O_{2max}$ and $\dot{W}_{peak}$ (10). This also agrees with findings showing that a

small to moderate PV reduction induced by heat stress or prolonged exercise does not decrease $\dot{Q}_{max}$ and $\dot{V}O_{2max}$ when assessed in normothermic conditions (26, 27). Our results also agree with previous studies investigating the acute effect of blood withdrawal on $\dot{V}O_{2max}$ when the exercise test is initiated within 3 h after phlebotomy (Fig. 4). A statistical analysis of these studies showed a linear decrease in $\dot{V}O_{2max}$ as a function of the BV removed with the x-intercept at ~150 mL (3, 4, 10-12, 28-31). This relationship was even stronger when restricting the comparison to studies initiating the exercise test within 1 h after phlebotomy (R^2 = .90), especially when employing a curvilinear fit (R^2 = 0.94). Thus, collectively, these studies indicate that compensatory mechanisms may enable the circulation to cope with a blood loss up to approximately 150 mL to preserve $\dot{V}O_{2max}$, with the adverse effects accelerating after suffering a larger blood loss. This threshold is probably dependent on the total BV of the subjects. This is supported by the fact that the two subjects with the largest individual reduction in $\dot{V}O_{2max}$ after removing 150 mL of blood (see Fig. 1) were among the three subjects with the lowest BV and Hb_{mass}, which may have implications for subjects with low BV, such as women and untrained men. It must be mentioned that $\dot{V}O_{2max}$ decreased non-significantly by 1.1% after BVR_{150mL}, within the measurement error of $\dot{V}O_{2max}$ for our subjects (1.9%). Therefore, it is plausible that the circulation starts responding negatively already to this small BV manipulation, but with the response being under the detection limit. Furthermore, although $\dot{V}O_{2max}$ and $\dot{W}_{peak}$ were unchanged after BVR_{150mL}, it is currently unknown whether endurance performance would change after a small acute blood loss since some studies indicate a more potent effect of BV manipulations on time trial performance than $\dot{V}O_{2max}$ (32).

<<Fig. 4 here>>

Compensatory mechanisms preserving $\dot{V}O_{2max}$

Blood loss may reduce systemic O_2 delivery and thereby $\dot{V}O_{2max}$ through two main mechanisms: 1) by reducing the Hb_{mass} that lowers the [Hb] and thus the CaO_2 and 2) by reducing the total circulating blood volume that impedes venous return, preload, and thus SV (2, 33). After BVR_{150mL}, the BV was unchanged, while the estimated CaO_2 was reduced by 4 $mL \cdot L^{-1}$ at maximal exercise. Therefore, it is likely that redistribution of extracellular fluid from the interstitial to the intravascular space re-established PV, secured euvolemia, and likely maintained the venous return and $\dot{Q}_{max}$. An effective contribution from the muscle

pump and vasoconstriction of capacitance vessels may also have maintained the central blood volume and thereby enabled a normal SV (26, 27). However, due to the hemodilution-induced reduction of CaO_2, the systemic O_2 delivery may still have been slightly reduced, potentially explaining the non-significant reduction in $\dot{V}O_{2max}$ of ~1%; especially since no compensatory change in peripheral O_2 extraction occurred, as indicated by the unchanged Vastus Lateralis TOI.

Physiological responses after a moderate blood loss (450 mL)

$\dot{V}O_{2max}$ was reduced by ~7% after withdrawing 450 mL blood, which is similar to previous findings, showing an average reduction of ~9% (range: 4-16%) when $\dot{V}O_{2max}$ was measured within 3 h after 450-500 mL blood donation (3, 4, 28-31). The reduction in $\dot{V}O_{2max}$ per milliliter of blood removed was 2.5-fold larger than after the 150 ml blood loss, which indicates that the adverse effects of blood loss accelerate with the volume of blood removed. The individual reduction in $\dot{V}O_{2max}$ ranged from 3-13%, meaning that a considerable variation in response to a clinical blood donation of one unit of blood can be expected. Interestingly, the reduction in $\dot{V}O_{2max}$ was 4.9 mL · min^{-1} per g Hb_{mass} removed from the circulation, which is almost identical to the slope between Hb_{mass} and $\dot{V}O_{2max}$ in subjects with a wide range in physical fitness and body size (4.4 - 4.8 mL · min^{-1} · g^{-1}) (2, 34, 35).

At rest, after removing 450 mL blood, [Hb] was reduced by 0.4 g · dL^{-1} compared to before the first phlebotomy. A net movement of fluid from the interstitial to the intravascular space was likely the cause of this decay. However, a contribution from post-exercise albumin retention, which increases oncotic pressure and therefore may have increased fluid retention within the intravascular space after exercise in the BVR_{150mL} trial, is also plausible (36), and may explain why the PV displayed a trend towards being increased (P = .083) before starting the second phlebotomy compared to at rest in control 2 (see arrow in Fig. 3E). Blood donations of 450 mL have previously been shown to decrease [Hb] by 0.3-0.4 g · dL^{-1} within 2 h (4, 37), although some studies observed a delayed response with decreased [Hb] first being evident the following day(s) (31, 38). By using the [Hb], Mora-Rodriguez et al. (2012) calculated that the BV was reduced by 287 mL 2 h after the withdrawal of 450 mL blood (37), in line with the findings in the current study after BVR_{450mL} (~300 mL). Therefore, after a blood donation of 450 mL, ~150 mL of the BV reduction is acutely compensated for by PV restoration, possibly through transcapillary fluid shifts.

The reduction in $\dot{V}O_{2max}$ after BVR_{450mL} was likely mediated by the combined effect of a reduced SV, as suggested by the reduced O_2 pulse, and a hemodilution-induced reduction of CaO_2, as estimated from [Hb] and SpO_2. It must be noted that the reduction in O_2 pulse may have been caused by the combined effect of a reduced SV and reduced CaO_2, since the latter can have evoked a reduction in the a-$\bar{v}O_2$ difference (O_2 pulse = SV · a-$\bar{v}O_2$ difference). However, some of the reduction in CaO_2 may have been counteracted by increased peripheral O_2 extraction fraction to maintain the a-$\bar{v}O_2$ difference at a similar level as we observed a trend towards decreased Vastus Lateralis TOI after BVR_{450mL} ($P = .080$), in line with previous findings (39). However, before concluding, these indications must be confirmed in catheterization studies since NIRS is limited to assessing a small tissue volume, since the TOI only trended to change, and due to the challenge of combining and interpreting data collected with distinct methodologies with different measurement units, such as O_2 pulse in mL and TOI in percent.

Very few investigations have measured $\dot{Q}_{max}$ after blood withdrawal. Ekblom et al. (1976) observed a 240 mL · min^{-1} reduction in $\dot{V}O_{2max}$ 24 h after the withdrawal of 800 mL blood (8). This was accompanied by an unchanged $\dot{Q}_{max}$ (determined by dye dilution) attributed to the "overnight" PV expansion indicated by the 1.7 g · dL^{-1} reduction in [Hb] and an unchanged BV determined by Cr^{51}-labelled RBCs. Therefore, the reduction in $\dot{V}O_{2max}$ was entirely attributed to reduced CaO_2, which lowered the systemic O_2 delivery. Conversely, when measured 1 h after the removal of 500 mL blood, $\dot{Q}_{max}$ was reduced by 4.5 L · min^{-1} (acetylene rebreathing) alongside a ~600 mL · min^{-1} reduction in $\dot{V}O_{2max}$ (30). The likely explanation for the different findings was that hypovolemia was not yet offset by PV expansion in the study by Krip et al. (1997), as confirmed using Evans blue dye (30). Therefore, in the early phases after moderate blood withdrawal (≥ 450 mL), such as in the present study, $\dot{V}O_{2max}$ is likely lowered due to reduced $\dot{Q}_{max}$ or the combined effect of reduced $\dot{Q}_{max}$ and CaO_2. Whereas, in the following days, when BV is fully restored, the reductions in systemic O_2 delivery and $\dot{V}O_{2max}$ are entirely attributed to reduced CaO_2 (8, 30, 40).

Methodological considerations

The order of trials was not randomized, and no subject blinding was carried out. Despite this, the post-exercise blood lactate concentration, the peak ventilation, and peak respiratory exchange ratio were similar between BVR and control trials, arguing for a similar exertion and against a nocebo effect after blood withdrawal. The calculations of intravascular volumes

during exercise assumes a stable Hb_{mass}, which can be challenged because of two reasons; 1) The Hb_{mass} can be underestimated when measured at rest in the seated position due to incomplete CO-mixing in blood, caused by venous pooling in capacitance vessels in the legs (41). These volumes are mobilized during exercise, such that the intravascular volumes calculated during exercise may have been slightly underestimated. 2) Exercise may also lead to splenic contraction, releasing blood with high hct into the circulation (42). This may represent up to 25% of the hemoconcentration during exercise and may cause an overestimation in the PV reduction when calculated from changes in [Hb] and hct (43, 44), such as in the present study. Despite this, calculated PV reductions from rest to maximal exercise based on [Hb] and hct have previously been found equivalent to that measured using radio-isotope-labeled human albumin (^{125}I-RISA) and RBCs (Cr^{51}), indicating that PV changes using this indirect method gives sufficient precision (20).

Conclusions

$\dot{V}O_{2max}$ was reduced more severely after an acute blood loss of 450 mL than after 150 mL, even after normalizing the decay in $\dot{V}O_{2max}$ to the volume of blood removed. This was likely caused by PV restoration, quickly ensuring euvolemia after the small but not after the moderate blood loss.

Additional information

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Author Contributions

Conception and design of the experiment: Ø.S., J.H., C.C.
Data collection: Ø.S., E.S.J., C.C.
Analysis of data: Ø.S., E.S.J.
Interpretation of data: Ø.S., J.H., C.C., E.S.J.
Writing the first draft: Ø.S.
Revising the manuscript: All authors.
All authors have read and approved the final version of the manuscript.

Conflict of interests

The authors declare that they have no conflicts of interest regarding the publication of this paper. There are no financial conflicts of interest to disclose. The results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation. The results of the present study do not constitute an endorsement by ACSM.

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Data availability statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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Figures

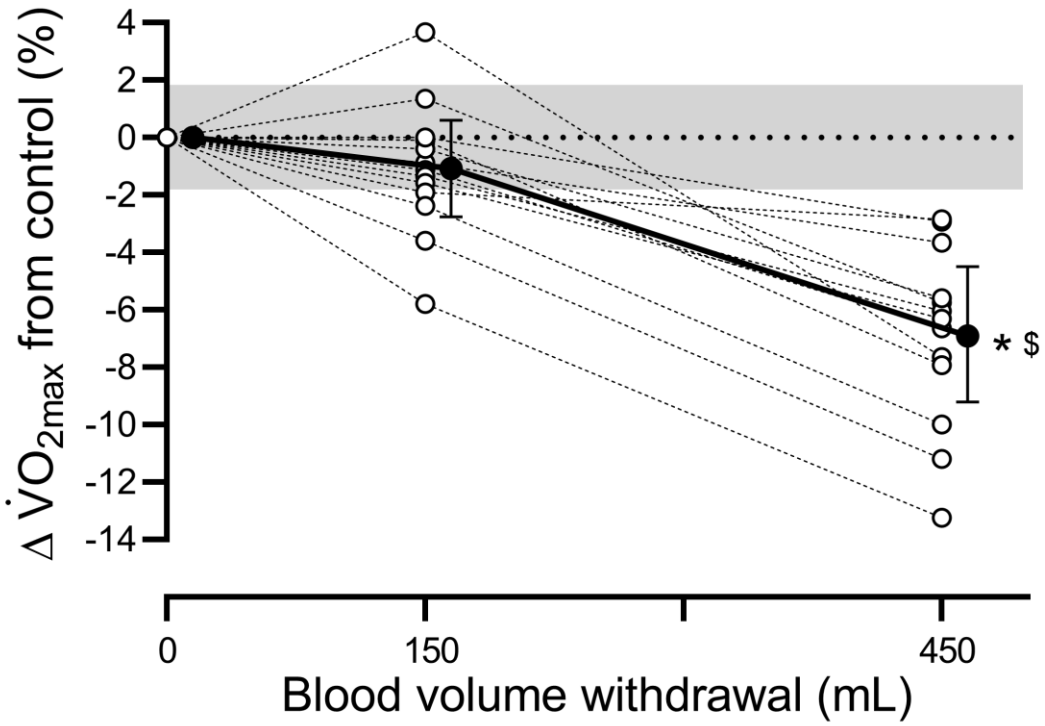

Fig. 1 The individual (white circles and dotted lines) and mean (black circles and solid line) differences in maximal oxygen uptake ($\dot{V}O_{2max}$) compared to the control trials as a function of the blood volume removed. Error bars indicate 95% confidence limits. The grey-shaded area indicates the coefficient of variation for $\dot{V}O_{2max}$ (± 1.9%). * Significant change from the control trial (P < .001). $ Significantly larger decrease after removing 450 mL compared to 150 mL blood (P < .001). N = 13

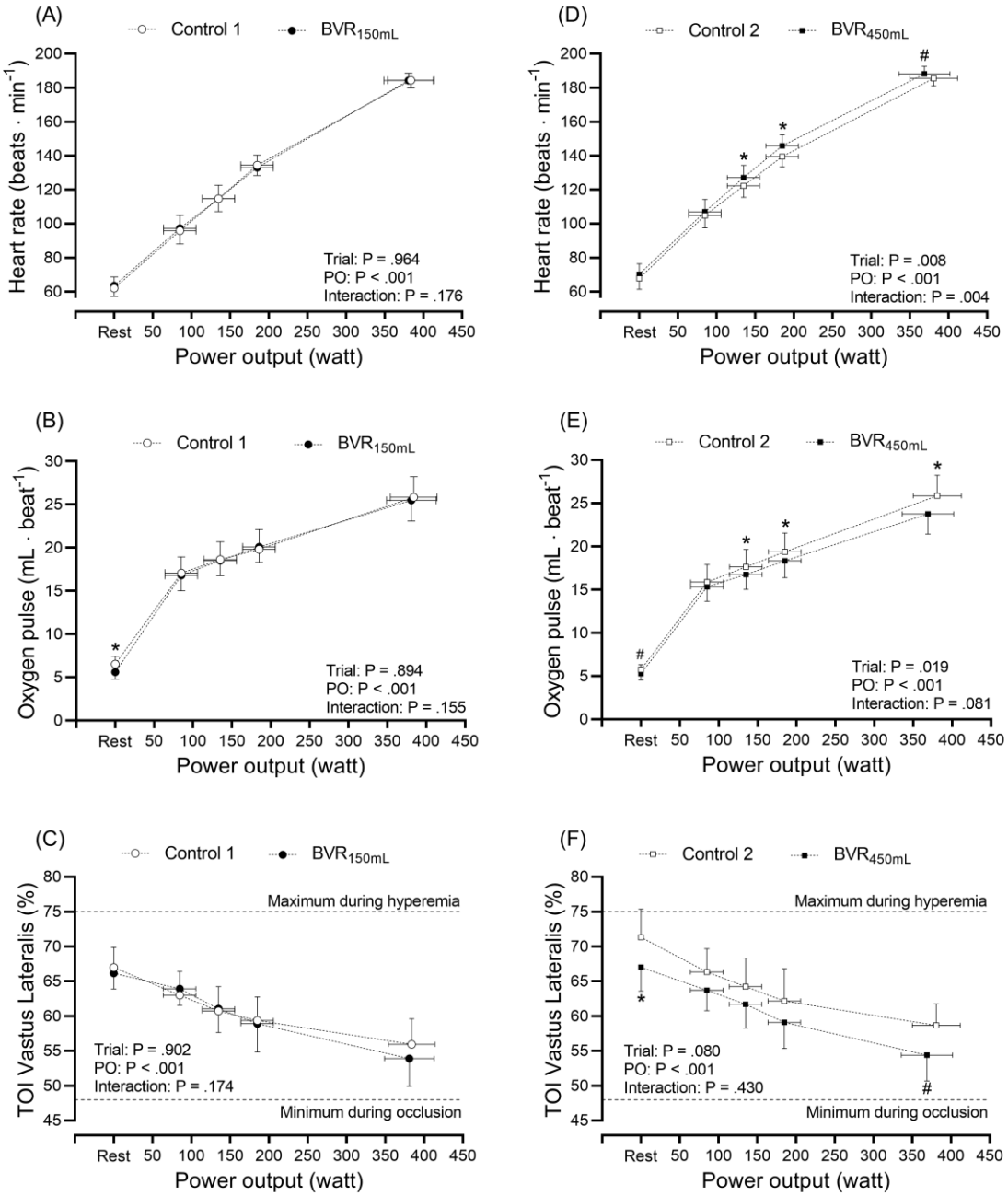

Fig. 2 The heart rate (A, D), oxygen pulse (B, E) and Vastus Lateralis tissue oxygenation index (TOI) (C, F) as a function of power output during submaximal and maximal exercise in the two control trials and the two blood volume reduction trials. Error bars indicate 95% confidence limits. The P-values for the ANOVA main effects of trial and power output (PO) as well as their interaction effect are inserted (submaximal POs only). * Significant change from the control trial ($P \leq .05$). # Trend towards a change from the control trial ($.05 < P \leq .10$). n = 12-13

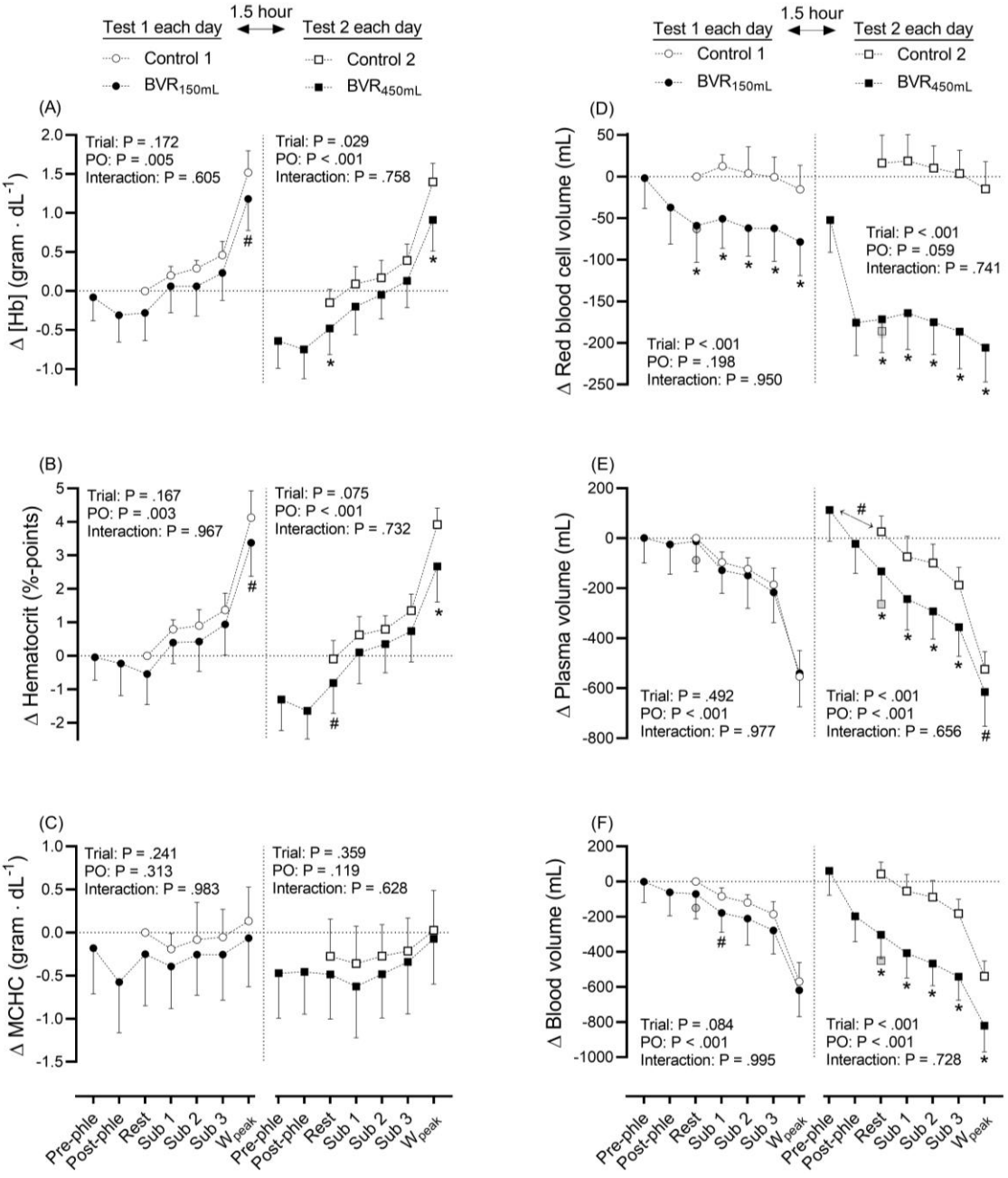

Fig. 3 Changes in hemoglobin concentration ([Hb]; A), hematocrit (B), mean corpuscular hemoglobin concentration (MCHC; C), red blood cell volume (D), plasma volume (E) and total blood volume (F) during the two control trials and the two blood volume reduction trials. All intravascular volumes were calculated using the changes in hematocrit and [Hb], and by assuming a stable hemoglobin mass (carbon monoxide rebreathing) apart from the reductions induced by phlebotomy and blood sampling. The grey symbols indicate the theoretical volumes at rest after phlebotomy if [Hb] and hematocrit had remained stable at the pre-phlebotomy level. All values are normalized to the resting measurement during

control 1. Error bars indicate 95% confidence limits. The P-values for the ANOVA main effects of trial and power output (PO) as well as their interaction effect are inserted (submaximal POs only). * Significant change from the control trial ($P \leq .05$). # Trend towards a change from the control trial ($.05 < P \leq .10$). N = 13

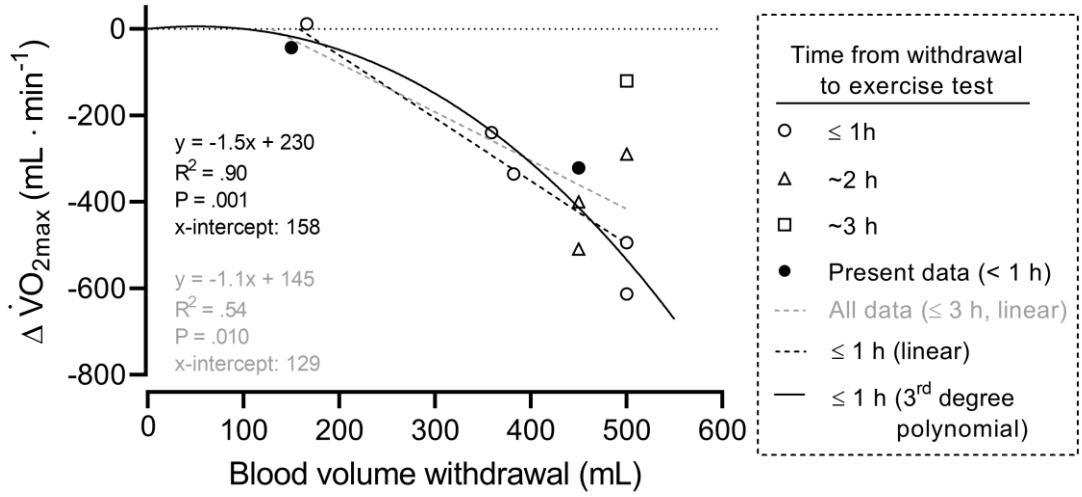

Fig. 4 The relationship between the magnitude of blood volume withdrawal and its impact on maximal oxygen uptake ($\dot{V}O_{2max}$) measured within the same day. The studies (3, 4, 10-12, 28-31) are organized according to the time from blood withdrawal to the initiation of the exercise. Linear regressions based on the data measured within 1 hour (black dashed line) and 3 hours (grey dashed line) after the blood withdrawal are depicted. A curvilinear fit (3rd degree polynomial) based on the data obtained within 1 hour is also displayed (continuous black line: $y = -0.0000008824x^3 - 0.002139x^2 + 0.2232x + 0$; $R^2 = 0.94$).

Tables

Table 1. Measurements obtained at maximal exercise, and body weight measured before each trial.

	First test each day			Second test each day		
	Control 1 (mean ± SD)	BVR_{150mL} (mean ± SD)	ES	Control 2 (mean ± SD)	BVR_{450mL} (mean ± SD)	ES
Peak power output (watt)	384 ± 50	381 ± 53	0.05	381 ± 51	369 ± 54***	0.22
$\dot{V}O_{2max}$ ($mL \cdot min^{-1}$)	4700 ± 667	4656 ± 707	0.06	4749 ± 694	4429 ± 697***	0.46
VE_{peak} ($L \cdot min^{-1}$)	195 ± 29	193 ± 33	0.05	195 ± 32	190 ± 34#	0.15
BF ($breaths \cdot min^{-1}$)	63 ± 14	63 ± 17	0.06	62 ± 10	62 ± 20	0.02
RER_{peak}	1.19 ± 0.05	1.19 ± 0.06	0.01	1.15 ± 0.05	1.16 ± 0.06*	0.30
$VE / \dot{V}O_2$	41.5 ± 3.0	41.6 ± 3.0	0.01	41.0 ± 3.4	42.9 ± 3.7***	0.54
$VE / \dot{V}CO_2$	35.1 ± 2.6	35.1 ± 3.0	0.01	35.8 ± 3.5	36.9 ± 3.5**	0.32
HR_{peak} ($beats \cdot min^{-1}$)	184 ± 8	184 ± 7	0.01	185 ± 7	188 ± 7#	0.35
O_2 pulse ($mL \cdot beat^{-1}$)	25.8 ± 3.9	25.5 ± 4.0	0.09	25.9 ± 4.0	23.8 ± 3.8***	0.54
[Hb] ($gram \cdot dL^{-1}$)	16.4 ± 1.0	16.0 ± 1.1#	0.32	16.3 ± 1.0	15.8 ± 1.1**	0.46
SpO_2 (%)	95 ± 2	95 ± 1	0.00	95 ± 1	95 ± 1	0.00
Estimated CaO_2 ($mL \cdot L^{-1}$)	212 ± 14	208 ± 14*	0.32	211 ± 14	205 ± 12**	0.47
Vastus lateralis TOI (%)	55.9 ± 6.1	53.9 ± 6.6	0.32	58.7 ± 5.1	54.4 ± 6.1#	0.76
$[La]_{peak}$ ($mmol \cdot L^{-1}$)	12.8 ± 1.4	12.7 ± 1.4	0.05	11.8 ± 1.5	11.5 ± 1.2	0.24
RPE	18.7 ± 1.2	19.0 ± 0.9	0.26	18.8 ± 0.8	19.3 ± 0.8#	0.52
Body weight (kg)	74.9 ± 6.6	75.1 ± 6.6	0.04	75.1 ± 6.4	75.2 ± 6.3	0.01

N = 13. ES, Cohen's *d* effect size (comparing the BVR trials with its respective control test); [Hb], hemoglobin concentration; HR_{peak}, peak heart rate; CaO_2, arterial O_2 content; $[La]_{peak}$, peak blood lactate concentration; RER_{peak}, peak respiratory exchange ratio; RPE, rating of perceived exertion using the Borg scale (6-20); SpO_2, capillary O_2 saturation (fingertip); TOI, tissue oxygenation index (n = 12); $\dot{V}CO_2$, carbon dioxide production; VE_{peak}, peak ventilation; $\dot{V}O_{2max}$, maximal oxygen uptake. *, ** and *** Significantly different from control ($P \leq .05$, $P < .01$ and $P < .001$, respectively). # Trend towards being different from control ($.05 < P \leq .10$). The body weight was measured before phlebotomy.