Phlebotomy eliminates the maximal cardiac output response to six weeks of exercise training

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Bonnet TC, Doucende G, Flick D, Jacobs RA, Nordsborg NB, Robach P, Walther G, Lundby C. Phlebotomy eliminates the maximal cardiac output response to six weeks of exercise training. Am J Physiol Regul Integr Comp Physiol 306: R752–R760, 2014. First published March 12, 2014; doi:10.1152/ajpregu.00028.2014.—With this study we tested the hypothesis that 6 wk of endurance training increases maximal cardiac output (Q˙max) relatively more by elevating blood volume (BV) than by inducing structural and functional changes within the heart. Nine healthy but untrained volunteers (V02max 47 ± 5 ml·min−1·kg−1) underwent supervised training (60 min; 4 times weekly at 65% V02max for 6 wk), and Q˙max was determined by inert gas rebreathing during cycle ergometer exercise before and after the training period. After the training period, blood volume (determined in duplicates by CO rebreathing) was reestablished to pretraining values by phlebotomy and Q˙max was quantified again. Resting echography revealed no structural heart adaptations as a consequence of the training intervention. After the training period, plasma volume (PV), red blood cell volume (RBVC), and BV increased (P < 0.05) by 147 ± 168 (5 ± 5%), 235 ± 64 (10 ± 3%), and 382 ± 204 ml (7 ± 4%), respectively. V02max was augmented (P < 0.05) by 10 ± 7% after the training period and decreased (P < 0.05) by 8 ± 7% with phlebotomy. Concomitantly, Q˙max was increased (P < 0.05) from 18.9 ± 2.1 to 20.4 ± 2.3 l/min (9 ± 6%) as a consequence of the training intervention, and after normalization of BV by phlebotomy Q˙max returned to pretraining values (18.1 ± 2.5 l/min; 12 ± 5% reversal). Thus the exercise training-induced increase in BV is the main mechanism increasing Q˙max after 6 wk of endurance training in previously untrained subjects.

adaptations; blood; cardiac output; Q; training

MAXIMAL CARDIAC OUTPUT (Q˙max) may increase within a few weeks of exercise training (49), and the underlying mechanisms leading to this are likely to be multifactorial. In the present study we aimed to determine the relative importance of training-induced changes in blood volume (BV) and structural and functional changes within the myocardium on the training-induced increases in Q˙max in untrained humans.

The greatest Q˙max measured in an endurance athlete (42.3 l/min) (15) is substantially higher than those found in healthy young volunteers, which usually is somewhere between 18 and 22 l/min (14, 28, 33). The higher Q˙max in athletes is entirely due to structural adaptations associated to exercise training but also to changes in BV, and accordingly, the enhanced Q˙max with exercise training may be the result of an interaction between the two. Indeed it has been suggested that training-induced increases in BV and hence venous return is the main parameter leading to an enhanced SV with training in untrained individuals. Endurance exercise training in untrained humans may increase BV by up to 10% within the first 14 days (50), which to a large extent is related to a rapid increase in plasma volume (PV). After several weeks of training, an expanded red blood
cell volume (RBCV) will also contribute to the BV increase (50). Numerous studies have confirmed that exercise training, as well as acute plasma volume expansion, increase submaximal SV in untrained healthy humans (23, 26, 34, 59), and at maximal exercise intensities, the general finding is that acute PV expansion also increases Q_{max} (8, 27, 58, 59). Although this strongly suggests that the greater SV_{max} and Q_{max} observed in moderately trained individuals may at least in part be related to their also higher BV, this does not address the relative importance of BV changes and structural adaptations within the heart to the augmentation of Q_{max} with training. To address this question experimentally for the first time, we quantified BVs and performed heart echocardiography before and after 6 wk of endurance training. Q_{max} was assessed before and after the training period, as well as after normalization of BV in the posttraining period by phlebotomy with the intent to offset potential BV-related effects on Q_{max} and thereby also allowing to decipher the magnitude of training-induced effects of altered heart structure and function on Q_{max}. With this study we test the hypothesis that 6 wk of endurance training increases Q_{max} mainly by elevating BV rather than inducing structural and functional changes within the heart.

**METHODS**

**Ethical approval.** The study was approved by the Ethical Committee for the Eidgenössische Technische Hochschule Zürich (EK 2011-N-51) and conducted in accordance with the Declaration of Helsinki. Before the start of the experiments, informed oral and written consents were obtained from all participants.

**Subjects.** To elicit a training response, subjects participating in any kind of training for more than three times per week were excluded as participants. Daily activities such as commuting to work by bike was not considered training. Nine healthy moderately active male volunteers (body weight = 78 ± 11 kg, age = 27 ± 3 yr, V_{O2max} = 47 ± 5 ml·min⁻¹·kg⁻¹) were recruited to participate in the study (Table 1). None of the subjects were taking or prescribed medication during the study. Dual-energy X-ray absorptiometer scan (Lunar iDXA, GE Healthcare, Madison, WI) were used to assess body composition before and after the study.

**Exercise testing and measurement of cardiac output.** To determine maximal power output \[W_{compl max} = W_{compl} + 30 \times (t/90),\] where \(W_{compl}\) is the last fully completed workload and \(t\) is the time sustained at the final workload and \(V_{O2 max}\), subjects performed incremental tests to exhaustion on an electronically braked cycle ergometer (Monark, Monark 839E, Varberg, Sweden) with continuous measurements of \(V_{O2}\) using an online gas collection system (Innomo M4000, Innovision, Odense, Denmark). The system was calibrated before each test. The exercise test consisted of 5 min of continuous exercise at 50, 100, and 150 W, respectively, where after the workload was increased by 30 W every 90 s until exhaustion. \(V_{O2 max}\) was determined as the highest value averaged over 30 s during the last 2 min. All subjects fulfilled the criteria set for achieving \(V_{O2 max}\) (1). Cardiac output was quantified by use of the Innomo M4000 (Innovision, Odense, Denmark), which is based on an inert gas rebreathing technique. Measurements are based on the assumption that pulmonary uptake of a blood soluble testing gas is proportional to pulmonary blood flow (29). The Innomo uses a test gas mixture containing 5% nitrous oxide (N₂O, soluble in blood and physiologically inert), 1% of the insoluble sulfur hexafluoride (SF₆), and 94% O₂. This is filled, together with ambient air, into the rebreathing bag before the onset of a measurement. The fraction of testing gas and volume of the bag are calculated by the Innomo based on the measured tidal volume and O₂ uptake to provide sufficient O₂ and allow for unrestricted ventilation during a rebreathing maneuver. When a measurement is initiated, the subject is switched from breathing room air to the closed circuit and rebreaths the testing gas. Photo-acoustic gas analyzers continuously quantify the gas concentrations in the rebreathing circuit. Pulmonary N₂O uptake is determined by a regression line over the N₂O concentration curve in three consecutive exspirations as soon as complete mixture is separated between residual pulmonary air and the gas in the rebreathing bag. The method has previously been validated showing a day-to-day reproducibility of 4.3% (18) and that the measured Q values are accurate compared with the direct Fick method (7). In our experience the Innomo tends to underestimate Q and that this is most likely due to an overlap in recirculation of the inert gas if multiple measurements are performed (55). In the current study the rebreathings were separated by several minutes with the intent to eliminate potential effects of recirculating inert gas. The Q/V₂O₂ relationship in the present experiments was 5.1 ± 0.4, which is in line with what is expected. If assuming a 10% hemoconcentration during the exercise trials the average systemic O₂ extraction corresponded to 94.7 ± 0.8%, which could however suggest a slight underestimation of Q. Subjects were familiarized to the rebreathing maneuver through two familiarization tests and further conducted 2–3 procedures to practice before each test. During the exercise trial Q was determined at 50 W and at maximal exercise before exhaustion (rebreathing procedure initiated at 5 heart rate beats below previously determined maximal heart rate).

**Echocardiographic image acquisition.** Images were obtained by fully trained operator (G. Walther) in the left lateral decubitus position after a 15-min resting period using a commercially available system (M7, Mindray, Shenzhen, P. R. China) with a 3.5-MHz sector scanning electronic transducer. Gains and filters were adjusted to eliminate background noise and allow for a clear tissue signal. Images were acquired in cine loops triggered to the QRS complex and saved digitally for subsequent off-line analysis as previously described (37).

**Standard and tissue Doppler imaging parameters.** Standard echocardiography consisted of two-dimensional, M-mode, and Doppler blood flow measurements, according the recommendations of the American Society of Echocardiography (45). LV mass was calculated by the formula of Devereux et al. (13). M-mode measurements were obtained in the parasternal long-axis view. Pulsed Doppler LV and right ventricle (RV) inflow (E and A waves) recordings were performed in the apical four-chamber view, with the sample volume at
the tip level of the mitral valves. SV was calculated as the product of the aortic root area and the integral of the aortic blood flow velocity recorded from a 5-chamber view. Q was calculated as the product of SV and HR. Tissue Doppler inflow during systolic and diastolic period was recorded in apical four-chamber view. We assessed wall motion velocities at the mitral annulus level on the septal and lateral walls. Data are presented as the average of peak myocardial systolic velocity (S') and diastolic velocities (E' and A'). The E/E' ratio was used as an index of LV filling pressure. To estimate LV afterload, LV systolic wall stress was calculated as 1.333 × SBP × (ESD/2), where SBP and ESD are systolic blood pressure and end-systolic diameter, respectively (51). For assessment of RV function, pulsed Doppler and M-mode were used in the apical four-chamber view to assess, respectively, E and A waves and tricuspid annular plane systolic excursion (TAPSE).

**Arterial elastance.** Effective arterial elastance (Ea), an index of ventricular-arterial coupling, was calculated as ESP/SV, where ESP represents end-systolic blood pressure (brachial SBP/2).

**Hematological parameters.** Total hemoglobin mass (Hbmax) was measured as previously described (56), using a modified version of a carbon monoxide (CO) rebreathing technique (5). All subjects rested for 20 min in a semirecumbent position before each measurement. Thereafter, 2 ml of blood were sampled from an antecubital vein via a 20-gauge Venflon (BD, Franklin Lakes, NJ) and analyzed immediately in quadruplicate for 1) percent carboxyhemoglobin (%HbCO) and Hb concentration ([Hb]) using a hemoximeter (ABL800, Radiometer, Copenhagen, Denmark); and 2) hematocrit with the micro-method (4 min at 13,500 rpm). After baseline collection, the subject breathed 100% O2 for 4 min to flush the nitrogen from the airways. The breathing circuit (previously flushed with O2) was then closed. A bolus 1.5 ml/kg of 99.997% chemically pure CO (CO N47, Air Liquide, Paris, France) was administrated into the breathing circuit. The subjects rebreathed this gas mixture for 10 min. Thereafter, an additional 2-ml blood sample was obtained and analyzed in quadruplicate. The change in %HbCO was used to calculate Hbmax, taking into account the amount of CO that remained in the rebreathing circuit at the end of the procedure (2.2%) (5). Total RBCV, BV, and PV were derived from measures of Hbmax, [Hb], and hematocrit (5). All CO rebreathing tests were performed by the same researcher. Pre- and postendurance training values reported here are the mean of duplicate measurements conducted on consecutive days. The coefficient of variation for Hbmax assessed from duplicate measures and expressed as the percent typical error (i.e., SD of difference scores/√2), was 3.84% at baseline and 2.12% after endurance training. However, one subject had a larger difference in values compared with the rest of the group at baseline. Without these values the typical error would have been 1.93% at baseline.

**Phlebotomy.** Before the last exercise test (conducted within 2–3 days after the posttest), the training-induced increase in BV was eliminated by means of phlebotomy. The amount of whole blood removed was exactly matched for each individual subject on the basis of duplicate determinations of Hbmax and on average amounted to 382 ± 204 ml. For this purpose a 20-gauge Venflon was placed in an antecubital vein and the blood withdrawn and discarded. The subjects then rested for an additional 10 min in the supine position and were then asked to move to the cycle ergometer and initiated the exercise protocol.

**Exercise training.** All subjects underwent 6 wk of supervised normoxic exercise training consisting of 60 min of cycle ergometer exercise performed every other day. The specific protocols are described elsewhere (46). Exercise training intensity was on average 65% of Vo2max for 60 min. In brief subjects performed a total of 20 training sessions over this period. Four different intensity profiles were circulated during the period to assure participant motivation and compliance. Profile 1 (total work = 234 kJ) consisted of a steady-state exercise, i.e., 60 min at 65% of Wmax. Profile 2 (total work = 242 kJ) started with 11.25 min (at 65% of Wmax) followed by 0.5 [130%]; 1.25 [50%]; 0.5 [130%]; 1 [50%]; 1.25 [50%]; 0.5 [130%]; 1.25 [50%]; 0.5 [130%]; 1 [50%]; 1.25 [50%]; 0.5 [130%]; 1.25 [50%]; 0.5 [130%]; 1 [50%]. Profile 3 (total work = 227 kJ) started with 3 min (at 50% of Wmax) followed by 3 [60%]; 3 [65%]; 3 [70%]; 3 [75%]; 3 [70%]; 3 [65%]; 3 [60%]; 3 [50%]; 3 [65%]; 3 [50%]; 3 [60%]; 3 [65%]; 3 [70%]; 3 [75%]; 3 [70%]; 3 [65%]; 3 [60%]; 3 [50%]; 3 [65%]. Profile 4 (total work = 248 kJ) started with 6 min (at 65% of Wmax) followed by 4 [75%]; 6 [65%]; 4 [75%]; 6 [65%]; 4 [75%]; 6 [65%]; 4 [75%]; 6 [65%]. Workloads were calculated from individual maximal workload determined at baseline testing. During the training sessions the subjects could themselves choose to be cooled by means of a fan. This was not controlled. Since exercise training in the heat can induce elevations in plasma volume (32), it cannot be excluded that this may have influenced the results slightly.

**Statistics.** A mixed model analysis with “Time” (Pre-, Post- Phlebotomy) and with specification of repeated measures for subject was performed in IBM SPSS versus 20 statistical software package. A post hoc analysis was performed if a significant main effect was observed using the Holm-Sidak adjustment for multiple comparisons. Unless otherwise stated results are means ± SD with n = 8. Significance set at P < 0.05.

**RESULTS**

**Maximal power output and Vo2max.** Wattmax increased (P < 0.05) by 66 ± 24 W (26 ± 12%) from pre- to postraining and was reduced (P < 0.05) by 24 ± 13 W (7 ± 4%) following the phlebotomy procedure compared with postraining (Table 1). When compared with pretraining values, Wattmax remained 42 ± 19 W (17 ± 10%) higher (P < 0.05) after the phlebotomy. Accordingly, Vo2max was increased (P < 0.05) by 358 ± 259 ml/min (10 ± 7%) from pre- to postraining and reduced (P < 0.05) 334 ± 270 ml/min (8 ± 7%) by phlebotomy compared with postraining. There was no statistical difference between pretraining and phlebotomy Vo2max.

**Hematological parameters.** Exercise training resulted in a 147 ± 168 (5 ± 5%), 235 ± 64 (10 ± 3%), and 382 ± 204 ml (7 ± 4%) increase (P < 0.05) in PV, RBCV, and BV, respectively (Table 1 and Fig. 1, A–C). The amount of whole blood removed during the phlebotomy procedure was exactly matched for each individual and on average equaled the gain in BV with training (382 ± 204 ml). Since the gain in PV and RCBV were not of equal magnitude following the training intervention, the phlebotomy procedure resulted in a 213 ± 120 and 170 ± 84 ml reduction in PV and RCBV, respectively. Therefore, in the normalized state after phlebotomy, PV and RBCV were 121 ± 91 (4 ± 3%) and 121 ± 91 ml (5 ± 3%) lower and higher, respectively, than before the training intervention.

**Cardiac echography and arterial elastance.** LV morphology, LV and RV systolic and diastolic function, as well as Ea, did not change as a function of the exercise training intervention, and this was also true after covarying for resting HR (Table 2). Aortic root diameter was 2.1 ± 0.1 and 2.1 ± 0.1 cm before and after training, respectively.

**Submaximal exercise data.** At 50 W HR tended to be reduced (P = 0.06) by 10 ± 7 beats/min after 6 wk of endurance training. After phlebotomy HR was increased (P < 0.05) by 15 ± 14 beats/min compared with postraining values. After normalization of BV HR thus remained 4 ± 15 beats/min higher (P < 0.05) than pretraining values. SV at 50 W was not
Maximal cardiovascular exercise data. Maximal HR (HRmax) was decreased (P < 0.05) following the training period by 3 ± 3 beats/min (Fig. 3). After the phlebotomy HRmax was increased (P < 0.05) by 3 ± 2 beats/min compared with the posttraining value. There was no difference in HRmax between pretraining and postphlebotomy values. Maximal SV (SVmax) was increased (P < 0.05) 10 ± 6 ml (10 ± 6%) as a consequence of the exercise training and was reduced by (P < 0.05) 15 ± 4 ml (16 ± 7%) after the phlebotomy procedures compared with posttraining. The correlation between percent change in BV and SV was R = 0.543 (P < 0.05). Qmax was increased (P < 0.05) by 1.5 ± 1.2 l/min (9 ± 6%) pre- to posttraining and was reduced (P < 0.05) by 2.3 ± 0.8 l/min (12 ± 5%) when subjects were phlebotomized. No statistical difference for Qmax was detected between pre- and postphlebotomy values. Percent changes in BV correlated with Qmax (R = 0.530, P < 0.05) (Fig. 2).

DISCUSSION

The major findings with the present study were that 1) 6 wk of endurance exercise training led to an increase in blood volume and Qmax but not to structural or functional alterations in heart properties and 2) Qmax was normalized to pretraining values when the training-induced increase in BV was restored to pretraining values by phlebotomy. Thus the overall conclusion from this study is that changes in BV are the main mechanism increasing Qmax in untrained humans following 6 wk of exercise training performed every second day for 60 min at an average 65% of Wattmax.

Blood volume and submaximal and maximal stroke volume. The observed parallel increase in BV and Qmax with exercise training, and subsequent reversal with phlebotomy supports our hypothesis that a major component, at least in the early phase of exercise training, explaining the increase in Qmax is related to changes in BV rather than to structural or functional adaptations within the myocardium. This is furthermore supported by the unchanged heart erophygram data reported in Table 2. It is clear, however, that the heart of an elite athlete is structurally different compared with an untrained but healthy individual. What remains an open question is whether these adaptations are the result of the strains induced on the heart through years of constant training, or whether these are a response to the exercise-induced increase in BV, i.e., a secondary effect. Genetic predisposition may be another factor of course.

Our findings that BV is an important contributor to the early increase in Qmax with training is in line with previous research, and it is generally accepted that the “Frank-Starling” mechanism is an important regulator of SV at rest as well as during exercise (47). The importance of BV and therefore venous return on exercise SV has in the past, however, only been investigated by administration of a PV expander or through the...
removal of whole blood. The present study extends previous work by actively restoring BVs after a training intervention period to pretraining values by means of phlebotomy and concurrent determinations of heart structure and function, and is hence the first to directly test the hypothesis that has only been deduced from previous studies. Our findings are in line with previous studies where it initially was demonstrated that at 60% of VO$_{2\text{max}}$ SV varied in proportion to changes in total blood volume as induced by blood removal and reinfusion (19). Kanstrup and Ekblom (27) subsequently demonstrated that the infusion of 700 ml dextran augments SV at rest and throughout exercise. This lead to the speculation that exercise training-induced increases in BV could be the underlying mechanism also augmenting SV following a training period. To test this experimentally Hopper and colleagues (26) increased BV in untrained individuals by means of dextran infusion to match the BV of endurance-trained subjects and observed an increase in SV from 130 to 144 ml, which was still lower than the 165 ml observed in their trained subjects. Although $Q$ in that study was assessed by CO$_2$ rebreathing, which is inaccurate at maximal exercise, it would seem that BV differences between endurance-trained subjects and untrained controls cannot explain the entire difference in SV. Mier et al. (34) tested whether PV expansion in untrained subjects at a magnitude also expected to be induced after 10 days of endurance training (~300 ml) would lead to similar increases in SV (as assessed by CO$_2$ rebreathing). As a result of the greater BV after PV expansion and with training, SV during submaximal cycling increased from control (110 ml) to 123 and 121 ml with PV expansion and training, respectively. In accordance here-with Krip and coworkers (28) induced similar blood volumes in trained and untrained subjects by either removing 500 ml of whole blood from trained subjects and by infusion of 500 ml of a dextran solution in untrained subjects. During submaximal and maximal exercise SV (determined by acetylene rebreathing) was increased in the untrained subjects and decreased in the trained subjects such that SV was similar for a given HR, suggesting that BV is the main determinant of exercise-training induced changes in SV. Collectively, the evidence thus far suggests that at least the early exercise training-induced changes in both submaximal and maximal SV are the consequence of an altered BV and not caused by functional changes within the myocardium itself of which may take years of training to achieve.

The present study also demonstrates that the exercise training facilitated decrease in submaximal HR is mainly the consequence of an altered BV as the phlebotomy procedure restored submaximal HR to pretraining values. It may also be worth noticing that maximal HR was decreased following the training period, which was normalized after phlebotomy. This could suggest that the frequent observed minor reduction in maximal HR of 1–2 beats/min with training (47) is not the result of an altered autonomic control or receptor density, but perhaps rather to filling times. Neuromuscular fatigue resistance may also be involved. The reduction in maximal HR has likely no physiological consequence for $Q_{\text{max}}$.

Of note is that whereas VO$_{2\text{peak}}$ was completely reverted to pretraining values following phlebotomy, this was not the case for Watt$_{\text{max}}$. This could suggest that at least some of the training-induced increases in Watt$_{\text{max}}$ were the result of by adaptations within the anaerobic pathways, which does not seem unreasonable considering the interval type of training protocol applied with numerous intervals corresponding to a workload of 130% of Watt$_{\text{max}}$. The fact that VO$_{2\text{peak}}$ was

### Table 2. Cardiovascular data before (pretraining) and after (posttraining) the 6-wk long training intervention

<table>
<thead>
<tr>
<th></th>
<th>Pretraining</th>
<th>Posttraining</th>
<th>$P$ Values</th>
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</thead>
<tbody>
<tr>
<td><strong>Left ventricular morphology</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Septal wall thickness, cm</td>
<td>0.9 ± 0.1</td>
<td>0.9 ± 0.1</td>
<td>0.58</td>
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<tr>
<td>Lateral wall thickness, cm</td>
<td>0.8 ± 0.1</td>
<td>0.9 ± 0.1</td>
<td>0.56</td>
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<tr>
<td>EDD, cm</td>
<td>5.4 ± 0.4</td>
<td>5.4 ± 0.3</td>
<td>0.74</td>
</tr>
<tr>
<td>ESD, cm</td>
<td>3.4 ± 0.3</td>
<td>3.3 ± 0.4</td>
<td>0.28</td>
</tr>
<tr>
<td>LV mass, g</td>
<td>174 ± 20</td>
<td>178 ± 22</td>
<td>0.15</td>
</tr>
<tr>
<td><strong>Left ventricular systolic function</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shortening fraction, %</td>
<td>38 ± 2</td>
<td>39 ± 3</td>
<td>0.23</td>
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<tr>
<td>Stroke volume, ml</td>
<td>69 ± 9</td>
<td>64 ± 12</td>
<td>0.10</td>
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<tr>
<td>Heart rate, beats/min</td>
<td>62 ± 7</td>
<td>69 ± 9</td>
<td>0.31</td>
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<tr>
<td>Cardiac output, l/min</td>
<td>4.2 ± 0.8</td>
<td>4.3 ± 0.8</td>
<td>0.25</td>
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<tr>
<td>Mean peak $S'$, cm/s</td>
<td>11.5 ± 0.9</td>
<td>12.0 ± 1.5</td>
<td>0.31</td>
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<tr>
<td>Systolic wall stress</td>
<td>253.7 ± 32.6</td>
<td>263.4 ± 30.7</td>
<td>0.11</td>
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<tr>
<td><strong>Left ventricular diastolic function</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Peak $E$, cm/s</td>
<td>80.8 ± 13.8</td>
<td>81.8 ± 9.5</td>
<td>0.19</td>
</tr>
<tr>
<td>Peak $A$, cm/s</td>
<td>46.3 ± 6.5</td>
<td>46.5 ± 9.1</td>
<td>0.38</td>
</tr>
<tr>
<td>$E/A$ ratio</td>
<td>1.8 ± 0.3</td>
<td>1.8 ± 0.2</td>
<td>0.66</td>
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<tr>
<td>Mean peak $E'$, cm/s</td>
<td>15.2 ± 1.3</td>
<td>15.6 ± 0.9</td>
<td>0.21</td>
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<tr>
<td>Mean peak $A'$, cm/s</td>
<td>8.3 ± 0.9</td>
<td>8.5 ± 1.4</td>
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<tr>
<td>$E/A'$ ratio</td>
<td>4.7 ± 0.8</td>
<td>4.7 ± 0.5</td>
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<td><strong>Right ventricle function</strong></td>
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<td>Peak $E$, cm/s</td>
<td>58.8 ± 12.1</td>
<td>50.7 ± 14.3</td>
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<tr>
<td>Peak $A$, cm/s</td>
<td>38.5 ± 7.2</td>
<td>36.3 ± 10.6</td>
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<tr>
<td>$E/A$ ratio</td>
<td>1.5 ± 0.1</td>
<td>1.4 ± 0.2</td>
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</tr>
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<td>TAPSE</td>
<td>2.8 ± 0.4</td>
<td>2.7 ± 0.3</td>
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<tr>
<td>Arterial elastance, mmHg/ml</td>
<td>0.60 ± 0.07</td>
<td>0.62 ± 0.11</td>
<td>0.84</td>
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</table>

Values are means ± SD. EDD, end-diastolic diameter; ESD, end-systolic diameter; LV Mass, left ventricular mass; TAPSE, tricuspid annular plane systolic excursion.
reverted to pretraining values by phlebotomy could suggest that adaptations, which could have occurred in the periphery (but which remains unknown in the present study) such as a shift in skeletal muscle fiber type distribution, augmented mitochondrial volume density and capillarization, are of only minor importance for exercise training-induced increases in VO2max. This has already been eluted to in previous animal work (12), and based on experimental (49) and theoretical (39) work, Qmax also seems the most important factor for VO2max in humans.

Heart ultrastructure, function, and exercise training. SV is augmented during exercise as a result of increases in ventricular end-diastolic volume and, to a lesser extent, a reduction in end-systolic volume. Elite endurance athletes possess elevated end-diastolic volumes (40). In the present study end-diastolic diameter was 5.4 cm before the training period and remained unchanged as a result of the training. Moreover, LV mass as calculated by Devereux et al. (13) was unchanged after the training intervention. In a group of 1,309 Italian athletes (73% male) of various disciplines LV end-diastolic diameter varied from 4.3 to 7.0 cm (mean, 5.5 cm) in men and markedly elevated LV chambers (>6.0 cm) were most common in athletes with higher body mass and in those participating in endurance sport (40). In the current study also LV wall thickness remained unchanged following the training (0.9 vs. 0.9 cm), which is in line with values observed in most elite endurance athletes, although values in the 1.3- to 1.6-cm range have been reported in a limited number of highly trained rowers or canoeists (42). Finally, also aortic root diameter (2.1 cm) remained unchanged in the current study. Although aortic root diameter is typically enhanced in strength-trained athletes (9), it remains debated whether this is also the case for endurance athletes (9, 41). It does seem reasonable to assume, however, that athletic training alone is not a common cause of

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Fig. 2. A: correlation between percent changes in blood volume (%) and submaximal stroke volume (%). B: maximal stroke volume (%). C: cardiac output (%). Filled triangles, open circles and filled circles denote pre- and posttraining, and phlebotomy values, respectively.
marked aortic root dilation (3). Resting HR, SV, and cardiac output remained unchanged with the intervention, and other functional measures of systolic function such as shortening fraction and peak $S'$ velocity were both within the reference values for healthy and trained individuals (38). Regarding diastolic function assessed by both conventional echocardiography ($E$, $A$, and $E/A$) and tissue Doppler imaging (peak $E'$ and $A'$), no alteration was observed after the training intervention. Moreover, no changes in LV filling pressures or LV postload were observed. Unfortunately RV volumes were not assessed in the current study, but no alteration of RV function or $E_a$ were detected. All echocardiographic data in the present study are in line with values obtained from untrained young subjects (37) and also similar to those obtained at rest in male “Ironman” triathletes (38) and even Olympic athletes (31). Also, none of the diastolic parameters were improved following 6 wk of endurance training in 32 overweight, obese, sedentary, or recreational active men (35). Accordingly, an active lifestyle could not hinder the age-depended decline in most of the above measures (44), and similarly one year of endurance training in heart failure patients (21) and Type 2 diabetics (31) did not cause improvements either. Some modest changes were however observed in previously untrained men more than 65 years of age after a 1-yr training intervention (20), and more recently also 8 wk of low-intensity training was able to restore diastolic function in obese men (53). Overall, however, it would seem that Doppler-derived measures of LV and RV systolic and diastolic function are generally not affected in healthy individuals to a great extent by short-term exercise training if at all.

Limitations to the conducted study include the fact that although blood volume was restored to resting pretraining levels, it remains uncertain to what extent the normalization was also preserved during exercise. This could have been verified by comparing Hct before and after the exercise test but was unfortunately not performed. It also remains unknown whether the findings from the present study, which is based on young healthy male volunteers, are transferrable to other populations. It should also be considered that the responses may have differed if a different training intervention had been applied. The applied training program consisted of a broad range of exercise intensities (50–130% of Wattmax), but if applying even higher intensities, which could have lead to higher cardiovascular strain, then perhaps also structural changes would have been observed. For untrained individuals, however, higher workloads would seem difficult to manage. Finally, the applied echocardiographic methods could have missed some small alterations in heart structure and function considering the small sample size in the present study. However, these techniques are used in routine and very good reproducibility has been demonstrated in previous works from our laboratory (4, 38) as well as by others (36). Moreover, other previous studies also reported no structural and/or functional changes in healthy untrained subjects after moderate intensity exercise training (16, 48).

The present study hence demonstrates that 6 wk of endurance exercise training is insufficient to induce structural changes within the myocardium, and that the training-induced elevation of maximal cardiac output is a consequence of the training-induced increase in BV. While it remains speculative, it must be assumed that the higher BV will facilitate an elevated preload, which subsequently facilitates a higher maximal cardiac output. Although the present investigation only used 6 wk of endurance training to provoke its results on cardiac output, similar results may be expected with longer term training also.
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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

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