Acute Exercise-Induced Changes in Cardiac Function Relates to Right Ventricular Remodeling Following 12-weeks Hypoxic Exercise Training

Short title: Acute Cardiac Responses *vs.* Cardiac Remodeling

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CONFLICT OF INTEREST/DISCLOSURES

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NEW & NOTEWORTHY

During exercise the right ventricle is exposed to a disproportionally higher wall stress than the left ventricle, which is further exaggerated under hypoxia. In this study, we showed that 12-week high-intensity running hypoxic exercise training induced right-sided structural remodeling, which was, in part, related to baseline cardiac increase in RV fractional area change to acute exercise. These data suggest that acute RV responses to exercise are related to subsequent right ventricular remodeling in healthy individuals upon hypoxic training.

ABSTRACT

Repeated ventricular exposure to alterations in workload may relate to subsequent cardiac remodeling. We examined whether baseline acute changes in right (RV) and left ventricular (LV) function relate to chronic cardiac adaptation to 12-week exercise training. Twenty-one healthy individuals performed 12-week high-intensity endurance running training under hypoxia (fraction of inspired oxygen: 14.5%). Resting transthoracic echocardiography was performed before and after the training programme to assess ventricular structure, function and mechanics (including strain-area/volume loops). In addition, we examined systolic cardiac function during recumbent exercise under hypoxia at baseline (heart rate of 110-120 bpm, 'stress echocardiography'). Fifteen individuals completed training (22.0±2.4y, 10 male). Hypoxic exercise training increased RV size, including diameter and area (all $p<0.05$). With exception of an increase in RV fractional area change ($p=0.03$), RV function did not change post-training (all $p>0.05$). Regarding the RV strain-area loop, lower systolic and diastolic slopes were found post-training ($p<0.05$). No adaptation in LV structure, function or mechanics were observed (all $p>0.05$). To answer our primary aim, we found that a greater increase in RV fractional area change during baseline stress echocardiography ($r=-0.67$, $P=0.01$) inversely correlated with adaptation in RV basal diameter following 12-week training. In conclusion, 12-week high-intensity running hypoxic exercise training induced right-sided structural remodeling, which was, in part, related to baseline increase in RV fractional area change to acute exercise. These data suggest that acute cardiac responses to exercise may relate to subsequent RV remodeling after exercise training in healthy individuals.

Keywords: athlete's heart; endurance exercise; hypoxia; echocardiography; speckle tracking echocardiography

INTRODUCTION

Exercise training results in remodeling of the heart, including chamber enlargement and hypertrophy.(33) Studies examining the impact of exercise training on cardiac remodeling have predominantly focused on left ventricular (LV) adaptation, with few studies revealing right ventricle (RV) changes to training.(8, 9, 18) To better understand the effects of exercise on RV and LV function, recent studies suggest a relative larger increase in wall stress for the RV *versus* LV during exercise.(22) These acute effects of exercise on cardiac function may be of importance. Indeed, cardiac remodeling seems mechanistically related to the repeated exposure to acute changes in wall stress. Therefore, in-exercise echocardiographic indices of cardiac function may (partly) relate to the presence of subsequent cardiac remodeling. However, no study directly examined this hypothesis in relation to exercise training and remodeling in humans.

Recently, the strain-area/volume loop has been introduced to allow for the assessment of simultaneous structure and strain across the cardiac cycle providing mechanical insight into cardiac function.(28) We found that post-surgery changes in LV strain-volume loop characteristics relate to subsequent cardiac remodeling in patients with aortic stenosis.(17) Therefore, these changes may serve as a proxy of changes in wall stress. Furthermore, we observed different RV loop characteristics in the 'four cornerstones' of the Mitchell classification of sports potentially due to their difference in cardiac structure and function.(28) Possibly, these differences in strain-area/volume loops may relate to cardiac remodeling to exercise training. Therefore, the strain-area loop, in conjunction with other measures of cardiac function, may provide insight into cardiac adaptation to exercise training.

The aim of this study was to relate pre-training changes in cardiac function during low-to-moderate-intensity exercise to subsequent adaptations to a 12-week hypoxic endurance exercise training program on cardiac structure, function and mechanics (i.e. longitudinal strain and

strain-area/volume loops) in healthy individuals. We specifically choose hypoxic exercise since, due to a smaller reduction in pulmonary vascular resistance compared to normoxic exercise(27), this type of exercise causes a higher RV afterload.(10, 11, 24, 26) Indeed, we showed that 45 minutes high-intensity running exercise under hypoxia lowers pulmonary acceleration time, increases right atrial size and lowers the late diastolic uncoupling of the RV strain-area loop compared to exercise under normoxia.(21) These echocardiographic markers support indirectly the presence of an increase in pulmonary artery pressure, and therefore, RV afterload. Accordingly, hypoxic exercise may exaggerate the disproportionate elevation in wall stress for the RV *versus* LV during exercise and may therefore lead to more rapid adaptations in the RV to exercise training allowing us to further explore our hypothesis.

METHODS

Study population

Twenty-one healthy individuals (fourteen males) were recruited for the study. Participants were eligible to take part in this study if they were able to run on a treadmill and that they did not engage in sport-related exercise for more than two hours a week at moderate-to-high intensity for the last six months. Exclusion criteria were a body mass index (BMI) <18 or >30 kg/m^2, any possibility of pregnancy, personal history of cardiovascular disease, a family history of cardiovascular death (<55y), exercise-limiting respiratory disease, physical (i.e. musculoskeletal) complaints making completion of the 12-week training program impossible, abnormal resting 12-lead electrocardiogram (ECG) and abnormalities observed on resting transthoracic echocardiography. The procedures were performed in accordance with institutional guidelines and conformed to the declaration of Helsinki. The study was approved by the Ethics Research Committee of the Liverpool John Moores University (18/SPS/065). Participants gave full written and verbal informed consent before participation.

Study design

In this prospective study, participants attended the laboratory on 35 separate occasions, see **Figure 1**. During the first visit, a medical screening was performed to determine eligibility of the potential participants. After signing informed consent, baseline measurements including echocardiographic assessment at rest were performed under normoxic conditions (FiO_2 20.9%). During visit 2, after 30 minutes of acclimation echocardiographic assessments at rest and during stress under hypoxic conditions (FiO_2 14.5%) were performed. These assessments were obtained in order to relate acute RV functional responses to exercise to chronic RV adaptation after 12 weeks of hypoxic training. Visit 3 to 34 comprised the individual sessions of the hypoxic training program. Visit 35 comprised follow-up measurements, including echocardiographic assessment at rest and were performed under normoxic conditions.

Baseline and follow-up measurements. Participants were examined for height (SECA stadiometer, SECA GmbH, Germany), weight (SECA scale, SECA GmbH, Germany), oxygen saturation (SpO_2, pulse oximetry; Ana Pulse 100, Ana Wiz Ltd., UK), 12-lead ECG (Cardiovit MS-2010, Schiller, Switzerland) and maximal oxygen consumption (VO_2max). Resting heart rate (HR, Polar, Kempele, Finland) and resting blood pressure (BP, Dinamap V100, GE Medical, Norway) were determined at the end of ten minutes of quiet rest in supine position. A standardized maximal cardiopulmonary exercise test (CPET, Oxycon pro, CareFusion, VS) for VO_2max assessment was conducted on a motorized treadmill (HP Cosmos, Nussdorf, Germany) after familiarization and a 10-min warm-up. VO_2max was defined as the highest value of a 30-s average(31), and attainment was verified according to previous recommend criteria.(13)

Training program. Participants took part in a 12-week normobaric hypoxic endurance exercise training program consisting of 2x45 minute sessions a week in the first four weeks and 3x45 minute sessions in the last eight weeks. This running exercise was performed on a motorized

treadmill at 3,000m simulated altitude (equivalent to FiO_2 14.5%) at high-intensity (85% of maximal heart rate).

Environmental chamber and safety. All training sessions were conducted in an environmental chamber (TISS, Alton, UK; Sportingedge, Bastingstoke, UK), which was set-up by a qualified technician. Normobaric hypoxia was achieved by a nitrogen dilution technique. Ambient temperature, carbon dioxide (CO_2) and oxygen (O_2) levels were controlled in all sessions (20°C; FiO_2 14.5%; CO_2 0.03%), whilst a Servomex gas analysis system (Servomex MiniMP 5200, Servomex Group Ltd., UK) was used inside the chamber to provide the researcher continuous information regarding O_2 and CO_2 levels. Acute mountain sickness symptoms (AMS, measured by Lake Louise Score (LLS)(30)) were monitored during testing and training sessions every 20 minutes. Subjects were removed from the environmental chamber if oxygen saturation levels dropped below 75% or severe AMS was suspected (LLS≥6).

Echocardiographic measurements

Echocardiographic assessments, prior to and post training program, were performed at rest ('rest') and during recumbent cycling to elevate heart rate allowing direct assessment of cardiac function during exercise ('stress', target HR 110-120 bpm). Rest and stress echocardiography were performed in the left lateral decubitus position on a supine cycle ergometer (Lode B.V.; Groningen, The Netherlands). For stress echocardiography, low-to-moderate-intensity exercise consisted of recumbent cycling at a cadence of ~60 revolutions per minute. All examinations were performed by one highly experienced sonographer (DO) using a Vivid E95 ultrasound machine (GE Medical, Horton, Norway), equipped with a 1.5-4.5 MHz transducer. Images were stored in raw digital imaging and communication in medicine (DICOM) format and were exported to an offline workstation (EchoPAC, version 203, GE Medical, Horton, Norway). Data-analysis was performed by a single observer with experience in echocardiography (GK) using three consecutive stored cycles with exception of strain-volume loops which were

analyzed from a single cardiac cycle. The observer was blinded for the timing (pre *vs*. post) under which echocardiography was performed.

Conventional measurements. Cardiac structural and functional measurements at rest and during low-to-moderate exercise were made according to the current guidelines for cardiac chamber quantification.(23) Regarding the right heart, we examined the following structural and functional indices: basal and mid-cavity end-diastolic diameters, RV end-diastolic area (RVEDA), RV end-systolic area (RVESA), RV outflow tract (RVOT) diameter at the proximal level in the parasternal long-axis (RVOT PLAX) and the proximal and distal portion in the parasternal short-axis (PSAX) view (RVOT1 and RVOT2, respectively), right atrial (RA) area, RV fractional area change (RVFAC), tricuspid annular plane systolic excursion (TAPSE) and tissue doppler imaging (TDI) of the tricuspid annulus ('s, e', a'). Regarding the left heart, the following structural and functional indices were determined: biplane LV end-diastolic volume (LVEDV), biplane LV end-systolic volume (LVESV), LV mass, relative wall thickness (RWT), LV wall thickness (IVSd, septal; PWd, posterior), LV internal diameter (LVIDd), LA diameter, LA volume, modified Simpson's left ventricular ejection fraction (LVEF), tissue Doppler imaging (TDI) of the mitral annulus (s', e' and a'), trans-mitral Doppler (E, A and E/A ratio). All RV and LV structural indices were allometrically scaled to body surface area (BSA) according to the laws of geometric similarity.(5)

Mechanics. Images were acquired specifically for offline speckle tracking analysis. This involved the optimization of frame rates between 40 and 90 frames s^{-1}, depth to ensure adequate imaging of the chamber of interest and compression and reject to ensure endocardial delineation. The RV focused and the apical two-chamber, four-chamber and long-axis view were utilized for the RV free wall (RVFWS) and LV global longitudinal strain (LVGLS), respectively. Valve closure times were determined from the respective pulsed wave Doppler signals. For both the RV and LV the myocardium was manually traced to include the septum

and adjusted so that the region of interest (ROI) incorporated all of the wall thickness, while avoiding the pericardium.(4, 35) The region of interest was divided into six myocardial segments, providing segmental strain curves. LV global longitudinal strain was obtained by averaging the 18 segments of the three separate apical LV views and global RV strain from three segments of the RV free wall. Where inappropriate tracking of segments was observed visually or detected by the system, retracing was performed until all segments were considered acceptable.

RV strain-area and LV strain-volume loops. The longitudinal strain-area/volume relationship (for methodology of derivation, see Supplemental 1, Oxborough *et al.*(28) and Hulshof *et al.*(14)) was assessed using the following parameters (**Figure 2**): (a) early linear slope during first 5% of volume ejection in systole (EarlySslope), (b) the overall linear slope during systole (Sslope) and (c) end-systolic peak global longitudinal strain (peak strain). In addition (un)coupling was termed to describe the relationship between systolic and diastolic strain for any given area/volume. By subtracting diastolic from systolic strain, the difference at any given area/volume was calculated. Uncoupling was assessed as the mean of the differences during (d) early diastole (early 2/3 of diastole [Uncoupling EarlyD]), (e) late diastole (late 1/3 of diastole [Uncoupling LateD]) and (f) overall (complete cardiac cycle). Furthermore, (g) the early linear slope during first 5% (EarlyDslope) and (h) late linear slope (LateDslope) during last 5% of volume increase in diastole.

In order to obtain intra-observer variability, 10 randomly selected echocardiograms were re-analyzed. Intra-class correlation coefficient (ICC) analysis was performed for the following measures: RV strain-area loop characteristics, RVEDA, RVESA, RVFAC, RV basal diameter, RV mid-cavity diameter, RVOT PLAX, RA area, IVSd, PWd, LVIDd.

Statistical measurements

Statistical analysis was performed using SPSS Statistics 25 (SPSS Inc., Chicago, IL, VS). All parameters were visually inspected for normality and tested with Shapiro-Wilk normality tests. Continuous variables were reported as mean ± standard deviation (SD) and categorical variables were presented as proportions. Paired-sampled T-tests were used to compare baseline and follow-up measurements, including echocardiographic indices, and to determine acute RV functional responses to exercise (augmentation in cardiac function between stress and rest echocardiography). Associations between acute RV functional responses to exercise (TDI s', RVFWS, TAPSE, RVFAC) and chronic RV adaptation (RV basal diameter, RV mid-cavity diameter, RVEDA) were analyzed by Pearson's correlation coefficient, in which 'acute' is defined as the change in RV function from rest to exercise and 'chronic' as change in structure pre- versus post-training program. For all tests, we assumed statistical significance at $p<0.05$.

RESULTS

Twenty-one participants were initially included in the study, of which six dropped-out (motivational issues n=4; health problems unrelated to the study n=2). Participants completed on average 30±2 training sessions (94% adherence) at an average 83.5% of their maximum HR. The fifteen participants who completed the study (22.0±2.4 years, ten men, 24.0±3.0 kg/m^2) showed a significant increase in VO_2max/kg (52±7 to 56±7 mL/min/kg, $p<0.001$) (**Table 1**). BMI and BSA did not significantly change ($p>0.05$) (**Table 1**). Mean SpO_2 during the individual 45 minutes high-intensity running exercise sessions of the hypoxic training program was 81±4%. At baseline, both right and left heart had normal geometry and all structural measurements were within normal ranges (**Table 2)**. There were no abnormal 12-lead ECG findings.

Cardiac adaptations to hypoxic exercise training

There was a significant increase in RV and RA size following the training intervention (all p<0.05) (**Table 2**). Exercise training caused an increase in RVFAC (p=0.03), whilst no other significant changes in RV function were observed (all p>0.05) (**Table 2**). In addition to a rightward shift of the strain-area loop (increased RVEDA), exercise training significantly decreased uncoupling and slopes of the RV strain-area loop (**Table 2, Figure 3A**). In contrast to the structural adaptation of the RV, exercise training did not alter LV structure (**Table 2**). Systolic LV function and mechanics, including LV loops, did not change following training (all p>0.05) (**Figure 3B**). Regarding diastolic function, A velocity decreased (p=0.002), resulting in an increased E/A ratio (p=0.005).

Acute exercise-induced changes in cardiac responses *versus* structural adaptation

Prior to training, all systolic indices for RV function (RVFWS, TDI s', RVFAC, TAPSE) significantly increased with acute exercise (all p<0.05) (**Table 3**). The RV strain-area loop characteristics did not significantly change with acute exercise (all p>0.05) (**Table 3**). The change in RVFAC with acute exercise showed a significant inverse correlation with changes in basal diameter post-training (r=-0.66, p=0.01) (**Figure 4**). The inverse relation indicates that a lesser increase in RVFAC with acute exercise is associated with greater RV structural adaptation to training. Changes in RVFWS, TDI s' and TAPSE with acute exercise did not correlate with RV structural indices (data in Supplemental 2). As strain-area loop characteristics did not significantly change with acute exercise, we did not perform correlations analysis on these data.

Intra-observer variability. ICC were as follows: RV free wall strain 0.96 (0.84-0.99), Sslope 0.92 (0.70-0.98), EarlySslope 0.84 (0.48-0.96), EarlyDslope 0.94 (0.79-0.99), LateDslope 0.95 (0.80-0.99), Uncoupling 0.87 (0.56-0.97), Uncoupling_EarlyD 0.86 (0.52-0.96), Uncoupling_LateD 0.88 (0.58-0.97), RVEDA 0.96 (0.87-0.99), RVESA 0.94 (0.78-0.99),

RVFAC 0.92 (0.72-0.98), RV basal diameter 0.91 (0.68-0.98), RV mid-cavity diameter 0.80 (0.38-0.95), RVOT PLAX 0.75 (0.27-0.93), RA area 0.99 (0.97-0.99), IVSd 0.67 (0.12-91), PWd 0.74 (0.25-0.93), LVIDd 0.79 (0.35-0.94).

DISCUSSION

The aim of our study was to relate pre-training changes in cardiac function during acute hypoxic exercise to subsequent adaptations to a 12-week hypoxic endurance exercise training program on RV cardiac structure, function and mechanics in healthy individuals. We present the following findings. First, hypoxic exercise training increased RV size, including diameter and area. Whereas measures of RV function remained largely unchanged, exercise training resulted in adaptations in RV mechanics, with less uncoupling and lessening of the systolic and diastolic slopes of the RV strain-area loop. Second, no adaptation in LV structure, function or mechanics were observed. Third, pre-training augmentation in RV fractional area change to acute hypoxic exercise was inversely related to cardiac remodeling of the RV following 12 weeks of hypoxic endurance training in healthy individuals. Taken together, our results demonstrate that acute cardiac responses of the RV to hypoxic exercise are related to subsequent RV remodeling upon 12-weeks of hypoxic exercise training in healthy, relatively untrained individuals.

Acute exercise-induced changes in cardiac responses *versus* structural adaptation

In this study, we tested the assumption that any potential disproportionate ventricular wall stress contributes to RV remodeling. Since assessment of cardiac wall stress during exercise is highly challenging and invasive, we examined cardiac (systolic) function during hypoxic exercise and explored whether these changes related to structural adaptation post-training. We found that augmentation in RV fractional area change to acute exercise is inversely related to RV size

following exercise training. In other words, small-to-modest (but not moderate-to-large) increases in RV systolic function during acute exercise relate to subsequent increases in RV structure post-training. One potential explanation for this observation may be that those individuals who had a blunted exercise-induced increase in RV fractional area change, were working at a higher afterload and hence received a greater stimulus for cardiac adaptation. Another potential explanation for this observation may relate to the structure of the RV. A smaller sized RV is less able to elevate measures of systolic RV function during exercise, and are therefore more susceptible for subsequent adaptation. Somewhat in line with this assumption, additional analysis revealed a positive relation between exercise-induced increases in RV fractional area change and RV size at baseline ($r=0.52$, $p=0.03$), indicating that individuals with smaller RV cavity size show a smaller elevations in RV systolic function during exercise. In contrast to RVFAC, other measures did not significantly correlate with adaptation to exercise training. A possible explanation for this may be that RVFWS, TAPSE and TDI s' respond differently to alterations in load compared to RVFAC.(32) These elevations in load may be central as a stimulus for subsequent cardiac adaptation to exercise. Moreover, RVFAC takes into account both radial and longitudinal functional whereas the other systolic functional indices only take the latter into account. The stress received by the RV may therefore better reflected by the augmentation in RVFAC to acute exercise compared to RVFWS, TAPSE and TDI s'.

Right ventricular adaptations to hypoxic exercise training

After 12 weeks of hypoxic exercise training, the right side of the heart showed structural adaptation concomitant with altered mechanics in the strain-area loop. Our observation of RV remodeling contrasts with others, who report the absence of RV adaption after an increase in training volume.(1, 7) Importantly, the lack of structural RV remodeling observed in these

previous studies is mainly observed when examining elite athlete populations, who already had a high level of training at baseline evaluation (e.g. they were not detrained for example during pre-season evaluation). Interestingly, the LV showed no evidence for adaptation after training. This agrees with a study by Arbab-Zadeh *et al.* (3) where they showed that after 12 months progressive and intensive marathon training in 12 previously sedentary subjects (mean age, 29±6 years), that RV size increased during the initial 3-month training period, but the LV only started to remodel after 6 months of training. The hypoxic exercise stimulus mainly effects RV afterload, and to a lesser extent LV afterload (10, 11, 24, 26, 27). Moreover, it may be that LV afterload is reduced during hypoxic exercise as a result of hypoxic induced peripheral vasodilation (12, 20). This may have amplified the disproportionate RV remodeling. However, due to the lack of a control group this remains speculative. Based on the lack of structural adaptation in the LV in this study, this may suggest that RV remodeling precedes LV remodeling in relatively untrained individuals. Future work, however, is required to better understand this phenomenon.

Previously, we have demonstrated changes in the strain-area loop in acute exercise settings (21, 28) but also marked differences in pulmonary hypertension populations (15, 16, 19) likely due to variation in loading conditions. We also demonstrated that 24-weeks of endurance exercise induced a modest rightward shift with a somewhat stronger coupling of the LV strain-volume loop (29). This is the first study, to our knowledge, that assessed RV strain-area loops following an exercise training in humans. We showed that training induced changes in RV mechanics concomitant to right-side structural adaptions. Specifically, lessening of the systolic and diastolic slope of the RV strain-area loop fits with the change in geometry of the RV, where the cavity size became larger. This is challenging to interpret but may be explained by the larger RV having greater unit area of myocardium requiring less deformation/contractility to facilitate the same stroke volume. Furthermore, we observed stronger coupling following training,

potentially suggesting the presence of a more dominant longitudinal contribution to area change in diastole compared to systole. This adaptation fits with previous cross-sectional findings, in that we previously observed that athletes with a sports discipline with low-static and high-dynamic components (IIIA Mitchell classification(25); e.g. high-intensity exercise as adopted in our study), showed more coupling in RV strain-area loops compared to other Mitchell classifications sports.(28) This could be suggestive for a sport discipline specific adaptation and the significant influence of variable loading conditions across disciplines on RV physiology. Moreover, the resemblance between the improved systolic-diastolic coupling following endurance training in the RV (this study) and LV (study by Oxborough *et al*. (29)) with increasing cavity sizes may indicate that a change in cardiac mechanics is not an isolated process but merely a consequence of cardiac structural remodeling due to exercise training. Future work, in larger cohorts assessing both RV and LV, is required to better understand this topic.

Perspectives

Challenging the cardiac system, e.g. through exercise, may be relevant in better understanding (patho)physiology. Indeed, exercise-induced troponin I elevation, independent from resting troponin I, predicts mortality and cardiovascular morbidity.(2, 6) In the present study, we found that exercise-induced changes in RV function relate to chronic RV adaptation. This concept, i.e. exploring cardiac responses to exercise, may be a potential strategy for future studies aiming to better understand cardiac (patho)physiology.

Limitations. We did not include a control group(s) who either; did not perform exercise or performed exercise under normoxic conditions. Whilst this may have provided additional insight into the role of hypoxia in mediating cardiovascular adaptations, we believe this does

not impact the primary finding of our study, that exercise training may lead to RV structural adaptation, which seems to relate, at least partly, to acute baseline exercise-induced changes in cardiac function. Another limitation is that we did not derive direct measures of pulmonary and systemic vascular resistance as this would require invasive procedures. This would have improved insight between the impact of hypoxia on RV and LV function in more detail. A further limitation is that we did not collect blood samples to assess hematocrit and hemoglobin. Although, the participants were exposed to very short durations of intermittent hypoxic exercise training session (maximum of 1 hour including acclimation), this may have led to a change in hematocrit and hemoglobin(34). In addition, the RV loop is based on area while volume would be more suitable given the complex RV geometry. However, the technique to derive the RV volume loops is not yet validated and will require 3D echocardiography. Finally, LV strain-volume loops were only constructed from an A4C view and not in the A2C and APLAX views.

CONCLUSION

12-week high-intensity running hypoxic exercise training induced right-sided structural remodeling, which was, in part, related to baseline cardiac increase in RV fractional area change to acute exercise. These data suggest that acute RV responses to exercise are related to subsequent right ventricular remodeling in healthy individuals upon hypoxic training.

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FIGURE LEGENDS

Figure 1. Overview of study design. Longitudinal data assessment (baseline and follow-up measurements including echocardiography) were performed under normoxic conditions whereas the training program was performed under hypoxic conditions. Additionally, during visit 2, an echocardiographic assessment was performed (after 30 minutes of acclimation) to obtain acute exercise induced changes in cardiac function to relate to chronic structural remodeling to hypoxic training.

Figure 2. Schematic overview of the RV strain-area loop and the derived characteristics. The black line represents the strain-area loop; the thick part represents the systolic phase and the thin part the systolic phase. ED, End-diastolic, EDA, end-diastolic area; ESA, end-systolic area; LD, late diastolic.

Figure 3. A) mean RV strain-area loops and B) mean LV strain-volume loops prior to ('Pre Systolic': black lines, 'Pre Diastolic': black dotted lines) and post ('Post Systolic': red lines, 'Post Diastolic': red dotted lines) 12-week hypoxic high-intensity running exercise training program. Error bars represent standard error of the mean.

Figure 4. Correlation between acute increase in RV fractional area change during first exercise session under hypoxia (visit 2) and increase in resting RV basal diameter at completion of the training protocol.

519 **APPENDICES**

520 **Supplemental 1. Strain-Area Loop – methods of derivation**

521 Private link: https://figshare.com/s/50feea09258bf0ed3377

522 DOI (public link, becomes active when manuscript is accepted):

523 https://doi.org/10.6084/m9.figshare.13379885.v2

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525 **Supplemental 2. Table Associations between acute functional responses to exercise and**

526 **chronic RV adaption**

527 Private link: https://figshare.com/s/47d96c3f89279238afce

528 DOI (public link, becomes active when manuscript is accepted):

529 https://doi.org/10.6084/m9.figshare.13379894.v1

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