Priming Cardiac Function with Voluntary Respiratory Maneuvers and Effect on Early Exercise Oxygen Uptake

Running Title: Priming Cardiac Function with Respiratory Maneuvers

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New & Noteworthy

We demonstrate that different breathing maneuvers can augment both right and left-sided cardiac output in healthy subjects. These maneuvers, when performed immediately before exercise, result in a pre-exercise ‘cardiodynamic’ increase in oxygen uptake ($\dot{V}O_2$) associated with a subsequent reduction in the 'cardiodynamic' $\dot{V}O_2$ normally seen during early exercise. We conclude that pre-exercise breathing maneuvers are a plausible tool worthy of additional study to prime $\dot{V}O_2$ kinetics and improve exercise tolerance in patients with cardiovascular disease.

ABSTRACT

Oxygen uptake ($\dot{V}O_2$) at exercise onset is determined in part by acceleration of pulmonary blood flow ($\dot{Q}p$). Impairments in the $\dot{Q}p$ response can decrease exercise tolerance. Prior research has shown that voluntary respiratory maneuvers can augment venous return, but the corollary impacts on cardiac function, $\dot{Q}p$, and early-exercise $\dot{V}O_2$ remain uncertain. We examined a) the cardiovascular effects of 3 distinct respiratory maneuvers (abdominal, AB; rib cage, RC and deep breathing, DB) under resting conditions in healthy subjects (*Protocol 1*, n=13) and b) the impact of pre-exercise DB on pulmonary O_2 transfer during initiation of moderate intensity exercise (*Protocol 2*, n=8). In *Protocol 1*, echocardiographic analysis showed increased RV and LV cardiac output (RVCO and LVCO, respectively) following AB (by +23±13 and +18±15%, respectively, P<0.05), RC (+23±16; +14±15%, P<0.05) and DB (+27±21; +23±14%, P<0.05). In *Protocol 2*, DB performed for 12 breaths produced a pre-exercise increase in $\dot{V}O_2$ (+801±254 ml·min^{-1} over ~ 6 s), presumably from increased $\dot{Q}p$, followed by a reduction in pulmonary O_2 transfer during early phase exercise (first 20 s) compared to the control condition (149±51 vs 233±65 ml, P<0.05). We conclude that (1) respiratory maneuvers enhance RVCO and LVCO in healthy subjects under resting conditions, (2) AB, RC and DB have similar effects on RVCO and LVCO, and (3) DB can increase $\dot{Q}p$ prior to exercise onset. These findings suggest that pre-exercise respiratory maneuvers may represent a promising strategy to prime $\dot{V}O_2$ kinetics and thereby to potentially improve exercise tolerance in patients with impaired cardiac function.

INTRODUCTION

Upon initiation of exercise, the pulmonary, cardiovascular, and muscular systems must synchronize to increase oxygen (O_2) flux into the mitochondria to enable sufficient ATP production through oxidative pathways. Inertia in the involved processes can result in a transitory mismatch between energetic demand and aerobic supply, a cumulative difference termed the "O_2 deficit" (1, 2). The rate at which O_2 uptake ($\dot{V}O_2$) increases immediately following exercise onset is a key determinant of the magnitude of the O_2 deficit and correspondingly is a determinant of exercise performance and tolerance (3, 4).

In healthy people performing moderate intensity exercise, the kinetics of the $\dot{V}O_2$ response to exercise (hereafter $\dot{V}O_2$-on kinetics) are limited by O_2 utilization in the working muscles rather than by O_2 delivery (5–9). Conversely, disease states that reduce O_2 delivery to working muscles are associated with a prolonged $\dot{V}O_2$-on response (10–15). In particular, people with impaired cardiac function (e.g. heart failure) may be unable to adequately augment ventricular function and may therefore fail to adequately increase cardiac output at the onset of exercise leading to slow $\dot{V}O_2$-on kinetics (15). Strategies to improve cardiac function at the onset of exercise may improve $\dot{V}O_2$-on kinetics and minimize O_2 deficit, thereby enhancing exercise tolerance in this population.

The "cardiodynamic phase" of early exercise (16, 17) is defined as the period of time during which increases in $\dot{V}O_2$, as measured at the mouth, are driven mainly by pulmonary blood flow ($\dot{Q}p$) augmentation rather than by increased muscular O_2 consumption (16–20). The increase in $\dot{Q}p$ immediately following exercise onset results from the integration of several processes including augmentation of venous return via respiratory-driven changes in intrathoracic (ITP) and intra-abdominal pressure (IAP) (21, 22). Results from studies using deliberate modulations of the "respiratory pump" by maneuvers indicate that abdominal (AB) and rib cage (RC) breathing can increase venous return (22–25) leading to greater alveolar O_2 transfer during the cardiodynamic phase of exercise (23). Accordingly, voluntary

77 modulation of respiratory mechanics may increase venous return, increase $\dot{Q}p$, and thus
78 enhance pulmonary $\dot{V}O_2$ but not locomotory muscle $\dot{V}O_2$. However, the impact of breathing
79 maneuver-induced increases in venous return on $\dot{Q}p$ and corollary pulmonary O_2 uptake has
80 not been rigorously defined. Furthermore, as the right and left ventricles lie in series
81 interposed by the low-resistance and high-capacitance pulmonary vascular bed, it is
82 unknown whether increases in right ventricular (RV) cardiac output and thus $\dot{Q}p$ immediately
83 result in commensurate increases in left ventricular (LV) cardiac output.

84 The objective of this study was to define the increase in cardiac output generated by
85 specific respiratory maneuvers at rest and during the onset of exercise, with the goal of
86 establishing that these voluntary modulations of the respiratory pump mechanism can
87 increase venous return and augment $\dot{Q}p$. We addressed this objective using two
88 complementary protocols involving healthy participants. In "*Protocol 1"*, we tested the
89 hypotheses that: (1) voluntary respiratory maneuvers (AB and RC) performed at rest would
90 improve RV and LV cardiac output (RVCO and LVCO, respectively); (2) "deep" breathing
91 maneuvers (DB), in which the respiratory pump mechanism is enhanced simply by taking
92 deeper breaths, have similar effects on RVCO and LVCO compared to AB and RC; and (3)
93 that there would be a time delay between the increases in RVCO and LVCO due to
94 capacitance properties of the pulmonary vascular bed. In "*Protocol 2"*, we aimed to
95 determine whether a 1-min bout of DB performed immediately prior to the initiation of
96 moderate intensity cycling exercise would reduce the increase in $\dot{V}O_2$ normally observed in
97 the early phase of exercise by producing a preemptive "cardiodynamic" increase in
98 pulmonary O_2 uptake prior to exercise onset.

99

100 **MATERIALS AND METHODS**

101 **Ethical approval**

These experiments were carried out in accordance with the 2013 version of the Declaration of Helsinki and were approved by the local institutional review board (Mass General Brigham Institutional Review Board; Protocol #2020P002299). Participants volunteered after giving written informed consent prior to starting data collection. Thirteen male participants without known cardiovascular disease took part in *Protocol 1*, and eight subjects (subjects 1-8) subsequently took part in *Protocol 2*.

Experimental Design

An overview of the experimental procedures is shown in ***Figure 1***. In *Protocol 1*, echocardiography to characterize cardiac structure and function was performed with subjects in the standard left lateral decubitus position. Echocardiograms were performed by a single highly experienced cardiac sonographer on a Philips EPIQ 7 ultrasound machine (Philips Healthcare, Cambridge MA, USA) using a focused protocol optimized for evaluation of cardiac structure and function. Two-dimensional imaging as well as pulsed-wave, continuous-wave, color, and tissue Doppler were performed from standard parasternal, apical, and subcostal positions. Two-dimensional and tissue Doppler frame rates were 25–75 Hz and >100 Hz, respectively, for all images. All data were stored digitally for subsequent analysis on commercially available software (Syngo Dynamics, Siemens Medical Solutions, Malvern, PA, USA).

Figure 1: Experimental Procedures

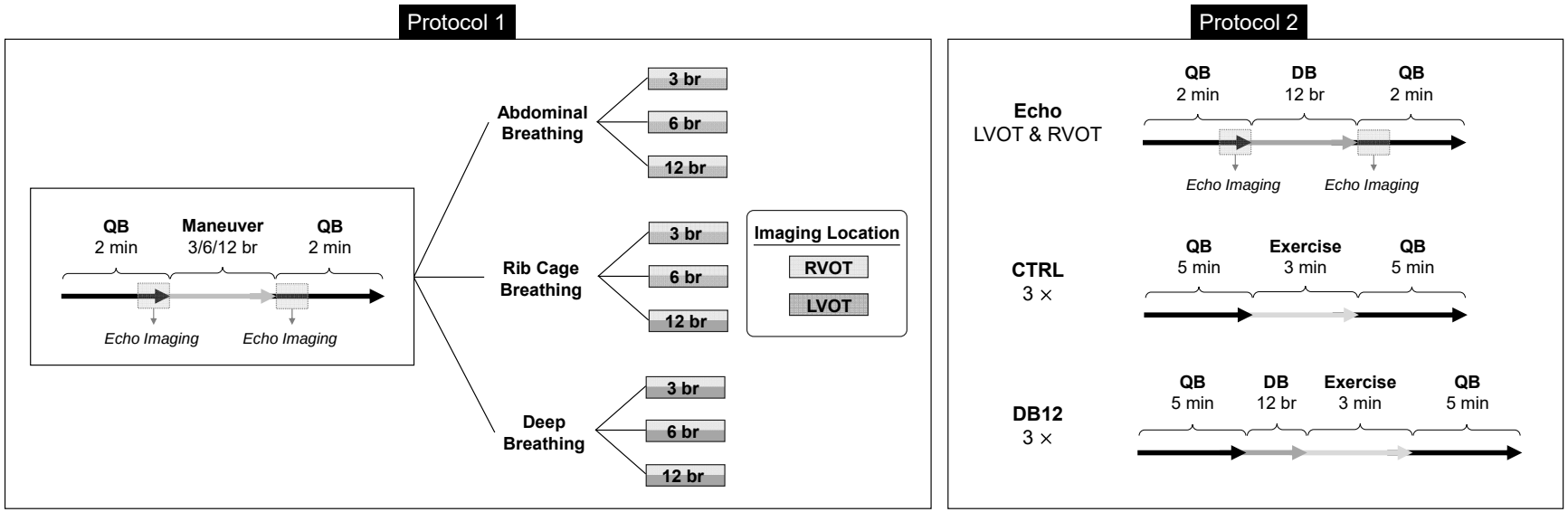
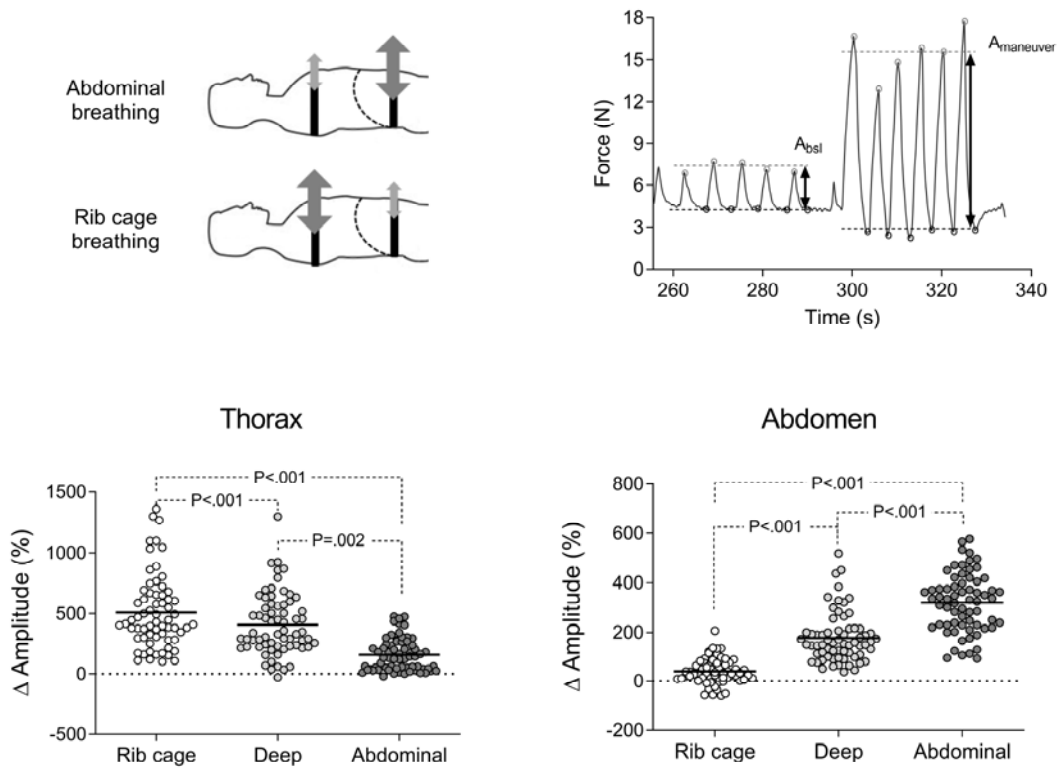


Figure 1: Overview of the experimental procedure. A schematic is shown outlining the experimental procedures performed in *Protocol 1* (resting assessment) and *Protocol 2* (transition to exercise with gas exchange measurement). QB = quiet breathing; RVOT=right ventricular outflow tract; LVOT=left ventricular outflow tract: DB = deep breathing maneuver; DB12 = deep breathing performed for 12 breaths. CTRL = control condition without pre-exercise breathing maneuver.

Participants were then familiarized with the respiratory maneuvers. For AB, they were instructed to breathe deeply by emphasizing abdominal excursion while minimizing rib cage movements. For RC, they were instructed to breathe deeply by emphasizing rib cage movements while minimizing abdominal excursion (***Figure 2A***). For DB, participants were instructed simply to breathe as deeply as possible without guidance as to a specific technique. Successful performance of each respiratory maneuver was confirmed using two respiratory belts (Go Direct® Respiration Belt, Vernier Software & Technology, Beaverton, OR, USA), each consisting of a force transducer attached to a nylon strap adjusted around the thorax (sternum level) and abdomen (umbilical level), respectively. Real-time signals of measured forces, which provide an estimate of changes in thoracic and abdominal excursion, were displayed on a screen via the dedicated software (Vernier Graphical Analysis v5.2.0-41, Vernier Software & Technology, Beaverton, OR, USA) to provide visual feedback in order to help participants optimize technique. Respiratory rate, set at 12 breaths per minute for all respiratory maneuvers, was guided by metronome.

142 **Figure 2: Experimental Respiratory Maneuvers**



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144 **Figure 2**: Overview of respiratory maneuvers. **A**: illustration of the techniques for abdominal
145 and rib cage breathing. Abdominal breathing consists of emphasizing the contribution of the
146 diaphragm, resulting in greater abdominal excursion; rib cage breathing consists of
147 emphasizing the contribution of the intercostal and accessory respiratory muscles, resulting
148 in greater rib cage excursion. **B**: graphical method used to quantify abdominal and thoracic
149 excursions during the respiratory maneuvers. The respiratory belts measured changes in
150 tension resulting from expansion and contraction of the abdomen and rib cage. The tracing
151 shown is an example of a force signal yielded by an abdominal belt. A_{bsl} is the average
152 amplitude over the six breaths preceding the maneuver, $A_{maneuver}$ is the average amplitude
153 over the number of breaths performed during the maneuver (3, 6 or 12). **C** and **D**: changes in
154 thoracic (C) and abdominal (D) excursion during rib cage, deep or abdominal breathing.
155 ΔAmplitude is the difference between A_{bsl} and $A_{maneuver}$ expressed in percentage. N=13.

Once participants demonstrated correct and consistent respiratory maneuver technique as confirmed by qualitative visual assessment of the abdominal and rib cage excursions tracings by a single experimented investigator, they each performed a series of transitions from quiet spontaneous breathing to RC, AB, and DB for sets of 3, 6 and 12 breaths (i.e. 15 s, 30 s and 1 min at 12 breaths·min^{-1}) while remaining in the left lateral decubitus position for optimal echocardiographic imaging. Abdominal and thoracic excursion was recorded continuously throughout the entire protocol and on-line traces were displayed to the participants as a form of visual feedback to optimize maneuver technique. Each respiratory maneuver was followed by a 2-min recovery period during which participants resumed quiet breathing to allow for restoration of cardiorespiratory parameters to baseline values. Participants executed the transitions in a randomized maneuver type order, but systematically in an ascending order as to the duration (from 3 to 12 breaths).

Pulsed-wave Doppler of blood flow in the RV outflow tract (RVOT) and LV outflow tract (LVOT) was performed both immediately prior to the initiation of each maneuver and immediately following the final expiration with a simultaneous breath hold at functional residual capacity. Each breathing maneuver was performed twice at a given duration (i.e. AB6) in order to record pulsed-wave Doppler of both RVOT and LVOT (i.e. once for the RVOT and once for LVOT). RVOT Doppler samples were acquired in the RV outflow view in the parasternal short-axis window with pulsed-wave Doppler sample volume just proximal to the level of the pulmonary valve. LVOT Doppler samples were acquired in the apical 5 or 3-chamber views with the sample volume approximately 0.5 cm proximal to the aortic annulus. RVOT and LVOT diameters were measured using zoomed-in images in the parasternal short axis and long axis, respectively. Heart rate (HR) was monitored throughout the protocol with 3-lead ECG integrated into the echocardiography machine.

Protocol 2

Eight participants completed *Protocol 2*. Exercise was performed on an upright cycle ergometer (Excalibur Sport, Lode B.V., Groningen, The Netherlands) with gas exchange measured on a breath-by-breath basis using a face mask (Hans Rudolph V2, Hans Rudolph, Inc., Shawnee, Kansas, USA) and a commercially available metabolic cart and gas exchange analyzer (Ultima CardiO2; Medgraphics Diagnostics, St. Paul, Minnesota, USA). Participants were fitted with respiratory belts as detailed in *Protocol 1*.

After 5 minutes of quiet breathing ('baseline' state), subjects performed a DB maneuver for twelve breaths (DB12). Participant respiratory rate was again guided by the metronome (12 breaths·min^{-1}), and real-time traces of abdominal and thoracic excursions were displayed as a visual feedback. The sequence was repeated twice to record pulsed-wave Doppler samples in both the RVOT and LVOT, in a randomized order, with at least 2 minutes in between to allow restoration of cardiorespiratory variables to baseline level. A 12-lead ECG tethered to the gas analyzer (Mortara Instrument X12+ wireless ECG transmitter, Milwaukee, Wisconsin, USA) monitored HR on a continuous basis.

Participants then performed transitions from baseline to constant-load moderate intensity exercise (100 W) for three minutes after having performed a DB12 maneuver immediately prior to exercise initiation; an identical protocol without the DB12 served as the control arm (CTRL). Each condition (i.e. initiation of exercise with DB12 and initiation of exercise without DB12) was performed in triplicate in randomized order. Each exercise sequence was followed by a 10-min recovery period. During the DB12 sequences, participants were asked to initiate exercise at the end of the last expiration. Throughout the procedure, breath-by-breath tidal volume (Vt), respiratory rate (RR), ventilation ($\dot{V}E$), end-tidal CO_2 ($PetCO_2$) and O_2 ($PetO_2$) pressure, and $\dot{V}O_2$ were obtained at the mouth from the metabolic cart.

Data analysis

Baseline echocardiographic measurements were performed according to American Society of Echocardiography / European Association of Cardiovascular Imaging guidelines (26). LV and left atrial volumes and the LV ejection fraction were calculated using the biplane method of discs (26). RVOT and LVOT diameters were measured in mid-systole using a zoomed-in view for maximal resolution.

To describe the mechanics of breathing during the respiratory maneuvers, the intra-breath peaks and nadirs of the two force signals derived from the abdominal and thoracic belts were identified manually for each transition sequence (***Figure 2B***). Peak and nadir values were averaged over 6 breaths during the quiet breathing period immediately preceding the maneuver and over the total number of breaths performed during each maneuver (3, 6 or 12). The difference between the average maximum and minimum excursion yielded abdominal and thoracic amplitudes. Percent changes in amplitudes between quiet breathing and each maneuver were then calculated.

RVOT and LVOT velocity time integrals (VTIs) from pre- and post-maneuver pulsed-wave Doppler recordings were measured on the aforementioned commercially-available echocardiographic analysis software. VTIs were manually traced and averaged over three successive beats for each maneuver. The analysis was performed by a single investigator blinded to the experimental conditions. Stroke volume (SV) was then calculated as:

$$SV = \pi r^2 \cdot VTI$$

where *r* is the measured radius of the RVOT and LVOT, respectively. The product of SV and HR yielded cardiac output (CO).

To assess changes in cardiorespiratory parameters during DB12 in *Protocol 2*, breath-by-breath signals of Vt, RR, $\dot{V}E$, $PetCO_2$, $PetO_2$, $\dot{V}O_2$ and HR were resampled to 1 Hz. The signals from all three repetitions were then aligned in time and averaged second-by-second to obtain a single signal per participant for each condition. The mean of each parameter computed during the last 10 s of quiet breathing and of DB12 were then

calculated to describe cardiopulmonary changes during DB12. To obtain parameters of $\dot{V}O_2$-on kinetics, the averaged $\dot{V}O_2$ signals for CTRL and DB12 were used to model the primary phase of $\dot{V}O_2$-on kinetics by fitting a single exponential function to the averaged $\dot{V}O_2$ data, starting at time (t) = 20 s:

$$\dot{V}O_2(t) = \dot{V}O_{2,QB} + A \cdot \left(e^{\frac{-(t-TD)}{\tau}}\right)$$

where $\dot{V}O_{2,QB}$ is the average $\dot{V}O_2$ at baseline during quiet breathing, A is the amplitude of the $\dot{V}O_2$ response (i.e. the difference between $\dot{V}O_{2,QB}$ and $\dot{V}O_2$ at the steady-state plateau), TD is the time delay (i.e. the time at which $\dot{V}O_2$ rises above $\dot{V}O_{2,QB}$) and τ is the time constant (i.e. the time taken to reach 63% of A).

To estimate the total volume of O_2 taken up at the alveolar level during the cardiodynamic phase (ΣVO_2), the area under the $\dot{V}O_2$ curve from t = 0 s to t = 20 s was calculated. This 20-s window was based on prior work which has shown that the rise in $\dot{V}O_2$ during the first 20 s of exercise is primarily driven by increases in $\dot{Q}p$ with negligible contributions from skeletal muscle metabolic activity (16, 18–20), although there may be some potential contributions from desaturated blood surging from the abdominal venous system into the right atrium (27).

Statistical analysis

Percent changes between baseline and post-maneuver measurements were calculated and compared for maneuver type using a one-way repeated measures ANOVA. Echocardiography measurements (CO, HR, SV) before and after each maneuver were compared using a two-way repeated measures ANOVA. Percent changes were calculated and compared for maneuver duration and measurement location (RVOT vs LVOT) using a two-way repeated measures ANOVA. All pairwise *post hoc* comparisons were performed with the Bonferroni correction.

Cardiopulmonary parameters collected in *Protocol 2* (Vt, RR, $\dot{V}E$, $PetCO_2$, $PetO_2$, $\dot{V}O_2$, HR, RVSV, LVSV, RVCO, LVCO) were compared between baseline and the post-DB12 maneuver using *Student's* paired t-test. $\dot{V}O_2$ kinetics parameters at exercise onset (ΣVO_2, A, τ, TD) were compared between the CTRL and DB12 runs using Student's paired t-test for each parameter. All data are reported as mean±SD. The probability that mean differences were greater than chance alone was reported as $p<0.05$. Statistical analyses were performed using SPSS Statistics 26 (IBM Corporation, Armonk, NY, 2011).

RESULTS

Participants

A total of 13 healthy males (mean age = 33.5 years, range 27-45) participated in *Protocol 1* (resting assessment), and 8 of these completed *Protocol 2* (exercise with gas exchange measurement). Participants' anthropometric measurements and cardiac structural and functional parameters are shown in ***Table 1***. Left ventricular size, wall thickness, and ejection fraction were normal in all participants. Left atrial volume and right ventricular chamber size were normal or mildly increased in all individuals. No subjects had valvular stenosis, and none had valvular regurgitation greater than mild in severity.

Table 1: Participant characteristics

Participants	
Age	33.5±5.2
Height (cm)	179.9±6.9
Weight (kg)	83.0±13.2
BSA (m^2)	2.0±0.2
Echocardiographic Parameters	
Resting heart rate (beats per minute)	62±8
Systolic blood pressure (mmHg)	118±11
Diastolic blood pressure (mmHg)	70±8
Interventricular septum (mm)	8.4±1.6
Posterior wall (mm)	9.3±1.0
LV end-diastolic dimension (mm)	49±5
LV end-systolic dimension (mm)	33±4
Left atrial volume (ml)	58±16
RV basal end-diastolic diameter (mm)	38±5
LV end-diastolic volume (ml)	113±24
LV end-systolic volume (ml)	47±11
LV ejection fraction (%)	58±3
LVOT diameter (mm)	22±1
RVOT diameter (mm)	25±2

BSA=body surface area; LVOT, RVOT=left and right ventricular outflow tract, respectively.

Values are mean (SD). N=13.

Thoracic and abdominal excursions

All respiratory maneuvers led to increased intra-breath swings in thoracic (by 159±136 [P<0.001], 510±303 [P<0.001] and 410±255% [P<0.001] on average for AB, RC and DB, respectively) and abdominal (by 320±119 [P<0.001], 41±52 [P=0.011] and 178±101% [P<0.001]) excursion. Among the three types of maneuvers, RC produced the greatest increase in thoracic swings (P<0.001 vs AB and DB) while abdominal swings were the greatest with AB (P<0.001 vs RC and DB, ***Table 1***).

Acute cardiac responses to respiratory maneuvers

Pre-maneuver heart rates (after the 'recovery' period of 2 minutes of quiet breathing – ***Figure 1***) did not differ across maneuver durations (***Supplemental Figure 1*** (https://doi.org/10.6084/m9.figshare.19091345). Pulsed-wave Doppler data collected both immediately before and immediately after each respiratory maneuver are reported in ***Table 2***, and changes compared to baseline in all conditions are represented in ***Figure 3***. All 6 and 12-breaths maneuvers produced an increase in RVCO resulting from increases in HR and RVSV (P<0.05 compared to baseline values). Similarly, 6 and 12-breath maneuvers increased the LVCO, driven primarily by increases in HR. In contrast, 3-breath maneuvers did not impact RVCO and produced only modest changes in LVCO (only with deep breathing), again driven in this case by an increase in HR.

297 **Table 2: Acute Cardiac Response to Respiratory Maneuvers Under Resting Conditions (Protocol 1)**

		Cardiac output [L·min^{-1}]			Stroke volume [ml]			Heart rate [beat·min^{-1}]		
		PRE	POST	P	PRE	POST	P	PRE	POST	P
AB3	L	4.5 (0.7)	4.9 (0.8)	0.059	78.4 (15.4)	79.7 (15.1)	0.488	57.8 (5.2)	61.8 (6.7)	0.007
	R	4.4 (1.0)	4.8 (1.3)	0.068	74.2 (15.5)	76.7 (20.0)	0.183	59.9 (5.5)	62.8 (7.2)	0.053
AB6	L	4.6 (0.9)	5.4 (1.2)	<0.001	80.1 (16.0)	81.5 (23.1)	0.472	58.0 (4.4)	67.1 (8.7)	<0.001
	R	4.4 (1.1)	5.4 (1.7)	<0.001	74.7 (18.0)	81.5 (23.1)	<0.001	59.5 (5.3)	66.0 (5.9)	<0.001
AB12	L	4.7 (0.8)	5.6 (1.2)	<0.001	81.6 (17.6)	84.5 (17.7)	0.132	58.3 (5.9)	66.4 (7.6)	<0.001
	R	4.5 (0.9)	5.7 (1.5)	<0.001	75.8 (16.7)	85.5 (21.2)	<0.001	59.6 (4.4)	66.2 (6.7)	<0.001
RC3	L	4.7 (1.2)	4.9 (1.2)	0.470	79.3 (21.4)	80.1 (19.8)	0.678	57.8 (4.4)	62.8 (6.2)	0.473
	R	4.6 (0.8)	4.7 (1.0)	0.463	76.9 (16.1)	78.0 (18.6)	0.546	59.8 (6.3)	61.8 (7.4)	0.442
RC6	L	4.7 (1.2)	5.3 (1.5)	0.001	78.1 (19.7)	81.8 (21.3)	0.050	58.2 (5.4)	67.2 (7.0)	<0.001
	R	4.6 (1.0)	5.6 (1.4)	<0.001	78.7 (17.8)	84.4 (18.5)	0.003	59.4 (6.0)	67.1 (7.3)	<0.001
RC12	L	4.8 (1.2)	5.5 (1.7)	0.002	79.4 (20.3)	81.5 (22.8)	0.257	57.9 (4.9)	65.5 (6.9)	<0.001
	R	4.8 (1.1)	5.9 (1.2)	<0.001	78.7 (17.9)	89.0 (18.7)	<0.001	59.1 (4.8)	67.4 (5.9)	<0.001
DB3	L	4.7 (0.8)	5.2 (1.0)	0.009	82.1 (15.3)	84.0 (16.6)	0.334	60.0 (7.3)	61.1 (6.8)	0.001
	R	4.7 (1.1)	4.8 (1.5)	0.514	78.5 (19.2)	79.7 (21.6)	0.522	60.4 (5.7)	61.5 (7.6)	0.184
DB6	L	4.7 (0.8)	5.8 (1.1)	<0.001	81.1 (15.5)	86.5 (16.6)	0.005	60.0 (6.3)	65.5 (7.5)	<0.001
	R	4.5 (1.1)	5.7 (1.9)	<0.001	76.7 (18.9)	84.4 (24.8)	<0.001	58.9 (5.6)	66.5 (8.1)	<0.001
DB12	L	4.6 (0.8)	5.6 (1.0)	<0.001	80.1 (15.4)	86.5 (16.1)	0.001	61.4 (5.8)	67.6 (8.5)	<0.001
	R	4.5 (1.1)	5.9 (1.8)	<0.001	77.4 (18.5)	87.2 (24.9)	<0.001	61.2 (7.3)	66.7 (5.3)	<0.001

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299 AB=abdominal breathing; RC=rib cage breathing; DB=deep breathing; associated numbers (e.g., AB3=AB for 3 breaths); L, R=Pulsed-wave
300 Doppler measurements collected in the left and right ventricular outflow tract, respectively. Values are mean (SD). N=13.

Figure 3: Effects of Breathing Maneuvers on Resting Cardiac Function

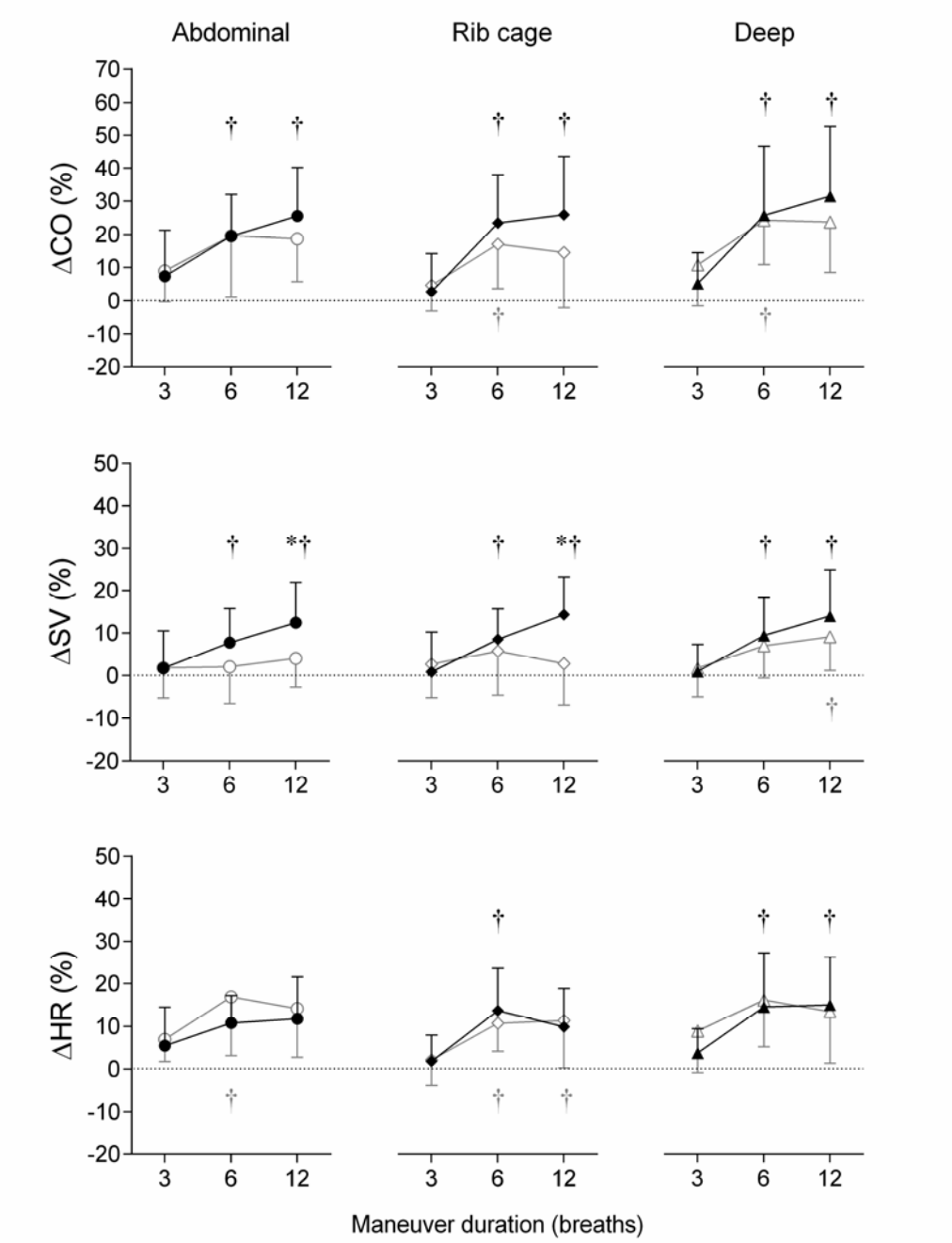

Figure 3: Cardiac output (CO), stroke volume (SV) and heart rate (HR) values derived from pulsed-wave Doppler interrogation of the left (panel A; grey boxes) and right (panel B, white boxes) ventricular outflow tracts. Values are percent changes after abdominal, rib cage or deep breathing maneuvers sustained for 3, 6 or 12 breaths. † different from 3 breaths; *different from LVOT; P<0.05. N=13.

When comparing the three durations within each type of maneuver, RVCO increased more with 6 and 12 breaths as compared to 3 in AB (+8±13 vs 20±13 vs 25±14 %, P=0.019 and P=0.003 respectively), RC (+3±11 vs 22±15 vs 25±17 %, P<0.001) and DB (+2±13 vs 24±21 vs 29±22 %, P<0.001). This was not systematically the case for LVCO, as only RC6 and DB6 produced greater changes than the corresponding 3-breath maneuvers [+5±8 vs 17±13 % (P=0.040) and +11±12 vs 24±13 % (P=0.024), respectively]. Similarly, RVSV increased more with 6 and 12 breaths than with 3 in AB (+3±9 vs 8±8 vs 13±9 %, P=0.048 and P=0.003, respectively), RC (+1±9 vs 8±8 vs 14±9 %, P=0.016 and P<0.001) and DB (+1±6 vs 9±9 vs 12±12 %, P=0.002 and P=0.001), while LVSV only changed with DB12 as compared to DB3 (+8±8 vs 2±7 %, P=0.048). The changes observed in RVSV did not differ from those found in LVSV, except during RC12 (+14±9 vs +3±9 % P=0.002) and AB12 (+12±9 vs +4±7 %, P=0.017), although these differences were not associated with a greater increase in RVCO compared to LVCO (+25±17 vs +13±17, P=0.069 and +25±14 vs +18±12 %, P=0.311, respectively).

Cardiopulmonary changes with DB12

Gas exchange analysis showed substantial cardiopulmonary changes with DB12 (***Figure 4***). During DB12, $\dot{V}E$ markedly increased compared to the baseline quiet breathing (by 31.3±8.1 $L \cdot min^{-1}$, P<0.001) due to an increase in Vt (by 3.0±0.8 L, P<0.001) that largely compensated for a decrease in RR (by 4.4±2.4 $breaths \cdot min^{-1}$, P=0.013). $PetCO_2$ gradually decreased (by 9.7±2.0 mm Hg, P<0.001) and $PetO_2$ increased (by 21.5±4.1 mm Hg, P<0.001). Upon initiation of the maneuver, $\dot{V}O_2$ peaked rapidly (within 5.9±0.6 s, increasing by 801±254 $ml \cdot min^{-1}$ above the baseline level), then gradually decreased to remain at a slightly higher level compared to quiet breathing by the end of DB12 (by 99±43 $ml \cdot min^{-1}$, P<0.001). Pre-exercise gas exchange without DB12 (CTRL arm) is shown in ***Figure 4***.

Figure 4: Physiological Responses to Deep Breathing Maneuvers Performed for Twelve Breaths Prior to Exercise

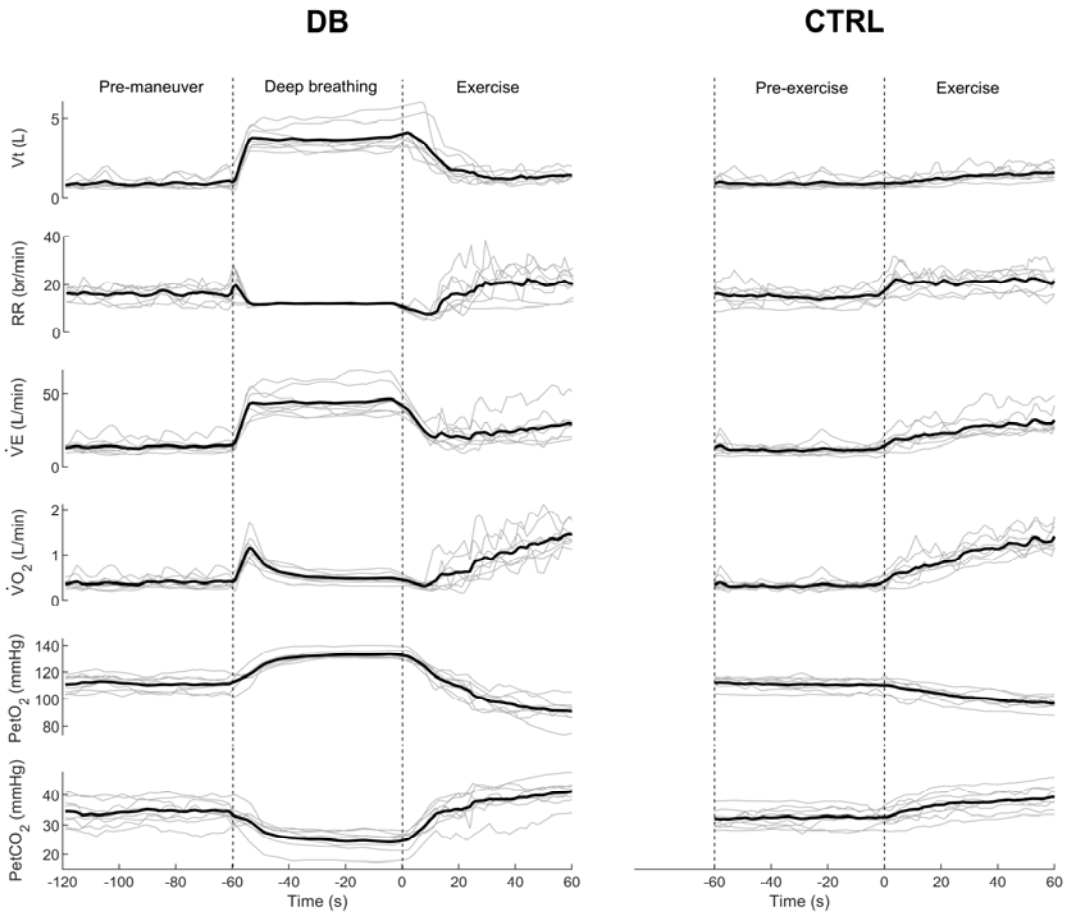

Figure 4: Cardiopulmonary response assessed by continuous gas exchange measurement during the deep breathing maneuver performed prior to exercise onset (DB; *left panel*) and in control condition (CTRL; *right panel*). Black line represents the averaged signal; grey lines are individual tracings. Vt= tidal volume, RR= respiratory rate, VE= ventilation, $\dot{V}O_2$= oxygen uptake, $PetO_2$ = end-tidal O_2 pressure and $PetCO_2$ = end-tidal CO_2 pressure. N=8.

342 Pulsed-wave Doppler measurements obtained immediately before and after DB12
343 showed an increase in RVSV (by 12±3%, P<0.001) and LVSV (by 9±10 %, P=0.005) with
344 concomitant increases in HR (76.8±7.6 vs 89.1±12.7 bpm, P=0.013) and thus increases in
345 RVCO (by 30±14%, P<0.001) and LVCO (by 27±17%, P=0.032). Changes produced by
346 DB12 did not differ in the seated (*Protocol 2*) compared to supine positioning (*Protocol 1*) in
347 HR (+16±11% vs 16±12 %, P=0.894), RVSV (+14±11 vs 12±3 %, P=0.609), LVSV (+10±9 vs
348 9±10 %, P=0.821), RVCO (+34±20 vs, 30±14 %, P=0.645) and LVCO (+25±16 vs 27±17 %,
349 P=0.725).

350

351 **$\dot{V}O_2$-on kinetics**

352 The time constant τ of the $\dot{V}O_2$-on from 20 s to 180 s (20.5±9.1 vs 19.8±10.1 s,
353 P=0.946) and $\dot{V}O_2$ amplitude (1169.8±89.1 vs 1179.1±98.2 ml·min^{-1}, P=0.307) remained
354 unchanged. ΣVO_2 during the first 20 seconds of exercise was substantially decreased
355 following DB12 compared to CTRL (149±51 vs 233±65 ml, P=0.003) (***Figure 5***).

Figure 5: Impact of Deep Breathing (DB) Maneuver on Oxygen Exchange During the Cardiodynamic Phase of Early Exercise

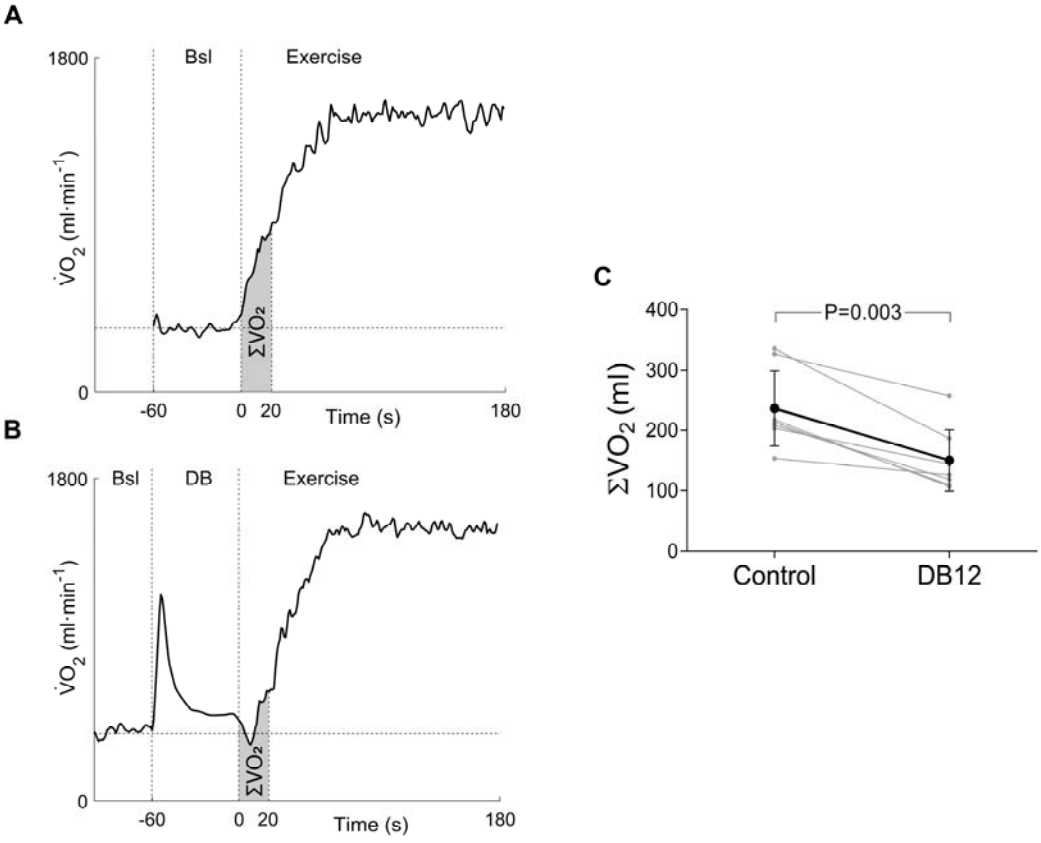

Figure 5: Total volume of oxygen exchanged at the mouth (ΣVO_2) during the 'cardiodynamic phase'. **A and B**: Illustration of the method. Black line represents the averaged oxygen uptake ($\dot{V}O_2$) signal in the control (A) and deep breathing (B) conditions. ΣVO_2 is defined as the area under the $\dot{V}O_2$ curve within the first 20 s of exercise, shown in grey. Bsl=baseline; DB=deep breathing. **C**: ΣVO_2 values in the control and DB conditions. Grey dots and lines are individual data, black symbols and line represent mean±SD. N=8.

DISCUSSION

This study was designed to examine the influence of respiratory maneuvers on cardiac function and $\dot{V}O_2$ uptake during the cardiodynamic phase of exercise. Key findings are summarized as follows. First, AB, RC and DB maneuvers performed under resting conditions for 6 and 12 breaths resulted in quantitatively similar increases in RVCO and LVCO. Second, dissociation between changes in RVSV and LVSV in response to the respiratory maneuvers were minimal. Finally, and of paramount importance, a 1-min bout of DB performed immediately prior to the onset of moderate intensity cycling reduced the increase in cardiodynamic $\dot{V}O_2$ typically observed during the early phase of exercise onset (first 20 s) without influencing the subsequent $\dot{V}O_2$-on response driven by the increase in muscle O_2 consumption. Collectively, these findings provide evidence that respiratory maneuvers can increase $\dot{Q}p$ and prime alveolar O_2 transfer prior to exercise.

Acute effects of respiratory maneuvers at rest

Acute cardiac responses to respiratory maneuvers

Previous experimental results suggested that voluntary amplification of the respiratory pump with AB and RC modulates femoral venous blood flow (21) and enhances blood displacements between the extremities and the trunk (22, 23, 25), indicating a potential for respiratory maneuvers to increase venous return. However, methodological constraints could not evaluate the impact of these interventions on cardiac function per se. Our findings confirm the hypothesis previously put forth and show for the first time that preload augmentation by respiratory maneuvers has a direct effect on RVCO and LVCO.

Elegant physiologic experiments dating back to the 1950s have shown that the fall in ITP during normal inspiration produces a decrease in right atrial pressure and widens the pressure gradient from the peripheral venous circulation to the right atrium, thus augmenting

venous return (28). With the RC breathing maneuver specifically, this seems to be the dominant mechanism, with more recent data confirming that deepening the inspiratory fall in ITP with RC breathing at rest augments femoral venous return (21) and results in greater intra-breath blood displacements between the trunk and the extremities during moderate exercise (23, 25). With AB in turn, in addition to this ITP-driven increase in the venous pressure gradient, the inspiratory descent of the diaphragm may also contribute to augmented venous return via the consequent increase in IAP (29–31). This effect is likely driven by compression of the hepatic and splanchnic vascular beds (30, 32), and experimental work has specifically demonstrated that greater IAP swings increase both splanchnic emptying (22, 24, 25) and IVC flow above the inlet of the hepatic vein (22, 30). In the present study, the increases in RVCO and LVCO produced by DB performed for 6 and 12 breaths were not different than those generated by AB and RC, suggesting that the act of breathing deeply can produce an increase in venous return to a similar magnitude as the more specific RC and AB techniques. We speculate that DB produced both greater splanchnic and femoral venous return through both greater diaphragmatic descent and rib cage expansion, respectively. Indeed, although we did not monitor IAP and ITP in this study, changes in abdominal and thoracic excursions observed with DB corroborate the contention that DB combines the mechanics of both AB and RC, i.e. greater diaphragmatic movements and greater rib cage expansion compared to quiet breathing (***Figures 2C & 2D***).

In addition to the mechanical effects resulting from the enhanced pumping mechanism, the metabolic cost of performing the breathing maneuvers also likely plays a role in the observed increases in RVCO and LVCO. A greater level of $\dot{V}E$ through greater contraction of the diaphragm and rib cage muscles would typically generate greater work of breathing and result in an increased metabolic load (33), and we speculate that this may be more functionally significant among diseased individuals whose functional ‘reserve’ is lower. In our study, $\dot{V}O_2$ measured throughout the DB12 run in *Protocol 2* remained elevated by the end of the maneuver (***Figure 4***), potentially reflecting the increased metabolic cost of

ventilation. This increase in $\dot{V}O_2$, in turn, would be expected to come with an associated increase in CO, as was observed.

Left vs right ventricular responses

We examined several maneuver durations (3, 6 and 12 breaths) to determine whether a dissociation between RV and LV responses to the respiratory maneuvers would be observed. Our initial hypothesis was that a maneuver-induced surge of blood from the peripheral circulation would affect RVSV ahead of LVSV, as the two ventricles are in series interposed by the pulmonary vasculature. We thus hypothesized that a temporal dissociation would be observed with the shortest maneuvers and progressively be reduced with time. Our analysis did not demonstrate a right-to-left difference in SV with either maneuver type when performed for 3 breaths (***Figure 3***), although neither technique at this duration increased RVSV or LVSV (***Table 2***). Comparison of the mean increases with 6 and 12 breaths suggests a slightly greater increase in RVSV compared to LVSV in all maneuver types (***Figure 3***), in line with prior work suggesting that the pulmonary vascular bed may act as a buffer for the blood volume acutely translocated from the periphery during transitions from rest to exercise (34). Although these differences were noted only with RC12 and AB12, potentially due to limited statistical power, we speculate that they could be the manifestation of a progressive pooling of blood in the low-resistance, high-capacitance pulmonary vasculature resulting from gradual capillary recruitment throughout the maneuver.

Cardiorespiratory adjustments to exercise onset

In *Protocol 2* we tested the hypothesis that DB performed immediately before a transition to moderate intensity exercise would produce a cardiodynamic increase in pulmonary O_2 uptake during the maneuver and thereby reduce the early increase in $\dot{V}O_2$ normally observed at the very beginning of exercise onset. Because the analysis of $\dot{V}O_2$-on kinetics requires the averaging of several transitions in order to improve signal-to-

noise ratio (35), we selected only one type of maneuver / duration amongst those examined in *Protocol 1* and focused on DB due to its relative ease of performance and on 12 breaths (DB12) to allow sufficient time for the maneuvers to generate an effect.

Pulmonary O_2 transfer within the cardiodynamic phase

The initiation of muscular exercise from a resting state is typically accompanied by near immediate cardiopulmonary adjustments to accelerate CO and optimize O_2 delivery to working muscles. The consequent increase in $\dot{Q}p$ leads to greater alveolar O_2 transfer through greater flow of reduced hemoglobin in the pulmonary capillaries, thus producing an early "non-metabolic" increase in pulmonary $\dot{V}O_2$ (16). Our analysis of pulmonary O_2 transfer at exercise onset indicates that the increase in $\dot{V}O_2$ within this cardiodynamic phase (i.e. first 20 s of exercise) was partly abolished with DB12, as shown by the reduction in ΣVO_2 in this condition compared to the control state (***Figure 5***). These results support the hypothesis that DB12 increased $\dot{Q}p$ ahead of exercise onset, thus partly reducing the acceleration of $\dot{Q}p$ normally responsible for the rise in $\dot{V}O_2$ in early exercise. This contention is further supported by the observed increase in RVSV and RVCO with DB12, directly documented with the echocardiographic measurements, as well as by the spike in $\dot{V}O_2$ seen upon initiation of DB12 (***Figure 4***). In the latter case, in addition to the aforementioned increase in $\dot{Q}p$, one potential contributor to this observed spike could be the sudden changes in lung gas stores due to changes in operational lung volume (36–39). However, while a contribution of this mechanism cannot be ruled out, its potential effect would likely occur only during the first breath of the maneuver, as changes in operational lung volume during subsequent breaths would be limited.

Further, in addition to the increased $\dot{Q}p$ prior to exercise onset, the hyperventilatory effect of DB12 may also contribute to the observed decrease in ΣVO_2. The voluntary increase in tidal volume with DB12, which resulted in a substantial increase in ventilation

despite a reduction in RR, produced a marked decrease in $PetCO_2$ throughout the maneuver (***Figure 4***). It is unlikely that this decrease *per se* would have affected $\dot{Q}p$, since previous experiments examining the effect of hyperventilation have reported similar increases in CO irrespective of changes in $PetCO_2$ (40). However, assuming that the observed fall in $PetCO_2$ reflects the dynamics of arterial PCO_2 levels, this decline may decrease the respiratory drive at the end of the maneuver (41), thus minimizing the pumping mechanism normally seen at exercise onset. This possibility is supported by the noticeable transitory drop in RR at the onset of exercise and could explain the brief simultaneous drop in $\dot{V}O_2$ that was observed (***Figure 4***).

Primary phase of $\dot{V}O_2$-on kinetics

The spike in $\dot{V}O_2$ upon initiation of the maneuver followed by the marked reduction in ΣVO_2 with DB12 both support the hypothesis that respiratory maneuvers can accelerate $\dot{Q}p$ ahead of exercise onset and thereby increase systemic O_2 delivery during exercise initiation. The influence of such mechanisms on muscle $\dot{V}O_2$-on kinetics is likely to be minimal in healthy individuals, as prior experimental data in this population point towards O_2 utilization at the muscle level rather than O_2 delivery as a limiting factor for the rate at which muscle O_2 consumption adjusts to moderate exercise (5–9, 42, 43). In line with these prior findings, the time constant of the primary phase did not differ between CTRL and DB12, suggesting similar overall O_2 utilization with and without DB. Similar observations of unchanged primary phase kinetics have been observed in a wide range of physiologic perturbations in healthy subjects, including interventions that increase O_2 transport such as hemodilution (44), administration of erythropoietin (45), inhalation of hyperoxic air (46), and priming exercise (47).

However, in disease states where convective O_2 transport is impaired, O_2 delivery to working muscles can limit $\dot{V}O_2$-on kinetics. Accordingly, individuals with conditions impairing

the cardiac response to exercise, such as in heart failure, demonstrate a slower increase in the $\dot{V}O_2$-on response during brief bouts of exercise (48–50). Importantly, the inability to adequately increase CO at the onset of exercise in these patients affects both the cardiodynamic phase, through a slower increase in $\dot{Q}p$, and the primary phase, through an impairment of O_2 delivery to the exercising muscles (51). Although the kinetics of the primary phase were unchanged in our healthy population, we demonstrate a cardiac response to the respiratory maneuvers and a subsequent reduction in the cardiodynamic phase due to the preemptive $\dot{Q}p$ acceleration. These mechanisms may represent a possible pathway for improving $\dot{V}O_2$-on kinetics in a diseased population, thereby reducing the O_2 deficit incurred during early exercise. The metabolic cost of the respiratory maneuvers may also be of particular importance in diseased individuals, and the net impact of this type of intervention in individuals with impaired cardiac function remains uncertain. Given the plausible mechanism suggested herein and the low cost and low risk of this technique, additional study is indicated to evaluate whether these results can be replicated in a diseased population and how these maneuvers may impact $\dot{V}O_2$-on kinetics, the early exercise O_2 deficit, and ultimately exercise capacity.

Limitations

Our study has several limitations. First, recruitment for this initial study was limited to male subjects, and future studies replicating these results among female subjects will be required. Similarly, this study involved only healthy subjects without cardiopulmonary disease, and the generalizability to diseased individuals remains to be established. Second, we chose not to estimate intra-thoracic and intra-abdominal pressure, considering the associated methodological constraint and potential obstacle for recruitment. Third, we did not correct $\dot{V}O_2$ for changes in lung O_2 stores, as doing so necessitates measuring the raw signals of O_2, CO_2 and airflow at the mouth and utilizing advanced experimental techniques

including optoelectronic plethysmography to monitor breath-by-breath variations in absolute lung volume. Fourth, in our measurement of ΣVO_2, we chose a fixed 20-s window to capture the typical duration for the cardiodynamic phase, based on previous reports (16, 20). Given the robust and consistent changes observed between DB12 and CTRL, however, it is unlikely that a slightly shorter or longer window would have produced fundamentally different results. Finally, RV and LV responses could not be assessed simultaneously with a single imaging probe, so we instead performed the RV and LV assessments sequentially in randomized order with an interceding 'rest' period.

CONCLUSIONS

Our findings suggest that in healthy male subjects at rest (1) respiratory maneuvers designed to amplify the respiratory pump mechanism can enhance RV and LV CO, (2) DB has similar effects as AB and RC on right and left ventricular CO; (3) dissociation between changes in RVSV and LVSV in response to the respiratory maneuvers are minimal. In addition, we demonstrate that a 1-min bout of DB performed prior to exercise initiation can increase $\dot{Q}p$ and alveolar O2 uptake, thus attenuating the cardiodynamic increase in $\dot{V}O_2$ typically seen in early exercise without impacting the subsequent metabolic $\dot{V}O_2$-on response. Demonstration of these physiologic properties in healthy subjects sets the stage for future studies aimed at examining the clinical utility of pre-exercise DB in patients with cardiovascular diseases that limit $\dot{Q}p$ acceleration at the onset of exercise resulting in impaired exercise capacity.

ADDITIONAL INFORMATION

Data Availability: Data from this study will be made available upon reasonable request to corresponding author.

Competing Interests: No authors report any conflicts of interest.

Author Contributions:

Conception and design of research: F.S., T.C., B.K. and A.B. Acquisition, analysis, and interpretation of data: F.S., T.C., J.C. B.P., J.S.G., M.M.W., B.K., A.B. Drafting manuscript and critical revision: F.S., T.C., J.C. B.P., J.S.G., M.M.W., B.K., A.B. All authors approved the final version of the manuscript. All authors agree to be accountable for all aspects of the work. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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724 moderate- intensity aerobic exercise training in chronic heart failure: Clinical and
725 pathophysiological correlates. *Int J Cardiol* 167: 2189–2195, 2013. doi:
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FIGURE CAPTIONS

Figure 1: Overview of the experimental procedure. A schematic is shown outlining the experimental procedures performed in *Protocol 1* (resting assessment) and *Protocol 2* (transition to exercise with gas exchange measurement). QB = quiet breathing; RVOT=right ventricular outflow tract; LVOT=left ventricular outflow tract: DB = deep breathing maneuver; DB12 = deep breathing performed for 12 breaths. CTRL = control condition without pre-exercise breathing maneuver.

Figure 2: Overview of respiratory maneuvers. A: illustration of the techniques for abdominal and rib cage breathing. Abdominal breathing consists of emphasizing the contribution of the diaphragm, resulting in greater abdominal excursion; rib cage breathing consists of emphasizing the contribution of the intercostal and accessory respiratory muscles, resulting in greater rib cage excursion. B: graphical method used to quantify abdominal and thoracic excursions during the respiratory maneuvers. The respiratory belts measured changes in tension resulting from expansion and contraction of the abdomen and rib cage. The tracing shown is an example of a force signal yielded by an abdominal belt. Absl is the average amplitude over the six breaths preceding the maneuver, Amaneuver is the average amplitude over the number of breaths performed during the maneuver (3, 6 or 12). C and D: changes in thoracic (C) and abdominal (D) excursion during rib cage, deep or abdominal breathing. ΔAmplitude is the difference between Absl and Amaneuver expressed in percentage. N=13.

Figure 3: Cardiac output (CO), stroke volume (SV) and heart rate (HR) values derived from pulsed-wave Doppler interrogation of the left (panel A; grey boxes) and right (panel B, white boxes) ventricular outflow tracts. Values are percent changes after abdominal, rib cage or deep breathing maneuvers sustained for 3, 6 or 12 breaths. † different from 3 breaths; *different from LVOT; $P<0.05$. N=13.

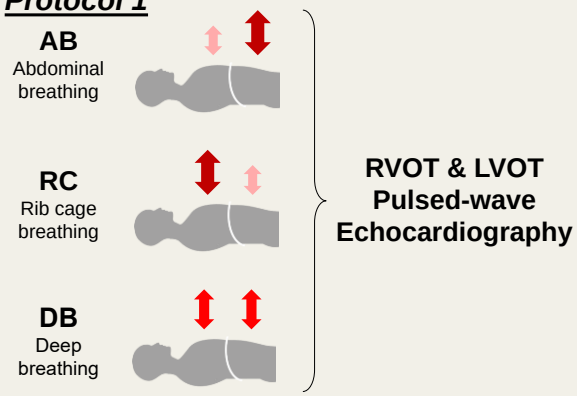
Figure 4: The cardiopulmonary response to the deep breathing maneuver performed prior to exercise onset and assessed by continuous gas exchange measurement is shown. Black line represents the averaged signal; grey lines are individual tracings. Vt=tidal volume, RR=respiratory rate, VE=ventilation, $\dot{V}O_2$=oxygen uptake, $PetO_2$=end-tidal O_2 pressure and $PetCO_2$=end-tidal CO_2 pressure. N=8.

Figure 5: Total volume of oxygen exchanged at the mouth (ΣVO_2) during the 'cardiodynamic phase'. A and B: Illustration of the method. Black line represents the averaged oxygen uptake ($\dot{V}O_2$) signal in the control (A) and deep breathing (B) conditions. ΣVO_2 is defined as the area under the $\dot{V}O_2$ curve within the first 20 s of exercise, shown in grey. Bsl=baseline; DB=deep breathing. C: ΣVO_2 values in the control and DB conditions. Grey dots and lines are individual data, black symbols and line represent mean±SD. N=8.

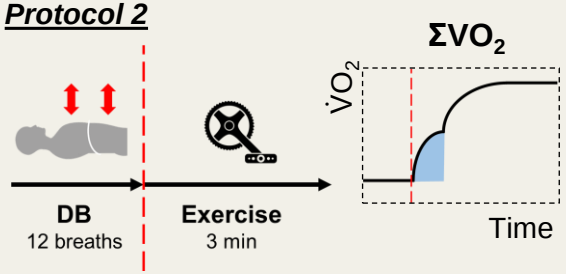
Priming Cardiac Function with Voluntary Respiratory Maneuvers and Effect on Early Exercise Oxygen Uptake

METHODS

Protocol 1

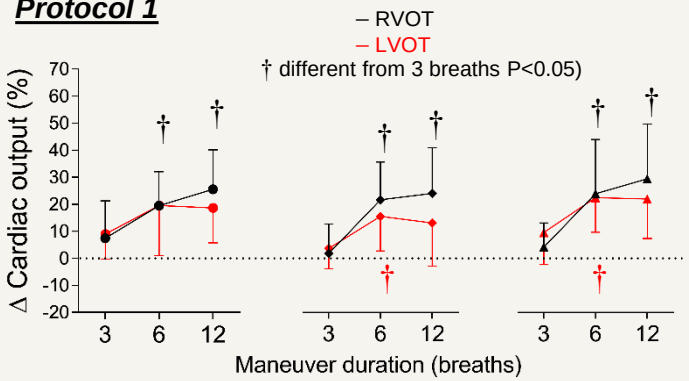


Protocol 2



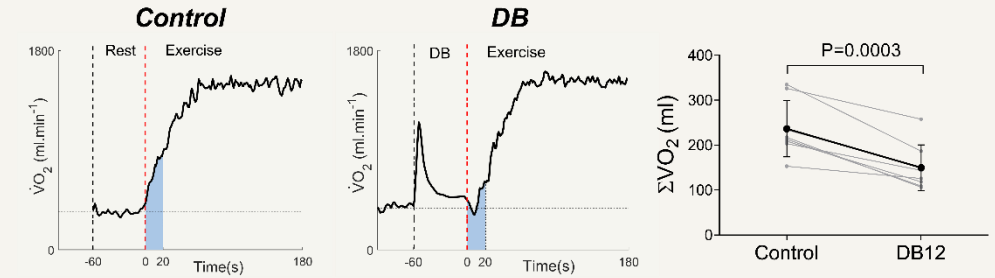
OUTCOME

Protocol 1



- All maneuvers increased cardiac output compared to resting conditions.
- Greater effect with 6 and 12 breaths

Protocol 2



- DB produced a spike in $\dot{V}O_2$ and subsequently reduced the cardiodynamic increase in $\dot{V}O_2$ during early exercise

CONCLUSION Respiratory maneuvers can prime pulmonary blood flow and improve alveolar O_2 transfer prior to exercise.

Table 1: Participant characteristics

Participants	
Age	33.5±5.2
Height (cm)	179.9±6.9
Weight (kg)	83.0±13.2
BSA (m^2)	2.0±0.2
Echocardiographic Parameters	
Resting heart rate (beats per minute)	62±8
Systolic blood pressure (mmHg)	118±11
Diastolic blood pressure (mmHg)	70±8
Interventricular septum (mm)	8.4±1.6
Posterior wall (mm)	9.3±1.0
LV end-diastolic dimension (mm)	49±5
LV end-systolic dimension (mm)	33±4
Left atrial volume (ml)	58±16
RV basal end-diastolic diameter (mm)	38±5
LV end-diastolic volume (ml)	113±24
LV end-systolic volume (ml)	47±11
LV ejection fraction (%)	58±3
LVOT diameter (mm)	22±1
RVOT diameter (mm)	25±2

BSA=body surface area; LVOT, RVOT=left and right ventricular outflow tract, respectively.

Values are mean (SD). N=13.

Table 2: Acute Cardiac Response to Respiratory Maneuvers Under Resting Conditions (Protocol 1)

		Cardiac output [L·min^{-1}]			Stroke volume [ml]			Heart rate [beat·min^{-1}]		
		PRE	POST	P	PRE	POST	P	PRE	POST	P
AB3	L	4.5 (0.7)	4.9 (0.8)	0.059	78.4 (15.4)	79.7 (15.1)	0.488	57.8 (5.2)	61.8 (6.7)	0.007
	R	4.4 (1.0)	4.8 (1.3)	0.068	74.2 (15.5)	76.7 (20.0)	0.183	59.9 (5.5)	62.8 (7.2)	0.053
AB6	L	4.6 (0.9)	5.4 (1.2)	<0.001	80.1 (16.0)	81.5 (23.1)	0.472	58.0 (4.4)	67.1 (8.7)	<0.001
	R	4.4 (1.1)	5.4 (1.7)	<0.001	74.7 (18.0)	81.5 (23.1)	<0.001	59.5 (5.3)	66.0 (5.9)	<0.001
AB12	L	4.7 (0.8)	5.6 (1.2)	<0.001	81.6 (17.6)	84.5 (17.7)	0.132	58.3 (5.9)	66.4 (7.6)	<0.001
	R	4.5 (0.9)	5.7 (1.5)	<0.001	75.8 (16.7)	85.5 (21.2)	<0.001	59.6 (4.4)	66.2 (6.7)	<0.001
RC3	L	4.7 (1.2)	4.9 (1.2)	0.470	79.3 (21.4)	80.1 (19.8)	0.678	57.8 (4.4)	62.8 (6.2)	0.473
	R	4.6 (0.8)	4.7 (1.0)	0.463	76.9 (16.1)	78.0 (18.6)	0.546	59.8 (6.3)	61.8 (7.4)	0.442
RC6	L	4.7 (1.2)	5.3 (1.5)	0.001	78.1 (19.7)	81.8 (21.3)	0.050	58.2 (5.4)	67.2 (7.0)	<0.001
	R	4.6 (1.0)	5.6 (1.4)	<0.001	78.7 (17.8)	84.4 (18.5)	0.003	59.4 (6.0)	67.1 (7.3)	<0.001
RC12	L	4.8 (1.2)	5.5 (1.7)	0.002	79.4 (20.3)	81.5 (22.8)	0.257	57.9 (4.9)	65.5 (6.9)	<0.001
	R	4.8 (1.1)	5.9 (1.2)	<0.001	78.7 (17.9)	89.0 (18.7)	<0.001	59.1 (4.8)	67.4 (5.9)	<0.001
DB3	L	4.7 (0.8)	5.2 (1.0)	0.009	82.1 (15.3)	84.0 (16.6)	0.334	60.0 (7.3)	61.1 (6.8)	0.001
	R	4.7 (1.1)	4.8 (1.5)	0.514	78.5 (19.2)	79.7 (21.6)	0.522	60.4 (5.7)	61.5 (7.6)	0.184
DB6	L	4.7 (0.8)	5.8 (1.1)	<0.001	81.1 (15.5)	86.5 (16.6)	0.005	60.0 (6.3)	65.5 (7.5)	<0.001
	R	4.5 (1.1)	5.7 (1.9)	<0.001	76.7 (18.9)	84.4 (24.8)	<0.001	58.9 (5.6)	66.5 (8.1)	<0.001
DB12	L	4.6 (0.8)	5.6 (1.0)	<0.001	80.1 (15.4)	86.5 (16.1)	0.001	61.4 (5.8)	67.6 (8.5)	<0.001
	R	4.5 (1.1)	5.9 (1.8)	<0.001	77.4 (18.5)	87.2 (24.9)	<0.001	61.2 (7.3)	66.7 (5.3)	<0.001

AB=abdominal breathing; RC=rib cage breathing; DB=deep breathing; associated numbers (e.g., AB3=AB for 3 breaths); L, R=Pulsed-wave Doppler measurements collected in the left and right ventricular outflow tract, respectively. Values are mean (SD). N=13.

Figure 1: Experimental Procedures

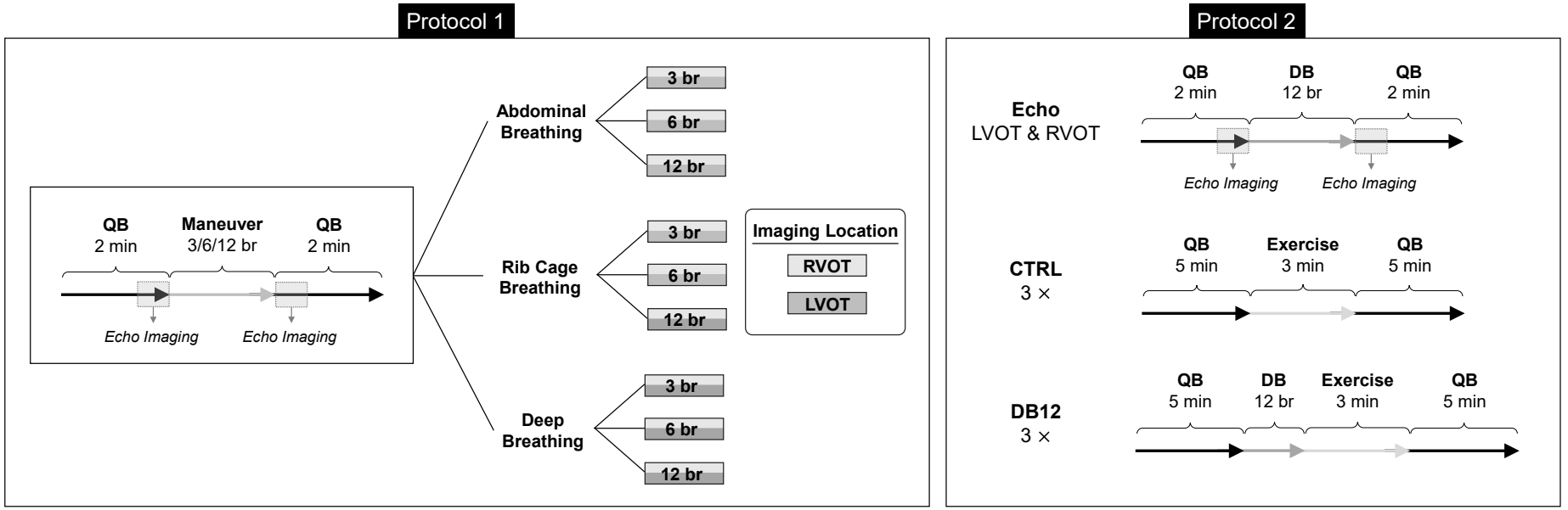


Figure 1: Overview of the experimental procedure. A schematic is shown outlining the experimental procedures performed in *Protocol 1* (resting assessment) and *Protocol 2* (transition to exercise with gas exchange measurement). QB = quiet breathing; RVOT=right ventricular outflow tract; LVOT=left ventricular outflow tract: DB = deep breathing maneuver; DB12 = deep breathing performed for 12 breaths. CTRL = control condition without pre-exercise breathing maneuver.

Figure 2: Experimental Respiratory Maneuvers

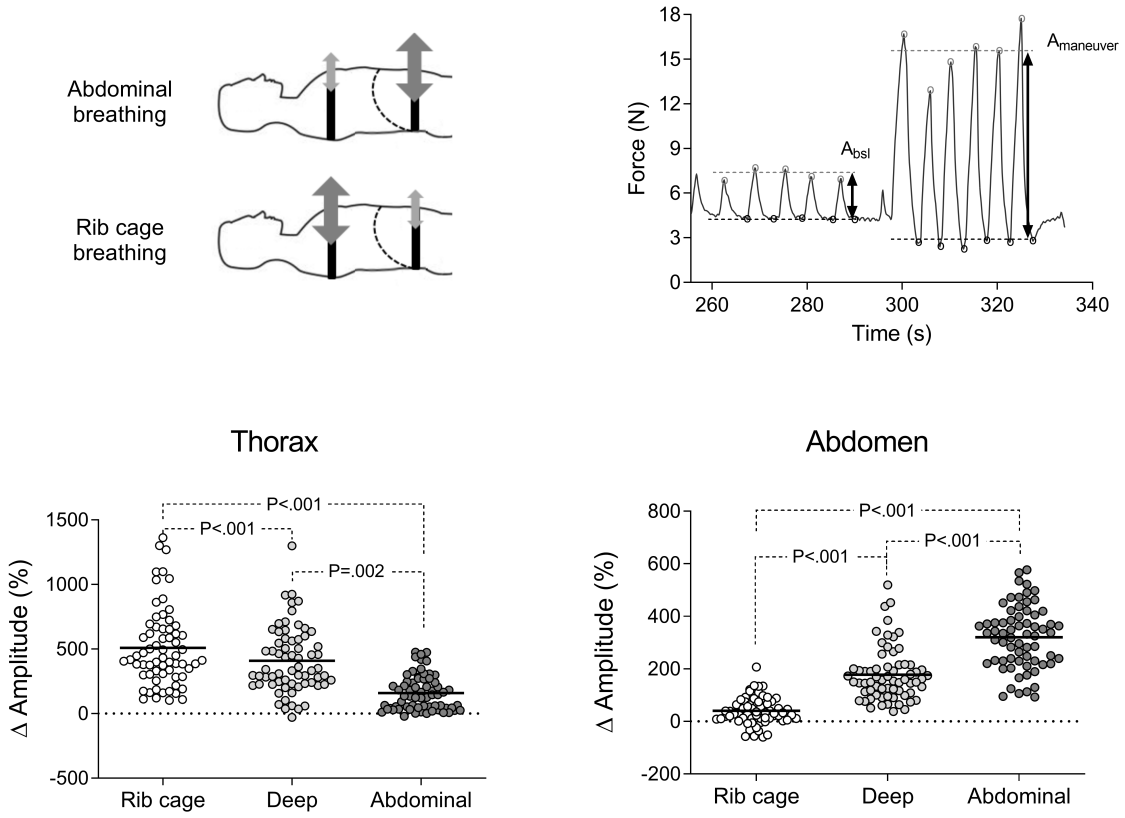


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Figure 3: Effects of Breathing Maneuvers on Resting Cardiac Function

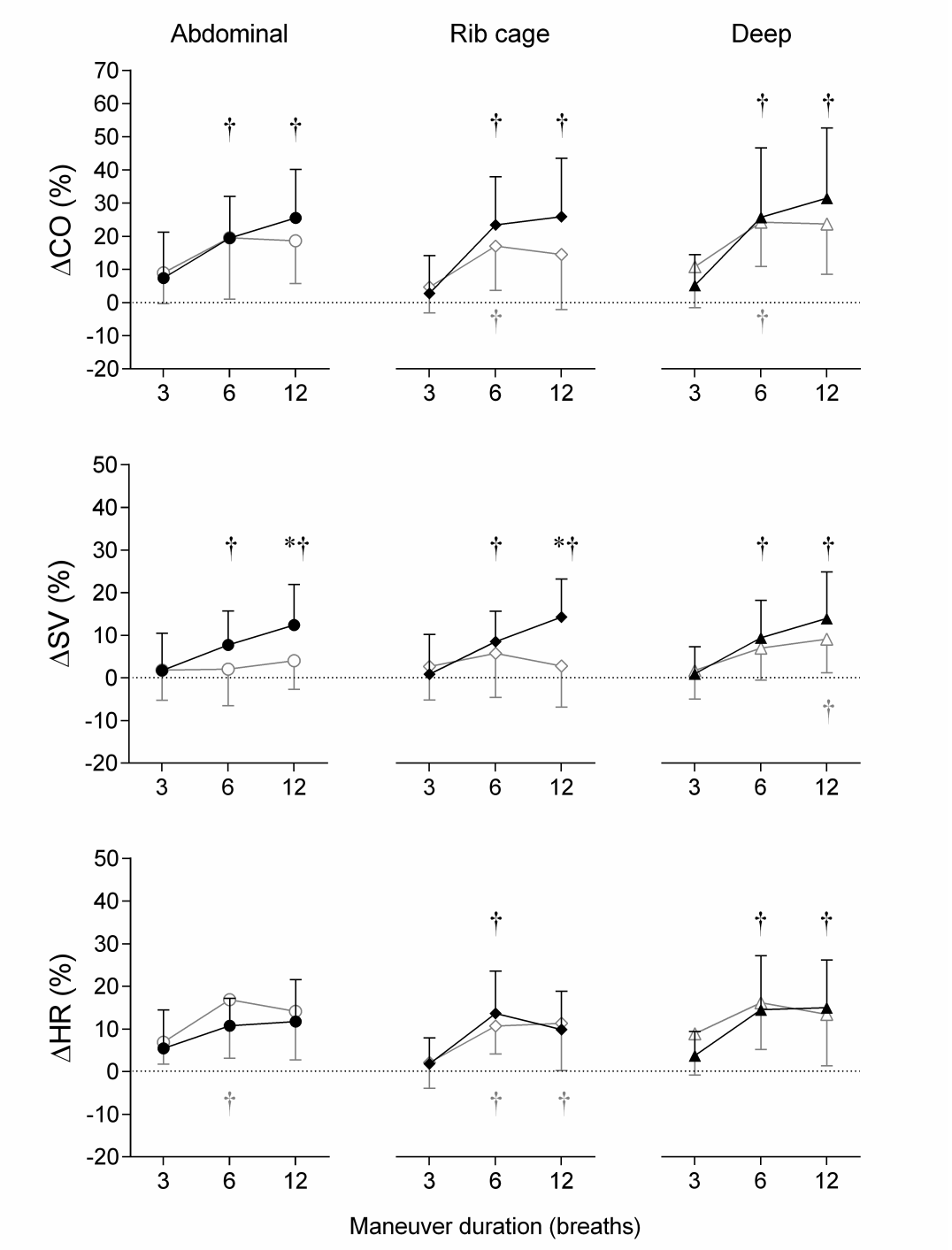

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Figure 4: Physiological Responses to Deep Breathing Maneuvers Performed for Twelve Breaths Prior to Exercise

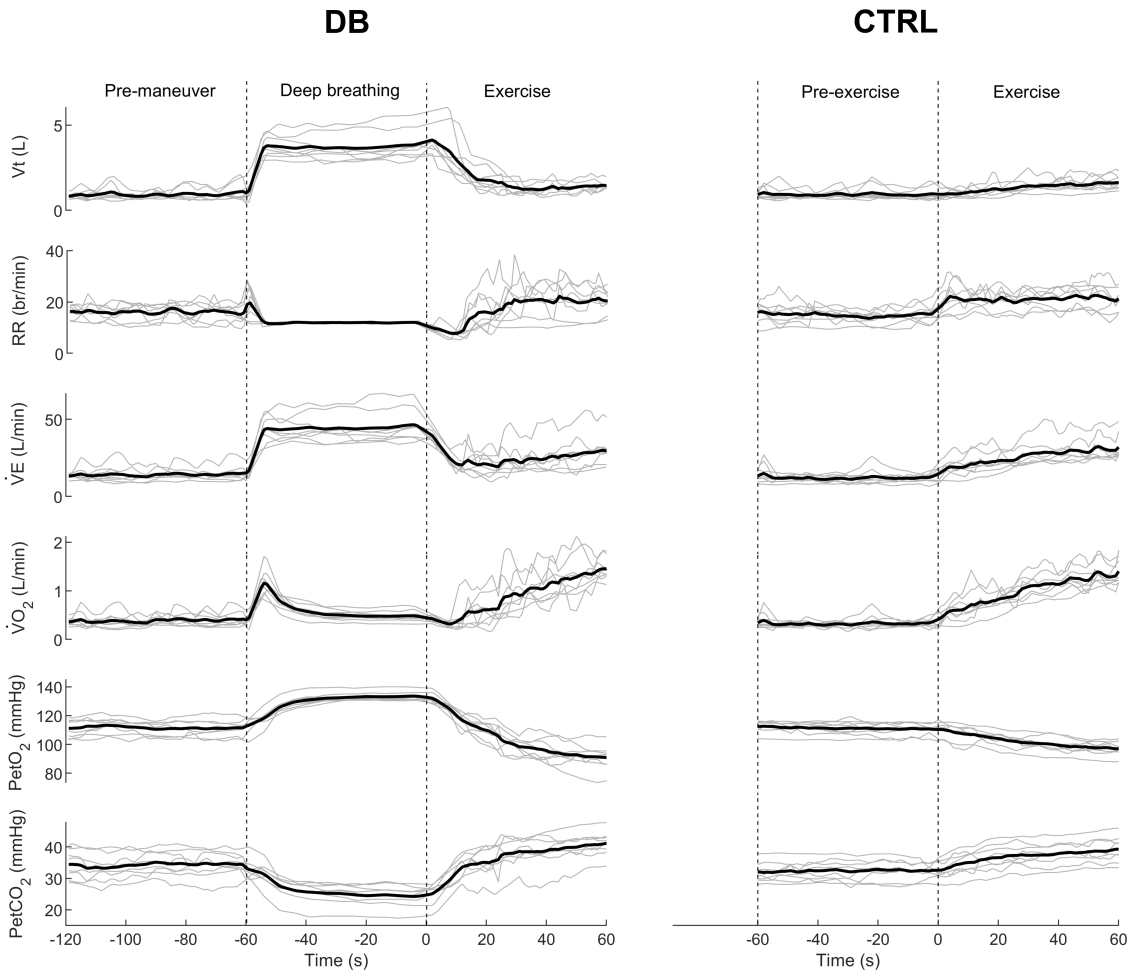
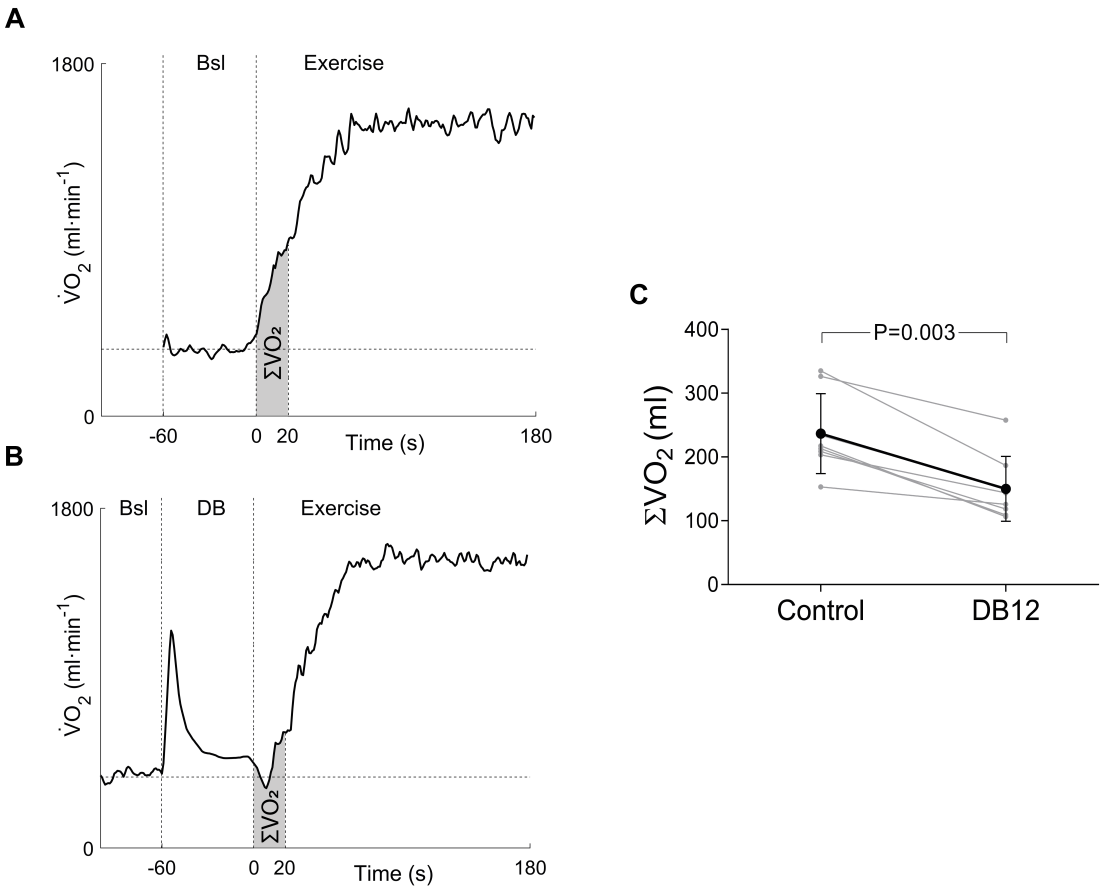


Figure 4: Cardiopulmonary response assessed by continuous gas exchange measurement during the deep breathing maneuver performed prior to exercise onset (DB; *left panel*) and in control condition (CTRL; *right panel*). Black line represents the averaged signal; grey lines are individual tracings. Vt = tidal volume, RR = respiratory rate, VE = ventilation, $\dot{V}O_2$ = oxygen uptake, $PetO_2$ = end-tidal O_2 pressure and $PetCO_2$ = end-tidal CO_2 pressure. N = 8.

Figure 5: Impact of Deep Breathing (DB) Maneuver on Oxygen Exchange During the Cardiodynamic Phase of Early Exercise



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