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Original Research

Influence of VO_2peak on Vagal Recovery Following Breathwork Interventions in ROTC Cadets

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Abstract

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Peak oxygen uptake (VO_2peak) provides a practical index of aerobic capacity when a physiological plateau is not verified; however, its association with acute autonomic recovery following structured breathwork remains unclear. This randomized controlled trial examined whether VO_2peak was associated with post-exercise parasympathetic recovery after accounting for breathwork group. Sixty-two participants (44 males, 18 females) were randomized to box breathing (BB), cyclic sighing (CS), or spontaneous breathing (SB) and completed a graded rowing exercise test to volitional exhaustion followed by a 3-min active recovery under their assigned breathing condition. High-frequency heart rate variability (lnHF-HRV) was assessed at baseline (Pre), 1–3 min post-exercise (Post1), and 4–6 min post-exercise (Post2). Heart rate recovery (HRR) was calculated at 1 and 3 min. Repeated-measures MANOVA and MANCOVA incorporating mean-centered VO_2peak (VO_2peak,c) as a continuous covariate were conducted. A significant Time × Group interaction was observed for lnHF-HRV in the unadjusted model (Pillai's Trace = .191, $F(4, 118) = 3.12$, $p = .018$, $\eta^2p = .096$), with a strong main effect of time ($p < .001$). After adjustment for VO_2peak,c, the Time × Group interaction remained significant (Pillai's Trace = .184, $F(4, 116) = 2.93$, $p = .024$, $\eta^2p = .092$), and the Time × VO_2peak,c interaction was not significant ($p = .314$). Post hoc comparisons indicated higher lnHF-HRV in BB and CS versus SB at Post1 and Post2 ($p < .01$), with no differences between BB and CS. HRR demonstrated a main effect of time ($p < .001$) without significant Group or VO_2peak,c effects. Structured breathwork was associated with more favorable parasympathetic recovery after adjustment for VO_2peak.

Keywords: Autonomic recovery, peak oxygen uptake, tactical physiology, parasympathetic reactivation, vagal modulation, respiratory interventions

Introduction

Maximal oxygen consumption (VO_{2max}) has been recognized as the gold standard for indicating aerobic fitness capacity[1] and serves as a strong predictor for aerobic performance and operational readiness in military populations.[2] In military domains, aerobic performance directly influences mission success, and in a report by the U.S. Army Public Health Command, cardiorespiratory endurance was shown to have strong correlations with key military tasks such as load carriage and casualty drags, when compared to strength only tests.[3] Knapik et al.[4] and Pihlainen et al.[5] similarly found that aerobic training largely produced improvements in military tasks and

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suggests that higher aerobic capacity supports sustained performance in the field. VO_{2max} is associated with enhanced body's ability to deliver and utilize oxygen during sustained exertion by integrating cardiovascular, pulmonary, and muscular efficiency.[6] Individuals with higher VO_{2max} values have been shown to exhibit superior cardiac output, capillary perfusion, and mitochondrial density[7] and given the demand for high intensity physical activity during tactical operations, aerobic capacity is a foundational aspect of long-term preservation and military preparedness.[8]

Beyond physical endurance and task efficiency in military populations, VO_{2max} scores have been strongly associated with autonomic nervous system (ANS) regulation, producing greater adaptability and vagal modulation reflected through heart rate variability (HRV) outputs.[9] HRV captures parasympathetic activity through the heart's ability to adjust to metabolic demand and reflects this shift via high-frequency HRV (HF-HRV). In combat-related environments, autonomic recovery is vital for reducing fatigue related injury risk and safeguarding cognitive performance. Depressed markers of HF-HRV have been shown to have associations with overreaching and impaired readiness.[10] However, in contrast, sustained aerobic training enhances vagal modulation and parasympathetic reactivation post exercise.[9,11] With these adaptations, tactical personnel may be able to transition more efficiently into recovery state from a sympathetically driven state, due to accelerated post-exercise parasympathetic reactivation.[12] Because structured breathing also targets vagally mediated recovery through respiratory modulation, it may provide an acute strategy for supporting the autonomic recovery processes associated with aerobic fitness

Controlled breathing techniques that have emerged over the last two decades, like box breathing (BB) and cyclic sighing (CS), can accelerate parasympathetic activation[13] and emotional regulation through paced respiration[14] following events requiring peak exertion. Both methods modulate respiratory sinus arrhythmia and show an elevation in HF-HRV, which reflects an increase in vagal outflow.[15] These methods are low-cost and easily adaptable in field environments that align with tactical protocols emphasizing acute recovery. However, effectiveness may differ based on the level of aerobic capacity and the individual's overall readiness. Individuals with greater aerobic capacity may enter recovery with greater vagal reserve and faster intrinsic parasympathetic reactivation, which could influence the magnitude of recovery observed during a structured breathing intervention. Research demonstrates that individuals with higher VO_{2max} exhibit quicker vagal reactivation following peak exertional activities.[9] Additionally, individuals demonstrate faster vagally related HRV return to baseline during recovery, namely due to smaller drops in HF-HRV immediately post-exercise, indicating a greater resilience to stress in individuals with greater aerobic fitness levels.[12]

In addition to HRV, heart rate recovery (HRR) is a commonly used indicator of cardiovascular health and autonomic function recovery. HRR is an easily captured reflection of heart rate decline after exertional activities and is primarily mediated by parasympathetic reactivation and sympathetic withdrawal.[16] Faster HRR has been associated with higher aerobic fitness, improved cardiovascular efficiency, and reduced mortality risk.[17] In military populations, delayed HRR has been linked to concerns surrounding elevated training strain, fatigue, and readiness decrements following repeated high-intensity tasks or prolonged exertion.[5] While higher VO_{2max} may indirectly support faster HRR through enhanced cardiac output and autonomic reserve,[9] its moderating influence on HRR is less consistent than for vagal-related HRV indices.[12] As such, HRR may better reflect short-term recovery demands, whereas HRV provides greater insight into underlying autonomic capacity following breathwork interventions.

These adaptations position VO_{2max} as a vital predictor of physiological resilience and responsiveness of the autonomic nervous system, not simply a marker of endurance capacity. Despite robust evidence supporting the role of VO_{2max} in aerobic performance and autonomic regulation, it remains unclear whether aerobic capacity moderates or has an influence on acute autonomic recovery, particularly in response to structured breathwork interventions within ROTC cadets and furthermore tactical populations. In the present investigation, aerobic capacity was operationalized as VO_2peak, defined as the highest oxygen uptake achieved during the graded rowing test, because a physiological plateau in oxygen uptake was not verified.

Accordingly, the aim of the present investigation was to determine whether VO_2peak was associated with HF-HRV and HRR recovery trajectories after accounting for assigned breathwork condition in ROTC cadets. It was hypothesized that higher VO_2peak would be associated with greater HF-HRV recovery following peak exertion.

Methods

A randomized controlled trial with mixed repeated measures was conducted to determine whether VO_2peak was associated with autonomic recovery after accounting for structured breathwork intervention in the acute phase following a graded exercise test to volitional exhaustion. Participants were randomly assigned to one of three intervention groups: BB, CS, or spontaneous breathing (SB). Randomization was accomplished using a computer-generated sequence in R (version 2025.05.1+513) with a block design of six and conducted prior to data collection to ensure balanced group allocation throughout recruitment. Participants were not blinded throughout the investigation because of the nature of the breathwork interventions. However, no participant was introduced to an interventional approach until baseline measurements were completed, thereby reducing the likelihood that assigned breathwork influenced baseline HRV metrics.

Approval was granted from the Concordia University of Chicago and Georgia Southern University's Institutional Review Board (H26016). The utilization of ROTC cadet information was approved by the U.S. Army Cadet Command and the Department of Defense Human Research Protections Office (HPRO). All research was performed following ethical standards of the Declaration of Helsinki and DoD Instruction 3216.02, "Protection of Human Subjects and Adherence to Ethical Standards in DoD-Supported Research". Additionally, this investigation was carried out fully in accordance with the ethical standards of the International Journal of Exercise Science.[18]

Participants

Participants were recruited from the Army's Reserve Officer Training Corps (ROTC) program at Georgia Southern University (n = 77, 53 males, and 24 females, aged 20.77 ± 2.75 years). All participants were screened for active ROTC status, cardiovascular, metabolic, or pulmonary risk, and no participants required a medical clearance when following updated guidance from the American College of Sports Medicine's preparticipation health screening[19] All inclusion and exclusion criteria were assessed prior to randomization. Individuals were excluded if they were pregnant, recently injured, or on a military medical profile that would violate their limited duty status due to physical strain and exertion. Participation in the study was voluntary and not directly influenced by ROTC leadership. Prior to beginning the study, cadets were informed that refusal to participate or withdrawal from the study would have no impact on their standing within the

ROTC program, and they could discontinue participation at any time. Participants were instructed to avoid vigorous-intensity exercise for 12 hours before testing to minimize residual fatigue and prolonged exercise-induced changes in autonomic activity. Participants were also instructed to refrain from caffeine for 4 hours before testing to reduce the acute cardiovascular and autonomic effects of recent ingestion. The shorter caffeine restriction served as a practical standardization period intended to limit recent caffeine intake rather than provide a complete caffeine washout.[20] Testing sessions were scheduled according to participant availability, and time of day was not standardized across participants. All participants in the investigation provided informed consent prior to the initiation of data collection. Ten participants were excluded due to technical issues with the heart rate strap's connectivity, resulting in data discrepancies. An additional five participants were excluded from analysis during data cleaning of HF-HRV metrics because outliers exceeding the ±3 SD criterion were present.

Protocol

Participants arrived and completed a standard physical activity readiness questionnaire for everyone (PAR-Q+), and a health history questionnaire (HHQ). PAR-Q+ and HHQ have demonstrated strong test-retest validity and reliability.[21] Their use aligns with guidance outlined by[19] for screenings and risk stratification and have been frequently published by the American College of Sports Medicine in their guidelines for exercise testing and prescription.[22] Following intake, participants' body mass and height were assessed using standard anthropometric procedures as outlined by the Anthropometric Standardization Reference Manual [23] Body mass was measured with participants in minimal clothing and shoes were removed for height assessments. Body mass was measured to the closest 0.1lb on a calibrated scale (Health O Meter 402KLCW; Health O Meter Professional, McCook, IL, USA), and 0.25 in using a standard non-digital stadiometer (Seca 213 Mobile Stadiometer, Seca GmbH & Co. KG, Hamburg, Germany).

Participants were fitted with a Polar H10 chest strap (Polar Electro, Kempele, Finland) a validated and reliable device for measuring R-R intervals during rest and high-intensity training with 99.4-99.6% agreement with electrocardiogram (ECG) recordings[24] to record heart rate and R–R intervals during supine and recovery HRV assessments. When properly positioned at the lower sternum while allowing for full skin contact, it can record inter-beat intervals at 1,000 Hz with minimal artifact.[25] Additionally, for optimal signal quality, the back of the strap was moisturized with Parker Aquasonic 100 Ultrasound Transmission Gel, allowing for minimal artifacts and ensuring stable conductivity when participants were in motion. Proper electrode contact and signal stability are particularly critical for acute HRV measurements, such as those collected during post-exercise recovery.

After fitting for VO_2 masks and Polar H10 straps, each participant completed a three-minute supine HRV measurement, as indicated in Figure 1. During this phase of testing, HRV was recorded during spontaneous breathing with zero coaching to establish baseline autonomic conditions with zero interventional influence. HRV was analyzed using Kubios HRV Premium software (Kubios Oy, Kuopio, Finland), which has been validated for short-term autonomic function, with high inter-rater and intra-rater reliability for HRV computation and excellent concurrent validity with R-R interval measurements.[26,27]

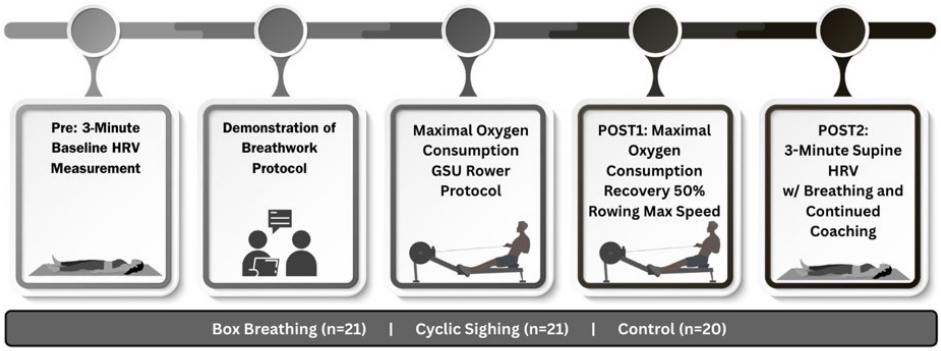


Figure 1. Experimental design and testing sequence.
Note. Following enrollment and group allocation (box breathing, cyclic sighing, or control), participants completed baseline supine HRV assessment, a graded exercise test to volitional exhaustion, and a 3-min active recovery at 50% of peak rowing wattage with the assigned breathing condition. Supine HRV recovery was assessed from 4–6 min post-exercise. Heart rate recovery was calculated at 1 min (HRR1) and 3 min (HRR3) post-exercise.

This investigation aimed at examining the differences between BB, CS, and SB. BB consisted of approximately six breaths per minute following Jerath et al.'s,[28] guidance of a four-second inhalation, a four-second inspiratory hold, a four-second exhalation, and a four-second expiratory hold (4-4-4-4). CS followed Balban et al.'s[13] framework of approximately 5.5 breaths per minute, utilizing a single deep inhalation, followed by a second shorter inhalation, and completion of the cycle with a prolonged exhalation. For SB, participants were not coached in any protocol; rather, they were instructed to breathe as they would normally. During supine measurements, all participants were monitored for movement to mitigate motion artifacts in the data. Considering that many study participants may not have been familiar with performing structured breathwork practices, those assigned to the breathwork conditions were advised to gradually progress toward the target cadence during the cooldown period immediately after the graded rowing exercise test. The BB group specifically were instructed to work up to the pattern warranted for investigation, 4-4-4-4, as a controlled study by[29] found that BB practices resulted in higher post-exercise heart rate and perceived exertion compared to other breathing patterns after high-intensity interval training. HRV was analyzed at baseline (Pre), one to three minutes post-exercise (Post1), and four to six minutes post-exercise (Post2).

Peak oxygen uptake was assessed using a graded rowing exercise test on a Concept2 Model D rower (Concept2 Inc., Morrisville, VT). Participants had prior exposure to rowing because it was incorporated into their regular ROTC physical training. Before the test, the investigator demonstrated proper technique and observed each participant during a brief familiarization trial, providing corrective feedback on the catch, drive, finish, and recovery phases. This familiarization promoted safe and consistent technique but was not a validated rowing-proficiency assessment; individual rowing experience and proficiency were not formally quantified. The rowing ergometer was selected because it was familiar and relevant to participants' regular physical training and provided a consistent whole-body modality. The modality has demonstrated reproducibility for assessment of aerobic fitness in Olympic-style rowing[30] and traditional rowing[31]. The protocol was also conducted in conjunction with a rowing-validation study to reduce participant burden and the risk of fatigue associated with completing an additional maximal test.

Before testing, the Concept2 rower was calibrated, and the drag factor was set at 135 for female participants and 145 for male participants; the drag factor remained constant throughout the test.[32]

As shown in Figure 2, the protocol began at 60 W for females and 100 W for males and increased by 30 W every 3 min. Participants achieved each increase through a self-selected combination of stroke rate and force per stroke while monitoring the ergometer display; stroke rate was not prescribed. Testing continued until participants were unable to maintain the prescribed workload or the required 10 o'clock–2 o'clock torso position, or until volitional fatigue[30,32]. Terminal rating of perceived exertion (RPE) was recorded using the Borg CR-10 scale[33]. RPE was available for 61 of 62 participants (median = 8, range = 4–10) and was treated as a descriptive measure rather than as independent confirmation of VO_2max.

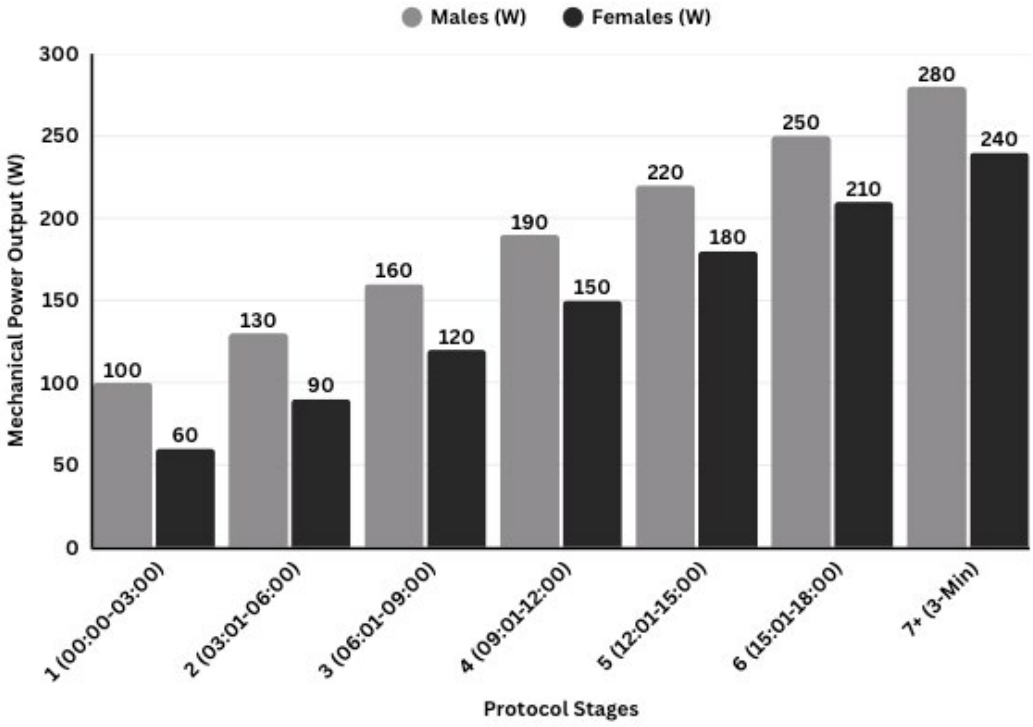


Figure 2. Graded Rowing Exercise Test Protocol.

Note. The graded rowing exercise test consisted of up to seven 3-min stages, with mechanical power output increasing by 30 W at each stage until a termination criterion was met. No participant completed the full 3 min of Stage 7. One participant entered Stage 7 and stopped after 0.04 min (approximately 2.4 s), and one participant stopped at the 18-min transition; therefore, no participant completed Stage 7 and continued at that workload. Gray bars represent male participants' workloads (drag factor = 145), and black bars represent female participants' workloads (drag factor = 135).

Oxygen uptake was measured using a VO_2Master Pro (VO_2 Master Health Sensors Inc., Vernon, BC, Canada), version 1.6.1. Prior validation studies have demonstrated strong agreement between the VO_2Master and laboratory-based metabolic carts, with reported correlations ranging from r = 0.93 to 0.97 relative to the ParvoMedics TrueOne 2400, absolute mean differences below 10% for VO_2, and coefficients of variation between 11.4% and 15.1%.[34] Additional validation has been reported during rowing-specific exercise testing on a Concept2 ergometer.[32] VO_2peak was operationally defined as the highest oxygen-uptake value recorded during the graded exercise test. Because a physiological plateau criterion was not evaluated, the outcome is reported as VO_2peak rather than VO_2max.

Immediately after terminating the graded rowing exercise test because a participant could no longer maintain the prescribed workload or torso position, or because of volitional fatigue, participants engaged in their assigned breathing condition during a 3-min active recovery at 50% of peak rowing wattage. During active recovery, participants were monitored and coached to maintain approximately four breaths per minute for box breathing and 5.5 breaths per minute for cyclic sighing. Participants assigned to BB or CS received brief verbal pacing cues during the initial minute to establish the target breathing rhythm. This guidance included approximately four to five coached respiratory cycles delivered by the principal investigator. Participants then continued the breathing protocol autonomously, with visual oversight used to confirm adherence to the prescribed pacing. Participants assigned to SB engaged in unstructured, spontaneous breathing throughout the 3-min recovery period.

Immediately following the 3-minute cooldown period, participants assigned to BB, CS, and SB were assisted from the graded rowing testing area to a padded table positioned in an adjacent area to reduce noise artifact. Next, participants were directed to lie in the supine position for three minutes. Data were continuously collected during the transition from the testing area to the table. To account for this position transitionary period, 45 seconds of HRV measurements corresponding to this period were discarded, which ensured adequate metrics were recorded and collected for analysis once the participant was in the supine body position for proper methodological considerations for assessment.[20] HRR was determined using absolute peak heart rate attained during the graded exercise test with recovery values recorded at the 1- and 3-minute mark (HRR1 & HRR3, respectively) following accepted methodological guidelines for recovery.[35]

Statistical Analysis

All statistical analyses were conducted using SPSS Statistics (Version 30; IBM Corp., Armonk, NY). Data were screened for missing values, outliers, and violations of distributional assumptions prior to inferential testing. Any participant with incomplete HRV data across time points was excluded from analyses. HF-HRV values were natural log transformed (lnHF-HRV) to improve normality and reduce heteroscedasticity prior to analysis, consistent with established recommendations for HRV data processing.[9,10] R-R intervals generated HF-HRV data and was then exported through Kubios Scientific, using artifact correction at a medium level and smoothness-prior method detrending set at $\lambda = 500$.[36]

Descriptive statistics were conducted for participant characteristics and outcome variables and are presented as means ± standard deviation for normally distributed data and as median and interquartile range (IQR) for non-normally distributed data. Baseline between-group differences, including VO_2peak, were evaluated using one-way analysis of variance (ANOVA) and are reported in Table 1. Baseline equivalence for age, height, weight, BMI, and VO_2peak across treatment groups was analyzed using one-way ANOVA, and a chi-squared test compared sex distribution.

VO_2peak was mean-centered before analysis. Mean-centered VO_2peak (VO_2peak,c) was calculated by subtracting the sample mean VO_2peak from each participant's measured value. Mean-centering preserved the original measurement units and allowed model coefficients to be interpreted at the sample mean aerobic capacity. VO_2peak,c was expressed as $mL \cdot kg^{-1} \cdot min^{-1}$ and was entered as a continuous covariate in the repeated-measures model. The Time × VO_2peak,c term tested whether changes in HF-HRV across measurement periods were associated with aerobic capacity after accounting for breathwork group. Changes in autonomic recovery were

analyzed using a 3 (Time: Pre, Post1, Post2) × 3 (Group: BB, CS, SB) mixed-model design. The primary effect of interest was the Time × Group interaction.

Changes in HF-HRV were evaluated using repeated-measures multivariate modeling rather than derived change scores to preserve within-subject variance and characterize recovery trajectories across time. Linear regression analyses were conducted as secondary analyses to evaluate time-specific associations between treatment group and HF-HRV while adjusting for VO_2peak. Due to attrition and data-quality exclusions, the final analyzed sample comprised 62 participants (BB = 21; CS = 21; SB = 20), which exceeded the a priori minimum of 42 participants established using G*Power 3.1. Therefore, the study was adequately powered to detect moderate effects (f = 0.25) at α = .05 with 80% power. Statistical significance was set a priori at $p \leq .05$, and partial eta squared (η^2p) was reported as a measure of effect size.

Results

Multivariate Effects on HF-HRV Recovery (Unadjusted Model)

A repeated-measures multivariate analysis of variance (MANOVA) was first conducted to examine the effect of Time (Pre, Post1, Post2) × Group (BB, CS, SB) on HF-HRV without inclusion of VO_2peak as a covariate. Pillai's Trace was selected as the primary multivariate test statistic because of its robustness to violations of multivariate assumptions.

The unadjusted MANOVA revealed a significant multivariate Time × Group interaction, indicating different HF-HRV recovery trajectories across breathing conditions before adjustment for VO_2peak. Specifically, Pillai's Trace demonstrated a significant interaction effect (Pillai's Trace = .191, $F(4, 118) = 3.12$, $p = .018$, partial $\eta^2 = .096$), supporting an intervention-related difference in autonomic recovery over time. A strong main effect of time was also observed (Pillai's Trace = .934, $F(2, 58) = 409.55$, $p < .001$), and visual inspection of estimated marginal means indicated that participants assigned to BB and CS exhibited attenuated reductions in HF-HRV at Post1 and greater recovery at Post2 compared with SB.

Table 1. Participant Characteristics.

	Total Group (n = 62)	**Box Breathing (BB, n = 21)**	**Cyclic Sighing (CS, n = 21)**	**Spontaneous Breathing (SB, n = 20)**	***p*-value**
Age (years)	22.0 (3)	20 (3)	20 (3)	20 (3)	.917
Weight (kg)	77.4 ± 15.4	75.4 ± 9.7	78.6 ± 17.2	78.2 ± 18.6	.763
Height (cm)	173.1 ± 9.0	172.3 ± 8.4	175.8 ± 10.8	171.1 ± 7.3	.224
BMI ($kg \cdot m^{-2}$)	25.7 ± 4.0	25.4 ± 3.4	25.2 ± 3.7	26.5 ± 5.0	.558
VO_2peak ($mL \cdot kg^{-1} \cdot min^{-1}$)	44.8 ± 10.0	43.1 ± 11.2	47.4 ± 8.78	44.4 ± 9.9	.265
Resting lnHF-HRV, ln(ms^2)	7.3 ± 1.1	7.8 ± 0.1	7.6 ± 1.0	7.4 ± 1.4	.450
Sex (% male)	71.0%	61.9%	81.0%	70.0%	.394

Note. p-values for continuous variables derived from one-way ANOVA.

Multivariate Effects on HF-HRV Recovery with VO_2peak Adjustment

To evaluate whether aerobic fitness was associated with HF-HRV recovery after accounting for intervention group, a 3 × 3 repeated-measures analysis of covariance was performed with

VO_2peak,c entered as a continuous covariate. The Time × VO_2peak,c interaction was not significant (Pillai's Trace = .040, $F(2, 57) = 1.18$, $p = .314$, partial $\eta^2 = .040$), indicating that the HF-HRV recovery trajectory was not significantly associated with VO_2peak after accounting for intervention group. The adjusted Time × Group interaction remained significant (Pillai's Trace = .184, $F(4, 116) = 2.93$, $p = .024$, partial $\eta^2 = .092$). The between-subject effect of VO_2peak,c across time points did not reach statistical significance, $F(1, 58) = 3.44$, $p = .069$, partial $\eta^2 = .056$, whereas the group effect remained significant, $F(2, 58) = 8.97$, $p < .001$, partial $\eta^2 = .236$. Thus, intervention-related differences in HF-HRV recovery remained statistically significant after adjustment for VO_2peak. A summary of the unadjusted and VO_2peak-adjusted models is presented in Table 2.

Table 2. Multivariate effects on HF-HRV recovery (MANOVA and VO_2peak-adjusted MANCOVA).

Model	Effect	Multivariate Statistic	$F(df_1, df_2)$	p	η_p^2
MANOVA (Unadjusted)	Time	Pillai's Trace = .934	$F(2,58) = 409.55$	< .001	.934
	Time × Group	Pillai's Trace = .191	$F(4,118) = 3.12$	.018	.096
MANCOVA (VO_2peak Adjusted)	Time	Pillai's Trace = .934	$F(2,57) = 404.21$	< .001	.934
	Time × Group	Pillai's Trace = .184	$F(4,116) = 2.93$	.024	.092
	Time × VO_2peak,c	Pillai's Trace = .040	$F(2,57) = 1.18$	.314	.040

Note. η_p^2 = partial eta squared.

For both the MANOVA and MANCOVA models, follow-up simple-effects analyses were conducted to examine the significant Group × Time interaction for HF-HRV. Bonferroni-adjusted pairwise comparisons of estimated marginal means indicated no between-group differences at baseline (Pre; all adjusted p-values > .05). At Post1 and Post2, both breathing intervention groups demonstrated significantly higher lnHF-HRV compared with the control condition (all adjusted p-values < .01), whereas no differences were observed between the two intervention groups. Within-group comparisons demonstrated a reduction in lnHF-HRV from Pre to Post1 across all groups ($p < .001$), followed by partial recovery at Post2, with intervention groups maintaining higher HF-HRV during recovery. Observed mean lnHF-HRV values and their corresponding standard deviations across the three measurement periods and breathing conditions are presented in Figure 3.

Association of Breathwork Intervention and Aerobic Capacity with HF-HRV

To characterize the contribution of VO_2peak to autonomic function, a simple linear regression was first conducted with VO_2peak entered as the sole predictor of baseline lnHF-HRV. VO_2peak explained 7.5% of the variance in baseline lnHF-HRV. Higher VO_2peak values were significantly associated with lower baseline lnHF-HRV values ($R^2 = .075$, $\beta = -.273$, $p = .032$).

Hierarchical linear regression was subsequently conducted at each measurement period to determine whether VO_2peak contributed to HF-HRV after accounting for breathwork condition. Model 1 included breathwork condition, and Model 2 added centered VO_2peak (VO_2peak,c) as a covariate.

Results of the baseline hierarchical regression models are presented in Table 3. Model 1 demonstrated no significant association between BB or CS and baseline lnHF-HRV ($R^2 = .027$, $p = .450$), supporting the absence of pre-existing group differences. Adding VO_2peak,c in Model 2 increased the explained variance to 8.2%; however, the overall model remained nonsignificant ($R^2 = .082$, $p = .174$), and VO_2peak,c did not reach statistical significance after accounting for intervention group ($\beta = -.238$, $p = .068$).

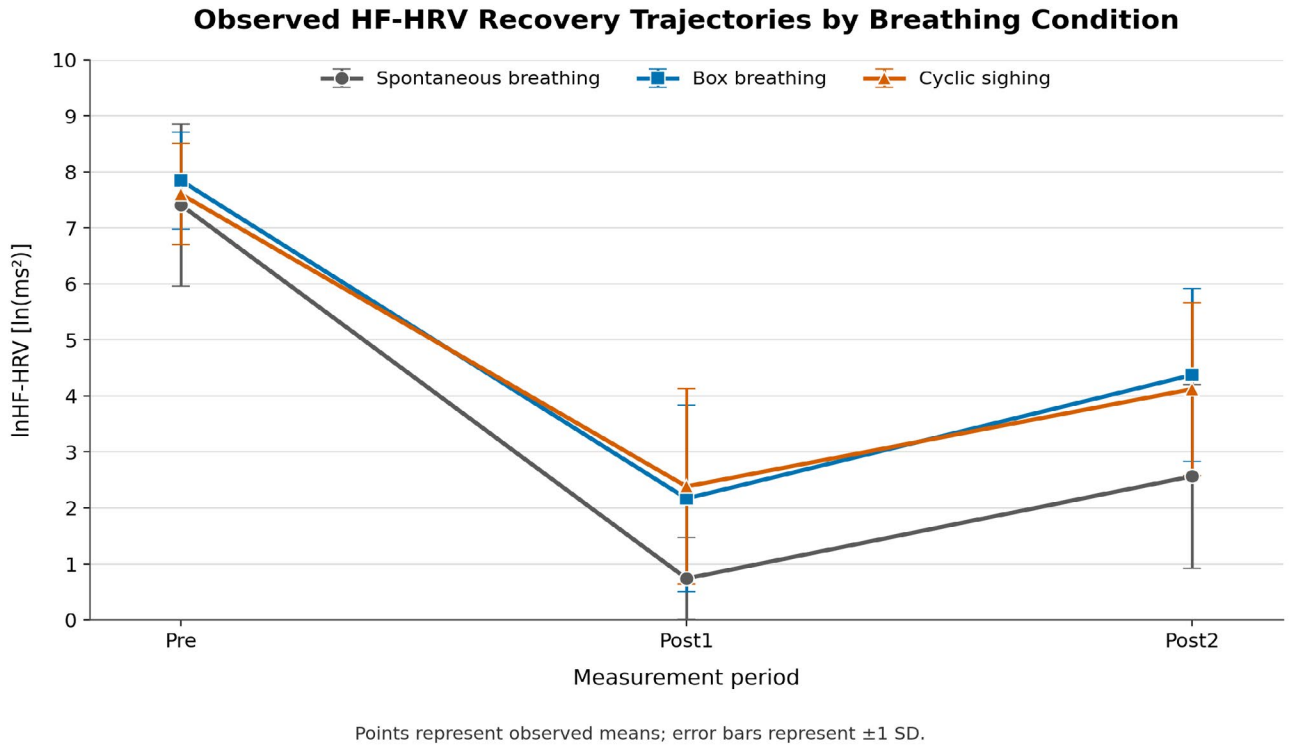


Figure 3. Observed HF-HRV Recovery Trajectories by Breathing Condition.

Note. Observed HF-HRV recovery trajectories according to breathing condition. Points represent unadjusted group means, and error bars represent ±1 standard deviation. Pre represents the baseline supine measurement, Post1 represents 1–3 minutes following the graded rowing exercise test, and Post2 represents 4–6 minutes following the test. HF-HRV values were natural-log transformed before analysis. Inferential conclusions were based on the repeated-measures models reported in Table 2.

Table 3. Linear Regression Models Predicting HF-HRV at Each Time Point Adjusted for VO_2peak.

Outcome (log HF-HRV)	Predictor	B	SE	β	p
Pre (Baseline)	Box Breathing vs Control	0.416	0.336	0.181	.221
	Cyclic Sighing vs Control	0.284	0.340	0.124	.407
	VO_2peak,c	-0.026	0.014	-0.238	.068
Post1 (1-3 min)	Box Breathing vs Control	1.420	0.460	0.420	.003
	Cyclic Sighing vs Control	1.679	0.465	0.497	<.001
	VO_2peak,c	-0.011	0.019	-0.064	.591
Post2 (4-6 min)	Box Breathing vs Control	1.778	0.480	0.487	<.001
	Cyclic Sighing vs Control	1.696	0.485	0.464	<.001
	VO_2peak,c	−0.040	0.020	-0.228	.052

Note. Control (spontaneous breathing) served as the reference group. HF-HRV values were log-transformed. VO_2peak was mean-centered (VO_2peak,c) and entered as a covariate in Model 2. Separate linear regression models were conducted for each time point. Statistical significance was set at α = .05.

Results of the Post1 linear regression models are presented in Table 4. Model 1 explained approximately 20% of the variance in HF-HRV (R^2 = .204, p = .001), with BB and CS predicting higher HF-HRV than SB. Adding VO_2peak,c in Model 2 produced a negligible increase in

explained variance (R^2 = .208), and VO_2peak,c was not significantly associated with HF-HRV at Post1 (p = .591).

Table 4. Post1 (1-3-min) Linear Regression Models Predicting HF-HRV (log).

	Predictor	B	SE	β	p	R^2
Model 1	Box Breathing	1.428	0.457	0.422	.003	.204
	Cyclic Sighing	1.643	0.457	0.486	< .001	
Model 2	Box Breathing	1.420	0.460	0.420	.003	.208
	Cyclic Sighing	1.679	0.465	0.497	<.001	
	VO_2peak,c	-0.011	0.019	-0.064	.591	

Note. Control (spontaneous breathing) served as the reference group. HF-HRV values were log-transformed. Model 1 included breathwork condition only. Model 2 added mean-centered peak oxygen uptake (VO_2peak,c) as a covariate. R^2 values represent total variance explained. Statistical significance was set a priori at α = .05.

Results of the Post2 linear regression models are presented in Table 5. Model 1 explained approximately 21% of the variance in HF-HRV (R^2 = .211, p < .001). Adding VO_2peak,c in Model 2 increased explained variance to R^2 = .261. The association between VO_2peak,c and HF-HRV approached but did not reach statistical significance at Post2 (B = −0.040, p = .052). The magnitude and significance of the breathwork coefficients remained similar after VO_2peak,c was added.

Table 5. Post2 (4-6-min) Linear Regression Models Predicting HF-HRV (log).

	Predictor	B	SE	β	p	R^2
Model 1	Box Breathing	1.808	0.492	0.495	<.001	.211
	Cyclic Sighing	1.557	0.492	0.426	.002	
Model 2	Box Breathing	1.778	0.480	0.487	<.001	.261
	Cyclic Sighing	1.696	0.485	0.464	<.001	
	VO_2peak,c	−0.040	0.020	-0.228	.052	

Note. Control (spontaneous breathing) served as the reference group. HF-HRV values were log-transformed. Model 1 included breathwork condition only. Model 2 added mean-centered peak oxygen uptake (VO_2peak,c) as a covariate. R^2 values represent total variance explained. Statistical significance was set a priori at α = .05.

Multivariate Effects on HRR Recovery (Unadjusted Model)

Heart rate recovery was analyzed using a 2 (Time: $HRR_1\Delta$, $HRR_3\Delta$) × 3 (Group: BB, CS, SB) repeated-measures model. A significant main effect of Time was observed, Pillai's Trace = .362, F(1, 59) = 33.51, p < .001, ηp^2 = .362, indicating that HRR differed across the recovery intervals. However, the Time × Group interaction was not significant, Pillai's Trace = .031, F(2, 59) = 0.95, p = .391, ηp^2 = .031, indicating that HRR trajectories did not differ between breathing conditions (Table 6).

Multivariate Effects on HRR Recovery with VO_2peak Adjustment

After adjustment for VO_2peak,c, the HRR results remained similar to those of the unadjusted multivariate model. There was a significant main effect of time (Pillai's Trace = .377, F(1, 58) = 35.06, p < .001, partial η^2 = .377), whereas the Group × Time interaction was not significant (Pillai's Trace = .019, F(2, 58) = 0.55, p = .578, partial η^2 = .019). The Time × VO_2peak,c interaction approached but did not reach statistical significance (Pillai's Trace = .058, F(1, 58) = 3.59, p = .063, partial η^2 = .058). Thus, acute breathing interventions did not differentially influence HRR in

this sample, although the near-significant VO_2peak,c trend and restricted fitness range warrant cautious interpretation.

Table 6. Multivariate effects on HRR (MANOVA and VO_2peak-adjusted MANCOVA).

Model	Effect	Multivariate Statistic	$F(df_1, df_2)$	p	η_p^2
MANOVA (Unadjusted)	Time	Pillai's Trace = .362	$F(1,59) = 33.51$	< .001	.362
	Time × Group	Pillai's Trace = .031	$F(2,59) = 0.95$	.391	.031
MANCOVA (VO_2peak Adjusted)	Time	Pillai's Trace = .377	$F(1,58) = 35.06$	< .001	.377
	Time × Group	Pillai's Trace = .019	$F(2,58) = 0.55$	.578	.019
	Time × VO_2peak,c	Pillai's Trace = .058	$F(1,58) = 3.59$	.063	.058

Note. η_p^2 = partial eta squared.

Discussion

The primary aim of this investigation was to determine whether VO_2peak was associated with acute autonomic recovery after accounting for breathwork intervention. BB and CS were associated with more favorable HF-HRV recovery trajectories than SB. The unadjusted MANOVA demonstrated a significant Time × Group interaction, and this effect remained significant after adjustment for VO_2peak,c, with minimal attenuation of effect size. The present models therefore show that intervention-related differences persisted after statistical adjustment for VO_2peak; they do not establish that the effects were independent of aerobic capacity in all populations.

Prior literature has associated higher VO_2max with quicker parasympathetic recovery and attenuated post-exercise HRV suppression.[12] Previous work has also shown that individuals with greater aerobic fitness may exhibit smaller immediate reductions in HF-HRV following maximal or near-maximal exercise.[9,37] and more rapid parasympathetic reactivation during recovery.[12] These relationships may be more apparent when aerobic capacity spans a wide range, including individuals with low fitness. In the present investigation, ROTC cadets' VO_2peak values occupied a relatively narrow portion of published age- and sex-based reference ranges.[22,38] This restricted variability likely reduced statistical power to detect associations between VO_2peak and recovery. Within this sample, VO_2peak may have functioned more as a general indicator of aerobic fitness than as a differentiating factor in responsiveness to breathwork.

From an applied perspective, aerobic capacity is an important contributor to autonomic function and recovery[12,39] and these findings suggest that BB and CS may be feasible low-cost recovery strategies requiring minimal training and no equipment. In military and tactical settings, either technique could be incorporated as a brief, approximately 3-minute paced-breathing component during an active cooldown following strenuous physical training, fitness testing, or repeated high-intensity tactical tasks. However, because the present sample represented a relatively narrow aerobic-fitness range, these findings should not be generalized to individuals at the lowest or highest ends of the fitness spectrum without additional study.

Several limitations should be considered. First, the relatively homogeneous, physically active ROTC sample and the restricted range of VO_2peak values likely reduced power to detect associations between aerobic capacity and recovery. Second, a physiological VO_2 plateau was not evaluated; therefore, the highest observed oxygen uptake is appropriately reported as VO_2peak rather than VO_2max. Terminal RPE was available for participants and ranged from 4 to 10, so it was retained as a descriptive measure and cannot independently verify maximal physiological effort. Third,

the acute design precludes inference regarding longer-term adaptations to breathwork practice. Longitudinal studies should determine whether baseline HRV, recovery kinetics, or training tolerance predict responsiveness to repeated breathwork.

Fourth, HRR was measured during an active rowing recovery rather than during passive, seated, or supine recovery. Although recovery intensity was standardized at 50% of each participant's peak rowing wattage, absolute workloads differed among participants and may have influenced the rate of heart-rate decline. The assigned breathing conditions were also performed during this interval. Therefore, the HRR findings reflect recovery under the combined conditions of active exercise, individualized absolute workload, and assigned breathing strategy and should not be generalized to standardized passive-recovery HRR protocols. Fifth, although HF-HRV was selected as the primary outcome because of the study's focus on vagally mediated recovery, LF-HRV was not included in the statistical analyses. The prescribed breathing frequencies of approximately 0.067 Hz for box breathing and 0.092 Hz for cyclic sighing fall within the conventional LF range of 0.04–0.15 Hz. Consequently, some respiratory-related vagal oscillations may have been represented within LF-HRV rather than HF-HRV. The exclusive use of HF-HRV may therefore provide an incomplete characterization of the autonomic response to slow breathing. Future analyses should incorporate LF-HRV, total power, time-domain measures, and directly assessed respiratory frequency. Sixth, time of day was not controlled for during this investigation, ROCT cadets had minimal time allowed for participation and the measurements were captured spanning throughout the duration of the day. Finally, respiratory frequency was prescribed for the BB and CS conditions but was not directly assessed in the SB group; therefore, breathing frequency could not be compared across groups. This limits our ability to determine the extent to which between-group differences in lnHF-HRV reflected direct respiratory modulation rather than differences in post-exercise parasympathetic recovery.

In conclusion, structured breathwork may enhance post-exercise autonomic recovery, as reflected by HF-HRV patterns. Intervention-related differences remained statistically significant after adjustment for VO_2peak, while VO_2peak was not significantly associated with the HF-HRV recovery trajectory in this physically fit ROTC cohort. Restricted variability in aerobic fitness and the absence of a verified VO_2 plateau limit broader inference. BB and CS may be practical recovery strategies for military settings, but additional research with a wider range of VO_2peak values and more comprehensive autonomic measures is needed.

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Conflict of Interest

The authors declare no professional relationships with companies or manufacturers that would benefit from the results of the present study. No financial or commercial conflicts of interest exist. The results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

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