

Parasympathetic Reactivation Following Maximal Exercise: Influence of Breathwork and Body Composition in ROTC Cadets

Original Research

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Abstract

Introduction: Autonomic recovery is critical for tactical readiness. While structured breathwork enhances parasympathetic reactivation, it is unclear whether body composition indices such as percent body fat (%BF) or fat-free mass index (FFMI) influence acute recovery following maximal exertion. This study examined whether %BF or FFMI moderate post-exercise autonomic recovery in ROTC cadets.

Methods: Fifty-two cadets (age 20.0 ± 3 yrs; %BF 28.5 ± 7.7 ; FFMI 18.4 ± 2.8) completed a maximal rowing test and were randomized to box breathing (BB, $n = 19$), cyclic sighing (CS, $n = 17$), or spontaneous breathing (SB, $n = 16$) during a 3-min active recovery. HF-HRV was measured at baseline, 1–3 min, and 4–6 min post-exercise. HRR was calculated at 1 and 3 min. Repeated-measures MANOVA and %BF- and FFMI-adjusted MANCOVA were performed.

Results: A significant main effect of time was observed for HF-HRV (Pillai's Trace = .934, $F(2,48) = 339.86$, $p < .001$). The Time \times Group interaction reached significance in the FFMI-adjusted model (Pillai's Trace = .190, $F(4,96) = 2.52$, $p = .046$). Time \times %BF and Time \times FFMI interactions were non-significant ($p \geq .271$). Regression analyses showed BB and CS predicted higher HF-HRV at Post1 ($R^2 = .193$) and Post2 ($R^2 = .224$; $p \leq .017$), whereas %BF and FFMI were not significant predictors. HRR demonstrated a main effect of time ($p < .001$) without group or body composition effects.

Conclusions: Structured breathwork enhanced post-exercise parasympathetic reactivation independent of %BF and FFMI. Body composition did not significantly influence acute autonomic recovery in this cohort.

Key Words: fat-free mass, box breathing, cyclic sighing, vagal tone, VO_{2max}

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Introduction

Autonomic recovery following peak exertion is a vital aspect in determining tactical readiness in the United States Military^{1–3}. Service members frequently engage in repeated bouts of high-intensity physical training and cognitive stressors, elevating sympathetic drive, autonomic nervous system regulation, potentially impairing parasympathetic reactivation, increasing cumulative fatigue, delaying physiological recovery, and increasing injury susceptibility. Therefore, laboratory methods like heart rate variability (HRV) and field assessments such as heart rate recovery (HRR) are widely accepted indicators for recovery efficiency, reflecting the balance between sympathetic withdrawal and parasympathetic reactivation, following these elevated

states^{4,5}. While aerobic capacity has traditionally been emphasized as a primary driver of post-exercise recovery efficiency, recent investigations suggest that body composition may exert an independent and meaningful influence on autonomic recovery dynamics^{3,6}.

Body composition has long been associated with operational performance across military populations. Lower body fat percentage (%BF) has consistently been linked to superior performance on combat-relevant fitness assessments in both males and females, while greater fat-free mass (FFM) and skeletal muscle mass (SMM) predict enhanced strength, power, and task completion efficiency⁷⁻⁹. In Reserve Officer Training Command (ROTC) cadets, %BF demonstrates an inverse relationship with multiple Army Combat Fitness Test events, whereas FFM explains a meaningful proportion of variance in total performance scores and individual strength and power tasks⁷. Collectively these findings suggest that FFM may be a more strongly associated indicator of readiness than total body mass or body mass index (BMI) alone, as BMI may confound physiological variability because it cannot differentiate between fat mass (FM) and FFM¹⁰⁻¹².

For this investigation, FFM refers to all non-adipose tissue components measured, such as skeletal muscle, organ tissue, and bone mineral content. SMM represents the contractile component of FFM; however, because dual-energy X-ray absorptiometry (DXA) derived FFM was used for this analysis, FFM is the primary index of relative muscularity examined.

Beyond physical performance, an individual's body composition appears to be substantially linked to post-exercise autonomic recovery, indicating that elevated %BF reduced parasympathetic activity and a delayed HRR^{3,13}. Conversely, greater FFM and lower %BF exhibit more favorable HRV profiles, reflecting enhanced vagal modulation and recovery capacity ($r = 0.30 - 0.40$) in young adult samples⁶. Furthermore, investigations in military firefighters demonstrate a strong inverse relationship between cardiorespiratory fitness, adiposity indexes, and autonomic markers ($r = -0.65$ for %BF)¹⁴. These findings reinforce the interdependence of body composition and physiological recovery. Collectively, these performance-oriented findings align with a broader autonomic demonstration of research that excess %BF is associated with altered sympathetic vagal balance and further impairment of cardiovascular recovery.

Autonomic dysfunction has been linked to excess adiposity and characterized by heightened sympathetic activity¹⁵ and significantly reduced parasympathetic tone leading to diminished HRV and a slower HRR following acute exercise ($p = .05$)¹⁶. Abdominal adiposity is associated with impaired vagal activity and delayed cardiovascular recovery in both males and females and appears to be a particularly detrimental aspect of recovery metrics due to central and visceral fat accumulation^{17,18}. These physiological consequences are especially relevant in military environments, where incomplete recovery between training bouts or missions may elevate injury risk, fatigue accumulation, and performance decrements^{1,3}.

Despite documented variability in body composition and autonomic responses across individuals, it remains unclear whether %BF or FFM independently influences parasympathetic recovery in an acute sense, following maximal exertion in military populations. BMI has well documented limitations and military and physically trained populations, primarily due to its inability to distinguish between fat mass and lean mass. As a result, BMI frequently misclassifies physically capable service members as overweight and fails to identify individuals with sufficient lean mass despite acceptable body weight^{11,12}. In response to the limitations of BMI in military populations, the fat-free mass index (FFMI) has emerged as a more functionally relevant indicator of physical fitness. FFMI is derived by normalizing fat-free mass to stature ($FFM/height^2$), thus allowing for the assessment of relative muscularity independent of total body mass or central adiposity, ultimately demonstrating more accuracy^{19,20}. Recently, Sergi et al.¹⁹ developed sex-specific FFMI normative values and proposed lower limit thresholds in Army personnel representing insufficient muscularity relative to height. These thresholds were defined relative to sex-specific normative FFMI reference ranges derived from using population-based distributional methods and demonstrated that a subset of service members classified as acceptable by BMI and %BF, exhibited FFMI values below recommended reference ranges. The authors of this study suggest this metric could serve as a modernized indicator of body composition relatively and suggest that FFMI may offer value beyond typical BMI and traditional %BF analysis when examining recovery capacity in tactical populations.

Structured breath work has been shown to influence autonomic regulation, particularly through techniques that enhance vagal tone. Cyclic sighing (CS), characterized by prolonged exhalations, reduces physiological arousal and increases parasympathetic activity, resulting in a more favorable HRV response²¹⁻²³. This technique may be relevant within tactical populations, where autonomic recovery following high-risk scenarios, or peak exertion is vital for

maintaining composure while also mitigating cognitive fatigue^{22,24}. Another breathing technique known as box breathing (BB), or tactical breathing, involves 4 distinct phases involving equal duration inhalations, breath holds, and exhalation²⁴⁻²⁶. This approach has demonstrated efficacy for stress regulation in high pressure tactical special operations settings. Despite growing evidence supporting breathwork induced improvements in autonomic regulation, and metabolic modulation, these effects following maximal exertion remain underexplored in tactical populations^{21,24,27}.

Despite evidence linking adiposity and relative muscularity to autonomic regulation in clinical and mixed populations, it remains unclear whether %BF and FFMI meaningfully influence acute parasympathetic recovery following maximal exertion in young, tactically trained personnel. Accordingly, the purpose of this investigation is to examine the extent to which %BF and calculated FFMI independently predict autonomic recovery following maximal exertion in ROTC cadets. This investigation addresses the main hypotheses that %BF would be inversely associated with HRV and HRR, whereas FFMI would demonstrate a positive association with these same recovery markers following maximal exertion. Defining the nature of these relationships may enhance future physiological monitoring strategies and inform individualized approaches in recovery training for service members and command teams aimed at optimizing unit readiness and resilience in military populations.

Methods

A randomized controlled trial with mixed repeated measures was conducted to determine if %BF and/or FFMI moderated the effect of autonomic recovery when paired with a structured breathwork intervention in the acute phase following maximal exertion. Participants were randomly assigned to one of three intervention groups: BB, CS, or spontaneous breathing (SB). Randomization was achieved using a computer-generated sequence in R (version 2025.05.1+513). A block design of six was planned prior to data collection to ensure balanced group allocation throughout recruitment. Participants were not blinded throughout the entirety of the investigation due to the nature of the breathwork interventions. However, until baseline breathing measurement was concluded, no participant was introduced to any interventional approach to ensure that baseline HRV metrics were not influenced.

Approval was granted from the appropriate university's Institutional Review Board (H26016) and was further 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".

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). The female participant distribution of the present sample is a slightly higher proportion of female cadets (~31%), compared to the normal representation within cadet commands of approximately 20-26% of commissioned officers in training²⁸. All participants were individually screened during an intake interview 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 protocols²⁹. Individuals were also screened to determine if they were pregnant, had a recent injury, or were on a military medical profile that would violate their limited duty status due to physical strain and exertion, with zero participant loss due to this criterion. 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 advised to avoid vigorous intensity exercise for 12-hours prior to testing and refrain from ingesting caffeine for 4 hours prior to testing, as this may have unwanted effects on HRV spectral analysis³⁰. All participants involved in the investigation provided informed consent. Ten participants were excluded due to technical problems with heart rate strap connectivity and resulting data discrepancies. An additional five participants were removed during data cleaning due to the presence of outliers exceeding ±3 SD criterion with the HF-HRV data. Lastly, 10 participants did not conduct the body composition analysis, leaving 52 participants in the final sample for this investigation.

Intake, Anthropometric Assessment, and Equipment Fitting

Participants arrived and completed a standard physical activity readiness questionnaire for everyone (PAR-Q+), health history questionnaire (HHQ), and university's informed consent. PAR-Q+ and HHQ have demonstrated strong test-retest validity and reliability³¹, align with guidance provided by Riebe et al.²⁹ 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 ³². Following intake, participants' body mass and height were assessed using standard anthropometric procedures as outlined by the Anthropometric Standardization Reference Manual ³³. Body mass was measured with participants in minimal clothing, and their 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 height to the nearest 0.25in 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 having 99.4-99.6% agreement with electrocardiogram recordings ³⁴ 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, this device can record inter-beat intervals at 1,000 Hz with minimal artifact ³⁵. Additionally, for optimal signal quality, the back of the strap was moisturized with Parker Aquasonic 100 Ultrasound Transmission Gel, allowing for minimal artifact and ensuring stable conductivity when participants were in motion. Proper electrode contact and signal stability are particularly critical for acute HRV measurements.

Dual-Energy X-ray Absorptiometry

Body composition was assessed utilizing Dual-Energy X-ray Absorptiometry (DXA; Hologic®, Bedford, MA, USA), which provides precise estimates of lean soft tissue, total and regional fat mass, and bone mineral content. DXA is widely regarded as a reference standard in body composition analysis and has demonstrated high test-retest reliability and strong validity for %BF assessments across athletic and tactical populations ³⁶. Typical coefficients of variation were below 2% for fat mass and lean mass estimates ^{37,38}

Prior to beginning DXA analysis, participants were instructed to remove all metal objects and scans were performed with participants wearing only minimal clothing. Following standard positioning protocols, participants were scanned in a supine position with arms placed at their sides and feet in a neutral position, with toes pointed towards the ceiling. All scans were performed by the same three trained technicians to reduce inter-rater variability. Daily quality control procedures were conducted along with calibration in accordance with manufacturer guidelines, prior to any data collection on the respective days.

Whole body %BF and fat-free mass (FFM) were extracted and calculated from DXA outputs. FFM index (FFMI) was calculated by normalizing FFM consistent with prior work in U.S. Army populations ¹⁹, as shown in Equation 1., and Equation 2 respectively:

Equation 1. Fat Free Mass

$$\text{FFM (kg)} = \text{Body Mass (kg)} \times \left(1 - \frac{\%BF}{100}\right)$$

Equation 2. Fat-Free Mass Index

$$\text{FFMI} = \frac{\text{Height (m)}^2}{\text{FFM (kg)}}$$

Heart Rate Variability & Heart Rate Recovery

After fitting for VO₂ masks and Polar H10 straps, each participant completed a three-minute supine HRV measurement, as indicated in Figure 1. During this phase of testing, spontaneous breathing with zero coaching was monitored 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 ^{39,40}.

Pre-Test Breathwork Protocol Demonstration

To evaluate autonomic recovery under different breathing conditions, participants were instructed in one of three breathwork protocols prior to testing, BB, CS, and spontaneous breathing (SB). In the BB group, participants were instructed to maintain approximately six breaths per minute following Jerath et al.'s 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) ²⁴. In the CS group, participants followed Balban et al.'s 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 exhalation²³. 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 VO₂max test. Specifically, the BB group were instructed to work up to the pattern warranted for investigation, 4-4-4-4, as a controlled study by Kasap and Aydin reported that BB practices resulted in higher post-exercise heart rate and perceived exertion compared to other breathing patterns after high-intensity interval training⁴¹. HRV was analyzed at baseline (Pre), one to three minutes post-exercise (Post1), and four to six minutes post-exercise (Post2).

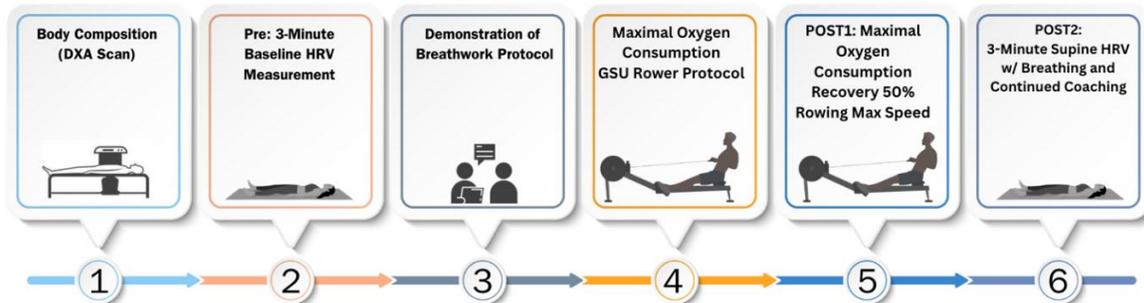


Figure 1. Experimental design and testing sequence.

Note. Following enrollment and group allocation (box breathing, cyclic sighing, or control), participants completed a body composition analysis via Dual-Energy X-ray Absorptiometry (DXA), followed by baseline supine HRV assessment, a graded exercise test to volitional exhaustion, and a 3-min active recovery at 50% of maximal rowing wattage while performing 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.

Maximal Oxygen Consumption

Maximal aerobic capacity was assessed utilizing a graded rowing protocol on a Concept2 Model D rower (Concept2 Inc., Morrisville, VT), which has been validated and deemed reproducible for assessing aerobic fitness in both Olympic style⁴² and traditional rowing practices⁴³, producing near perfect correlation with peak VO₂ performance with reliability confirmed during incremental testing.

Before testing began, the Concept2 Rower was calibrated to ensure accuracy, with a drag factor set at 135 for female participants and 145 for males⁴⁴. As shown in Table 1, the protocol commenced at an initial workload of 60W for females and 100W for males, with increases of 30W every three minutes. Exercise testing continued until participants were unable to maintain the required 10 o'clock – 2 o'clock torso position or they reached volitional fatigue, defined as inability to sustain the prescribed workload^{42,44}. Each participant recorded their rate of perceived exertion at the end of the VO₂max test to confirm maximal exertion using the BORD CR-10 scale⁴⁵.

Table 1. Maximal Oxygen Consumption Protocol

Stage	Time Interval	Males (W)	Female (W)
1	00:00 – 03:00	100	60
2	03:01 – 06:00	130	90
3	06:01 – 09:00	160	120
4	09:01 – 12:00	190	150
5	12:01 – 15:00	220	180
6	15:01 – 18:00	250	210
7	≥18:00 (3-min)	280	240

Note. The maximal oxygen consumption (VO₂max) protocol consisted of a staged graded exercise test with progressive increases in workload across seven stages. Each stage lasted three minutes, with mechanical power output (W) increasing incrementally until volitional exhaustion. The final stage (7+) reflects participants who continued beyond the sixth stage until termination criteria were met and any continual stages will add 3-min

Maximal oxygen uptake was measured using a VO2Master Pro (VO2 Master Health Sensors Inc, Vernon, BC, Canada), version 1.6.1. Prior validation studies have demonstrated strong agreement between the VO₂ Master 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 VO₂, and coefficients of variation between 11.4% and 15.1%⁴⁶. Additional validations have been demonstrated during rowing-specific exercise testing on a Concept2 ergometer⁴⁴

Post 1 Breathwork Protocol

Immediately after reaching VO₂max or failing to maintain the prescribed torso positioning^{42,44}, participants engaged in their respective breathing condition for a three-minute cooldown at 50% of their maximal rowing wattage. During the cooldown phase, all participants were monitored and coached to maintain the respiratory rate of ~4 breaths per minute for BB and ~5.5 breaths per minute for CS. During the recovery interval, participants assigned to the breathing interventions were provided brief verbal pacing cues from the principal investigator during the initial minute to facilitate establishment of the target breathing rhythm, covering approximately four to five respiratory cycles. After this familiarization period, participants continued the breathing protocol autonomously, with visual oversight used to confirm adherence to the prescribed pacing. Participants assigned to the control condition (SB) engaged in unstructured, spontaneous breathing throughout the 3-minute recovery period.

Post 2 Breathwork Protocol

Immediately following the 3-minute cooldown period, participants assigned to BB, CS, and SB were assisted from the VO₂max testing area to a padded table positioned in an adjacent area to reduce noise artifact³⁰. Here, participants were directed to lie in the supine position for a minimum of three minutes. Data was continuously collected during the transition from the testing area to the table. However, 45-seconds of data recorded during this transitional period were discarded, which ensured anomalous HRV measurements resulting from participant movement during this period time were excluded from analysis. This approach ensured adequate data were recorded and collected for analysis once the participant returned to supine body position while meeting appropriate methodological considerations for data assessment³⁰. HRR was determined using absolute peak heart rate attained at VO₂max with recovery values recorded at the 1- and 3-minute mark (HRR1 & HRR3, respectively) following accepted methodological guidelines for recovery⁴⁷.

Statistical Analysis

All statistical analyses were conducted using SPSS Statistics (Version 30; IBM Corp., Armonk, NY). Data were screened for missing values, outliers, and distributional assumptions prior to inferential testing. Any participant with incomplete HRV data across time points were excluded from analyses. HF-HRV values were natural log transformed to improve normality and reduce heteroscedasticity prior to analysis, consistent with established recommendations for HRV data processing^{48,49}

Participant characteristics and outcome variables and are presented as means \pm standard deviation for normally distributed data and as median and interquartile range (IQR) for non-normally distributed data. Baseline between-group differences, including VO₂max, were evaluated using one-way analysis of variance (ANOVA) and are reported in Table 2. Baseline equivalence for age, height, weight, BMI, FFMI, and VO₂max across all groups were analyzed using a one-way ANOVA, and sex distribution were assessed via Chi-square analysis.

Changes in autonomic recovery were analyzed using a 3 (Time: Pre, Post1, Post2) \times 3 (Group: BB, CS, SB) mixed design model with centered %BF (BFc) or centered FFMI (FFMIc) included as a continuous covariate to determine whether an individual's body composition or relative muscularity influenced recovery responses. The primary effect of interest was the Time \times Group interaction. R-R intervals generated HF-HRV data, which was then exported through Kubios Scientific software (Kubios Oy, Kuopio, Finland) using artifact correction at a medium level and smoothness-prior method detrending set at $\lambda = 500$ ⁵⁰.

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 to support multivariate findings and to evaluate time-specific associations between treatment group, with the control as the comparator group, and HF-HRV while adjusting for %BF and FFMI. Due to attrition and data quality exclusions, the final analyzed sample comprised 52 participants (BB = 19; CS = 17; SB = 16), which exceeded the *a priori* minimum of 42 total participants originally identified using G*Power 3.1. Therefore, the study was

adequately powered to detect moderate effects ($f = 0.25$) at $\alpha = 0.05$ with 80% power. Partial eta squared (η^2_p) was reported as a measure of effect size.

Results

Participant characteristics are presented in Table 2. Additionally, sex-specific characteristics at baseline along with descriptive HRV and HRR values across recovery time points are provided in Supplemental Table 1. Fifty-two participants completed the protocol (BB = 19, CS = 17, SB = 16). No significant group differences were observed for age, body mass, height, BMI, FFMI, VO_{2max} , or sex distribution (all $p > .05$). A significant group difference was observed for body fat percentage ($p = .036$); however, values were within expected ranges for the sample and were subsequently accounted for in adjusted analyses.

Table 2. Participant Characteristics.

	Total Group (n = 52)	Box Breathing (BB, n = 19)	Cyclic Sighing (CS, n = 17)	Spontaneous Breathing (SB, n = 16)	p- value
Age (yrs)	20.0 (3)	20 (3)	20 (3.5)	20 (3)	.949
Weight (kgs)	77.6 ± 14.4	75.2 ± 9.9	80.8 ± 16.5	77.2 ± 16.8	.979
Height (cm)	173.0 ± 9.1	171.7 ± 8.2	176.5 ± 11.1	171.0 ± 7.0	.076
BMI (kg*m ²)	25.8 ± 3.8	25.6 ± 3.6	25.7 ± 3.6	26.2 ± 4.5	.504
BF%	28.5 ± 7.7	29.2 ± 9.9	27.6 ± 5.8	28.7 ± 6.7	.036*
FFMI	18.4 ± 2.8	17.9 ± 2.6	18.6 ± 2.5	18.6 ± 3.4	.512
VO_{2max}	43.8 ± 10.4	41.7 ± 11.4	46.4 ± 9.4	43.6 ± 10.1	.591
Sex (%Male)	69.23	57.89	82.35	68.75	.283

Note. p-values for continuous variables derived from one-way ANOVA. * A significant between-group difference was observed for BF%; this variable was mean-centered and included as a covariate in adjusted models.

Multivariate Effects on HF-HRV Recovery

Multivariate effects for HF-HRV are summarized in Table 3. In the unadjusted MANOVA, a strong main effect for time was observed (Pillai's Trace = .934, $F(2,48) = 339.86$, $p < .001$, $\eta^2_p = .934$), indicating that across recovery intervals, there were substantial changes in HF-HRV. However, while the Time × Group interaction approached statistical significance ($p = .053$), there were no differences in HF-HRV between breathing groups at any timepoint.

After adjustment for %BF, the main effect for time remained significant ($p = < .001$) while the Time × Group interaction remained non-significant, though slightly attenuated. The Time × BFc interaction was not significant ($p = .271$), indicating that there was no moderation of HF-HRV recovery across time when accounting for %BF.

Similarly, in the FFMI-adjusted model, the main effect for time demonstrated statistical significance (Pillai's Trace = .935, $p < .001$) and the Time × Group interaction also reached statistical significance (Pillai's Trace = .190, $F(4,96) = 2.52$, $p = .046$, $\eta^2_p = .095$). However, the Time × FFMIc interaction was not significant ($p = .550$), suggesting that FFMI did not influence HF-HRV across the three timepoints assessed.

Table 3. Multivariate effects on HF-HRV recovery (MANOVA and BF%/FFMI -adjusted MANCOVA)

Model	Effect	Multivariate Statistic	F(df₁, df₂)	p	η^2_p
MANOVA (Unadjusted)	Time	Pillai's Trace = .934	$F(2,48) = 339.86$	<.001	.934
	Time × Group	Pillai's Trace = .180	$F(4,98) = 2.43$.053	.090
MANCOVA (BF% Adjusted)	Time	Pillai's Trace = .934	$F(2,47) = 333.17$	<.001	.934
	Time × Group	Pillai's Trace = .177	$F(4,96) = 2.33$.061	.089
	Time × BFc	Pillai's Trace = .054	$F(2,47) = 1.34$.271	.054
MANCOVA (FFMI Adjusted)	Time	Pillai's Trace = .935	$F(2,47) = 340.69$	<.001	.935
	Time × Group	Pillai's Trace = .190	$F(4,96) = 2.52$.046	.095
	Time × FFMIc	Pillai's Trace = .025	$F(2,47) = 0.61$.550	.025

Note. η^2 = partial eta squared.

Regression Models Predicting HF-HRV

To characterize the contribution of %BF and FFMI to autonomic recovery, a hierarchical linear regression with three models was conducted at each timepoint, comparing baseline breathwork conditions (Model 1), further incorporating BFc (Model 2) and FFMIc (Model 3).

At baseline, none of the linear regression models significantly predicted HF-HRV. In Model 1, neither BB nor CS differed from spontaneous breathing, explaining minimal variance in HF-HRV ($R^2 = .027$). The inclusion of centered body fat percentage in Model 2 did not meaningfully improve model fit ($R^2 = .030$), and %BF was not a significant predictor. Similarly, Model 3 incorporating FFMIc explained a slightly greater proportion of variance ($R^2 = .051$), though FFMI was not a significant predictor. These findings as shown in Table 4, indicate no baseline differences in HF-HRV attributable to breathwork condition or body composition.

Table 4. Baseline (Pre) linear regression models predicting HF-HRV (log)

	Predictor	B	SE	β	<i>p</i>	<i>R</i>²
Model 1	Box Breathing	.461	.392	.195	.245	.027
	Cyclic Sighing	.259	.403	.107	.523	
Model 2	Box Breathing	.458	.396	.194	.254	.030
	Cyclic Sighing	.267	.407	.110	.515	
	BFc	.007	.021	.048	.738	
Model 3	Box Breathing	.415	.394	.176	.297	.051
	Cyclic Sighing	.253	.402	.104	.531	
	FFMIc	-.063	.058	-.154	.283	

Note. Control (spontaneous breathing) served as the reference group for treatment comparisons. HF-HRV values were log-transformed prior to analysis. Model 1 included breathwork condition only (box breathing and cyclic sighing). Model 2 included breathwork condition with centered maximal oxygen consumption (VO_{2c}) entered as a covariate. Separate linear regression models were conducted for each time point. R^2 values represent total variance explained by each model. Statistical significance was set a priori at $\alpha = .05$.

During Post1, immediately following peak exertion, breathwork condition significantly predicted HF-HRV. In Model 1, both BB and CS were associated with significantly higher HF-HRV compared to SB, accounting for approximately 19% of the variance ($R^2 = .193$). The addition of BFc in Model 2 did not meaningfully alter the proportion of variance explained ($R^2 = .195$), and %BF was not a significant predictor. Similarly, Model 3 including FFMIc did not improve the overall contribution of the model ($R^2 = .194$), with FFMI remaining non-significant (Table 5). Across models, the magnitude and significance of breathwork effects remained stable.

Table 5. Post1 (1-3-min) linear regression models predicting HF-HRV (log)

	Predictor	B	SE	β	<i>p</i>	<i>R</i>²
Model 1	Box Breathing	1.543	.497	.469	.003	.193
	Cyclic Sighing	1.467	.510	.435	.006	
Model 2	Box Breathing	1.547	.502	.471	.003	.195
	Cyclic Sighing	1.458	.516	.432	.007	
	BFc	-.009	.027	-.041	.751	
Model 3	Box Breathing	1.555	.505	.473	.003	.194
	Cyclic Sighing	1.469	.515	.435	.006	
	FFMIc	.017	.075	.030	.821	

Note. Control (spontaneous breathing) served as the reference group for treatment comparisons. HF-HRV values were log-transformed prior to analysis. Model 1 included breathwork condition only (box breathing and cyclic sighing). Model 2 included breathwork condition with centered maximal oxygen consumption (VO_{2c}) entered as a covariate. Separate linear regression models were conducted for each time point. R^2 values represent total variance explained by each model. Statistical significance was set a priori at $\alpha = .05$.

In Post2, where participants returned to a supine position, breathwork conditions continued to significantly predict HF-HRV. In Model 1, both BB and CS were associated with higher HF-HRV relative to SB, explaining approximately

22% of the variance ($R^2 = .224$). Model 2 demonstrated a modest increase in explained variance with the inclusion of BFc ($R^2 = .246$); however, %BF was not considered a significant predictor. In Model 3, FFMlc did not contribute meaningfully to the model ($R^2 = .224$) and was not associated with HF-HRV. As observed at Post1, breathwork effects remained robust across all models (Table 6).

Table 6. Post2 (4-6-min) linear regression models predicting HF-HRV (log)

	Predictor	B	SE	β	p	R^2
Model 1	Box Breathing	1.986	.536	.550	<.001	.224
	Cyclic Sighing	1.365	.550	.368	.017	
Model 2	Box Breathing	1.968	.534	.545	<.001	.246
	Cyclic Sighing	1.402	.549	.378	.014	
	BFc	.034	.029	.150	.239	
Model 3	Box Breathing	1.996	.544	.552	<.001	.224
	Cyclic Sighing	1.366	.556	.368	.018	
	FFMlc	.014	.080	.022	.836	

Note. Control (spontaneous breathing) served as the reference group for treatment comparisons. HF-HRV values were log-transformed prior to analysis. Model 1 included breathwork condition only (box breathing and cyclic sighing). Model 2 included breathwork condition with centered maximal oxygen consumption (VO_{2c}) entered as a covariate. Separate linear regression models were conducted for each time point. R^2 values represent total variance explained by each model. Statistical significance was set a priori at $\alpha = .05$.

Multivariate Effects of Heart Rate Recovery

Multivariate effects for HRR are presented in Table 7. In the unadjusted MANOVA, a significant main effect of time was observed (Pillai's Trace = .732, $F(2,48) = 65.42$, $p < .001$, $\eta^2_p = .732$), reflecting progressive heart rate recovery across intervals. However, the Time \times Group interaction failed to reach significance ($p = .355$), suggesting no difference in HRR between any of the three intervention groups. After adjustments for %BF and FFMI, the main effect for time remained significant across all models (all, $p \leq .001$) while there were no significant effects for any interaction terms included in the models (all, $p > .350$). This indicates that breathing condition, body composition, and relative muscularity did not alter HRR trajectories.

Collectively, these results demonstrate that structured breathwork strategies may significantly influence parasympathetic recovery in ROTC cadets, as indicated by group-level differences in post-exercise HF-HRV over time. Moreover, the influence of structured breathwork interventions on parasympathetic recovery did not appear to be impacted by indices of adiposity or fat-free mass in these participants. Conversely, HRR was driven primarily by time and not differentially altered by conditions such as breathing intervention or body composition metrics.

Table 7. Multivariate effects on HRR (MANOVA and BF%/FFMI-adjusted MANCOVA)

Model	Effect	Multivariate Statistic	$F(df_1, df_2)$	p	η^2_p
MANOVA (Unadjusted)	Time	Pillai's Trace = .732	$F(2,48) = 65.42$	<.001	.732
	Time \times Group	Pillai's Trace = .087	$F(4,98) = 1.11$.355	.043
MANCOVA (BF% Adjusted)	Time	Pillai's Trace = .732	$F(2,47) = 65.33$	<.001	.732
	Time \times Group	Pillai's Trace = .084	$F(4,96) = 1.09$.368	.043
	Time \times BFc	Pillai's Trace = .041	$F(2,47) = 0.99$.382	.040
MANCOVA (FFMI Adjusted)	Time	Pillai's Trace = .732	$F(2,47) = 65.06$	<.001	.732
	Time \times Group	Pillai's Trace = .086	$F(4,96) = 1.12$.351	.045
	Time \times FFMlc	Pillai's Trace = .037	$F(2,47) = 0.90$.413	.037

Note. η^2 = partial eta squared.

Discussion

The primary findings suggest that structured breath work significantly enhances parasympathetic reactivation immediately following maximal exertion. This is indexed by HF-HRV, whereas %BF and FFMI did not independently moderate trajectories in autonomic recovery within this sample of ROTC cadets. Across all models, time domain was the most dominant effect on both HF-HRV and HR, this confirms expected physiological recovery patterns following peak exercise. However, while BB and CS demonstrated meaningful associations with HF-HRV neither %BF nor

FFMI explained additional variance in recovery beyond that accounted for by time and breathing condition during early and late recovery windows. It is postulated that acute autonomic recovery may be more strongly influenced by immediate regulatory behaviors rather than static body composition characteristics in young, operationally trained populations.

No significant moderation effects were observed for %BF nor FFMI moderated HF-HRV recovery across time in the multivariate models. Similarly, neither %BF nor FFMI independently predicted HRR trajectories within this investigation. Therefore, does however contrast with prior work linking elevated adiposity to reduce vagal tone and delayed recovery and civilian and mixed tactical cohorts. Given the relatively young age and physical capability of the present ROTC sample, the authors feel this may be an explanation for this discrepancy⁵¹. Participants demonstrated %BF consistent with expected values for trained collegiate-aged cadet populations, alongside mean VO₂max values that fall within good to excellent normative value classification ranges for young adults³². Collectively, there is potential for physiological variability limitation that are necessary to detect body composition driven differences in short term recovery, based on the characteristics captured in this investigation.

Similarly, FFMI values observed in this investigation presented largely centered within established normative ranges derived from DXA-based analysis. Mean FFMI results aligned closely to the 50th and 75th percentile ranges that Sergi et al.¹⁹ reported in recent sex-specific reference data involving U.S. Army personnel. Furthermore, 15% participants fell below the proposed lower thresholds associated with physical readiness concerns proposed by Sergi (2025) (males < 17.0 kg·m⁻²; females < 14.0 kg·m⁻²). This distribution indicates that most participants possessed relative muscularity that was sufficient for reducing the likelihood that low lean mass status contributed to impaired autonomic recovery. The combination of adequate aerobic capacity, with moderate adiposity, and relatively preserved muscularity likely constrained the contrast required for either %BF or FFMI to show significance as predictors of acute autonomic recovery, specifically after maximal exertion^{48,51}.

Regression analysis demonstrated that breathing condition rather than body composition metrics primarily predicted HF-HRV during Post1 and Post2. Both BB & CS were suggested to be associated with significantly greater HF-HRV at these timepoints, relative to SB. Additionally, BB Exhibited the strongest associations in the Post2 window of recovery. These patterns align with mechanistic models of vagal reactivation, wherein slow, controlled breathing enhances baroreflex sensitivity and promotes parasympathetic dominance during recovery²⁴. Moreover, the absence of significant associations between %BF, FFMI, and HF-HRV at any time point reinforces that respiratory control remains highly modifiable in acute parasympathetic recovery, even when accounting for individual differences in body composition and muscularity as indexed by %BF and FFMI.

HRR demonstrated more of a distinct and complementary pattern, relative to HRV. HRR was driven almost exclusively by time with no evidence that breathing conditions, %BF, or FFMI have any effect in alteration of recovery processes. Divergence between HRV and HRR supports the conceptual distinction between these metrics. Specifically, HRR reflecting rapid sympathetic withdrawal and sinoatrial node responsiveness and HF-HRV capturing a more nuanced parasympathetic modulation^{5,47}. As such, HRV may be a more responsive observational metric in the acute sense, while HRR appears to reflect a more time-dependent recovery process that is less sensitive to short-term behavioral modulation⁴⁸.

From an applied perspective, these findings highlight the value of markers such as HF-HRV when investigations seek to detect intervention specific recovery effects. This is particularly relevant in tactical environments as subtle differences in autonomic regulation may accumulate more rapidly across repeated stress exposures that service members are often exposed to². Collectively structured breath work represents a low cost, easily deployable strategy for behavior change that may enhance parasympathetic recovery immediately following peak exertion^{23,24}. However, body composition metrics maybe a more informative when looking at chronic disease readiness assessments as compared to acute recovery in younger tactical populations^{3,14}. Additionally, it is important to recognize that the findings here are constrained by the limited sample size and specific population investigated. This investigation primarily encompassed operationally fit ROTC cadets and does not reflect the broader spectrum of the U.S. Army population, or the military branches in general. In a general sense older service members, individuals with higher %BF, lower VO₂max, and lower FFMI must be accounted for, and future investigations should extend investigations across broader age ranges and fitness profiles, to fully capture the diversity of the total military population^{1,2}.

Sex-based differences further complicate the relationship between body composition and autonomic recovery. Males typically possess greater absolute FFM and lower %BF, which has been associated with faster early-phase HRR and higher vagally mediated HRV indices^{6,52}. While women often demonstrate substantial relative training adaptations, they may experience greater physiological strain during equivalent task demands, particularly when higher %BF or unfavorable fat distribution is present^{2,8}. However, substantial overlap in body composition and autonomic responses exists within each sex, suggesting that sex-based averages may mask meaningful interindividual variability^{4,6,12}. These differences between sex highlight the importance of examining body composition as a continuous physiological construct rather than relying on categorical classifications or sex-based assumptions alone.

Several limitations should be acknowledged when interpreting the findings of this investigation. Variability in breathwork adherence throughout both Post1 and Post2 measurement periods may have influenced external validity, particularly early in these phases. HRV is particularly influenced by external noise, participant's hydration status, and ongoing psychological stressors³⁰. Prior practice of breathing techniques among some participants could not be ruled out, potentially affecting comparisons between groups. An additional consideration is the potential influence of time of day on post exercise autonomic recovery. The participant sessions were scheduled based on availability and were not standardized by the time of day. However, prior work indicates that parasympathetic reactivation following maximal exercise may be attenuated in the evenings compared with morning for HRV dash derived indices⁵³. Consistent with this literature HRR did not differ between groups in the present investigation; however, with unstandardized times of testing HF-HRV responses may have additional variability due to current design. Furthermore, another consideration relating to female specific Physiology is fluctuations in HRV across the menstrual cycle. This has been reported with reductions in vagally mediated indices commonly observed during mid to late luteal phase⁵⁴. However, with this investigation menstrual cycle phases were not controlled for or included as a covariant.

Additionally, with the use of a 3×3 repeated measures ANOVA, the reduced group sizes may have limited statistical power and sensitivity to detect interaction effects between group and time. Mixed model designs are particularly sensitive to sample size imbalances and assumption violations. As such, non-significant findings should be interpreted cautiously as subtle physiological differences may not have reached statistical significance due to limited power rather than a true absence of effect⁵⁵.

The inclusion of only ROTC cadets versus a sample with more varied military experience limits generalizability within all tactical populations, and the short intervention duration and requirement to perform a newly learned skill under post maximal-exertion fatigue may have limited full expression of autonomic recovery within the participants. Future investigations in self-selected breathing patterns to determine whether preferences of breathwork carry more weight are warranted. Additionally, the supine position used in the current investigation does not replicate a tactically applicable resting position during real-world scenarios; therefore, a more realistic recovery position, like "Taking a knee", should be evaluated against the supine position to evaluate whether similar autonomic recovery and efficacy outcomes are achieved.

Conclusion

The present investigation demonstrated that structured breath work interventions, specifically BB and CS, enhance parasympathetic reactivation following maximal exertion when monitoring HF-HRV. However, including %BF or FFM as predictors did not influence autonomic recovery in the acute sense independently. The findings highlight an important distinction between modifiable behavioral strategies which may influence autonomic regulation and physiological characteristics, which exert greater influence on performance capacity and overall readiness profiles. Importantly, the cohort in this investigation demonstrated body composition and aerobic capacity values aligning with or exceeding normative ranges for collegiate aged army personnel. This included mean VO_{2max} values with classifications in the good to excellent ranges and FMI distributions centered between the 50th and 75th percentile reference ranges. While all service members are required to meet physical fitness standards, the ROTC sample in the present investigation represents a relatively homogeneous, higher functioning subgroup within the broader context of all military populations. Additionally, the greater military population includes wider variability including age, adiposity, aerobic capacity, and relative muscularity. Consequently, our findings may not be as generalizable with service members later in their service tenure, or those that are near their minimum readiness thresholds. Future investigations should extend into a more diverse population to better understand how body composition metrics interact with autonomic recovery under specific operational stressors and maximal exertion activities. The findings support the use of structured breath work for the broader population level to optimize the total force.

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Conflicts 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.

Author Declaration Regarding AI Use. Artificial intelligence tools were used only for minor editorial assistance. No artificial intelligence tools were used for data analysis, data generation, or interpretation. The authors assume full responsibility for all aspects of the manuscript.

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Supplemental Table 1. Sex-specific baseline characteristics and descriptive HRV and HRR responses across measurement time points.

	Time Point	Males (n = 36)	Females (n = 16)	Group (n = 52)
Age (yrs)	Baseline	21.1 ± 2.9	20.4 ± 2.5	20.8 ± 2.8
Weight (kgs)	Baseline	81.7 ± 13.2	68.5 ± 13.3	77.6 ± 14.4
Height (cm)	Baseline	176.6 ± 7.3	164.4 ± 6.4	173.0 ± 9.1
BMI (kg*m ²)	Baseline	26.1 ± 3.6	25.3 ± 4.4	25.8 ± 3.8
BF%	Baseline	25.1 ± 5.3	32.6 ± 6.5	28.5 ± 7.7
FFMI	Baseline	19.4 ± 2.4	15.9 ± 1.9	18.4 ± 2.8
VO _{2max}	Baseline	47.9 ± 8.8	34.6 ± 7.3	43.8 ± 10.4
HF-HRV (log)	Pre	7.5 ± 1.1	8.0 ± 1.2	7.7 ± 1.1
HF-HRV (log)	Post1	1.7 ± 1.7	2.0 ± 1.3	1.8 ± 1.6
HF-HRV (log)	Post2	3.5 ± 1.7	4.2 ± 1.6	3.7 ± 1.8
HRR	HRR ₁	-56.3 ± 30.2	-44.1 ± 44.3	-52.6 ± 35.2
HRR	HRR ₃	-62.6 ± 31.4	-58.2 ± 50.1	-61.2 ± 37.7

Note. Values are presented as mean ± SD. Data are provided for descriptive purposes to characterize the sample and observed responses by sex. No sex-stratified inferential analysis was performed. HRR values represent the absolute change in heart rate (bpm) from peak exercise at 1 min (HRR₁) and 3 min (HRR₃) post-exercise.