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RESEARCH ARTICLE

Autonomic effects of simulated periodic breathing in patients with chronic heart failure on contemporary guideline-directed therapy

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Abstract

Purpose The aim of this study was to evaluate sympathetic responses to experimentally induced periodic breathing/Cheyne-Stokes respiration (PB/CSR)-like patterns in optimally treated patients with chronic heart failure (CHF) compared with matched healthy controls.

Methods Muscle sympathetic nerve activity (MSNA) was recorded in 22 patients with systolic CHF (63 ± 9 years; left ventricular ejection fraction $31 \pm 8\%$) and 10 age- and sex-matched healthy controls during spontaneous breathing and during two experimental protocols designed to reproduce PB/CSR-like oscillations: a short-cycle protocol (five cycles of 30-s apnea/30-s recovery breathing) and a long-cycle protocol (five cycles of 30-s apnea/60-s recovery breathing).

Results At baseline, MSNA was comparable between patients with CHF and controls, whereas heart rate variability indices were reduced in CHF. Compared with spontaneous breathing, simulated PB/CSR—averaging apnea and recovery phases—was associated with modest reductions in MSNA, with no significant differences between protocols or groups ($p > 0.05$). Both maneuvers elicited reproducible oscillatory patterns in MSNA, characterized by increases during apnea and decreases during recovery breathing, consistent with PB/CSR-like entrainment. However, patients with CHF exhibited weaker and more temporally dispersed coupling between MSNA and cardiorespiratory variables compared with controls.

Conclusions In optimally treated CHF, baseline sympathetic activity is comparable to that of healthy controls, and PB/CSR-like breathing primarily induces phase-locked oscillatory modulation of MSNA rather than sustained sympathetic activation. Impaired autonomic-hemodynamic coupling may represent a key mechanism underlying the clinical relevance of abnormal breathing patterns in CHF.

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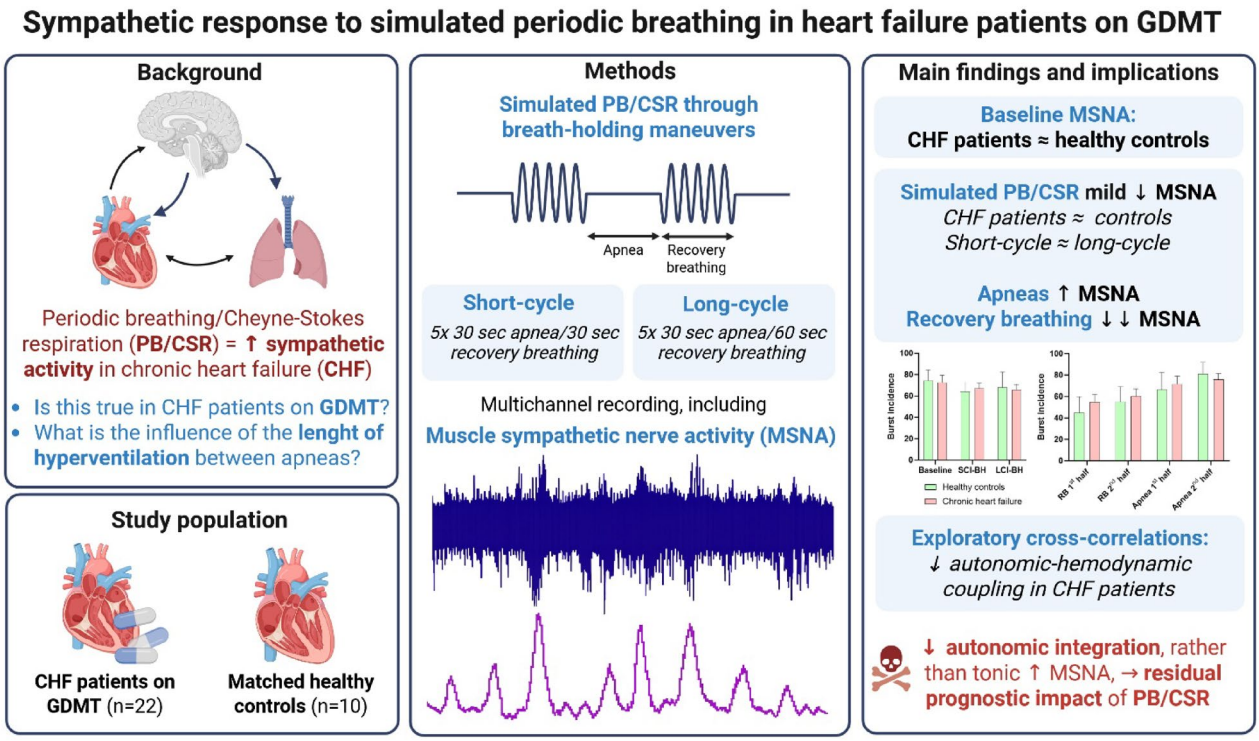
Graphical abstract



Central Illustration. Sympathetic response to simulated PB/CSR in patients with CHF on GDMT. CHF: chronic heart failure; GDMT: guideline-directed medical therapy; PB/CSR: periodic breathing/Cheyne-Stokes respiration; MSNA: muscle sympathetic nerve activity

Keywords Heart failure · Autonomic nervous system · Muscle sympathetic nerve activity · MSNA · Breath holding · Periodic breathing · Cheyne-Stokes respiration · Central apnea

Abbreviations

BMI	Body mass index
BRS	Spontaneous baroreflex sensitivity
BH	Breath holding
CHF	Chronic heart failure
eGFR	Estimated glomerular filtration rate
HRV	Heart rate variability
LCl	Long cycle
LVEF	Left ventricular ejection fraction
MSNA	Muscle sympathetic nerve activity
NT-proBNP	N-terminal pro-B-type natriuretic peptide
RB	Recovery breathing
SCl	Short cycle

Introduction

Chronic heart failure (CHF) is a prevalent and complex clinical syndrome characterized by impaired cardiac output and/or elevated filling pressures [1], which trigger compensatory neurohormonal responses, including increased sympathetic nerve activity (SNA) to the heart and resistance vessels and withdrawal of cardio-vagal (parasympathetic) drive, as assessed from heart rate variability (HRV) metrics [2, 3]. Despite being compensatory in the acute phase, in the chronic setting this sympatho-vagal imbalance contributes to disease progression, arrhythmogenesis, and adverse outcomes [4].

Beyond cardiovascular dysfunction, many patients with CHF exhibit disordered respiratory control, manifesting as central apneas, periodic breathing (PB), and Cheyne–Stokes respiration (CSR) [5, 6]. The pathophysiology of these phenomena involves neurally-driven instability in respiratory control, with associated hemodynamic and respiratory gases fluctuations, which are believed to trigger intermittent surges in SNA. However, the precise mechanisms remain incompletely understood. Indeed, only one study has directly measured muscle sympathetic nerve activity (MSNA) during spontaneous CSR in patients with CHF, demonstrating a phase-locked oscillatory modulation, with increased MSNA during apneas and reduced MSNA during hyperventilation phases [7]. Importantly, that study enrolled only patients

with advanced CHF ($n = 14$, New York Heart Association [NYHA] functional class III/IV and left ventricular ejection fraction [LVEF] 20% ± 2%) and predated the current era of guideline-directed medical therapy (GDMT) [7], which includes anti-neurohormonal medications known to positively modulate the autonomic function [8]. Given the challenges of recording MSNA during diurnal spontaneous PB/CSR in stable patients with CHF on modern therapy, its simulation through breath holding (BH) may be a reproducible model [9].

The pathophysiological significance of PB/CSR in CHF remains controversial. Earlier studies suggested that PB/CSR contributes to sustained sympathetic activation and adverse prognosis in CHF [10, 11]. However, more recent evidence has challenged the view that PB/CSR is uniformly maladaptive. In particular, the hyperventilation phases of PB/CSR, often prolonged in CHF, may exert sympatho-inhibitory effects through lung stretch-mediated and hemodynamic mechanisms [12], potentially counterbalancing apnea-induced sympathetic excitation. Therefore, the net autonomic impact of PB/CSR may reflect the balance between opposing physiological influences. Furthermore, in healthy young individuals, simulated PB/CSR increased MSNA only when the hyperventilation phase was as long as the apnea phase [9]. By contrast, patients with CHF typically exhibit prolonged hyperventilation phases, proportional to disease severity, as a consequence of longer circulatory delay [5, 13]. However, whether these differences in hyperventilation length similarly affect MSNA responses in patients with CHF is unknown.

Therefore, the present study aimed to investigate autonomic responses to simulated PB/CSR, using BH, in patients with CHF on GDMT compared with matched healthy controls. The specific objectives were: (1) to verify whether simulated PB/CSR in patients with CHF receiving contemporary GDMT is associated with net sympathetic activation, net inhibition, or predominantly phasic oscillatory modulation of sympathetic outflow; (2) to test whether hyperventilation length modulates MSNA responses in CHF; (3) to investigate which factors predominantly determine MSNA changes during PB/CSR.

Methods

Between May 2023 and January 2025, patients ≥ 18 years-old with a diagnosis of CHF and LVEF < 50%, who were on stable treatment for at least three months, were prospectively screened at the outpatient clinic of our center (Fondazione Monasterio, Pisa, Italy). Patients were eligible if they could remain recumbent comfortably for the protocol period (up to 3 consecutive hours). Exclusion criteria included clinical instability at the time of the examination (i.e., requiring pharmacologic/mechanical circulatory support, ventilatory support or continuous oxygen therapy, intravenous diuretics); acute coronary syndrome, acute decompensated heart failure, or changes in CHF treatment within 3 months before enrollment; severe renal dysfunction (estimated glomerular filtration rate [eGFR] < 30 mL/min/1.73 m^2); major psychiatric and neurological disorders (e.g., neuromuscular and neurodegenerative disorders, neuropathies); active cancer; or inability to provide consent. To provide a more faithful representation of contemporary CHF populations, atrial fibrillation and diabetes mellitus were not used as exclusion criteria.

Within 3 days, all patients underwent a comprehensive cardiovascular and autonomic assessment, including a baseline recording and BH maneuvers (as detailed below), a standard 2D-Doppler echocardiography (EPIQ7, Philips, Andover, Massachusetts, USA), 24-h cardiorespiratory monitoring (Somté, Compumedics, Abbotsford, Australia) for detection and classification of apneas, and a biohumoral evaluation, including hemoglobin, eGFR, and N-terminal pro-B-type natriuretic peptide (NT-proBNP) levels, as previously described [6].

For comparison, a group of sex- and age-matched healthy controls was also enrolled to undergo autonomic assessment and BH maneuvers. Only individuals without known chronic medical conditions and not on any chronic medication were included.

The study protocol (part of the project: "Predictors of sympathetic nerve activity in patients with systolic heart failure: role of central apneas, sleep, impaired cardiac function and feedback resetting. The SNAP-HF study") was approved by the local Institutional Review Board (*Comitato Etico Regionale per la sperimentazione clinica – Sezione Area Vasta Nord Ovest, CEAVNO*), and conducted in accordance with the Declaration of Helsinki. Informed consent was obtained from all participants.

Autonomic and cardiorespiratory assessment

Following an initial clinical evaluation and a familiarization visit, each patient underwent a multiparametric recording under quiet conditions between 11 a.m. and 4 p.m. Patients were instructed to refrain from smoking and consuming coffee, tea, or alcohol for 12 h before the study, avoid heavy meals and physical activity for six hours before the study, and empty their bladder before the protocol. Prescribed medications were not altered. All recordings were performed with the subject in a semi-seated position on a chair with a 45° reclined backrest. Participants were instructed to remain silent, breathe normally, and stay awake.

MSNA was measured using microneurography at the left common peroneal nerve with a standardized technique [14]. To identify the course of the nerve, electrical pulses

(2–10 mA; Stimulus Isolator, ADInstruments, Sydney, Australia) were delivered via a 2-mm smooth surface probe at the level of the fibular head. Once the optimal stimulation site was located, a sterile insulated tungsten microelectrode (impedance 1–5 MΩ, Frederick Haer and Co., Bowdoin, United States) was inserted into the skin, and a reference electrode with a larger uninsulated tip was placed 1 cm away. Weak electrical stimulation delivered through the active microelectrode (200 µs, 1 Hz, 0.01–1.0 mA) guided advancement of the tip into the nerve. Evoked muscle twitches at ≤ 0.02 mA indicated penetration of a muscle fascicle. Neural activity was amplified (gain $2*10^4$, bandpass 0.3–5.0 kHz) via an isolated amplifier and headstage (NeuroAmpEX, ADInstruments), then digitized and stored on a computer (10 kHz sampling rate). The electrode was manually adjusted until MSNA bursts, defined as consistently pulse-synchronous nerve activity, were encountered (Fig. 1). In addition to MSNA, each recording included: surface ECG (three thoracic electrodes; BioAmp, PowerLab, ADInstruments, Sydney, Australia); beat-to-beat finger blood pressure (BP) via plethysmography (Finapres Medical Systems BV, Enschede, Netherlands); respiratory rate (band transducer, ADInstruments); minute ventilation and end-tidal carbon dioxide ($etCO_2$) measured through a mouthpiece connected to a breath-by-breath gas analyzer (Cosmed, Rome, Italy); and oxygen saturation (SaO_2) via pulse oximetry (SET Radical, Masimo, Irvine, California, USA).

All signals were acquired using a data acquisition and analysis system (PowerLab 16SP™ and LabChart, ADInstruments). MSNA burst detection was performed using a previously validated and freely available analysis toolbox [15]. The raw neurogram was processed by calculating the root mean square (RMS) signal using a 0.2 s time constant. Burst identification was based on amplitude thresholds and time-windows as previously described [16]. To reduce baseline fluctuations (e.g., owing to external noise or patient motion), optional detrending of the RMS signal was applied. Following automated detection, all identified bursts were visually inspected, and manual editing was performed when necessary. Bursts were classified as invalid and removed if they were consistent with artifacts, including short-duration noise or interference unrelated to sympathetic neural activity. Burst frequency (burst per minute) and incidence (burst/100 heartbeats) were then calculated and reported. As previously described [17] and detailed in the Supplemental Material, HRV and spontaneous cardiovagal baroreflex sensitivity (BRS) were derived from ECG and BP signals in patients in spontaneous sinus rhythm. Owing to the influence of respiration on heart rate (HR), HRV, and BRS were assessed only during baseline spontaneous breathing.

Respiratory maneuvers

After stable recordings of all physiological signals was obtained, a 10-min baseline recording was conducted. The average values recorded in the last five minutes were used for analysis.

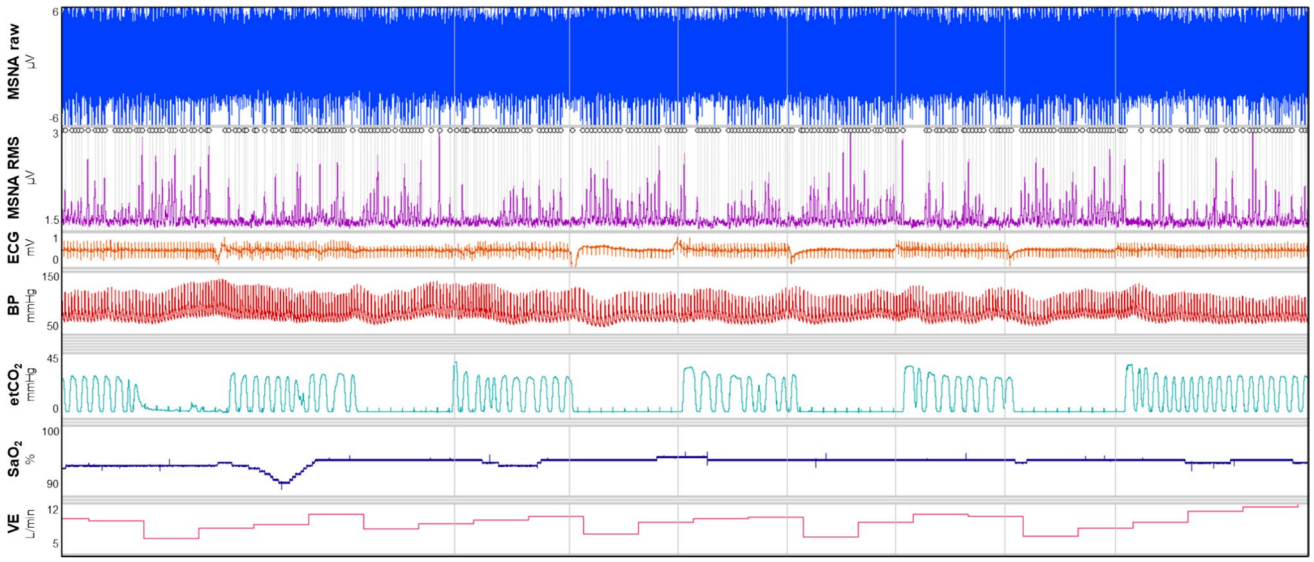


Fig. 1 Example of a multiparametric recording, including muscle sympathetic nerve activity (*MSNA*), during simulated periodic breathing. As displayed, all the signals were recorded simultaneously using LabChart software (ADInstruments). The following signals are shown in the figure from top to bottom: (1) raw MSNA; (2) root-mean-square (*RMS*) MSNA; (3) surface ECG; (4) beat-to-beat finger blood pressure (*BP*); (5) end-tidal carbon dioxide ($etCO_2$); (6) oxygen saturation (SaO_2); (7) minute ventilation (*VE*)

After baseline, patients underwent two respiratory maneuvers to simulate PB/CSR (Fig. 1). Each maneuver consisted of five volitional end-inspiratory apneas lasting 30 s (i.e., BH), each separated by recovery breathing (RB) phases of either 30 s (short cycle BH, SCl-BH) or 60 s (long cycle BH, LCl-BH). A visual interface guided patients through each phase. The two maneuvers were separated by a 5-min spontaneous breathing period.

Specifically, RB refers to the spontaneous resumption of breathing following BH, without externally imposed control of respiratory rate, tidal volume, or ventilatory pattern. Although compensatory hyperventilation may occur after apnea physiologically, RB in this protocol was not intended to reproduce the specific morphology of spontaneous PB/CSR hyperventilation phase, but rather to permit a physiological return toward baseline conditions between apneas. Two RB durations were tested to assess whether shorter recovery intervals might limit autonomic recovery and promote cumulative sympathetic activation.

Statistical analysis

Statistical analyses were performed using SPSS (version 25.0, 2017, IBM Statistics, Armonk, New York, USA) and R software (version 3.4.0). A two-tailed p-value ≤ 0.05 was considered statistically significant. Quantitative variables were tested for normality using the Kolmogorov–Smirnov test and reported as mean ± standard deviation (SD) for normally distributed data or median (interquartile range) for skewed distributions. Categorical variables were presented as frequencies (n) and percentages (%). For signal comparisons across the SCl-BH and LCl-BH maneuvers, measurements were averaged across each apnea and RB phase, as well as their first and second half. To assess the contribution of hemodynamic and respiratory gas oscillations to MSNA, HR, BP, $etCO_2$, and SaO_2 were sampled at the end of each apnea (i.e., at the first breath of RB, following the previous apnea) and RB (i.e., at the last breath of RB, preceding the following apnea). Independent t-tests or Mann–Whitney U tests were applied for between-group comparisons. The main effects of respiratory maneuvers, patient group, and their interaction were examined using mixed model analysis of variance (ANOVA) for repeated measures, while Bonferroni correction was used for post hoc comparisons.

Exploratory cross-correlation analysis was performed to estimate the temporal relationship between MSNA and cardiorespiratory signals. To minimize biases in lag estimation, this analysis was restricted to a subset of participants (five patients with CHF and five healthy controls) since requiring high-quality, continuous recordings of all signals throughout the maneuvers. All signals were up-sampled to 2 kHz, mean-subtracted, and standardized (Z-score) to allow direct comparison. Cross-correlation was computed over ± 30 s, and the lag corresponding to the maximum absolute correlation within ± 10 s (i.e., ≈10 cardiac cycles) [18] was used as the estimated delay, with negative lags indicating MSNA precedes cardiorespiratory changes. To evaluate whether observed lags were more tightly clustered than expected by chance, we quantified lag dispersion using the median absolute deviation (MAD), which is robust to outliers and small sample sizes [19]. Statistical significance was tested using a nonparametric permutation approach with 1000 iterations, in which the cardiovascular signal was randomly shuffled to remove temporal structure while preserving amplitude characteristics. Observed MAD values were compared against this null distribution, with lower p-values indicating consistent, non-random temporal alignment [20]. Finally, as detailed in the Supplemental Material, further exploratory analyses compared cardiorespiratory-autonomic coupling metrics and lag consistencies between patients with CHF and controls.

Results

Study population

Out of 38 patients with CHF and 12 healthy controls meeting the entry criteria initially selected, high quality MSNA data across the experimental protocol were obtained in 22 patients (aged 63 ± 9 years, 82% males, mean LVEF 31 ± 8%, median NT-proBNP 616 [185–1247] ng/L) and 10 healthy controls (aged 60 ± 13 years, 90% males), which were considered for analysis in the study (Table 1).

Patients with CHF had an ischemic etiology in half cases, were mildly symptomatic (NYHA class I/II in 77% and class NYHA III 23% of the cases), and received GDMT as testified by the prescription rate of β-blockers (91%), angiotensin receptor-neprilysin inhibitors (ARNI, 81%), mineralocorticoid receptor antagonists (MRA, 82%), and sodium-glucose cotrasporter-2 inhibitors (SGLT2i, 54%) (Supplemental Table 1). At 24-h cardiorespiratory monitoring, the median daytime and nighttime apnea–hypopnea index (AHI) were 8 (2–18) event/hour and 13 (7–33) event/hour, respectively, with 8 patients (37%) showing moderate-to-severe central apnea (i.e., AHI ≥ 15 event/hour, with > 50% of events being central apneas) during the night.

Baseline autonomic and cardiorespiratory measurements

At baseline assessment, patients with CHF and controls were well balanced in terms of anthropometric data, though patients with CHF had lower BP and SaO_2 ($p < 0.05$).

HRV and BRS were not available in five cases (22%) owing to either atrial pacing ($n = 2$), atrial fibrillation

Table 1 Baseline characteristics of the study population

Variables	Healthy controls $n=10$	Patients with CHF $n=22$	P
Age, years	60 ± 13	63 ± 9	0.307
Males, *n* (%)	9 (90)	18 (82)	1.000
BMI, Kg/m^2	26 ± 3	27 ± 6	0.667
Heart rate, bpm	64 ± 9	65 ± 9	0.833
Minute ventilation, L/min	12 ± 4	13 ± 7	0.280
SaO2, %	97 ± 1	95 ± 2	**0.006**
$etCO_2$, mmHg	36 ± 4	34 ± 4	0.332
Systolic BP, mmHg	132 ± 21	115 ± 21	0.068
Diastolic BP, mmHg	76 ± 10	71 ± 15	0.414
Mean BP, mmHg	95 ± 14	84 ± 17	**0.036**
SDNN, ms	34 ± 11	26 ± 10	0.097
RMSSD, ms	25 ± 9	16 ± 10	**0.021**
BRS, ms/mmHg	5.4 ± 0.4	4.5 ± 2.3	0.322
HF power, ms^2	261 (71–310)	28 (21–118)	**0.012**
LF power, ms^2	399 (195–661)	111 (43–309)	**0.021**
LF/HF	2.8 ± 2	3.9 ± 3.5	0.385
Burst incidence (burst/100 heartbeats)	74 ± 14	73 ± 14	0.879
Burst frequency (burst/min)	46 ± 6	46 ± 9	0.935

Values are mean ± SD, median (interquartile interval), or *n* (%)

HRV and BRS data were reported for 17/22 patients with CHF, owing to either atrial pacing ($n=2$), atrial fibrillation ($n=2$), or frequent ectopic beats ($n=1$)

BMI body mass index, *BP* blood pressure, *BRS* baroreflex sensitivity, *CHF* chronic heart failure, *$etCO_2$* end-tidal carbon dioxide pressure, *HF* high-frequency, *LF* low-frequency, *MSNA* muscle sympathetic nerve activity, *RMSSD* root mean square of successive normal-to-normal intervals differences, *SaO_2* oxygen saturation, *SDNN* standard deviation of normal-to-normal intervals

Bold value indicates statistically significant

($n=2$), or frequent ectopic beats ($n=1$). While HRV metrics (namely root mean square of successive differences [RMSSD], high frequency [HF], and low frequency [LF] powers) were higher in healthy controls compared with patients with HF ($p<0.05$), no significant difference was observed as regards BRS and baseline MSNA (all $p>0.05$) (Table 1 and Fig. 2).

Autonomic responses to simulated PB/CSR

Compared with baseline (spontaneous free breathing), simulated PB/CSR (including apneas and RB phases) was associated to a slight increase in mean BP and to a decrease in MSNA burst frequency and incidence (all p values <0.01) (Table 2 and Fig. 3). No significant difference was found comparing either maneuvers (SCl-BH versus LCl-BH) or subgroups (controls versus patients with CHF) (all Bonferroni-adjusted p values for pairwise comparisons >0.05).

When considered separately, apnea and RB phases increased and decreased $etCO_2$, respectively, with only mild effects on SaO_2 (ANOVA main effect p value <0.001). Both SCl-BH and LCl-BH maneuvers were effective in simulating PB/CSR-like MSNA entrainment, which increased during apneas and decreased during RB (all p values <0.001) (Table 2). In particular, the highest and lowest MSNA values were observed during the second half of apnea and the first half of RB, respectively (Fig. 4). No significant difference was noted between healthy controls and patients with CHF in any phase (all Bonferroni-adjusted p values for pairwise comparisons >0.05).

Cross-correlation analysis

In healthy controls, during both SCl-BH and LCl-BH, systolic BP fluctuations followed MSNA, with significant negative lags during SCl-BH (median −0.35 s, $p=0.037$) and LCl-BH (median −1.87 s, $p<0.001$), and moderate correlation strengths ($r\approx0.31$–0.39). For the R-R interval, a significant positive lag was observed during SCl-BH (median + 0.55 s, $r=0.37$, $p=0.017$), indicating that changes in HR preceded MSNA oscillations. Cross-correlations between MSNA and $etCO_2$ showed increased correlation strength during BH maneuvers but no consistent or statistically significant temporal coupling (Fig. 5 and Supplemental Table 2). In patients with CHF, MSNA-BP relationships were characterized by greater variability in lag

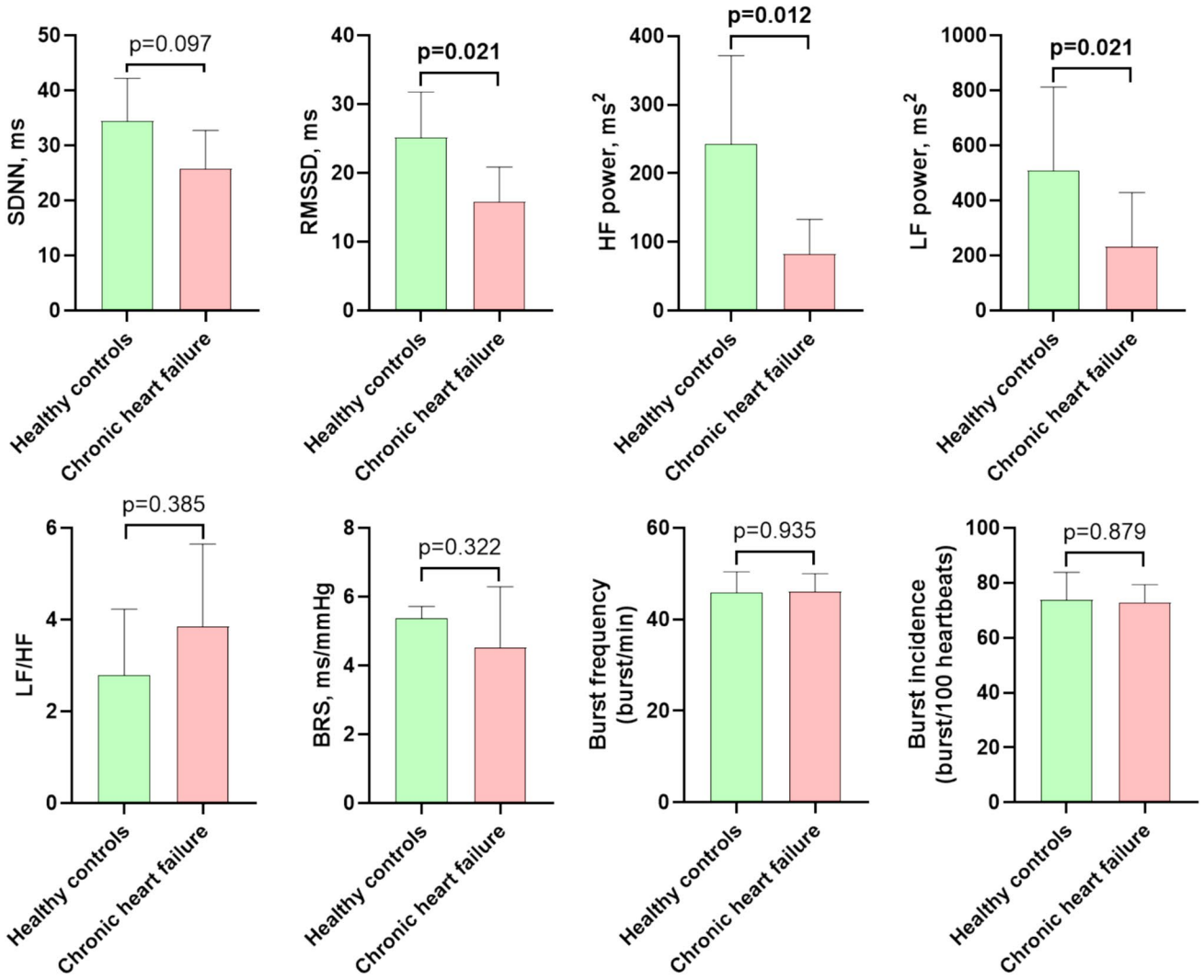


Fig. 2 Baseline autonomic data in healthy controls and patients with chronic heart failure (*CHF*). At baseline, healthy controls showed a greater time- and frequency domain heart rate variability compared to patients with CHF. No difference was noted for baroreflex sensitivity (BRS) and muscle sympathetic nerve activity (*MSNA*) measures. *HF* high-frequency, *LF* low-frequency, *RMSSD* root mean square of successive normal-to-normal intervals differences, *SDNN* standard deviation of normal-to-normal intervals

timing, with statistical significance limited to systolic BP during LCl-BH (median −1.70 s, $r=0.29$, $p=0.002$). Associations between MSNA and HR or $etCO_2$ were weaker and temporally inconsistent across BH maneuvers (Fig. 5 and Supplemental Table 2).

Discussion

This is the first study to investigate the effects of simulated PB/CSR on MSNA in patients with CHF receiving GDMT compared with matched healthy controls. The principal findings were: (1) baseline BRS and MSNA levels did not differ significantly between patients with CHF and matched controls, despite lower HRV in the CHF group; (2) averaging apnea-recovery breathing phases, simulated PB/CSR led to a mild decrease in MSNA, which was comparable between patients with CHF and healthy controls and independent of RB duration; (3) the respiratory maneuvers used in this study effectively simulated PB/CSR and entrained oscillatory MSNA patterns in both groups, with peaks during apnea and nadirs during RB; and (4) exploratory cross-correlation analysis revealed weaker and less temporally consistent cardiorespiratory-autonomic coupling in patients with CHF compared with healthy controls (Central Illustration).

Table 2 Respiratory gases, hemodynamic, and autonomic response to short-cycle (SCl-BH) and long-cycle breath holding (LCl-BH) maneuvers in healthy controls and patients with chronic heart failure (CHF)

Variables	Group	Baseline	SCl-BH			LCl-BH			ANOVA p values		
			Whole	Apnea	RB	Whole	Apnea	RB	Phase	Group	Phase*group
SaO_2, %	Healthy	97 ± 1	97 ± 1	96 ± 1	98 ± 2	97 ± 1	96 ± 1	98 ± 1	0.017	0.016	0.768
	CHF	95 ± 2	96 ± 1	95 ± 2	97 ± 1	96 ± 1	95 ± 2	96 ± 4			
$etCO_2$, mmHg	Healthy	36 ± 4	36 ± 5	40 ± 6	32 ± 5	36 ± 9	40 ± 6	33 ± 5	< 0.001	0.482	0.982
	CHF	34 ± 4	35 ± 5	39 ± 6	31 ± 3	36 ± 7	39 ± 6	32 ± 4			
Minute ventilation, L/min	Healthy	12 ± 4	–	–	14 ± 3	–	–	15 ± 3	0.024	0.139	0.885
	CHF	13 ± 7	–	–	17 ± 6	–	–	17 ± 4			
Heart rate, bpm	Healthy	64 ± 9	66 ± 10	66 ± 9	67 ± 8	65 ± 11	65 ± 11	64 ± 10	0.049	0.914	0.610
	CHF	65 ± 9	65 ± 10	65 ± 9	66 ± 9	66 ± 10	65 ± 10	65 ± 9			
Mean BP, mmHg	Healthy	95 ± 14	108 ± 18	107 ± 17	110 ± 17	103 ± 16	103 ± 15	102 ± 13	0.028	0.036	0.007
	CHF	84 ± 17	91 ± 23	90 ± 24	93 ± 15	91 ± 14	91 ± 13	92 ± 13			
Burst incidence (burst/100 heartbeats)	Healthy	74 ± 14	64 ± 11	76 ± 14	51 ± 15	68 ± 17	79 ± 18	61 ± 18	< 0.001	0.558	0.138
	CHF	73 ± 14	67 ± 10	75 ± 11	58 ± 14	66 ± 10	74 ± 11	61 ± 11			
Burst frequency (burst/min)	Healthy	46 ± 6	42 ± 5	50 ± 9	33 ± 9	43 ± 9	50 ± 10	38 ± 9	< 0.001	0.651	0.142
	CHF	46 ± 9	44 ± 7	48 ± 8	39 ± 9	43 ± 7	47 ± 9	40 ± 8			

Values are mean ± SD

BP blood pressure, *$etCO_2$* end-tidal carbon dioxide pressure, *RB* recovery breathing, *SaO_2* oxygen saturation

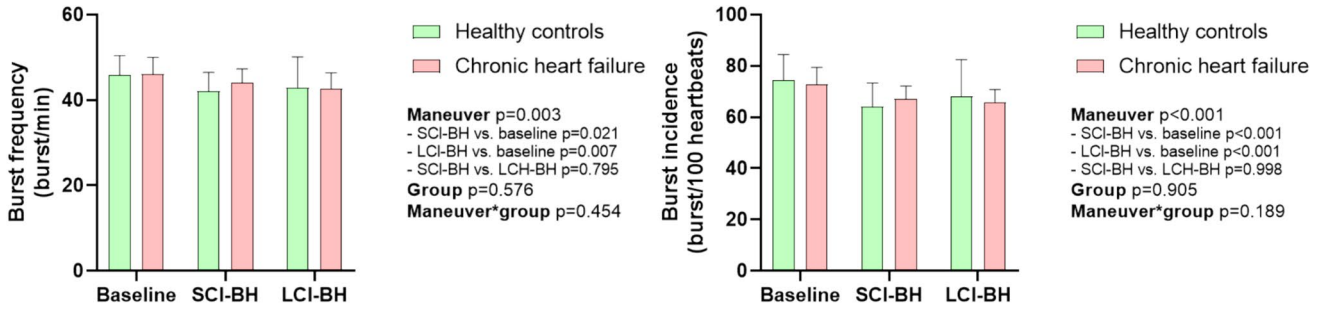


Fig. 3 Impact of short-cycle (*SCl-BH*) and long-cycle breath holding (*LCl-BH*) maneuvers on muscle sympathetic nerve activity (*MSNA*). Compared with baseline, both respiratory maneuvers were associated with mild reduction in MSNA in both healthy controls and patients with chronic heart failure. No significant difference was noted comparing either SCl-BH versus LCl-BH or healthy controls versus patients with CHF

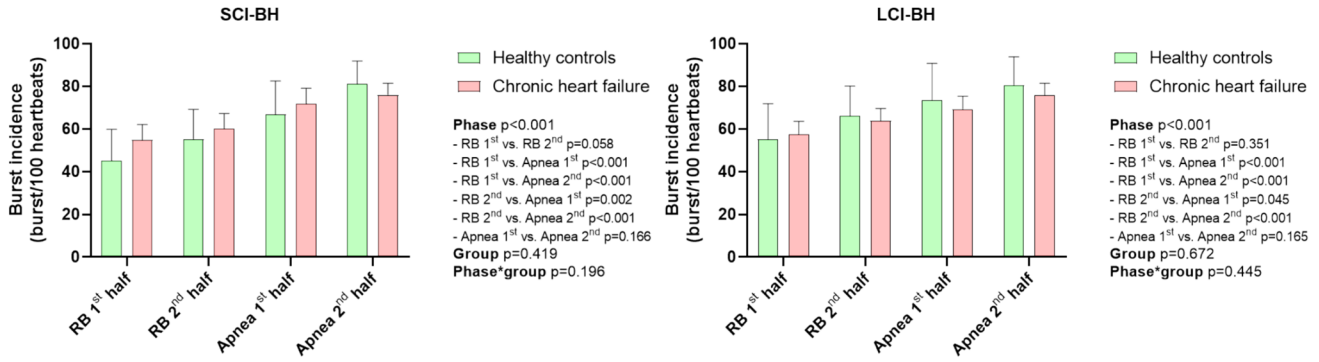


Fig. 4 Impact of apneas and recovery breathing (*RB*) phases of short-cycle (*SCl-BH*) and long-cycle breath holding (*LCl-BH*) maneuvers on muscle sympathetic nerve activity (*MSNA*). During both maneuvers, MSNA showed an oscillatory pattern, with peaks during the second half of apneas and nadir during the first half of RB. No significant difference was noted between healthy controls and patients with chronic heart failure in any phase

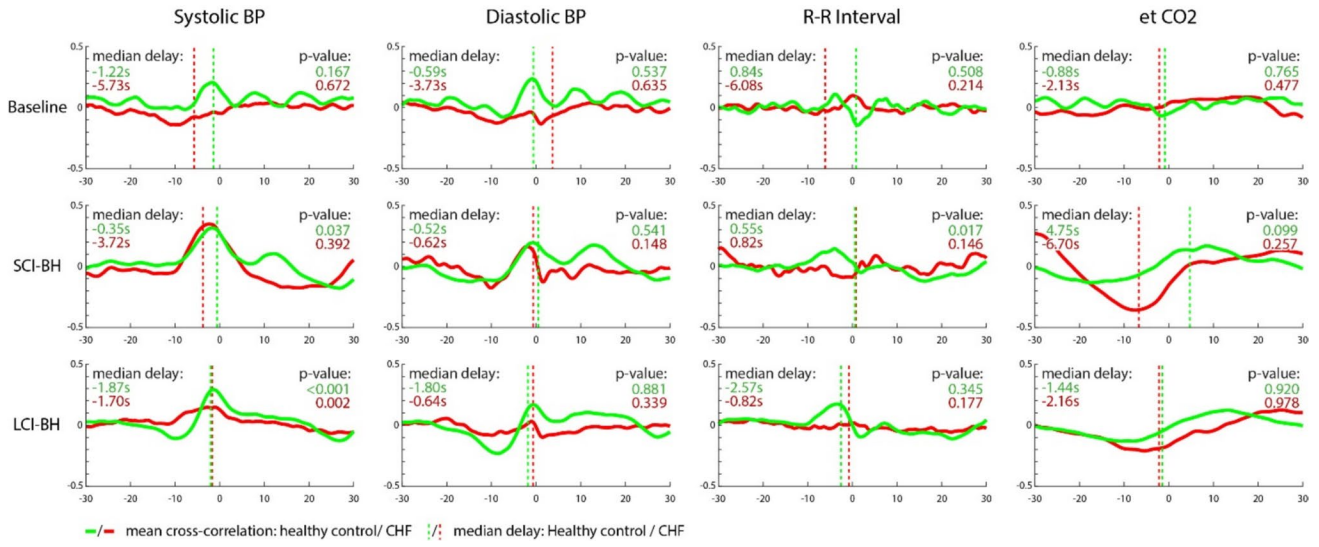


Fig. 5 Cross-correlations between muscle sympathetic nerve activity and blood pressure (*BP*), R-R interval, and end-tidal CO_2 ($etCO_2$) for baseline, short-cycle (*SCl-BH*) and long-cycle breath holding (*LCl-BH*) maneuvers in healthy controls and patients with chronic heart failure (*CHF*). The cross-correlation average among subjects and trials is displayed as a solid line in green color for the healthy control and in red color for CHF, the median signals lag is indicated by the broken vertical line

Baseline autonomic function in optimally-treated heart failure

Although elevated sympathetic drive is traditionally considered a hallmark of CHF [3], our data did not demonstrate marked differences in baseline MSNA between patients with CHF and carefully matched healthy controls. The substantial overlap in MSNA distributions between groups suggests that, if a true difference exists, it is likely to be modest rather than large. While the study may have been underpowered to exclude subtle effects, the absence of a clear signal across multiple MSNA indices and experimental conditions argues against major differences between the groups in this cohort. Importantly, controls were specifically matched for key determinants of sympathetic activity, including age, sex, and BMI, reducing the likelihood that baseline imbalance explains the findings [21].Our cohort consisted predominantly of stable, well-compensated patients receiving contemporary GDMT, which may attenuate neurohormonal activation compared with historical CHF populations. Accordingly, these findings raise the possibility that resting sympathetic outflow in modern treated CHF may be less markedly abnormal than previously described, aligning with a recent larger observational cohort study [21].

Similarly, cardiovagal BRS did not significantly differ between patients and controls. Although, by contrast with the historical description of reduced BRS in CHF [22], this finding is in line with more modern data: among 425 patients with CHF on GDMT (mean age 65 years, median LVEF 32%, 94% on beta-blockers) the median BRS value (7 ms/mmHg) [17] was not different from that (6.8 ms/mmHg) documented among 164 healthy individuals in the 50–60 years-old age group in a different study [23]. However, while ageing and other physiological variables may affect baroreflex function [23], severely reduced BRS (particularly for values $\leq$4.9 ms/mmHg) remains an independent predictor of poor prognosis in patients with CHF even on GDMT [17].

Finally, the lower HRV metrics observed in patients with CHF versus controls confirms once again that impairment in the tonic parasympathetic cardiac modulation persists in the majority of patients with CHF despite GDMT, as recently shown also in case of recovered LVEF [24].

Autonomic effects of simulated PB/CSR

The respiratory maneuvers used in this study successfully reproduced the cyclical MSNA oscillations typically observed during spontaneous PB/CSR, validating this approach as a safe and consistent controlled model. In both patients with CHF and controls, sympathetic bursts were phase-locked to the apnea-RB cycle, peaking during late apnea and declining during early RB. Though similar, this pattern slightly differs from a previous report in patients with CHF with spontaneous CSR [7], in which MSNA nadirs fell during the second rather than the first half of hyperventilation. Though definitive conclusions could not be drawn, different time delays due to different circulation time/cardiac output, or uneven cortical influences between simulated and spontaneous PB/CSR may be involved.

While MSNA increased during spontaneous PB/CSR [7], simulated PB/CSR induced a modest reduction in MSNA compared with baseline in the present study. Although a definitive explanation could not be extrapolated from our data, uneven study populations or unaccounted for physiological confounders between spontaneous and simulated PB/CSR may have contributed. Accordingly, while baseline MSNA in patients with CHF was not elevated compared with matched healthy controls, by contrast with earlier reports [25, 26], the modest reduction in MSNA observed during simulated PB/CSR was similarly present in healthy subjects. This suggests that the attenuation during the maneuver is unlikely to be treatment-specific, and more plausibly reflects a physiological response to the breathing protocol itself. Of note, the length of RB phases did not significantly affect MSNA response, contradicting preliminary findings in young healthy individuals in which SCl-BH – but not LCl-BH – was associated with a modest increase in MSNA [9]. Again, between-the-study comparisons warrants caution considering the different patient age and baseline MSNA.

Notwithstanding, from a clinical perspective it should be underscored that—even in absence of sustained sympathetic overactivity—the observed oscillatory sympathetic activity may underly the detrimental consequences of PB/CSR: repetitive adrenergic surges during apneic phases could indeed trigger arrhythmias, augment myocardial oxygen demand, and activate maladaptive remodeling in the failing heart [27]. Interventions that attenuate oscillatory breathing, e.g., targeting chemoreceptors [28], may therefore enhance autonomic resilience and mitigate these risks.

Determinants of autonomic oscillations during simulated PB/CSR

Cross-correlation analysis provided quantitative insight into the temporal organization linking MSNA to cardiorespiratory variables during simulated PB/CSR. In healthy controls, MSNA oscillations exhibited moderate-to-strong coupling with BP, with significant negative lags during BH maneuvers indicating that sympathetic burst timing consistently preceded systolic BP fluctuations. This temporal pattern supports an efferent mechanism whereby oscillatory sympathetic vasoconstrictor outflow contributes directly to the generation of BP variability during apnea-rebreathing cycles. While MSNA is classically

more closely associated with diastolic BP under resting conditions [29], these findings suggest that during simulated PB/CSR, systolic BP may exhibit stronger temporal coupling with MSNA. Although the limited sample size does not allow definitive conclusions to be drawn, this may reflect the combined influence of transient hemodynamic changes and the use of cross-correlation analysis, which emphasizes temporal alignment rather than beat-to-beat MSNA-BP relationships. On the other hand, coupling between MSNA and HR was weaker and less consistently time-ordered, suggesting that MSNA-driven effects on vascular tone dominate over chronotropic modulation under these conditions.

Patients with CHF demonstrated reduced temporal consistency between MSNA and cardiovascular signals. While this does not directly measure autonomic 'flexibility,' greater variability in the timing of cardiovascular-autonomic coupling may reflect reduced efficiency or stability of autonomic regulation under dynamic conditions. In a healthy system, reproducible temporal coordination between sympathetic activation and vascular responses may support effective moment-to-moment hemodynamic adaptation, whereas a more inconsistent relationship could indicate impaired integration between neural signals and cardiovascular effectors.

It should be acknowledged that conventional assessments of sympathetic neurovascular transduction and sympathetic baroreflex modulation typically rely on burst-by-burst analyses relating MSNA to BP amplitude changes or regression-based MSNA-BP relationships [30, 31]. By contrast, the cross-correlation approach used in this study was specifically intended to assess temporal coordination between oscillatory autonomic and cardiovascular signals during simulated PB/CSR, and therefore provides complementary rather than directly comparable physiological information. In this respect, the shorter delays observed between MSNA and BP compared with previously reported physiological latencies [32, 33] likely reflect methodological rather than physiological differences. Prior studies estimated delays related to discrete burst-to-pressure relationships under steady-state conditions, whereas our cross-correlation approach identifies the time shift of maximal coupling between continuous oscillatory signals during dynamic simulated PB/CSR. These measures are therefore not directly equivalent.

Respiratory gas variables exhibited weaker and less temporally ordered relationships with MSNA in both groups. The absence of significant lags and the modest correlation strengths likely reflect limited chemoreflex engagement during simulated PB/CSR, as SaO_2 and $etCO_2$ oscillations remained small and did not reach levels typically associated with robust sympathetic activation. The overall short duration of respiratory maneuvers (limited to 5 apnea-RB cycles per maneuver), together with buffering by body O_2 and CO_2 stores [34, 35], may have further dampened chemoreflex-driven sympathetic responses, potentially underestimating the contribution of respiratory gas fluctuations to MSNA modulation. Dedicated studies incorporating larger gas perturbations or targeted chemoreflex stimulation will be required to clarify the contribution of chemoreflex pathways to sympathetic dysregulation in patients with CHF receiving GDMT.

Limitations

Considering the technical difficulties in obtaining high-quality MSNA recordings and the long duration of the protocol, only compliant and well-compensated individuals were included. This may limit the applicability of our findings to patients unable to adequately undergo MSNA recording and/or BH maneuvers (e.g., those with more severe clinical conditions or not receiving GDMT). The relatively small sample size, inherent to the technical complexity of microneurography, may limit statistical power, particularly for detecting subtle between-group differences. Therefore, negative findings should be interpreted cautiously and confirmed in larger cohorts, with different demographic characteristics (age, sex, ethnicity), etiology, HF phenotypes, and comorbidities.

Our cohort was mainly composed of males. Although both CHF with LV systolic dysfunction and PB/CSR are more prevalent in male versus female patients, recent evidence suggests that the prognostic relevance of PB/CSR may be greater in females [36], potentially owing to sex-differences in autonomic chemoreflex response [37]. Therefore, the existence of sex-differences in autonomic response to PB/CSR remains to be investigated by this approach.

Simulated PB/CSR was performed in awake subjects under controlled experimental conditions and relied on volitional BH maneuvers, that do not fully reproduce the complexity of spontaneous PB/CSR. As such, cortical influences related to wakefulness, task engagement, and voluntary respiratory control may have affected autonomic responses, potentially differing from those observed during spontaneous PB/CSR or occurring during sleep, a condition in which other mechanisms may be involved, such as rostral fluid shift, changes in cortical influences on the brainstem, degree of chemoreflex activity [5, 38]. Similarly, contrary to spontaneous PB/CSR, end-inspiratory rather than end-expiratory apneas were employed in the present study due to higher feasibility. Although both end-inspiratory and end-expiratory apneas have been shown to entrain MSNA, uneven hemodynamic consequences due to intrathoracic pressure swings may not be excluded [39]. Moreover, RB between apneas was spontaneous and not externally instructed, and therefore should not be interpreted as a volitional breathing maneuver. As such, the observed respiratory pattern was not intended

to replicate the waxing-waning oscillations characteristic of spontaneous PB/CSR.

Owing to technical limitations, to avoid bias, cross-correlation analyses were conducted only in a subset of participants with high-quality, artifact-free recordings across all maneuvers. Therefore, the related findings should be considered hypothesis-generating rather than conclusive. Finally, the present study was not designed to assess clinical outcomes such as arrhythmia, hospitalization, or mortality. Therefore, the clinical implications of the observed physiological alterations remain speculative and require validation in outcome-driven studies.

Conclusions

In patients with CHF receiving contemporary GDMT, baseline MSNA and cardio-vagal BRS did not differ from that of matched healthy controls. Controlled respiratory maneuvers effectively reproduced phase-locked oscillations in MSNA, simulating the pattern observed during spontaneous PB/CSR. Compared with healthy controls, patients with CHF exhibited weaker and less temporally consistent cross-correlations between MSNA and hemodynamic oscillations, indicating cardiovascular-autonomic coupling may be impaired despite comparable baseline sympathetic outflow. This suggests that, even in the context of modern therapies that improve neurohormonal and sympathovagal function, the integration between cardiorespiratory and autonomic functions remain compromised in CHF. This may explain the residual prognostic impact of PB/CSR in these patients. Further studies are warranted to evaluate interventions aimed at restoring cardiorespiratory-autonomic coupling and/or stabilizing breathing patterns in patients with CHF as potential strategies to mitigate these risks.

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Declarations

Conflict of interest None.

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