

Original Article

## Acute effects of Rajyoga meditation on heart rate variability in older trained practitioners and younger novices: A quasi-experimental repeated measures study

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Received: 26 May 2025

Accepted: 16 November 2025

EPub Ahead of Print: 16 March 2026

Published:

DOI

10.25259/IJPP\_302\_2025

Quick Response Code:



### ABSTRACT

**Objectives:** Engaging in meditation contributes to both mental and physical well-being. This quasi-experimental repeated measures Study aimed to investigate the impact of Rajyoga meditation (RM) on acute, reproducible changes in heart rate variability (HRV) markers, reflecting enhanced parasympathetic activity in trained and untrained individuals.

**Materials and Methods:** This study included 45 participants, comprising 21 healthy trained meditators (10 females/11 males) and 24 healthy untrained individuals (5 females/19 males). The trained group ( $n = 21$ ), who practiced RM daily for at least 1 year (30–60 min/session, 7 days/week, with  $\geq 90\%$  adherence), was recruited from the Brahma Kumaris Meditation Centre in Durgapur, India, and the untrained group ( $n = 24$ , no prior meditation experience), the electrocardiogram (ECG) was recorded for 30 min in 3 phases, 10 min before, 10 min during and 10 min after meditation for both the trained and untrained participants. Relative risk (RR) intervals were extracted from the ECG for the analysis of HRV. The Bonferroni test was employed as a *post hoc* analysis following a non-parametric Friedman test to assess statistical differences among the phases.

**Results:** During meditation, a significant increase in HRV parameters, including the standard deviation of RR intervals (SDNN), low-frequency component, total power and long-term variability ( $SD_2$ ) was observed compared to pre- and post-meditation phases in both trained and untrained individuals (Friedman test with Bonferroni correction,  $P < 0.01$ ). *Post hoc* analysis confirmed that for these parameters, values during meditation were significantly higher than both pre- and post-meditation values ( $P < 0.01$ ), while no significant difference was found between pre- and post-meditation phases ( $P > 0.01$ ), indicating a reversible effect. Conversely, a significant decrease in the inverse average length of acceleration/deceleration segments and the percentage of short segments was observed during meditation compared to before and after meditation in both groups ( $P < 0.01$ ), with values returning to baseline post-meditation. The secondary analysis, using Quade's nonparametric analysis of covariance to control for the significant age difference between the trained and untrained groups, using age as a covariate, revealed that the magnitude of change ( $\Delta$ ) in HRV from pre- to during-meditation was significantly greater in the trained group across nearly all key HRV parameters. Most notably, trained practitioners exhibited a 154.6% greater increase in root mean square of successive differences (Trained:  $\Delta = 10.57 \pm 4.36$  ms; Untrained:  $\Delta = 4.15 \pm 1.84$  ms;  $P < 0.001$ ) and a 54.7% greater increase in SDNN (Trained:  $\Delta = 14.24 \pm 2.70$  ms; Untrained:  $\Delta = 9.20 \pm 2.55$  ms;  $P < 0.001$ ), indicating a significantly enhanced parasympathetic and overall autonomic response to meditation. This pattern of greater responsiveness among trained participants was consistent across the majority of parameters, including  $SD_1$  (154.5% greater increase) and  $SD_2$  (43.6% greater increase).

**Conclusion:** These results suggest that RM promotes a shift in sympathovagal balance towards parasympathetic dominance, highlighting its potential benefits for autonomic regulation.

**Keywords:** Autonomic nervous system, Electrocardiogram, Heart rate variability, Rajyoga meditation

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## INTRODUCTION

Meditation, referred to as all practices of physical as well as mental self-regulation, is known to influence the behavioural and psychological functioning of the central nervous system and the autonomic nervous system (ANS).<sup>[1,2]</sup> There has been a significant development in research investigating the effectiveness of meditation linked with heart and mind diseases. Until now, numerous medical practices have been employed to detect cardiovascular disease. The analysis of heart rate variability (HRV), based on electrocardiographic recordings, is a widely used approach to monitor ANS activity.<sup>[3,4]</sup> Higher HRV (e.g. standard deviation of RR intervals [SDNN], root mean square of successive differences [RMSSD] and high frequency [HF]) indicates enhanced parasympathetic tone and cardiovascular health.<sup>[5,6]</sup> HRV represents the fluctuation of heart rate from beat to beat and can be readily investigated based on RR interval series obtained from the electrocardiogram (ECG), an accessible and inexpensive recording.<sup>[3]</sup> It is widely accepted that psychological distress can influence various physiological processes and increase the risk of disease.<sup>[7]</sup> Further evidence links depression to increased morbidity and mortality from cardiovascular disease (CVD).<sup>[8]</sup> Stress and the associated imbalance in the ANS may play a role in sudden cardiac death in people with CVD.<sup>[9]</sup> Recent studies have demonstrated a significant positive connection between psychological distress and CVD, such as heart attack and stroke.<sup>[10,11]</sup> Therefore, interventions that can mitigate the effects of stress, such as deep breathing, meditation and other relaxation techniques, may be convenient in managing psychological distress while decreasing cardiovascular morbidity and mortality.<sup>[12,13]</sup> Meditation practices, such as mindfulness and transcendental meditation, have been shown to increase HRV, suggesting improved autonomic regulation and potential stress modulation.<sup>[5,6,14,15]</sup> Rajyoga meditation (RM), taught by the Brahma Kumaris, is an open-eyed practice involving positive affirmations and compassionate mind training, hypothesised to enhance vagal tone through relaxation and emotional regulation.<sup>[6,16]</sup> Unlike other meditation forms, RM's accessibility and structured audio guidance make it suitable for novices and trained practitioners. This study investigates the effects of RM on HRV across different age groups. Given that HRV is a known biomarker of autonomic flexibility and is often diminished in stress-related conditions, these findings may offer preliminary physiological insights into its potential mechanisms for stress management.<sup>[17]</sup> However, the therapeutic potential for stress-specific conditions requires direct future investigation with validated psychological measures. Alterations in breathing rate, posture and mental state are the primary contributors to heart rate variations during the practice of meditation and yoga. These variations are mediated to a large extent by the ANS, which consists of the sympathetic nervous system

(SNS) and the parasympathetic nervous system (PNS), with the SNS being active during states of excitement and stress ('fight or flight') and the PNS during states of rest ('rest and digest').<sup>[5]</sup> HRV can be analysed using a variety of methods, which can be categorised as linear techniques (time and frequency domain (FD) measures) and nonlinear (NL) techniques (fractal measures, entropy measures and Poincaré plot representations).<sup>[3,18-21]</sup> FD methods are designed to determine how the spectral power of HRV is distributed in different bands. The typical bands are called very low frequency, low frequency (LF) and HF bands (or components). The activity of the SNS and PNS is reflected in the LF power range (0.04–0.15 Hz), whereby the SNS has a more significant impact than the PNS. However, heart rate fluctuations in the HF band (0.15 Hz–0.4 Hz) essentially reflect the activity of the PNS. The increase in LF power and LF/HF ratio, often a source of conflicting interpretation, was observed alongside other markers of relaxation. This supports the contemporary view that LF power and LF/HF ratio during meditation may not solely reflect sympathetic activity but rather a complex mix of autonomic inputs, including parasympathetic modulation and baroreflex function, likely influenced by the controlled breathing and focused attention inherent to the practice.<sup>[20,22,23]</sup>

In studies on meditation, HRV parameters are scrutinised to establish whether meditation affects the ANS. While it is recognised that HRV parameters are influenced by complex autonomic interplay and can be correlated.<sup>[24]</sup> The specific and acute effects of RM on this interplay remain to be fully characterised. Therefore, the primary aim of this study was to investigate the immediate impact of a single session of RM on a comprehensive set of linear and non-linear HRV markers.

## MATERIALS AND METHODS

### Study design and participant recruitment

A quasi-experimental repeated measures study was conducted, encompassing ECG data collection from trained and untrained Rajyoga meditators. RM, as taught by the Brahma Kumaris, is an open-eyed meditation practice focusing on positive affirmations, self-awareness and connection to inner peace, typically guided by audio or instructor prompts to foster relaxation and mental clarity.<sup>[6]</sup> Trained participants ( $n = 21$ , 10 females/11 males, age range 36–72 years, mean  $52.76 \pm 9.56$  years) were recruited from the Brahma Kumaris Meditation Centre in Durgapur, India. They had practised RM daily for at least 1 year, with sessions lasting 30–60 min/day, 7 days/week, and  $\geq 90\%$  adherence (missing no more than 3–4 sessions/month), verified by consultation with meditation centre instructors. Untrained participants ( $n = 24$ , 5 females/19 males, age range 18–35 years, mean  $25.92 \pm 3.42$  years), with no prior meditation or mindfulness

experience, were recruited from the National Institute of Technology Durgapur (NITD). Following ethical approval from the Institute Ethics Committee (Ref. No. NITD/IEC/3-25 dated May 2, 2025), subject recruitment was initiated. Healthy adult volunteers of either sex, aged between 18 and 70 years, were enrolled in the study after obtaining informed written consent. While meditation is known to influence the ANS activity, it remains unclear whether long-term practice alters the acute physiological response. This study tests the hypothesis that experienced Rajyoga meditators exhibit a significantly greater and distinct HRV response during meditation compared to novices, even after accounting for age differences.

### Inclusion and exclusion criteria

Eligible participants were healthy adults aged 18–70 years, of either sex, with no history of cardiovascular, neurological or psychiatric disorders. Trained participants required at least 1 year of daily RM practice (30–60 min/day,  $\geq 90\%$  adherence). Untrained participants had no prior meditation experience. All participants reported no regular smoking (no cigarette or tobacco use in the past 6 months) and no regular alcohol consumption ( $\leq 1$  drink/week, e.g. 330 mL beer or 150 mL wine, in the past 6 months), confirmed through self-reported questionnaires. Exclusion criteria included use of medications affecting HRV (e.g. beta-blockers, antidepressants), regular smoking or alcohol use or inability to comply with the study protocol.

### Procedure

Participants were advised to have adequate sleep ( $\geq 6$  h) the night before recordings, consume a light meal approximately 2 h prior, refrain from caffeine-containing beverages (e.g. tea, coffee and energy drinks) and avoid strenuous physical activity for 24 h prior. On arrival at the laboratory, height and weight were measured using standard procedures to calculate body mass index (BMI). ECG recordings were collected for 30 min (10 min each for pre-meditation, during meditation and post-meditation phases) using a lead-II configuration.

### Meditation methodology

In this study, two groups of participants have been selected for Brahma Kumaris RM. The first group consisted of trained practitioners ( $>1$  year of daily practice) of Brahma Kumaris Rajyoga, a technique involving open-eyed meditation on soul-consciousness. The second group consisted of meditation novices. RM pertains to the Brahmakumaris school of thought, which serves as the foundation for this specific type of meditation. Distinguishing itself from other meditation techniques, RM is unique in that it involves

practising meditation with open eyes.<sup>[6]</sup> While many meditation techniques involve closed-eye practices, such as focusing on thoughts, counting breaths, chanting mantras or maintaining silence, RM follows a distinct approach. In RM, practitioners are guided to perceive themselves as eternal souls, represented as points of light, situated between the eyebrows. During meditation, they direct their gaze towards an external symbol, which is considered a symbolic representation of the Supreme Soul or God.<sup>[16,25]</sup> This study aimed to quantify and compare the acute HRV responses to a standardised, guided RM session in trained practitioners and novices. Specifically, we sought to determine whether the magnitude of autonomic change elicited by this guided practice differed based on prior meditation experience.

### Dataset collection

The ECG was recorded from the subjects of a group of 21 healthy trained participants from the Brahma Kumaris Meditation Centre, Durgapur (10 females/11 males; mean age  $\pm$  SD:  $52.76 \pm 9.56$  years), who had been practicing RM daily for at least 1 year (30–60 min/day, 7 days/week, with  $\geq 90\%$  adherence). The control group consisted of 24 healthy untrained participants (5 females/19 males; mean age  $\pm$  SD:  $25.92 \pm 3.42$  years) from the NITD (research scholars). The ECG was continuously acquired for 30 min from the subjects in a seated position, and the following protocol was applied during these 30 min: 10 min of recording before meditation was started, 10 min of meditation and 10 min after meditation was stopped. Between 10 and 20 minutes of the recording, i.e. during the meditation phase, all participants listened to the same Rajayoga-guided meditation audio recording, intended as a compassionate mind training audio exercise. The audio, delivered through standardised audio equipment in a quiet, controlled environment, consisted of: (1) an introduction (0–2 min) with instructions to sit comfortably, maintain open eyed focus and breathe slowly; (2) positive affirmations (2–7 min) encouraging thoughts of inner peace, self-worth and compassion (e.g. ‘I am a peaceful soul’, ‘I radiate love and kindness’) and (3) guided visualisation and silence (7–10 min) to internalise a serene state. This audio, designed as a compassionate mind training exercise, aimed to foster relaxation and positive emotional states.<sup>[25]</sup> Untrained participants received brief pre-session instructions to follow the audio prompts.

The laboratory experimental setup [Figure 1] was placed in a room kept at a constant temperature of  $26^{\circ}\text{C}$ . The ECG was recorded in a standardised manner. The subjects were instructed to sit on a chair. The ECG was recorded using the BIOPAC MP45 system using pre-gelled Ag/AgCl electrodes placed on the extremities, and Einthoven’s lead II of the ECG was recorded and digitised at a sampling rate of 2000 Hz.

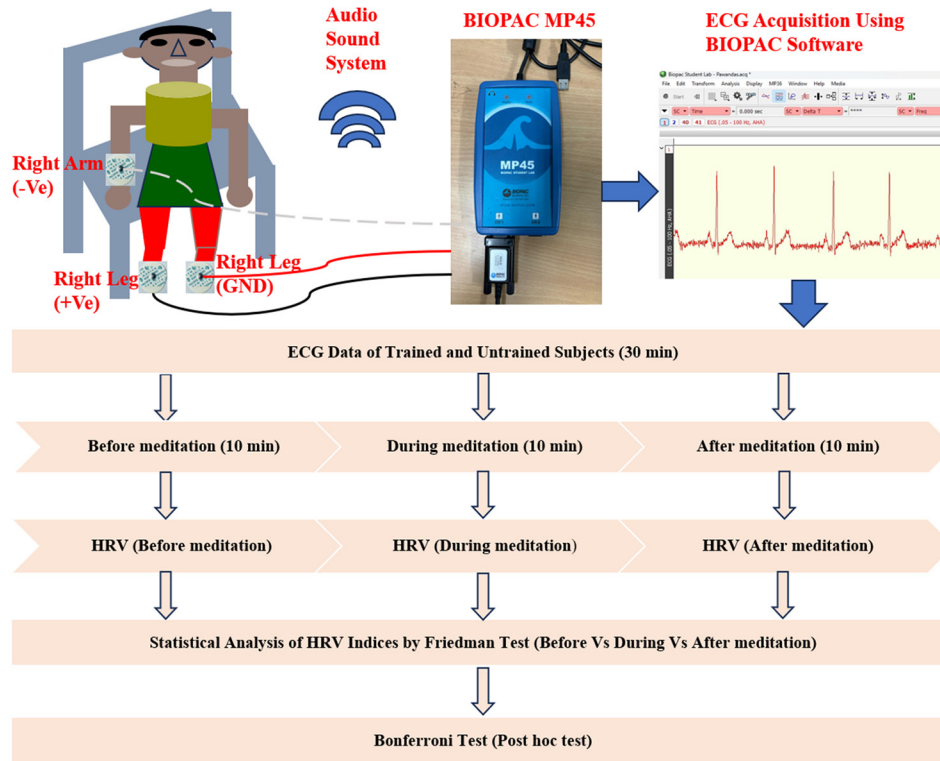


Figure 1: Schematic representation of the study protocol.

### Analysis of HRV

HRV analyses were conducted using the PhysioZoo HRV software<sup>[26]</sup> and the data were processed using MATLAB 2023a. The PhysioZoo user interface consists of two main modules – Peak Detection (for processing electrophysiological signals and extracting RR time series) and HRV analysis (calculating HRV measures from RR intervals). The default RQRS peak detector was utilised in the peak detection module, employing the following settings: A refractory period of 0.280 s was employed. The maximum RR interval was 2.4 s. A typical heart rate of 75 beats/min was used. The QRS duration was set to a typical value of 0.07 s. The QT duration was established at a typical value of 0.35 s. The typical QRS peak-to-peak amplitude was specified as 750  $\mu\text{V}$ . A minimum QRS peak-to-peak amplitude of 130  $\mu\text{V}$  was considered. The RQRS forward-search duration of the window is set at 80% of the average QRS complex time. To enhance the robustness of R-peak detection and effectively address false-positive and false-negative detections using the RQRS peak detector, it examines both minimal and maximal extremal points in the window, accommodating various ECG polarities.

It computes the median difference between signal values at Q-onset and extremal amplitudes. Larger median difference for maximal extremal points designates them as R-peaks; otherwise, minimal points are chosen. Table 1 shows the HRV

metrics of the time domain (TD), FD and NL analysis.<sup>[26]</sup> All HRV metrics are computed in the HRV analysis modules. The RR interval data were first detrended ( $\lambda = 10$ ) to remove any linear trend, and the power spectral density was computed using the fast Fourier transform-based Welch algorithm with 50% overlap.

### Statistical analysis

All statistical analyses were performed using Python (version 3.11.5) with the SciPy and Pandas libraries. First, once the HRV parameters had been extracted from the recorded ECGs, the normality of the data was examined by applying the Shapiro–Wilk test. This test did not confirm the normality of the distribution of the data. The results are presented as medians along with their interquartile ranges due to the absence of a Gaussian distribution for any of the parameters. For within-group comparisons across the three phases (pre-meditation, during meditation and post meditation), the non-parametric Friedman test was employed to assess differences in HRV parameters. After the Friedman test, *post hoc* Bonferroni correction was conducted for pairwise comparisons to determine the phases during which the HRV markers differed significantly. In the case of the Bonferroni correction, the corrected significance level ( $\alpha_{\text{corr}} = \alpha/3$ ) is calculated by dividing the desired overall significance level (denoted as  $\alpha$ ) by the number of

comparisons being performed. This adjustment minimises the risk of Type I errors when conducting multiple comparisons.

To analyse the difference in the meditation response between the trained and untrained groups. This analysis tested the hypothesis that the magnitude of the autonomic response to meditation differs based on prior experience. For this, the change score ( $\Delta$ ) for each HRV parameter was calculated as  $\Delta = \text{During meditation} - \text{pre meditation}$ . To control for the significant age disparity between the trained and untrained cohorts, between-group comparisons of these  $\Delta$  values were performed using Quade's nonparametric analysis of covariance (ANCOVA), with age included as a covariate. This method adjusts the ranks of the dependent variable ( $\Delta$  HRV) for the covariate (age) before performing a non-parametric test on the adjusted ranks, thus providing an age-adjusted comparison of the meditation response.

<b>Table 1:</b> HRV metrics of time domain, frequency domain and nonlinear analysis.		
Time domain metrics	AVNN (ms)	Average RR interval duration
	SDNN (ms)	Standard deviation of RR intervals
	RMSSD (ms)	Square root of the mean squared differences between consecutive RR intervals
	PIP (%)	Percentage of inflection points in the RR interval time series
	PSS (%)	Percentage of short segments
	IALS (n.u.)	Inverse average length of the acceleration/deceleration segments
Frequency domain metrics	LF (ms <sup>2</sup> )	Power in LF band (0.04–0.15 Hz)
	HF (ms <sup>2</sup> )	Power in HF band (0.15–0.4 Hz)
	TP (ms <sup>2</sup> )	The density spectra's total power ( $\leq 0.4$ Hz)
Non-linear metrics	SD <sub>1</sub> (ms)	Short-term variability in the RR intervals
	SD <sub>2</sub> (ms)	Long-term variability in the RR intervals
	SampEn (n.u.)	Sample entropy (keeping the value of embedding dimension (m=2) and distance (r=0.2))
The reported values represent the median within the range of the 25 <sup>th</sup> –75 <sup>th</sup> percentiles. AT: Analysis technique, TD: Time domain, FD: Frequency domain, NL: Nonlinear method, Pre: Before meditation, Med: During meditation, Post: After meditation. Level of significance: * $P < 0.05$ , ** $P < 0.01$ . AVNN: Average RR interval duration, SDNN: Standard deviation of RR intervals, RMSSD: Root mean square of successive differences, PIP: Percentage of inflection points, PSS: Percentage of short segments, IALS: Inverse average length of acceleration/deceleration segments, LF: Low frequency, HF: High frequency, TP: Total power, RR: Relative risk, HRV: Heart rate variability		

## RESULTS

### Demographic profile and baseline metrics

Participant demographics and baseline HRV characteristics are mentioned in Table 2. Data presented as mean  $\pm$  SD. As expected by the study design, the trained meditation group was significantly older than the untrained group ( $52.76 \pm 9.56$  vs.  $25.92 \pm 3.42$  years,  $P < 0.0001$ ). This is an inherent characteristic of the study population, as achieving 'trained' status in RM requires many years of practice. The untrained group was significantly taller ( $169.3 \pm 7.6$  vs.  $159.7 \pm 14.2$  cm,  $P = 0.015$ ) and heavier ( $67.4 \pm 11.1$  vs.  $61.1 \pm 10.0$  kg,  $P = 0.039$ ), but there was no significant difference in BMI between groups ( $P = 0.866$ ). At baseline, the untrained group showed significantly higher SDNN ( $50.9 \pm 22.2$  vs.  $38.0 \pm 13.9$  ms,  $P = 0.039$ ) and SD<sub>2</sub> ( $66.3 \pm 28.7$  vs.  $46.5 \pm 16.4$  ms,  $P = 0.012$ ), consistent with known age-related declines in HRV. There were no significant baseline differences in average RR interval duration, RMSSD, or SD<sub>1</sub>.

### Within-group differences in meditation-induced HRV changes

Tables 3 and 4 convey a comparison of the HRV features (TD, FD and NL methods) in the group of trained subjects and in the group of untrained subjects, respectively, under the three different phases of the test protocol. Figures 2 and 3 depict the HRV indices for trained and untrained subjects, respectively, encompassing data points from all individuals.

**Table 2:** Baseline demographic and HRV characteristics of trained and untrained groups.

Characteristic	Trained (n=21)	Untrained (n=24)	Mann-Whitney U-test
Demographic			P-value
Age (years)	52.76 $\pm$ 9.56	25.92 $\pm$ 3.42	<0.0001**
Weight (kg)	61.05 $\pm$ 10.04	67.36 $\pm$ 11.10	0.0386
Height (cm)	159.71 $\pm$ 14.24	169.29 $\pm$ 7.63	0.0153*
BMI (kg/m <sup>2</sup> )	24.26 $\pm$ 4.80	23.56 $\pm$ 4.10	0.8658
Baseline HRV metrics			
AVNN (ms)	809.68 $\pm$ 79.85	747.56 $\pm$ 107.66	0.0511
SDNN (ms)	38.04 $\pm$ 13.88	50.88 $\pm$ 22.17	0.0386*
RMSSD (ms)	33.23 $\pm$ 20.32	36.72 $\pm$ 20.09	0.4773
SD <sub>1</sub> (ms)	23.51 $\pm$ 14.38	25.98 $\pm$ 14.21	0.4773
SD <sub>2</sub> (ms)	46.50 $\pm$ 16.40	66.29 $\pm$ 28.74	0.0117*
BMI: Body mass index, P-values from Mann-Whitney U test, level of significance: * $P < 0.05$ , ** $P < 0.01$ . AVNN: Average RR interval duration, SDNN: Standard deviation of RR intervals, RMSSD: Root mean square of successive differences, HRV: Heart rate variability			

**Table 3:** Statistical analysis for the trained subjects.

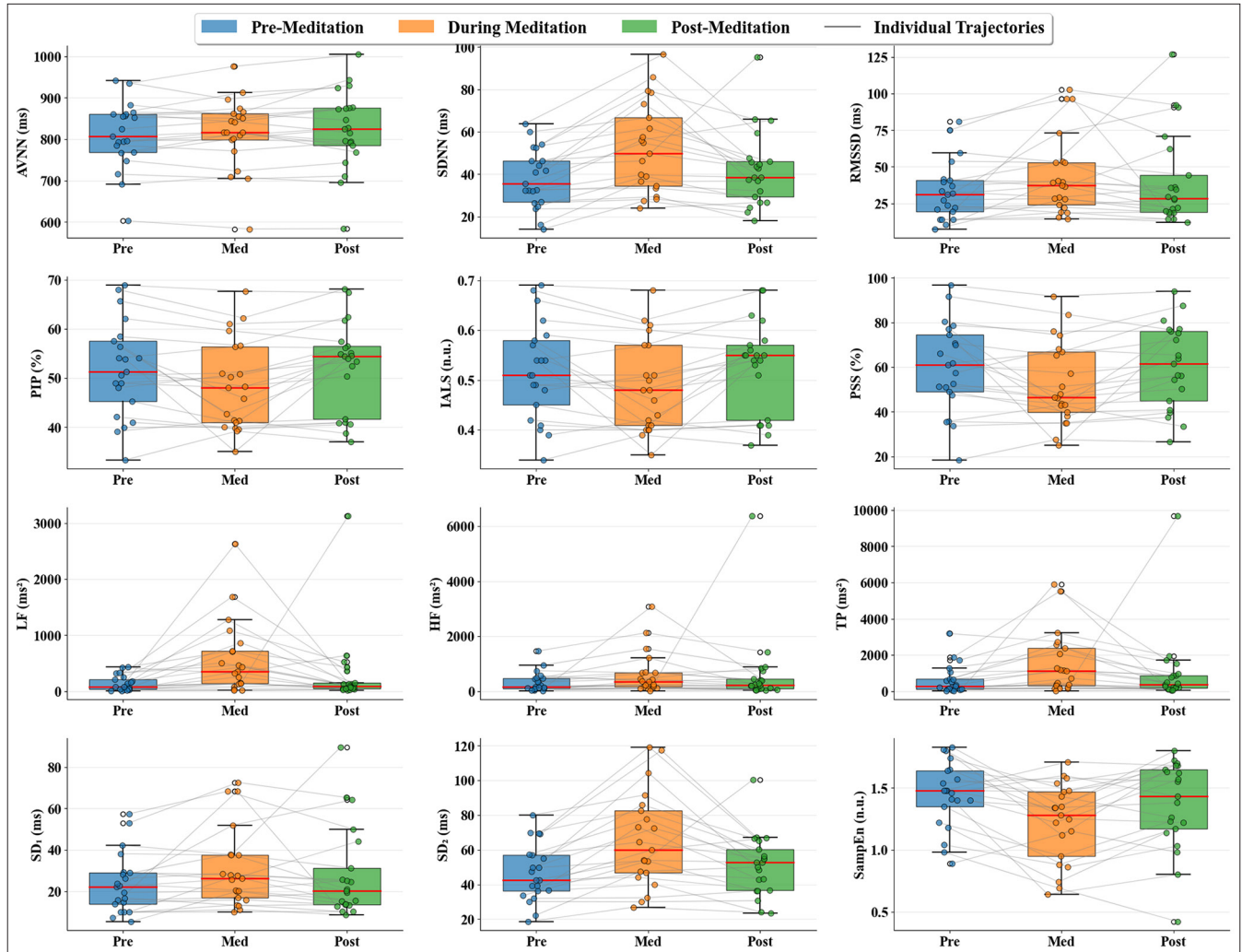
AT	Median (25–75% interquartile range)			Friedman test	Bonferroni <i>post-hoc</i> tests		
	Pre	Med	Post		Pre-med	Pre-post	Med-post
TD							
AVNN (ms)	806.57 (767.82–857.82)	817.16 (792.66–857.35)	815.75 (783.66–874.27)	0.4667	1	0.6511	1
SDNN (ms)	32.63 (26.86–47.81)	45.11 (34.15–62.95)	38.41 (28.65–45.73)	<0.0001**	0.0001**	1	<0.0001**
RMSSD (ms)	27.3 (19.08–39.96)	29 (23.53–43.43)	27.49 (19.50–38.16)	0.0497	0.1922	1	0.0619
PIP (%)	51.31 (42.07–57.8)	42.7 (39.71–52.38)	54.39 (40.93–58.51)	0.0119*	0.0619	1	0.0164*
IALS (n.u.)	0.51 (0.42–0.58)	0.43 (0.4–0.52)	0.55 (0.41–0.59)	0.0020**	0.0194	1	0.0030**
PSS (%)	61.81 (44.62–75.26)	43.54 (35.03–65.68)	61.66 (40.52–76.24)	0.0048**	0.0406	1	0.0060*
FD							
HF (ms <sup>2</sup> )	211.21 (89.82–484.43)	353.58 (141.77–673.91)	214.86 (108.56–419.71)	0.0119*	0.0619	1	0.0164*
LF (ms <sup>2</sup> )	89.75 (32.16–222.98)	320.45 (135.51–710.49)	92.41 (49.22–168.38)	0.0017**	0.0020**	1	0.0261
TP (ms <sup>2</sup> )	351.25 (134.84–789.41)	725.3 (290.67–2142.39)	364.38 (198.70–878.48)	0.0001**	0.0003**	1	0.0011**
NL							
SD <sub>1</sub> (ms)	19.31 (13.5–28.27)	20.52 (16.64–30.73)	19.45 (13.8–26.99)	0.0497	0.1922	1	0.0619
SD <sub>2</sub> (ms)	42.4 (35.68–57.50)	59.89 (46.12–83.41)	49.93 (36.54–59.99)	<0.0001**	<0.0001**	0.3684	0.0101*
SampEn (n.u.)	1.48 (1.4–1.64)	1.34 (1.14–1.49)	1.55 (1.22–1.68)	0.0497	0.0619	1	0.1922

The reported values represent the median within the range of the 25<sup>th</sup> to 75<sup>th</sup> percentiles. AT: Analysis technique, TD: Time domain, FD: Frequency domain, NL: Nonlinear method, Pre: Before meditation, Med: During meditation, Post: After meditation. Level of significance: \* $P < 0.05$ , \*\* $P < 0.01$ . AVNN: Average RR interval duration, SDNN: Standard deviation of RR intervals, RMSSD: Root mean square of successive differences, PIP: Percentage of inflection points, PSS: Percentage of short segments, IALS: Inverse average length of acceleration/deceleration segments, LF: Low frequency, HF: High frequency, TP: Total power

**Table 4:** Statistical analysis for the untrained subjects.

AT	Median (25–75% interquartile range)			Friedman test	Bonferroni <i>post-hoc</i> tests		
	Pre	Med	Post		Pre-med	Pre-post	Med-post
TD							
AVNN (ms)	699.57 (637.32–802.46)	690.8 (665.69–803.1)	687.27 (644.03–792.7)	0.3528	0.4467	1	1
SDNN (ms)	43.55 (30.70–51.83)	52.97 (40.51–61.88)	40.04 (31.06–52.33)	0.0003**	0.0015**	1	0.0015**
RMSSD (ms)	26.88 (20.45–37.99)	32.31 (27.10–38.86)	25.32 (16.2–35.17)	0.0076**	0.0911	1	0.0073*
PIP (%)	45.31 (40.43–49.11)	38.13 (30.7–44.89)	44.78 (36.12–47.83)	0.0003**	0.0009**	1	0.0027**
IALS (n.u.)	0.45 (0.41–0.49)	0.38 (0.31–0.45)	0.45 (0.36–0.48)	0.0001**	0.0004**	1	0.0017**
PSS (%)	50.03 (32.35–59.46)	30.17 (17.49–48.07)	44.54 (27.21–62.53)	<0.0001**	0.0005**	1	0.0001**
FD							
HF (ms <sup>2</sup> )	279.96 (153.38–429.70)	376.5 (291.31–571.23)	291.29 (119.67–470.04)	0.0094**	0.0116*	1	0.0627
LF (ms <sup>2</sup> )	189.16 (118.92–350.88)	391.26 (202.02–774.10)	198.11 (87.88–417.58)	<0.0001**	<0.0001**	1	<0.0001**
TP (ms <sup>2</sup> )	504.34 (274.54–974.03)	943.46 (545.27–1358.54)	556.79 (262.16–884.96)	<0.0001**	0.0001**	1	<0.0001**
NL							
SD <sub>1</sub> (ms)	19.01 (14.47–26.88)	22.86 (19.17–27.5)	17.91 (11.46–24.89)	0.0076**	0.0911	1	0.0073*
SD <sub>2</sub> (ms)	57.54 (40.76–67.68)	71.4 (53.92–82.39)	52.76 (42.65–70.45)	0.0003**	0.0015**	1	0.0015**
SampEn (n.u.)	1.43 (1.15–1.62)	1.27 (1.0–1.55)	1.35 (1.29–1.6)	0.0098**	0.1266	1	0.0088*

The reported values represent the median within the range of the 25<sup>th</sup>–75<sup>th</sup> percentiles. AT: Analysis technique, TD: Time domain, FD: Frequency domain, NL: Nonlinear method, Pre: Before meditation, Med: During meditation, Post: After meditation. Level of significance: \* $P < 0.05$ , \*\* $P < 0.01$ . AVNN: Average RR interval duration, SDNN: Standard deviation of RR intervals, RMSSD: Root mean square of successive differences, PIP: Percentage of inflection points, PSS: Percentage of short segments, IALS: Inverse average length of acceleration/deceleration segments, LF: Low frequency, HF: High frequency, TP: Total power



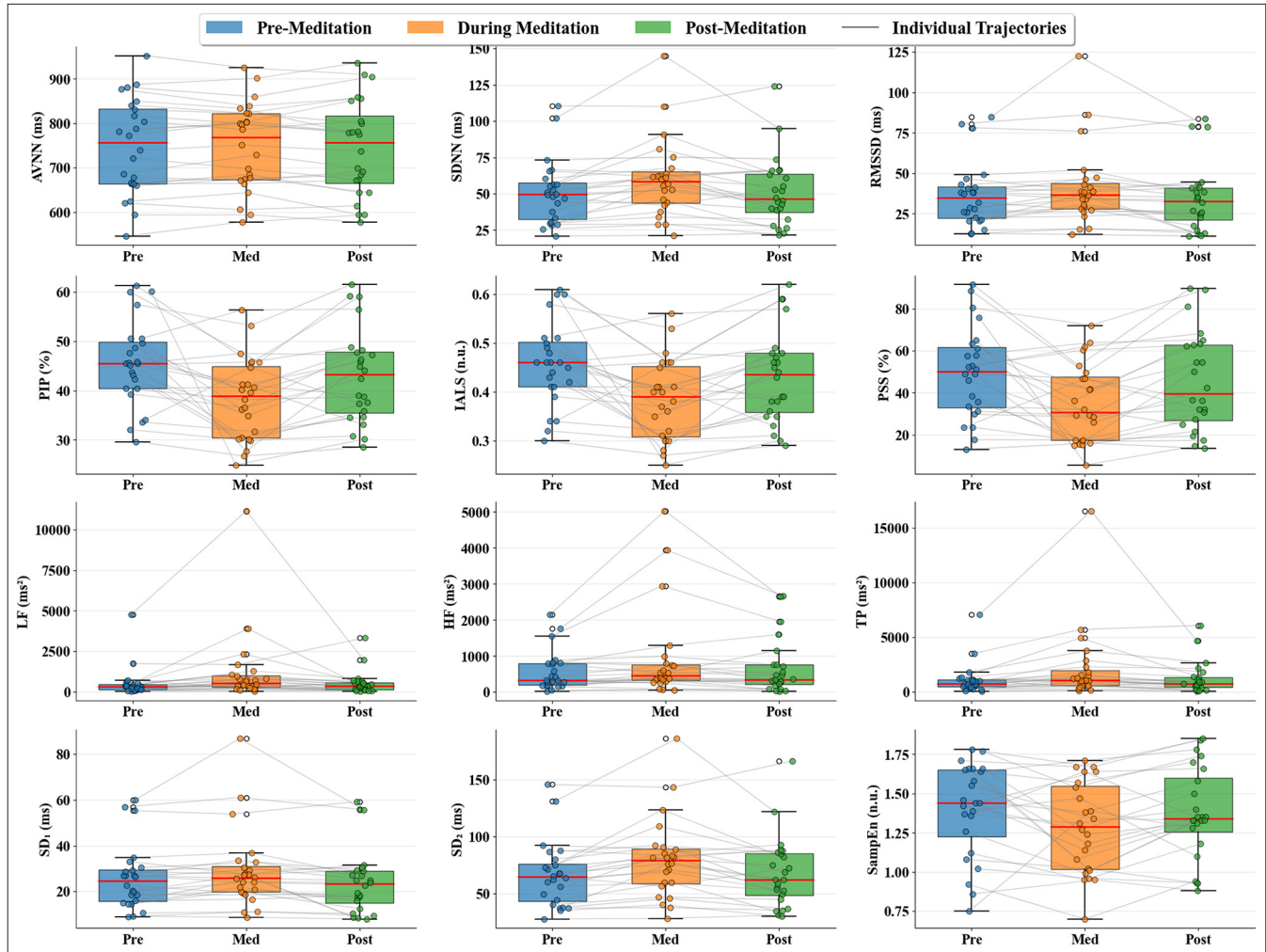
**Figure 2:** Linear and nonlinear heart rate variability indices of trained subjects ( $n = 21$ ) before, during and after meditation encompassing data points from all individuals. Each circle denotes an individual subject's data point.

From these figures and tables, we can say that a significant main effect of meditation was observed across both cohorts. The Friedman test revealed that during meditation, significant increases occurred in HRV parameters, including the SDNN, LF component, total power (TP) and long-term variability ( $SD_2$ ) compared to both pre- and post-meditation phases ( $P < 0.01$ ). *Post hoc* analysis confirmed that these values during meditation were significantly higher than both pre- and post-meditation values ( $P < 0.01$ ), while no significant difference was found between pre- and post-meditation phases ( $P > 0.01$ ), indicating a transient, reversible effect specific to the meditation practice. Conversely, a significant decrease in the inverse average length of acceleration/deceleration segments (IALS) and the percentage of short segments (PSS) was observed during meditation compared to before and after meditation in both groups ( $P < 0.05$ ), with values returning to baseline post-meditation. In untrained subjects, a significant within-group

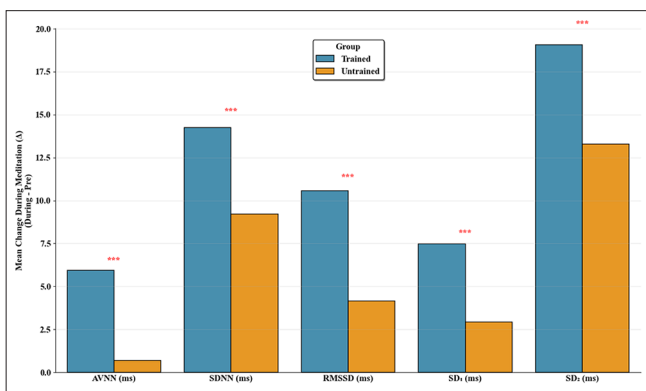
difference was observed in short-term variability ( $SD_1$ ) and SampEn ( $P < 0.05$ ), a change that was not significant in trained subjects. This suggests that novice meditators may undergo a different pattern of autonomic adjustment during their first meditation experience compared to the stable, habituated response of long-term practitioners.

### Between-group differences in meditation-induced HRV changes

After controlling for age using Quade's nonparametric ANCOVA, trained practitioners demonstrated significantly greater meditation-induced HRV changes compared to untrained novices across most of the HRV parameters examined ( $P < 0.001$ ). Table 5 shows the between-group comparisons of meditation-induced HRV changes using Quade's ANCOVA (age-adjusted). Figure 4 shows the age-adjusted magnitude of HRV changes during meditation



**Figure 3:** Linear and nonlinear heart rate variability indices of untrained subjects ( $n = 24$ ) before, during, and after meditation encompassing data points from all individuals. Each circle denotes an individual subject's data point.



**Figure 4:** Age-adjusted magnitude of heart rate variability changes during meditation in trained versus untrained practitioners.

**Table 5:** Between-group comparisons of meditation-induced HRV changes using Quade's ANCOVA (age-adjusted).

Parameter	Trained group ( $n=21$ )	Untrained group ( $n=24$ )	Quade's ANCOVA test
	Mean $\Delta \pm$ SEM	Mean $\Delta \pm$ SEM	P-value
AVNN (ms)	5.95 $\pm$ 5.68	0.70 $\pm$ 4.35	<0.001
SDNN (ms)	14.24 $\pm$ 2.70	9.20 $\pm$ 2.55	<0.001
RMSSD (ms)	10.57 $\pm$ 4.36	4.15 $\pm$ 1.84	<0.001
SD <sub>1</sub> (ms)	7.48 $\pm$ 3.09	2.94 $\pm$ 1.30	<0.001
SD <sub>2</sub> (ms)	19.07 $\pm$ 3.71	13.28 $\pm$ 3.49	<0.001

$\Delta$ : During meditation - Pre meditation. AVNN: Average RR interval duration, SDNN: Standard deviation of RR intervals, RMSSD: Root mean square of successive differences, HRV: Heart rate variability, ANCOVA: Analysis of covariance, SEM: Standard error of the mean

in trained versus untrained practitioners. As detailed in Table 5 and Figure 4, the most substantial differences were observed in parameters reflecting PNS activity. Trained

meditators showed a 154.6% greater increase in RMSSD (Trained:  $\Delta = 10.57 \pm 4.36$  ms; Untrained:  $\Delta = 4.15 \pm 1.84$  ms;

$P < 0.001$ ) and a 154.5% greater increase in  $SD_1$  (Trained:  $\Delta = 7.48 \pm 3.09$  ms; Untrained:  $\Delta = 2.94 \pm 1.30$  ms;  $P < 0.001$ ), indicating markedly enhanced vagally mediated modulation during meditation.

Significant between-group differences were also evident in measures of overall autonomic regulation. Trained practitioners exhibited a 54.7% greater increase in SDNN (Trained:  $\Delta = 14.24 \pm 2.70$  ms; Untrained:  $\Delta = 9.20 \pm 2.55$  ms;  $P < 0.001$ ) and a 43.6% greater increase in  $SD_2$  (Trained:  $\Delta = 19.07 \pm 3.71$  ms; Untrained:  $\Delta = 13.28 \pm 3.49$  ms;  $P < 0.001$ ), suggesting more robust overall autonomic flexibility and long-term variability regulation.

## DISCUSSION

This study provides clear evidence that a single session of RM induces acute, significant and reversible changes in cardiac autonomic regulation in both experienced practitioners and complete novices. Significant increases in time-domain (SDNN, RMSSD), frequency-domain (HF, TP) and NL ( $SD_1$ ,  $SD_2$ ) HRV metrics during meditation ( $P < 0.01$ , Friedman test, Bonferroni-corrected) indicate enhanced parasympathetic activity, consistent with prior studies on meditation-induced autonomic modulation.<sup>[3,17,27]</sup> The absence of pre- versus post-meditation differences ( $P > 0.01$ ) confirms reversible effects. We cautiously interpret the LF band changes. While they increased in both groups, modern literature warns against interpreting LF power as a sole sympathetic marker.<sup>[20,22,23]</sup> In this context, its concurrent rise with TP and SDNN is better interpreted as an increase in overall autonomic regulatory output, integral to the focused attention of meditation.<sup>[6,20]</sup> The RM audio, incorporating compassionate mind training through positive affirmations (e.g. 'I am a peaceful soul'), likely contributed to these effects by fostering relaxation and emotional resilience.<sup>[6,25]</sup> The decreased value of the SampEn in both trained and untrained subjects during meditation indicates a more regular and less complex HRV pattern.<sup>[24]</sup> This could be indicative of a decline in dynamical complexity during the meditation period.<sup>[3,28]</sup> The degree of fragmentation (percentage of inflection points, IALS and PSS) decreases during meditation in both trained and untrained groups. This observed trend indicates a positive outcome during meditation.<sup>[21,29]</sup> Between-group comparisons revealed baseline differences, with the untrained group showing higher SDNN ( $50.88 \pm 22.17$  ms vs.  $38.04 \pm 13.88$  ms,  $P = 0.0386$ ) and  $SD_2$  ( $66.29 \pm 28.74$  ms vs.  $46.50 \pm 16.40$  ms,  $P = 0.011$ , Mann-Whitney U test), potentially due to their younger age.

### Key findings and interpretation

To rigorously control for the confounding effect of age, Quade's nonparametric ANCOVA was performed on the change scores ( $\Delta = \text{Med} - \text{Pre}$ ) for key HRV parameters.

This study provides compelling evidence that meditation experience fundamentally alters autonomic responsiveness. The most striking finding was the 154.6% greater increase in RMSSD among trained practitioners, representing a substantial enhancement of parasympathetic activation during meditation.<sup>[20]</sup> This suggests that long-term Rajyoga practice may induce neuroplastic adaptations within the vagal circuit, potentially through strengthened efferent vagal outflow or improved cardiorespiratory coupling.<sup>[27,30,31]</sup> The parallel 154.5% greater increase in  $SD_1$  further supports this interpretation, as both RMSSD and  $SD_1$  are established markers of short-term, vagally mediated HRV.<sup>[32]</sup> The consistency of these findings across multiple parasympathetic indices strengthens the conclusion that experienced meditators develop an enhanced capacity for parasympathetic engagement during practice. The significant between-group differences in SDNN (54.7% greater increase) and  $SD_2$  (43.6% greater increase) suggest that meditation experience enhances not only paraspecific parasympathetic activation but also overall autonomic flexibility.<sup>[33]</sup> The greater increase in  $SD_2$ , which reflects long-term variability patterns, particularly indicates that trained practitioners develop more robust regulatory capacity across multiple time scales.<sup>[32]</sup>

Altogether, our results suggest that meditation has a beneficial effect on cardiovascular regulation by intensifying parasympathetic activity.

### Comparison with existing literature and mechanisms

Our findings on the acute autonomic effects of RM align with a growing body of research on diverse meditation practices, which consistently demonstrate increased HRV and a shift towards parasympathetic dominance during practice.<sup>[32,33]</sup> The observed increase in SDNN, RMSSD and HF power in our cohort is consistent with studies on Vipassana<sup>[23]</sup> and Kundalini Yoga<sup>[5]</sup> suggesting a common physiological signature of the meditative state characterised by enhanced autonomic regulation and metabolic efficiency.

The unique contribution of our study lies in its rigorous demonstration of this effect within the framework of standardised, open-eyed RM and its specific investigation into the impact of long-term training. While other studies have noted differences between meditators and non-meditators,<sup>[19]</sup> a significant confounding factor has often been the age disparity between groups. By employing Quade's ANCOVA to control for age, we provide robust, methodologically sound evidence of a significantly greater parasympathetic response.

### Study limitations and directions for future research

This study has a few limitations that should be considered when interpreting the results. First, the study predominantly

consisted of male participants, potentially introducing gender bias and hindering generalisability to female populations. A key limitation is the significant age disparity between groups ( $P < 0.01$ ), which may influence baseline HRV differences (e.g. lower SDNN/SD<sub>2</sub> in trained participants) due to age-related autonomic decline. While age-adjusted Quade's nonparametric ANCOVA analyses mitigated this confound, future studies should match groups by age to eliminate this limitation. The male dominance in the untrained group (19/24) is a limitation, as sex is a known factor influencing HRV, and this may affect the generalisability of the findings. This study did not directly assess stress levels (e.g. via subjective scales or cortisol measurements), relying instead on HRV as an indirect marker of stress-related autonomic modulation. Future research should include validated stress assessments to directly evaluate RM's impact on stress-related conditions. Future studies should use age- and sex-matched groups, subjective measures and longitudinal designs to explore RM's long-term effects. Despite these limitations, the study's findings offer valuable preliminary evidence that can guide future prospective studies in this field. Further research should address these limitations and aim for a more comprehensive understanding of the relationship between meditation and cardiovascular outcomes.

## CONCLUSION

This study demonstrates that a single session of RM induces significant, acute alterations in cardiac autonomic regulation, characterised by a pronounced increase in overall HRV and a shift towards parasympathetic dominance. The most critical finding, robustly confirmed after controlling for the confounding effect of age, is that long-term trained practitioners exhibit a quantitatively and significantly greater magnitude of this response compared to novices. This suggests that long-term practice enhances the functional capacity of the ANS to respond to the meditation stimulus, potentially conferring resilience against age-related autonomic decline. All the aforementioned findings imply enhanced parasympathetic activity while dynamical complexity and irregularity were reduced during meditation periods. These findings advocate that meditation has the potential to induce a relaxation response and promote a more relaxed state compared to the phases before and after meditation. These findings underscore the potential of RM as a practice for improving autonomic regulation.

**Acknowledgements:** We would like to express our sincere gratitude to BK. Sanjay and BK. Snigdha from the Rajyoga Meditation Centre, for their invaluable support in facilitating the participation of meditators in this study. We sincerely thank all participants for their generous time and involvement in this research. We are grateful to BK Chandrani for her valuable assistance and to BK. Shrikant of the

Rajyoga Education and Research Foundation (RERF) for granting us permission to recruit local RM practitioners.

**Ethical approval:** The research/study was approved by the Institutional Review Board at the Institutional Ethical Committee for Human Research, National Institute of Technology Durgapur, India, approval number NITD/IEC/3-25, dated 2nd May 2025.

**Declaration of patient consent:** The authors certify that they have obtained all appropriate patient consent forms. In the form, the patients have given their consent for their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

**Financial support and sponsorship:** Nil.

**Conflicts of interest:** There are no conflicts of interest.

**Use of artificial intelligence (AI)-assisted technology for manuscript preparation:** The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

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**How to cite this article:** Ranjan V, Kucera JP, Singh R, Ganguly A, Halder S. Acute effects of Rajyoga meditation on heart rate variability in older trained practitioners and younger novices: A quasi-experimental repeated measures study. *Indian J Physiol Pharmacol*. doi: 10.25259/IJPP\_302\_2025