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Longitudinal Changes in Acute Heart Rate Variability Responses to Sequential Relaxation Techniques During a Six-Month Hatha Yoga Intervention

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Abstract

Background and Objectives: Evidence regarding longitudinal changes in acute heart rate variability (HRV) responses to sequential relaxation and breathing techniques remains limited. This study examined these responses during six months of continued Hatha yoga practice. *Materials and Methods*: Thirty-five experienced female yoga practitioners participated in a six-month, single-group longitudinal study with twice-weekly supervised sessions. At baseline and after 2, 4, and 6 months, participants underwent a standardized 20-min electrocardiogram (ECG) recording protocol comprising preparation rest, Yoga Nidra, Ujjayi breathing, and final rest. Changes in RR interval (time between consecutive R peaks), low-frequency (LF), high-frequency (HF), and LF/HF ratio transition responses were evaluated using repeated-measures analysis of variance (ANOVA) with Bonferroni correction. Friedman tests provided sensitivity analyses. *Results*: Two of 20 longitudinal analyses showed nominally significant RR interval transition differences involving Ujjayi breathing ($p = 0.017$ and $p = 0.005$), but neither remained significant after correction for multiple testing (adjusted $p = 0.341$ and $p = 0.093$). No significant longitudinal effects were identified for LF, HF, or LF/HF. Sensitivity analyses yielded consistent findings. *Conclusions*: No longitudinal HRV transition effects remained statistically significant after multiple-testing correction. The exploratory RR findings do not establish physiological adaptation to continued yoga practice. Controlled studies incorporating respiratory monitoring and counterbalanced experimental sequences are needed.

Keywords: heart rate variability; Hatha yoga; Yoga Nidra; Ujjayi breathing; autonomic regulation; controlled breathing; longitudinal study

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1. Introduction

Heart rate variability (HRV) is a non-invasive measure of beat-to-beat variation in cardiac rhythm and is widely used to investigate autonomic regulation and cardiovascular responses to physiological and behavioral interventions. Yoga combines postural, breathing, and meditative practices and has increasingly been investigated as a mind–body intervention with potential effects on physical and psychological health [1–3]. Cardiovascular measures, including heart rate, blood pressure, and HRV, have therefore been frequently used to evaluate physiological responses to yoga practice [4–7].

HRV reflects complex autonomic modulation of cardiac rhythm and is commonly analyzed in the frequency domain, in which heart rate oscillations are divided into very-low-frequency (VLF), low-frequency (LF), and high-frequency (HF) bands [8–10]. LF and HF are commonly used frequency-domain measures in short-term HRV recordings [10,11]. HF is predominantly associated with respiratory-related parasympathetic modulation, although it should not be regarded as an exclusive measure of cardiac vagal activity [8,10]. In contrast, LF reflects multiple physiological influences, including autonomic and baroreflex mechanisms, and should not be interpreted as a direct measure of sympathetic activity [8,10,12]. Similarly, although the LF/HF ratio has historically been used as an index of sympathovagal balance, this interpretation has important physiological and methodological limitations, and the ratio should not be considered a direct measure of sympathetic-to-parasympathetic activity [8,12]. Therefore, in the present study, LF and LF/HF were analyzed as spectral HRV measures without assigning them exclusively to sympathetic or parasympathetic activity.

Yoga interventions of markedly different durations have been investigated, ranging from the acute effects of a single session to interventions lasting several months [13–15]. However, findings regarding HRV responses to longer-term yoga practice remain inconsistent. For example, Papp et al. reported changes in HF and LF/HF following an 8-week Hatha yoga intervention, whereas Hewett et al. did not observe an increase in HF following a 16-week Bikram yoga program [16,17]. Six months of regular yoga practice has also been associated with changes in indices of cardiac autonomic regulation [18]. Overall, evidence regarding the effects of yoga on HRV remains heterogeneous. An earlier systematic review found insufficient evidence for consistent HRV effects [19], while a more recent systematic review and meta-analysis reported improvements in several HRV indices but substantial heterogeneity and methodological limitations [20]. Differences in intervention duration, study populations, yoga styles, and experimental protocols may contribute to variability among reported findings.

HRV responses may also depend on the specific component of yoga being performed. Postural practice, meditation, and controlled breathing impose different physiological and respiratory demands and may consequently produce different cardiovascular and HRV response patterns [6,21–23]. Yoga Nidra is primarily a guided meditative relaxation practice, whereas Ujjayi involves deliberate regulation of breathing and airflow. Recent evidence suggests that Yoga Nidra may influence cardiovascular parameters, although methodological limitations remain in the available literature [24]. Slow and controlled breathing can also acutely modify cardiovascular function and HRV [25–27]. Importantly, respiratory rate can substantially influence the distribution of HRV. During slow or resonance-frequency breathing, respiratory oscillations may occur within the conventional LF frequency range, resulting in changes in LF, HF, and LF/HF that cannot be interpreted using simple sympathetic–parasympathetic assumptions [28]. Consequently, HRV responses observed during Ujjayi breathing should be interpreted in the context of deliberate respiratory modulation rather than as direct indicators of changes in sympathetic activity. Examining transitions between relaxation, meditation, and controlled breathing may therefore provide information about how acute HRV response patterns vary across these physiological states.

Previous yoga–HRV studies have generally focused on overall intervention effects or responses to individual yoga techniques, while considerably less is known about whether acute within-session HRV responses change over a prolonged period of regular practice. In particular, longitudinal data examining repeated responses to the same fixed sequence of rest, meditation, controlled breathing, and recovery remain limited. Studying these within-person response patterns may help characterize whether acute cardiovascular responses remain stable or change with continued yoga experience. Because the techniques were performed in a fixed sequence, the present study was designed to evaluate longitu-

dinal changes in transition responses rather than to determine the relative superiority of individual techniques.

Importantly, Bertisch et al. [29] investigated long-term practitioners of yogic breathing and reported that differences in respiratory sinus arrhythmia between practitioners and controls observed during spontaneous breathing were not maintained under standardized breathing conditions. These findings highlight the importance of respiratory patterns and suggest that sustained cardiovascular adaptations associated with repeated yoga practice remain incompletely understood. Furthermore, whether continued yoga practice influences the magnitude of acute HRV responses to sequential relaxation and breathing techniques in previously experienced practitioners has not been adequately established.

The present study aimed to characterize longitudinal changes in acute HRV transition responses during an additional six months of supervised Hatha yoga practice in women with previous yoga experience. Specifically, within-participant differences in RR interval, LF, HF, and the LF/HF between predefined stages of a fixed sequence comprising preparation rest, Yoga Nidra, Ujjayi breathing, and final rest were examined at baseline and after 2, 4, and 6 months.

Because participants were already experienced practitioners and the available evidence does not establish a consistent direction of longitudinal HRV change in this population, the investigation was exploratory rather than based on a prespecified directional hypothesis [16,29]. The primary research question was whether the magnitude of these predefined within-session transition responses differed across the four assessments. A secondary exploratory question was whether continued yoga practice was associated with a reduction in the magnitude of acute HRV responses to relaxation and controlled breathing, potentially reflecting increasing familiarity or adaptation to the experimental techniques. However, such a pattern could also arise from changes in the preceding resting conditions or repeated exposure to the assessment protocol rather than physiological adaptation itself. No single HRV parameter was designated as the primary outcome, and the findings were interpreted in the context of multiple testing and the fixed experimental sequence.

2. Materials and Methods

2.1. Study Design

This prospective, single-group longitudinal study followed experienced Hatha yoga practitioners for an additional six months of supervised practice. Participants attended two supervised Hatha yoga classes per week. Baseline characteristics of participants were obtained during the first meeting. Electrocardiogram (ECG) measurements were obtained at baseline and after 2, 4, and 6 months of the intervention. All assessments were conducted in the same laboratory using the same ECG equipment and standardized testing sequence and all yoga sessions were led by the same certified yoga teacher. The study did not include a non-yoga control or comparison group; accordingly, longitudinal findings were interpreted as changes observed during continued yoga practice rather than as causal effects attributable to yoga alone. Attendance was checked at supervised sessions for organizational purposes. Participants received verbal instructions concerning yoga practice outside the supervised sessions; however, no home yoga practice was prescribed, and adherence to these instructions was not systematically monitored.

2.2. Participants

Recruitment and data collection for the present cohort took place during 2016–2018. Enrollment was staggered, and assessments were scheduled at baseline and after 2, 4, and 6 months relative to each woman's individual intervention start.

Only women were included to obtain a homogeneous study population, as yoga participation is more common among women than men [30]. The study protocol was informed by a previously published preliminary investigation involving 13 women [31]. The present longitudinal study included a separate cohort of 35 women, with no participant overlap between the two investigations. Thirty-five healthy women were recruited through advertisements displayed at a local yoga studio. All participants were already regular Hatha yoga practitioners before enrollment.

Women were eligible if they met all of the following criteria: (a) healthy women aged 18–39 years, (b) regular Hatha yoga practice twice weekly, (c) normal blood pressure (BP) (systolic BP $\leq$ 130 mmHg and diastolic BP $\leq$ 85 mmHg), (d) regular menstrual cycles, (e) at least six months of Hatha yoga experience. Body mass index (BMI) was calculated from measured height and weight and recorded as a descriptive baseline characteristic. BMI was not used as an inclusion or exclusion criterion. Before each assessment, participants were instructed to consume only a light breakfast and to refrain from chocolate, tea, coffee, caffeinated beverages, and alcohol for 24 h before testing [11,32].

Participants were excluded if they: (a) had any contraindications for physical activity, (b) used regular medication, (c) were pregnant, (d) participated in other structured exercise programs, (e) had any diagnosed cardiovascular, metabolic, neurological, or other chronic diseases, (f) reported a major stressful life event during the study period [33].

This study's protocol was approved by Kaunas regional biomedical research ethics committee (BE-2-36) and adhered to the tenets of the Declaration of Helsinki. All participants received detailed information regarding the study procedures and potential risks before providing written informed consent.

2.3. Yoga Intervention

The six-month intervention consisted of approximately 50 scheduled supervised Hatha yoga classes, generally offered twice weekly. Attendance was checked at supervised sessions for organizational purposes; however, participant-specific attendance records were not retained for research analysis. Consequently, participant-specific attendance rates and cumulative intervention exposure could not be quantified for the present analysis. Sessions were led by a certified yoga teacher with 10 years of experience and lasted approximately 60–90 min. Each class followed the same general structure: approximately 5 min of pranayama, 10–15 min of warm-up, 40–60 min of asana practice, and 5–10 min of Savasana [34]. The exact asana sequence varied between classes, as occurs in routine Hatha yoga practice, but included seated, standing, balance, spinal-mobility, back-extension and relaxation postures. Representative poses included: Sukhasana, Tadasana, Adho Mukha Svanasana, Bhujangasana, Virabhadrasana I–III, Trikonasana, Vrksasana, Marjaryasana, Balasana and Savasana [31]. Postures were adapted to individual ability, and participants were instructed to coordinate movement with breathing and to avoid forcing postures.

2.4. Experimental Protocol

Participants attended two supervised yoga classes per week. Each participant had an individual meeting and underwent ECG assessment.

For each assessment, ECG electrodes were placed according to the study recording configuration. Participants remained supine throughout a 20 min fixed-sequence protocol consisting of consecutive 5 min stages: preparation rest, Yoga Nidra meditation, Ujjayi breathing exercise and final rest (Figure 1). Supine relaxation and Ujjayi breathing exercise have been used in previous yoga research [21,35].

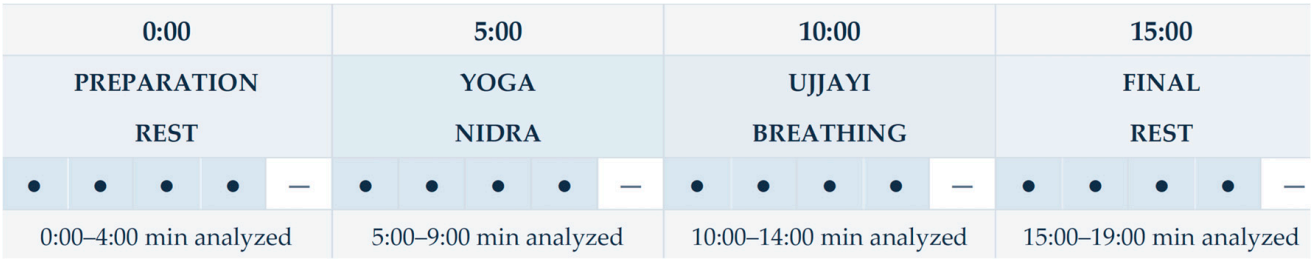


Figure 1. Standardized 20-min relaxation protocol and electrocardiogram (ECG) parameter extraction windows. Continuous ECG recording across four consecutive 5-min phases; analysis was performed using first 4 min of each phase. Colors distinguish the four experimental stages; black circles indicate the analyzed minutes, and dashes indicate the minutes excluded from analysis.

During the five-minute Yoga Nidra stage, participants remained in a supine position and listened to a prerecorded audio-guided relaxation session. The same audio recording was used at all four assessments (baseline and after 2, 4, and 6 months) to ensure consistency in the content and delivery of instructions. During the subsequent five-minute Ujjayi breathing stage, participants were instructed to breathe through the nose while producing a "ha" sound in the throat during exhalation. Breathing was performed at each participant's own comfortable pace, without a prescribed respiratory frequency. Respiratory rate and breathing depth were not recorded.

2.5. Procedures

The experimental protocol was designed to examine whether acute HRV transition responses within the same fixed sequence changed across six months of continued Hatha yoga practice [15,18]. The principal longitudinal question was therefore whether the magnitude of each predefined transition response differed across assessment time points.

All measurements were performed in a dedicated, quiet ECG laboratory with only one participant and investigator present. Laboratory temperature was maintained at 20–24 °C and 40–60% relative humidity [11]. The same equipment and protocol were used at all assessments.

At baseline, a laboratory technician measured age, height, body mass, body mass index, systolic and diastolic blood pressure and resting heart rate (Table 1).

Table 1. Baseline characteristics of participants.

Characteristic	N	Mean ± SD	95% CI
Height, m	35	1.70 ± 0.06	1.68 to 1.72
Weight, kg	35	62.11 ± 10.53	58.50 to 65.70
Body mass index, kg/m^2	35	21.37 ± 3.22	20.26 to 22.48
Age, years	35	29.80 ± 5.60	27.88 to 31.72
Systolic blood pressure, mmHg	35	110.60 ± 10.01	107.16 to 114.04
Diastolic blood pressure, mmHg	35	68.06 ± 9.23	64.89 to 71.23
Resting heart rate, bpm	35	66.00 ± 8.69	63.02 to 68.99

Values are presented as means ± standard deviations and 95% confidence intervals. SD, standard deviation; CI, confidence interval; bpm, beats per minute; N, number of participants.

2.6. ECG Recording Device

ECG was recorded using a wireless 10-lead Bluetooth ECG recorder M-Trace PC (M4Medical, Lublin, Poland) [36]. ECG data were analyzed using the Kaunas-Load ECG analysis system, version WI.6, developed in the Institute of Cardiology at the Lithuanian

University of Health Sciences (Kaunas, Lithuania) [37]. Recorded signals were transferred from the ECG device to the analysis system via Bluetooth.

2.7. HRV ECG Parameters

Frequency-domain HRV analysis was performed using the Kaunas-Load ECG analysis system, version WI.6 (Kaunas, Lithuania). The software displays conventional LF (0.04–0.15 Hz) and HF (0.15–0.40 Hz) spectral powers (ms^2) separately from frequency-band-specific alpha-related coefficients. Previous work using the Kaunas-Load system describes alpha-related indices associated with spectral slopes on logarithmic axes [38]. The LF and HF values retained in the present research dataset were reported as Tg(α) coefficients and should not be interpreted as absolute spectral-power values. The LF/HF variable was obtained separately from the software output and was not calculated by dividing the reported Tg(α) coefficients. Examination of two illustrative original software intervals showed that the displayed LF/HF was consistent with the ratio of conventional LF and HF spectral powers, not with division of the separately displayed alpha-related coefficients. This explains how LF/HF can exceed 1 when the LF Tg(α) coefficient is numerically below the HF Tg(α) coefficient. However, the exact numerical implementation of Tg(α) and the formal equivalence of that historical label to the software's alpha notation could not be independently verified. The illustrative intervals were not assumed to reproduce the original historical extraction windows. A continuous four-minute interval comprising the first four minutes of each five-minute experimental stage was selected for HRV analysis, consistent with the documented study method. Bourdillon et al. [39] provide methodological support for four-minute recordings of selected frequency-domain HRV measures, although their protocol included an initial settling period before analysis. In the present study, the analysis windows began at the onset of each experimental stage; therefore, early transitional and potentially nonstationary physiological responses may have influenced the HRV estimates. The original manual processing settings could not be independently reconstructed.

The analyzed outcomes were RR interval, LF, HF, and LF/HF ratio. Transition variables (Δ) were calculated as the difference between predefined protocol stages: Preparation rest to Yoga Nidra (ΔYn–Pr), Yoga Nidra to Ujjayi breathing (ΔUb–Yn), Ujjayi breathing to Final rest (ΔFr–Ub), between Ujjayi breathing and preparation rest (ΔUb–Pr) and between preparation rest and final rest (ΔFr–Pr). These variables quantify change across stages within the fixed sequence; they do not isolate technique-specific effects because each stage followed the preceding stage in the same order.

2.8. Data Analysis

Statistical analysis was performed using IBM SPSS Statistics Version 27.0 (IBM Corp., Armonk, NY, USA) and Microsoft Excel Version 2608 (Microsoft Corporation, Redmond, WA, USA). Data are presented as mean ± SD, unless otherwise specified. Longitudinal changes were evaluated in 35 participants with measurements obtained at baseline and after 2, 4 and 6 months.

For each participant and assessment, changes in heart rate variability (HRV) parameters between predefined experimental stages were calculated as difference scores (Δ). Five transitions were examined: preparation rest to Yoga Nidra (ΔYn–Pr), Yoga Nidra to Ujjayi breathing (ΔUb–Yn), Ujjayi breathing to Final rest (ΔFr–Ub), preparation rest to Ujjayi breathing (ΔUb–Pr), and preparation rest to final rest (ΔFr–Pr). Four parameters were analyzed for each transition: RR interval, LF, HF and LF/HF. Five predefined transitions and four HRV outcomes yielded 20 longitudinal outcome–transition analyses.

Longitudinal differences across the four assessments were examined using repeated-measures analysis of variance (ANOVA). The Shapiro–Wilk test was used to screen the distribution of transition scores at each assessment, while Mauchly's test was applied to evaluate the assumption of sphericity. When sphericity was violated ($p < 0.05$), Greenhouse-Geisser correction was applied. Effect sizes were expressed as partial eta squared (partial η^2).

For post hoc comparisons between assessment times, mean paired differences were reported with Bonferroni-adjusted p-values and simultaneous 95% confidence intervals (CIs), accounting for the six possible pairwise comparisons within each outcome-transition combination. To address multiple testing across the 20 longitudinal omnibus analyses, an additional Bonferroni adjustment was applied to their p-values. Both unadjusted and Bonferroni-adjusted omnibus p-values were reported.

For outcome-transition combinations exhibiting departures from normality according to the Shapiro–Wilk test ($p < 0.05$ at one or more assessments), Friedman tests were performed as sensitivity analyses. These nonparametric analyses were used to assess the consistency of the findings obtained from repeated-measures ANOVA. Friedman test results were reported using chi-square statistics, degrees of freedom, and p-values.

The familywise significance level was set at $\alpha = 0.05$. Omnibus effects were considered statistically significant only when their Bonferroni-adjusted p-values were below 0.05. Pairwise differences identified within outcome-transition combinations whose omnibus effects did not remain significant after correction across the 20 analyses were interpreted as exploratory.

Because the study was exploratory, included multiple interrelated HRV outcomes and overlapping transitions, and lacked a control group, the findings were interpreted cautiously without attributing longitudinal differences specifically to yoga practice.

3. Results

3.1. Longitudinal Changes in HRV Transition Responses

Longitudinal changes in heart rate variability (HRV) transition responses were evaluated across baseline and 2, 4 and 6 month assessments using repeated-measures analysis of variance (ANOVA). Twenty outcome-transition combinations were examined, comprising RR interval, low-frequency (LF), high-frequency (HF), and the LF/HF ratio across five predefined transitions. Following identification and correction of an error in the two-month ΔLF transition variable for the Ujjayi breathing to Final rest transition, two omnibus analyses demonstrated nominally significant longitudinal effects ($p < 0.05$), both involving RR interval changes during transitions into Ujjayi breathing.

However, neither remained statistically significant after Bonferroni adjustment across the 20 omnibus analyses (Supplementary Table S1). Accordingly, the pairwise findings are interpreted as exploratory.

3.1.1. Preparation Rest to Yoga Nidra Transition

For the transition from preparation rest to Yoga Nidra (ΔYn–Pr), no statistically significant longitudinal differences were identified for any of the analyzed HRV parameters (Table 2). The repeated measures ANOVA yielded $F(2.42, 82.35) = 1.121$, $p = 0.338$, partial $\eta^2 = 0.032$ for ΔLF, with Greenhouse–Geisser-adjusted degrees of freedom following a significant Mauchly's test of sphericity ($p = 0.013$). No significant longitudinal effects were observed for ΔHF ($F(3, 102) = 0.284$, $p = 0.837$, partial $\eta^2 = 0.008$), ΔLF/HF ($F(3, 102) = 2.345$, $p = 0.077$, partial $\eta^2 = 0.065$), or ΔRR ($F(3, 102) = 0.889$, $p = 0.450$, partial $\eta^2 = 0.025$).

Table 2. Heart rate variability changes from preparation rest to Yoga Nidra during six-month period (Δ (Yn–Pr)).

Parameters	Baseline	2 Months	4 Months	6 Months
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
ΔLF, Tg(α)	−0.02 ± 1.01	−0.13 ± 1.18	0.14 ± 0.83	−0.30 ± 1.18
ΔHF, Tg(α)	0.13 ± 0.62	0.14 ± 0.68	0.01 ± 0.78	0.09 ± 0.66
ΔLF/HF	−0.16 ± 0.43	−0.12 ± 0.31	0.01 ± 0.31	−0.07 ± 0.32
ΔRR, s	0.00 ± 0.03	0.01 ± 0.02	0.01 ± 0.03	0.01 ± 0.03

LF, low-frequency; HF, high-frequency; RR, interval between two consecutive R peaks.

The previously reported difference in ΔLF between 4 and 6 month assessments did not remain statistically significant after Bonferroni adjustment (mean paired difference, 0.438; simultaneous 95% CI, −0.085 to 0.961; adjusted $p = 0.149$). Similarly, the difference in ΔLF/HF between baseline and 4 months was not significant after adjustment (mean paired difference, −0.165; simultaneous 95% CI, −0.353 to 0.022; adjusted $p = 0.111$). The corresponding stage-level HRV measurements are presented in Tables 3 and 4.

Table 3. Heart rate variability parameters during preparation rest across the six-month follow-up.

Parameters	Baseline	2 Months	4 Months	6 Months
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
LF, Tg(α)	−1.06 ± 0.78	−1.07 ± 0.88	−1.12 ± 0.71	−1.00 ± 0.75
HF, Tg(α)	−1.20 ± 0.51	−1.40 ± 0.54	−1.29 ± 0.76	−1.22 ± 0.56
LF/HF	0.83 ± 0.50	0.79 ± 0.50	0.79 ± 0.42	0.83 ± 0.52
RR, s	0.92 ± 0.12	0.91 ± 0.11	0.89 ± 0.13	0.89 ± 0.13

LF, low-frequency; HF, high-frequency; RR, interval between two consecutive R peaks.

Table 4. Heart rate variability parameters during Yoga Nidra across the six-month follow-up.

Parameters	Baseline	2 Months	4 Months	6 Months
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
LF, Tg(α)	−1.08 ± 0.72	−1.20 ± 0.90	−0.98 ± 0.69	−1.30 ± 0.99
HF, Tg(α)	−1.07 ± 0.59	−1.26 ± 0.73	−1.27 ± 0.53	−1.13 ± 0.56
LF/HF	0.67 ± 0.31	0.68 ± 0.38	0.79 ± 0.46	0.76 ± 0.38
RR, s	0.92 ± 0.12	0.91 ± 0.11	0.90 ± 0.13	0.90 ± 0.13

LF, low-frequency; HF, high-frequency; RR, interval between two consecutive R peaks.

3.1.2. Yoga Nidra to Ujjayi Breathing Transition

For the transition from Yoga Nidra to Ujjayi breathing (ΔUb–Yn), a nominally significant longitudinal effect was observed for ΔRR ($F(3, 102) = 3.553$, $p = 0.017$, partial $\eta^2 = 0.095$). Bonferroni-adjusted pairwise comparisons indicated differences between baseline and 4 months (mean paired difference, −0.0223 s; simultaneous 95% CI, −0.0438 to −0.0008 s; adjusted $p = 0.038$) and between baseline and 6 months (mean paired difference, −0.0238 s; simultaneous 95% CI, −0.0459 to −0.0017 s; adjusted $p = 0.029$). These results indicate that the negative RR transition differences were smaller in magnitude at the later assessments.

However, the omnibus effect did not remain statistically significant after Bonferroni correction across the 20 longitudinal analyses (adjusted $p = 0.341$). Therefore, these pairwise differences should be interpreted as exploratory rather than confirmatory evidence of longitudinal change.

No statistically significant longitudinal effects were identified for ΔLF ($F(3, 102) = 0.910$, $p = 0.439$, partial $\eta^2 = 0.026$), ΔHF ($F(3, 102) = 1.051$, $p = 0.373$, partial $\eta^2 = 0.030$), or

ΔLF/HF ($F(3, 102) = 0.679$, $p = 0.567$, partial $\eta^2 = 0.020$). The transition scores and corresponding stage-level measurements are presented in Tables 5 and 6.

Table 5. Heart rate variability changes from Yoga Nidra to Ujjayi breathing during six month period (Δ (Ub-Yn)).

Parameters	Baseline	2 Months	4 Months	6 Months
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
ΔLF, Tg(α)	−0.69 ± 1.62	−0.42 ± 1.71	−0.86 ± 1.67	−0.37 ± 1.73
ΔHF, Tg(α)	0.06 ± 0.63	0.20 ± 0.87	0.30 ± 0.58	0.15 ± 0.56
ΔLF/HF	0.87 ± 0.65	0.81 ± 0.68	0.73 ± 0.57	0.74 ± 0.66
ΔRR, s	−0.06 ± 0.05 #*	−0.05 ± 0.06	−0.04 ± 0.04 *	−0.04 ± 0.05 #

* Significant difference between baseline and 4 months; # significant difference between baseline and 6 months (Bonferroni-adjusted pairwise $p < 0.05$). These comparisons are exploratory because the corresponding omnibus effects did not remain significant after Bonferroni correction across the 20 longitudinal analyses. LF, low-frequency; HF, high-frequency; RR, interval between two consecutive R peaks.

Table 6. Heart rate variability parameters during Ujjayi across the six-month follow-up.

Parameters	Baseline	2 Months	4 Months	6 Months
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
LF, Tg(α)	−1.76 ± 1.18	−1.62 ± 1.22	−1.84 ± 1.37	−1.67 ± 1.23
HF, Tg(α)	−1.01 ± 0.35	−1.06 ± 0.45	−0.98 ± 0.37	−0.98 ± 0.51
LF/HF	1.54 ± 0.63	1.49 ± 0.72	1.52 ± 0.67	1.51 ± 0.78
RR, s	0.86 ± 0.12	0.87 ± 0.11	0.86 ± 0.12	0.86 ± 0.12

LF, low-frequency; HF, high-frequency; RR, interval between two consecutive R peaks.

3.1.3. Ujjayi Breathing to Final Rest Transition

For the transition from Ujjayi breathing to final rest (ΔFr–Ub), the recalculated ΔLF values did not demonstrate a statistically significant longitudinal effect ($F(3, 102) = 0.313$, $p = 0.816$, partial $\eta^2 = 0.009$). The corrected mean ΔLF values were 0.70 ± 1.48 at baseline, 0.52 ± 1.65 at 2 months, 0.44 ± 1.71 at 4 months, and 0.38 ± 1.84 at 6 months. The previously reported significant pairwise differences involving the 2-month assessment were not supported following correction of the underlying transition variable.

No statistically significant longitudinal differences were observed for ΔHF ($F(3, 102) = 0.687$, $p = 0.562$, partial $\eta^2 = 0.020$), ΔLF/HF ($F(3, 102) = 0.174$, $p = 0.914$, partial $\eta^2 = 0.005$), or ΔRR ($F(3, 102) = 1.131$, $p = 0.340$, partial $\eta^2 = 0.032$). The corrected transition scores are presented in Table 7, and the corresponding final-rest measurements are presented in Table 8.

Table 7. Heart rate variability changes from Ujjayi breathing to final rest during six month period (Δ(Fr-Ub)).

Parameters	Baseline	2 Months	4 Months	6 Months
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
ΔLF, Tg(α)	0.70 ± 1.48	0.52 ± 1.65	0.44 ± 1.71	0.38 ± 1.84
ΔHF, Tg(α)	−0.23 ± 0.50	−0.26 ± 0.69	−0.37 ± 0.63	−0.39 ± 0.57
ΔLF/HF	−0.60 ± 0.52	−0.65 ± 0.64	−0.57 ± 0.63	−0.58 ± 0.72
ΔRR, s	0.06 ± 0.05	0.05 ± 0.06	0.05 ± 0.04	0.05 ± 0.06

LF, low-frequency; HF, high-frequency; RR, interval between two consecutive R peaks.

Table 8. Heart rate variability parameters during final rest across the six-month follow-up.

Parameters	Baseline	2 Months	4 Months	6 Months
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
LF, Tg(α)	−1.06 ± 0.85	−1.10 ± 0.82	−1.40 ± 0.87	−1.29 ± 1.07
HF, Tg(α)	−1.24 ± 0.53	−1.32 ± 0.56	−1.35 ± 0.62	−1.37 ± 0.53
LF/HF	0.94 ± 0.44	0.84 ± 0.41	0.95 ± 0.49	0.92 ± 0.48
RR, s	0.93 ± 0.11	0.92 ± 0.11	0.91 ± 0.12	0.91 ± 0.12

LF, low-frequency; HF, high-frequency; RR, interval between two consecutive R peaks.

3.1.4. Preparation Rest to Ujjayi Breathing Transition

For the transition from preparation rest to Ujjayi breathing (ΔUb–Pr), a nominally significant longitudinal effect was identified for ΔRR ($F(3, 102) = 4.593$, $p = 0.005$, partial $\eta^2 = 0.119$). Bonferroni-adjusted pairwise comparisons indicated differences between baseline and 4 months (mean paired difference, −0.0268 s; simultaneous 95% CI, −0.0523 to −0.0012 s; adjusted $p = 0.036$) and between baseline and 6 months (mean paired difference, −0.0327 s; simultaneous 95% CI, −0.0599 to −0.0055 s; adjusted $p = 0.011$).

However, the omnibus effect did not remain statistically significant after Bonferroni adjustment across the 20 longitudinal analyses (adjusted $p = 0.093$). Accordingly, these pairwise findings are considered exploratory.

No statistically significant longitudinal differences were observed for ΔLF ($F(3, 102) = 0.119$, $p = 0.949$, partial $\eta^2 = 0.003$), ΔHF ($F(3, 102) = 0.433$, $p = 0.729$, partial $\eta^2 = 0.013$), or ΔLF/HF ($F(3, 102) = 0.097$, $p = 0.962$, partial $\eta^2 = 0.003$). The corresponding transition scores are presented in Table 9.

Table 9. Heart rate variability changes from preparation rest to Ujjayi breathing during six-month period (Δ(Ub–Pr)).

Parameters	Baseline	2 Months	4 Months	6 Months
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
ΔLF, Tg(α)	−0.70 ± 1.41	−0.54 ± 1.65	−0.72 ± 1.47	−0.67 ± 1.38
ΔHF, Tg(α)	0.19 ± 0.56	0.34 ± 0.78	0.31 ± 0.75	0.24 ± 0.60
ΔLF/HF	0.71 ± 0.71	0.70 ± 0.71	0.74 ± 0.66	0.67 ± 0.74
ΔRR, s	−0.06 ± 0.06 *#	−0.04 ± 0.06	−0.04 ± 0.06 *	−0.03 ± 0.05 #

* Significant difference between baseline and 4 months; # significant difference between baseline and 6 months (Bonferroni-adjusted pairwise $p < 0.05$). These comparisons are exploratory because the corresponding omnibus effects did not remain significant after Bonferroni correction across the 20 longitudinal analyses. LF, low-frequency; HF, high-frequency; RR, interval between two consecutive R peaks.

3.1.5. Preparation Rest to Final Rest Transition

For the transition from preparation rest to final rest (ΔFr–Pr), no statistically significant longitudinal differences were identified for any of the HRV parameters (Table 10). The repeated measures ANOVA yielded $F(3, 102) = 0.666$, $p = 0.575$, partial $\eta^2 = 0.019$ for ΔLF; $F(2.39, 81.23) = 0.760$, $p = 0.492$, partial $\eta^2 = 0.022$ for ΔHF, using Greenhouse-Geisser correction following a significant Mauchly's test ($p = 0.035$); $F(3, 102) = 0.558$, $p = 0.644$, partial $\eta^2 = 0.016$ for ΔLF/HF; and $F(3, 102) = 1.533$, $p = 0.211$, partial $\eta^2 = 0.043$ for ΔRR.

Table 10. Heart rate variability changes from preparation rest to final rest during six-month period (Δ (Fr–Pr)).

Parameters	Baseline	2 Months	4 Months	6 Months
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
ΔLF, Tg(α)	0.00 ± 1.01	−0.02 ± 1.17	−0.28 ± 1.14	−0.29 ± 1.09
ΔHF, Tg(α)	−0.04 ± 0.60	0.09 ± 0.61	−0.06 ± 0.90	−0.15 ± 0.52
ΔLF/HF	0.11 ± 0.46	0.05 ± 0.43	0.17 ± 0.34	0.09 ± 0.37
ΔRR, s	0.00 ± 0.04	0.01 ± 0.04	0.01 ± 0.04	0.02 ± 0.05

LF, low-frequency; HF, high-frequency; RR, interval between two consecutive R peaks.

The previously reported difference in ΔRR between baseline and 6 months did not remain statistically significant after Bonferroni adjustment (mean paired difference, −0.0175 s; simultaneous 95% CI, −0.0383 to 0.0034 s; adjusted $p = 0.148$). The corresponding transition scores are presented in Table 10.

Friedman sensitivity analyses were performed for nine outcome-transition combinations for which the Shapiro–Wilk test indicated departures from normality at one or more assessments. The findings were consistent with the primary repeated-measures ANOVA results with respect to nominal statistical significance. Nominally significant longitudinal differences were observed for ΔRR during the Yoga Nidra to Ujjayi breathing transition ($\chi^2(3) = 13.080$, $p = 0.004$) and the preparation-rest-to-Ujjayi transition ($\chi^2(3) = 9.549$, $p = 0.023$). The remaining seven Friedman tests yielded nonsignificant results (all $p \geq 0.231$). Neither nominally significant RR result remained significant after Bonferroni adjustment across the 20 longitudinal outcome-transition analyses (adjusted $p = 0.089$ and $p = 0.456$, respectively). These findings support the consistency of the primary exploratory results across parametric and nonparametric approaches, although they do not establish that all ANOVA assumptions were satisfied.

3.1.6. Overall Longitudinal Findings

Across the 20 longitudinal outcome-transition analyses, two RR parameters demonstrated nominally significant omnibus effects, both involving transitions into Ujjayi breathing. However, neither effect remained significant after Bonferroni correction across the complete set of 20 analyses. No nominally significant longitudinal omnibus effects were identified for LF, HF or LF/HF, and most transition measures did not demonstrate statistically significant longitudinal differences.

The complete omnibus ANOVA statistics, assumption checks, effect sizes, and Bonferroni-adjusted *p*-values are provided in Supplementary Table S1. Given the exploratory design, multiple interrelated outcomes, and overlapping transition definitions, the observed RR differences should not be interpreted as confirmatory evidence of physiological adaptation attributable to continued yoga practice.

4. Discussion

The present study investigated longitudinal changes in acute heart rate variability (HRV) responses to a standardized sequence of preparation rest, Yoga Nidra, Ujjayi breathing and final rest during six months of continued Hatha yoga practice in women with previous yoga experience. The principal finding was that none of the 20 longitudinal outcome-transition analyses remained statistically significant after Bonferroni correction for multiple testing. Although nominally significant longitudinal differences were identified in RR interval transitions involving Ujjayi breathing, these findings did not survive correction across the complete set of analyses. No nominally significant longitudinal effects were observed for LF, HF, or LF/HF transition responses. Furthermore, Friedman sensitivity

analyses yielded findings consistent with the primary repeated-measures ANOVA results regarding nominal statistical significance. These results should be considered in the context of the heterogeneous findings reported in previous yoga-HRV research and the methodological challenges associated with interpreting cardiovascular responses during controlled breathing [8,10,16,17].

The exploratory RR findings require consideration. Nominally significant longitudinal differences were observed for the transitions from Yoga Nidra to Ujjayi Breathing and from preparation rest to Ujjayi Breathing. In both transitions, the negative RR differences were smaller in magnitude at the later assessments than at baseline. However, examination of the stage-level measurements indicates that these changes were not primarily associated with an increase in RR interval during Ujjayi breathing. Mean RR values during Ujjayi remained relatively consistent across the four assessments (0.86–0.87 s), whereas RR intervals during preparation rest decreased from 0.92 s at baseline to 0.89 s at 4 and 6 months. Consequently, the smaller negative transition differences may have been driven largely by changes in the preceding reference stages rather than by changes during Ujjayi breathing. This distinction is important because HRV and heart period measures are influenced by multiple physiological and contextual factors, and differences between experimental conditions cannot automatically be attributed to the subsequent technique [8,10]. Furthermore, these longitudinal RR findings did not remain significant after Bonferroni correction across all 20 analyses and should therefore be regarded as exploratory.

Respiratory modulation provides another important consideration when interpreting the observed RR responses. Ujjayi breathing involves deliberate regulation of airflow and respiratory timing, potentially affecting heart period through respiratory sinus arrhythmia, cardiovascular reflex mechanisms, and interactions between respiratory and cardiac rhythms. Previous research has shown that voluntary slow breathing can modify heart rate dynamics and HRV, although the magnitude and nature of these responses depend on breathing frequency, respiratory depth, and experimental conditions [23,25,40]. Because respiratory rate, tidal volume, and inspiratory-to-expiratory ratio were not recorded in the present study, it is not possible to establish whether differences in respiratory behavior contributed to the observed RR transition patterns. In addition, repeated exposure to the same experimental sequence may have influenced familiarity with the protocol, anticipatory responses, or the degree of relaxation achieved during the preceding stages. These potential influences cannot be distinguished from changes associated with continued yoga practice in an uncontrolled longitudinal design. Accordingly, the nominal RR findings should not be interpreted as evidence of technique-specific autonomic adaptation.

No statistically significant longitudinal omnibus effects were identified for LF or LF/HF transition responses. The physiological interpretation of these parameters requires particular caution. LF reflects contributions from several interacting cardiovascular regulatory mechanisms, including baroreflex-related oscillations, rather than exclusively representing sympathetic activity. Similarly, the LF/HF ratio cannot be regarded as a direct quantitative measure of sympathovagal balance [8,10,12]. These limitations are particularly relevant during experimentally modified breathing, because respiratory oscillations may shift into the conventional LF frequency range when breathing becomes sufficiently slow. Consequently, changes in LF or LF/HF during Ujjayi breathing may reflect respiratory characteristics and cardiorespiratory coupling rather than changes in sympathetic or parasympathetic nervous system activity [8,23,25]. Although the present analysis did not identify statistically significant longitudinal effects for these spectral measures after correction, this finding should not be interpreted as evidence of physiological equivalence between assessments.

Likewise, no statistically significant longitudinal changes were identified for HF transition responses. HF is commonly associated with respiratory-related cardiac vagal modula-

tion, but its interpretation depends on respiratory frequency and measurement conditions. When breathing slows below approximately 0.15 Hz, respiratory-related oscillations may extend into the LF band, reducing the usefulness of conventional frequency-band boundaries for interpreting autonomic activity [8,10,23]. Thus, the absence of detectable longitudinal changes in HF does not necessarily indicate unchanged parasympathetic regulation, particularly because breathing was not measured during the experimental protocol. These considerations support the recommendation that HRV investigations involving controlled breathing should include simultaneous respiratory monitoring and interpret spectral parameters in relation to the actual respiratory pattern [8,11].

Previous studies examining yoga interventions and HRV have reported heterogeneous findings. Papp et al. [17] in an eight-week Hatha yoga pilot study, observed an improvement in the time-domain measure pNN50 during nighttime recordings, whereas LF, HF and LF/HF did not show significant changes. Hewett et al. [16] investigated a 16-week Bikram yoga intervention in stressed and sedentary adults and found no significant between-group improvement in HF. In that trial, higher attendance was nevertheless associated with favorable changes in selected cardiovascular risk-related measures. These findings illustrate that the observed physiological outcomes may depend on the selected HRV parameter, recording conditions, participant characteristics, intervention duration, and adherence to practice. More broadly, existing reviews have emphasized variability in yoga intervention protocols and methodological limitations that complicate comparisons between studies [19,20]. Therefore, the predominantly nonsignificant findings of the present study are not necessarily inconsistent with the wider literature, particularly given differences in study design and outcome selection.

An important characteristic of the present investigation is that participants had already practiced Hatha yoga for at least six months before enrollment. Unlike studies evaluating the initiation of yoga among previously inactive individuals, the present study examined an additional six months of supervised practice among experienced practitioners. Prior experience may have influenced baseline cardiovascular responses, familiarity with controlled breathing and the magnitude of additional changes detectable during follow-up. However, the present data cannot determine whether the absence of significant longitudinal effects reflects previous physiological adaptation, limited statistical power, individual variability, or the absence of substantial changes. In particular, nonsignificant findings should not be interpreted as proof of physiological stability or equivalence. Differences in participant experience, intervention characteristics and methodological approaches should therefore be considered when comparing the present findings with those of previous yoga intervention studies [16,17,20].

The fixed sequence of experimental stages represents another important methodological consideration. Yoga Nidra always followed preparation rest, Ujjayi breathing always followed Yoga Nidra, and final rest always followed Ujjayi. Repeating the same sequence at all four assessments enabled longitudinal evaluation of a standardized protocol, but prevented the separation of technique-specific responses from order-dependent and carry-over effects. Accordingly, the transition from Yoga Nidra to Ujjayi breathing cannot demonstrate that Ujjayi independently induced a particular cardiovascular response, while the transition from Ujjayi to final rest may incorporate effects carried over from controlled breathing. The interpretation of these transitions is further complicated by their mathematical dependence: several difference scores share the same stage-level measurements or represent combinations of adjacent stage differences. Although Bonferroni correction addresses multiple statistical testing, it does not eliminate this structural dependence. Analyses of the original stage-level measurements using longitudinal models that account for repeated observations may provide a complementary approach to understanding these relationships.

The study has several methodological strengths. The same participants were assessed repeatedly across four time points over six months, and ECG recordings were obtained using the same equipment and standardized laboratory sequence. The analysis included both individual stage-level measurements and predefined transition scores, allowing examination of whether apparent transition changes reflected changes in the preceding or subsequent experimental stage. In addition, the corrected statistical analysis incorporated Bonferroni adjustment for multiple comparisons, assessment of sphericity, and Friedman sensitivity analyses for outcomes showing departures from normality. Although the sensitivity analyses yielded results consistent with the nominal ANOVA findings, this agreement does not establish that all parametric assumptions were satisfied. The use of repeated measurements and a fixed protocol supports the descriptive value of the study while remaining insufficient for causal inference.

Several limitations must be acknowledged. First, the lack of a non-yoga control group prevents attribution of the observed longitudinal patterns specifically to continued yoga practice.

Temporal changes, repeated testing, seasonal variation, lifestyle factors, and other unmeasured influences could not be excluded. Second, the fixed order of the experimental stages prevented separation of technique-specific effects from potential order and carry-over influences. Third, respiratory rate, tidal volume, and respiratory timing were not measured, limiting the physiological interpretation of RR interval and frequency-domain HRV measures, particularly during Ujjayi breathing. The importance of respiratory recording and appropriate measurement conditions has been emphasized in methodological guidance for HRV research [8,11]. During self-paced Ujjayi breathing, respiratory frequency may have fallen below 0.15 Hz, shifting respiration-related oscillations into the conventional LF band. Because breathing frequency and depth were not recorded, this possibility could not be assessed and limits the physiological interpretation of LF, HF, and LF/HF.

Fourth, HRV parameters were extracted from four-minute analysis windows within each five-minute experimental stage. Although standardized analysis durations were applied across visits, these windows may have included transient physiological responses following transitions between techniques. The possibility that nonstationarity influenced the spectral HRV estimates cannot be excluded. Previous methodological literature has emphasized the importance of recording duration, signal quality, and comparability of measurement conditions in HRV analysis [10,11]. A sensitivity analysis using the final four minutes of each experimental stage was not performed. Consequently, the extent to which spectral HRV estimates depended on the placement of the four-minute window or on immediate post-transition responses could not be determined. Fifth, menstrual-cycle phase and time of day were not incorporated as analytical covariates. These factors may contribute to physiological variation, and their potential influence could not be evaluated in the present study.

Additional limitations concern intervention exposure and participant characteristics. The participants were experienced yoga practitioners, limiting the generalizability of the findings to individuals initiating yoga practice. Although the supervised sessions followed a consistent general structure, the exact sequence of asanas varied between classes. Furthermore, additional yoga practice and other physical activity outside the supervised sessions were not systematically monitored. Consequently, possible differences in habitual physical activity and changes in exercise behavior could not be quantified or controlled. This limitation is relevant because physical activity and training exposure may influence cardiovascular regulation and HRV, while intervention adherence may influence the outcomes of yoga studies [10,16]. Although BMI was not an eligibility criterion, the study included participants across different BMI categories. As BMI was not incorporated as a covariate in the

exploratory longitudinal analyses, its potential contribution to interindividual variability in HRV responses cannot be excluded. Finally, the modest sample size, lack of an a priori power calculation for the present exploratory analyses, and examination of multiple correlated outcomes and transitions limited the precision and interpretability of the statistical findings. Although Bonferroni correction reduced the familywise risk of false-positive findings, it also reduced the ability to detect small effects. The analyses used predefined transition scores rather than a unified mixed-effects model of stage-level measurements across assessments. Because several transition scores share stage-level values, the associated dependencies were not fully modeled, limiting stage-specific longitudinal inference. Future studies should evaluate assessment time, experimental stage, and interaction within a common mixed-effects framework.

Another methodological limitation concerns the software-derived frequency-domain HRV parameters. The LF and HF values were reported as Tg(α) coefficients, whereas the LF/HF values were obtained separately from the software. Although examination of illustrative software outputs suggests that LF/HF corresponds to the conventional spectral-power ratio rather than the quotient of Tg(α) coefficients, the underlying mathematical transformations and their exact implementation in the historical analyses could not be independently verified. Consequently, the original coefficients cannot be fully reproduced or directly equated with conventional LF and HF spectral-power measures. This limits the reproducibility, comparability, and physiological interpretability of these measures.

Future investigations should incorporate randomized and appropriately matched comparison groups and counterbalanced experimental sequences to distinguish technique-related effects from order and carry-over influences. Simultaneous recording of respiration and ECG would allow HRV responses to be interpreted in relation to breathing frequency and depth, particularly during Ujjayi practice [8,23,40]. Future studies should also document supervised-session attendance, additional physical activity and relevant physiological or behavioral covariates. Adequate sample-size planning, prospective identification of primary outcomes, standardized ECG analysis windows, and longitudinal statistical models incorporating assessment time and experimental stage would strengthen the evaluation of acute HRV responses during prolonged yoga interventions.

Overall, the present findings indicate that no longitudinal HRV transition effects remained statistically significant after Bonferroni correction across the 20 predefined analyses. The nominal RR findings involving Ujjayi breathing may have been influenced, at least in part, by changes in the preceding reference-stage RR values. These observations demonstrate the importance of evaluating stage-level measurements alongside transition scores and considering respiratory behavior when interpreting acute HRV responses. The study contributes exploratory longitudinal information about responses to a standardized sequence in experienced Hatha yoga practitioners, but controlled studies are needed to determine whether continued yoga practice produces identifiable physiological adaptations.

5. Conclusions

In women with previous Hatha yoga experience, no statistically significant longitudinal changes in acute HRV transition responses were identified across six months of continued twice-weekly supervised practice after Bonferroni correction for multiple testing. Although nominally significant differences in RR interval transitions involving Ujjayi breathing were observed, these findings did not remain significant after adjustment across the 20 longitudinal analyses and should therefore be interpreted as exploratory.

The findings suggest that most measured HRV transition responses did not demonstrate detectable longitudinal differences over the study period. However, the absence

of a statistically significant effect should not be interpreted as evidence of physiological equivalence or the absence of adaptation.

Furthermore, the fixed order of the relaxation and breathing stages, absence of a control group and lack of respiratory recordings prevent attribution of the observed patterns to specific yoga techniques or autonomic mechanisms. Future studies incorporating appropriate comparison groups, counterbalanced experimental sequences, respiratory monitoring, and prespecified statistical analyses are necessary to determine whether continued yoga practice influences acute cardiovascular responses to relaxation and controlled breathing.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/medicina62122334/s1, Table S1: Longitudinal repeated-measures analysis of variance (ANOVA) of heart rate variability (HRV) transition scores.

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