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RESEARCH ARTICLE

Time sequence of autonomic changes induced by daily slow-breathing sessions

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Abstract
Objective Daily sessions of slow-breathing (6 breaths/min) significantly reduced 24-h ambulatory blood pressure (ABP) in patients with mild hypertension and this effect persisted at least 6 months after the interruption of sessions. The sequence of changes induced by slow-breathing (SB) daily sessions on the modulation of ambulatory blood pressure, renal resistive index, heart rate variability (HRV), and baroreflex sensitivity (BRS) was thus investigated in a randomized, controlled clinical trial.
Methods Thirty-seven patients (30–75 years, grade I essential hypertension), untreated with antihypertensive drugs, were randomized to daily sessions (30 min) of music-guided SB (<10 breaths/min) (intervention, $n = 24$) or simple relaxation (control, $n = 13$). Office and ambulatory blood pressure monitoring renal Doppler ultrasound, assessment of BRS (sequence method and spectral analysis), and HRV (spectral power in the high- and low-frequency bands) were performed at baseline, and after 1, 4, and 8 weeks. Mixed model analysis was conducted on derived variables given by the difference between each measurement and the baseline value within subjects.
Results After 1 week, the intervention enhanced the parasympathetic modulation (high-frequency power; at least $p < 0.05$ vs both control and baseline) and reduced renal vascular resistance ($p < 0.05$ for both comparisons); after 1 month, the enhancement of BRS ($p < 0.05$ for both comparisons at both methods) paralleled a significant reduction in 24 h ABP ($p < 0.05$ for all comparisons).
Interpretation Repeated daily session of music-guided SB increased parasympathetic modulation and decreased renal resistive index early in the study. These changes were being followed by a positive modulation of BRS and blood pressure reduction.

Keywords Hypertension · Prevention and control · Complementary therapies · Breathing exercises · Autonomic nervous system · Baroreflexes

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Introduction

An increase in sympathetic activity, with a possible reduction in parasympathetic tone, is present in borderline hypertension and contributes to the maintenance of sustained hypertension [1]. The reduction of breathing rate from 16 breaths/min to less than 10 breaths/min for at least 2 min enhances parasympathetic tone and/or reduces sympathetic activation [2], being and is followed by increased baroreflex sensitivity (BRS) [3, 4] and BP reduction [3, 5]. Although these changes promptly vanish after the restoration of a normal breathing rate, a reduced chemoreflex sensitivity was observed in pranayama yoga trainees who learned to breathe slowly and deeply, mobilizing in sequence the diaphragm and the lower and upper chest [6]. Electronic devices able to guide breathing rate were thus developed and tested in hypertensive patients with favourable results [7–13], and this approach is now considered among other non-pharmacologic approaches to treat hypertension [14]. However, although there is evidence to suggest that slow breathing (SB) has positive effects on the autonomic nervous system at the acute level [15], it remains unknown whether these effects are long-term when treatment is provided over a number of weeks.

In a single study in which patients were assigned to SB daily sessions, this antihypertensive effect was found to cover all of the 24-h period measured by ambulatory blood pressure monitoring and to persist for at least 6 months after the termination of the sessions [13]. Therefore, the present randomized controlled study was performed to investigate whether the voluntary act of performing daily slow-breathing exercises can target the autonomic nervous system inducing chronic changes in the modulation of BP, renal resistive index (RRI), heart rate variability (HRV), and BRS in mild hypertension.

Methods

Subjects

The study protocol was approved by the Review Committee and Ethics Committee of the Azienda Ospedaliera Universitaria Careggi (Prot. n. 2009/0004365), and all subjects gave informed consent. Inclusion criteria were patients 30–75 years old with a previous diagnosis of grade I essential hypertension and not receiving antihypertensive medications [16]. Exclusion criteria included coronary heart disease, heart failure, use of beta-blockers, cerebrovascular disease, diabetes mellitus, chronic respiratory disease, pregnancy, neoplasm, altered night-time sleep pattern due to shift work, and recent weight change. Patients who regularly performed athletic activities were also excluded

Study design and protocol

The study had a controlled, parallel design with a randomization ratio of 2:1 (intervention:control) because of an expected higher drop-out rate in the intervention group. Participants in the control group ($n = 15$) listened to slow music to provide/encourage a period of relaxation without the the manipulation of breathing. Participants in the intervention group ($n = 30$) were instructed to perform daily sessions of music-guided slow (<10 breaths/min)"abdominal" breathing with a 1:2 inspiration: expiration duration ratio (pranayama yoga) as previously described [13]. Breathing technique was taught by a certified practitioner (AF) in a single 2-hour session. The session with the therapist was repeated at each follow-up visit after investigations.

None of the hypertensive participants in the study were on antihypertensive drug therapy. All subjects were instructed to repeat daily (at home) 30-min relaxation sessions in the supine position at least 3 hours after lunch and to record frequency and duration of home sessions on a diary card. Follow-up visits in laboratory were scheduled at 1, 4, and 8 week intervals. Measurements of BP and RRI, as well as assessments of HRV and BRS, were performed at baseline and at all follow-up visits. Compliance with interventions was monitored in both groups using participant diary cards.

Measured variables and autonomic assessment

Hemodynamic assessments

(*a*) *Blood pressure and heart rate* (*HR*) 24 h ambulatory BP was measured using validated devices (SpaceLabs 90207, Spacelabs Healthcare Co., Issaquah, WA) programmed to take measurements at 15 and 20 min intervals over the day and the night, respectively, as previously reported [13].

(*b*) *Renal resistive index* Two subgroups of subjects (10 in the intervention group and 10 in the control group, matched for age and gender) underwent assessment of RRI (at baseline and at follow-up visits) as previously reported [17]. Briefly, patients were placed in a supine position and the B-Mode and the Doppler measurements of RRI were performed using a Siemens G60S (Toshiba Aplio) machine with a 3.5-MHz sector transducer. The RRI was calculated in both kidneys according to the following formula: [(peak systolic velocity − minimum diastolic velocity)/peak systolic velocity] [17]. RRI values from the left and right sides were averaged for analysis. All measurements were taken in the late morning by the same examiner (MB).

Autonomic assessments

Subjects were examined in the late morning, under standardized conditions in a quiet room with at a comfortable temperature set to (25 °C). Participants were not permitted coffee or tea on the day of the study. After 30 min of quiet rest, subjects underwent continuous recordings of ECG, beat-to-beat non-invasive BP by Nexfin monitor (BMEYE B.V, Amsterdam, The Netherlands), and respiration rate during 10 min of spontaneous breathing and during 10 min of deep breathing defined as 6 cycles/min. The patient was then asked to stand up quickly within 3–4 s and ECG registration was continued over a period of 60 s. All recordings were made in a random order.

Offline, data were selected and exported on a personal computer for analysis of BRS and HRV

BRS assessments

Sequence method (*time domain*) The time series of RR interval and systolic BP (SBP) recorded during spontaneous breathing were scanned with software capable of

identifying the sequence of at least three cardiac cycles in which both SBP and pulse interval are either steadily increasing (up) or decreasing (down); the variation threshold was fixed at 1 mmHg for SBP and 6 ms for pulse interval [18]. BRS values are expressed as combined values for up and down sequences. Separate values for up and down sequences were also calculated.

Spectral method (*frequency domain*) Time series of the tachogram and systogram were visually selected and used for analysis. The α-index (α) (the gain in the relationship between the RR period and SBP variability), was obtained by means of simultaneous spectral analysis of RR and SBP variability, with the calculation being made from the square root of the ratio between RR and SBP variability in the two major bands of LF (α-LF) and HF (α-HF). The values of the α-index were validated when the coherence between fluctuations in the RR interval and SBP was greater than 0.5 [19].

HRV assessments

HRV during inspiration and expiration (*time domain*) Measurements of HR response to deep breathing, the subject was placed in a seated position. Respiration rate for a period of 30 sec and baseline ECG recording were done. Then, subjects were asked to perform slow and deep inspiration followed by slow and deep expiration such that each breathing cycle lasted for 10 s, consisting of 6 breathing cycles/min. The difference between the highest HR recorded during deep inspiration and the lowest HR recorded during deep expiration was measured per cycle.

HR response to standing (*time domain*) For HR response to standing, an ECG was recorded in the supine position. The subject was instructed to stand up within 3 s. The ECG was continuously recorded during the procedure. The ratio between the RR interval of the 30th beat and the 15th beat (30:15 ratio) after standing was calculated. The average value of the two tests was then determined.

Spectral method (*frequency domain*) After 15 min of supine rest, and ECG was recorded for short-term HRV analysis following the procedures recommended by the Task Force [20]. The low-frequency and high-frequency components of HRV were assessed by frequency analysis of the sequential R wave to R intervals of the ECG obtained from short-term recordings of 5 min [20, 21]. LF_{RR} and HF_{RR} were expressed in normalized units, which represent the relative value of each power component in proportion to the total power minus the very low-frequency (VLF) component. The LF_{RR}/HF_{RR} ratio was then calculated.

Statistical analysis

Continuous variables are expressed as mean ± standard deviation (SD) when normally distributed. The predetermined sample size of groups was calculated for providing at least 80 % power (β) with $p < 0.05$ (α) based on previous results with home SBP measurements taking a 5-mmHg difference in population means with $\sigma = 5.5$ mmHg. Categorical variables were compared with Fisher's exact test.

Factors associated with compliance to treatment with daily SB sessions (defined as subjects who completed the study in the intervention group) were preliminarily investigated by logistic regression analysis in the subgroup of subjects assigned to intervention. Age, gender, body mass index (BMI), smoking habit (yes or no), office SBP, diastolic BP (DBP), and HR were included as independent variables.

Statistical analysis was conducted on derived variables given by the difference between each measurement and the baseline values associated with each subject. A linear mixed-effect model was fitted by restricted maximum likelihood. Random effects were identified and repeated measurements were taken for these individuals, while fixed effects were specified for experimental conditions. A linear time trend was also included in the model terms. A further model was fitted allowing interaction terms between time and experimental conditions.

Multivariate linear regression was then used to identify variables independently associated with changes of 24 h SBP and RRI. The three measures of BRS sensitivity (sequence method in the time domain and α-indexes in the two major bands of LF, α-LF, and HF, α-HF) were found to be affected by serious multi-colinearity (variance inflation factor VIF >3). Independent variables included in the models were office SBP, BRS assessed by the sequence method (Seq), HRV during deep breathing (HRVdeep), HRV after standing (HRVortho), LF_{RR}/HF_{RR} ratio, with adjustment for age, HR, and breathing rate at rest.

Results are expressed as an odds ratio (OR) with 95 % confidence limits (95 % CI). A value of $p < 0.05$ was taken as the minimum level of statistical significance throughout the paper. Statistical analysis was performed using the Statistical Package for Social Science (SPSS, version 21.0, SPSS Inc., Chicago, IL) software package.

Results

From January 2009 to September 2010, 243 patients with essential hypertension were screened for enrollment in the study. From that group, 198 patients did not meet the inclusion criteria or met one or more of the exclusion criteria. Of the 30 patients assigned to the intervention SB group, 6 refused to continue the protocol and were excluded from the intervention group after their initial enrollment. Of the 15 patients assigned to the placebo SB (P-SB),

Table 1 Baseline characteristics of patients randomized to control or intervention

Variable	Control	Intervention	*p*
Male/female (*n*/*n*)	4/9	7/17	–
Age (years)	53.4 ± 9.63	51.5 ± 9.12	0.551
Smokers (*n*)	0	0	–
BMI (kg/m^2)	24.2 ± 3.49	23.5 ± 2.15	0.454
Office SBP (mmHg)	144 ± 13	145 ± 11	0.786
Office DBP (mmHg)	96 ± 7	94 ± 6	0.409
Office HR (bpm)	83 ± 14	79 ± 12	0.409
Blood glucose (mg/dl)	0.86 ± 0.09	0.90 ± 0.11	0.401
Total cholesterol (mg/dl)	191 ± 35	188 ± 34	0.809
Creatinine (mg/dl)	0.79 ± 0.14	0.74 ± 0.15	0.361
Creatinine clearance (ml/min)	94 ± 13	95 ± 19	0.964

BMI body mass index, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *HR* heart rate, *GFR* glomerular filtration rate

two could not be reached at the time of follow-up. Therefore, 37 patients completed the protocol: 24 in the SB group and 13 in the P-SB group. Clinical characteristics were comparable between the two groups (Table 1). The main reasons for participant dropout were: family activity ($n = 1$), or work responsibility ($n = 7$). At logistic regression analysis, exclusion was not influenced by gender, age, smoking habit, baseline SBP or DBP, HR, or allocation to SB group. The 24 patients in the SB group self-reported a high compliance with daily slow-breathing treatment, with 6.6 ± 0.8 sessions of breathing exercises per week (range 5–7) during the first week of the study and 5.7 ± 0.9 sessions(range 4–7) during the last week. Diary cards revealed a similar compliance with listening to music in the control group.

Hemodynamic changes

Blood pressure Ambulatory recordings were of good technical quality in all participants. At the end of the study, the mean 24 h SBP and DBP were both significantly reduced compared to baseline values in the intervention group (120 ± 8 mmHg vs 129 ± 8; 77 ± 9 vs 82 ± 7; $p < 0.001$ for both). In the control group, no significant changes were observed at the 2 month follow up (128 ± 10 mmHg vs 128 ± 10; 84 ± 8 vs 85 ± 8; ns for both). A significant reduction in 24 h SBP in the intervention group was detectable at the 1-month follow-up visit (125 ± 7 mmHg; $p < 0.05$) (Table 2). Mixed model analysis of changes vs baseline revealed a significant time per group interaction on 24 h SBP ($p < 0.01$) (Fig. 1).

Renal resistive index At the 2-month follow-up the RRI was reduced in the intervention group (0.54 ± 0.05 vs 0.63 ± 0.05; $p < 0.001$). The reduction vs baseline values was already significant at the 1-week visit (0.58 ± 0.06; $p < 0.05$). No significant changes were observed in the control group. The effect of the intervention on RRI was confirmed by mixed model analysis (time per group interaction, $p = 0.003$) (Fig. 1).

Autonomic cardiovascular regulation

BRS In the intervention group BRS at the 2-month follow-up increased compared to baseline for both the sequence method (12.17 ± 3.26 vs 8.84 ± 3.34 ms/mm Hg; $p < 0.001$) and spectral analysis (13.94 ± 4.25 vs 9.39 ± 3.61 for α-HF and 8.46 ± 3.44 vs 5.91 ± 3.51 for α-LF; $p < 0.001$ for both). Mixed model analysis revealed a significant time per group interaction for all methods of assessment ($p < 0.05$) (Table 3).

HRV Assessment of changes in HRV performed in the time domain (during standing and during deep breathing) revealed a significant effect of intervention even at the 1-week visit ($p < 0.05$), with the effect of intervention progressively increasing during follow-up ($p < 0.001$ for time per group interaction). In the frequency domain, subjects assigned to intervention showed a significant increase of the spectral HF_{RR} band at 1 week ($p = 0.03$), a response which further increased at 1 month ($p < 0.001$). A significant decrease of the LF_{RR} band was observed in the intervention group at the 2-month visit ($p < 0.001$). Overall whole a significant reduction of the LF_{RR}/HF_{RR} ratio was evident at the 1-month visit ($p < 0.001$) with a significant time per group interaction ($p < 0.001$). No changes were observed in the control group. The main results are shown in the Fig. 1.

Interaction between hemodynamic and autonomic changes

At multivariate linear regression 24 h SBP changes were found to be affected by changes in BRS (sequence method, β coefficient −0.180) and sympathetic tone (LF_{RR}/HF_{RR} ratio, β coefficient of 0.309, $p < 0.001$). Changes of RRI (1 unit) were mostly affected by changes in the sympathetic tone (LF_{RR}/HF_{RR} ratio with β coefficient of 0.322, $p < 0.001$; HRV after standing, β coefficient −0.279, $p < 0.001$; HRV during deep breathing, β coefficient −0.251, $p < 0.003$) (Table 4).

Discussion

According to our findings, the voluntary act of performing daily slow-breathing exercises induce stable changes in the autonomic cardiovascular control before the reduction of ambulatory BP. More precisely, the early enhancement of

Table 2 Blood pressure changes in control ($n = 24$) and intervention ($n = 13$) groups

Variable	Time (w)	Control ($n = 24$)		Intervention ($n = 13$)		Mixed model analysis		
		Mean ± SD	Variation (95 % CI)	Mean ± SD	Variation (95 % CI)	Time	Gr	$T \times$ Gr
(A) Systolic blood pressure (mmHg)								
Office	0	146 ± 12	0	145 ± 11	0			
	1	143 ± 11	−3.231 (−7.286 to 0.825)	144 ± 12	−1.333 (−4.318 to 1.651)	–	–	–
	4	143 ± 14	−2.615 (−6.405 to 1.175)	140 ± 11	−5.029 (−7.818 to −2.239)	–	–	–
	8	145 ± 12	−1.231 (−5.134 to 2.673)	137 ± 9	−7.863 (−10.736 to −4.990)	–	–	–
						0.412	0.088	0.050
24 h	0	128 ± 10	0	129 ± 8	0			
	1	127 ± 9	−0.824 (−4.336 to 2.688)	126 ± 10	−2.677 (−5.341 to −0.012)	–	–	–
	4	129 ± 10	0.476 (−1.872 to 2.824)	125 ± 7	−3.300 (−5.092 to −1.507)	–	–	–
	8	128 ± 10	−0.517 (−3.826 to 2.792)	120 ± 8	−7.812 (−10.264 to −5.361)	–	–	–
						0.003	0.001	0.001
Day time	0	134 ± 11	0	133 ± 10	0			
	1	132 ± 8	−2.376 (−7.107 to 2.355)	130 ± 13	−2.715 (−6.197 to 0.767)	–	–	–
	4	134 ± 8	−0.941 (−4.495 to 2.613)	128 ± 9	−4.367 (−6.982 to −1.752)	–	–	–
	8	133 ± 9	−1.186 (−5.831 to 3.458)	125 ± 10	−8.121 (−11.539 to −4.703)	–	–	–
						0.083	0.001	0.059
Night time	0	118 ± 12	0	118 ± 9	0			
	1	117 ± 11	−1.429 (−4.943 to 2.086)	115 ± 9	−2.766 (−5.353 to −0.179)	–	–	–
	4	118 ± 13	−0.470 (−4.245 to 3.305)	115 ± 7	−2.791 (−5.57 to −0.013)	–	–	–
	8	120 ± 11	1.349 (−3.481 to 6.178)	111 ± 9	−6.633 (−10.187 to −3.078)	–	–	–
						0.571	0.015	0.008
(B) Diastolic blood pressure (mmHg)								
Office	0	95 ± 8	0	94 ± 6	0			
	1	93 ± 8	−2.308 (−5.395 to 0.779)	92 ± 8	−1.833 (−4.105 to 0.439)	–	–	–
	4	93 ± 8	−1.462 (−4.052 to 1.129)	92 ± 8	−2.244 (−4.151 to −0.338)	–	–	–
	8	93 ± 7	−1.538 (−4.470 to 1.393)	89 ± 7	−5.003 (−7.160 to −2.846)	–	–	–
						0.001	0.468	0.219
24 h	0	85 ± 8	0	82 ± 7	0			
	1	85 ± 9	−0.654 (−3.372 to 2.063)	77 ± 18	−1.535 (−3.579 to 0.508)	–	–	–
	4	84 ± 7	−1.019 (−2.675 to 0.637)	81 ± 6	−1.610 (−2.829 to −0.392)	–	–	–
	8	84 ± 8	−1.394 (−4.659 to 1.871)	77 ± 9	−4.812 (−7.215 to −2.409)	–	–	–
						0.003	0.479	0.832
Day time	0	92 ± 11	0	87 ± 8	0			
	1	90 ± 11	−1.815 (−4.744 to 1.113)	85 ± 10	−1.747 (−3.902 to 0.408)	–	–	–
	4	90 ± 10	−1.220 (−3.192 to 0.751)	84 ± 8	−2.121 (−3.572 to −0.670)	–	–	–
	8	90 ± 10	−1.978 (−5.761 to 1.805)	81 ± 10	−5.349 (−8.133 to −2.565)	–	–	–
						0.305	0.188	0.498
Night time	0	75 ± 11	0	73 ± 9	0			
	1	74 ± 9	−0.972 (−4.274 to 2.329)	71 ± 9	−1.508 (−3.938 to 0.922)	–	–	–
	4	75 ± 9	−0.116 (−3.284 to 3.053)	71 ± 7	−1.712 (−4.044 to 0.620)	–	–	–
	8	75 ± 10	0.344 (−3.653 to 4.341)	69 ± 10	−3.882 (−6.823 to −0.940)	–	–	–
						0.861	0.090	0.504

parasympathetic tone at 1 week is followed by an increase of BRS at the 1-month follow-up.

The voluntary reduction of breathing rate from 16 to less than 10 acts/minute for at least 2 min is consistently followed by an acute BP reduction [3, 5] associated with BRS increase [3, 4], enhanced parasympathetic and vagal tone, and/or reduction of sympathetic activation [2, 22]. In the absence of information regarding cardiovascular

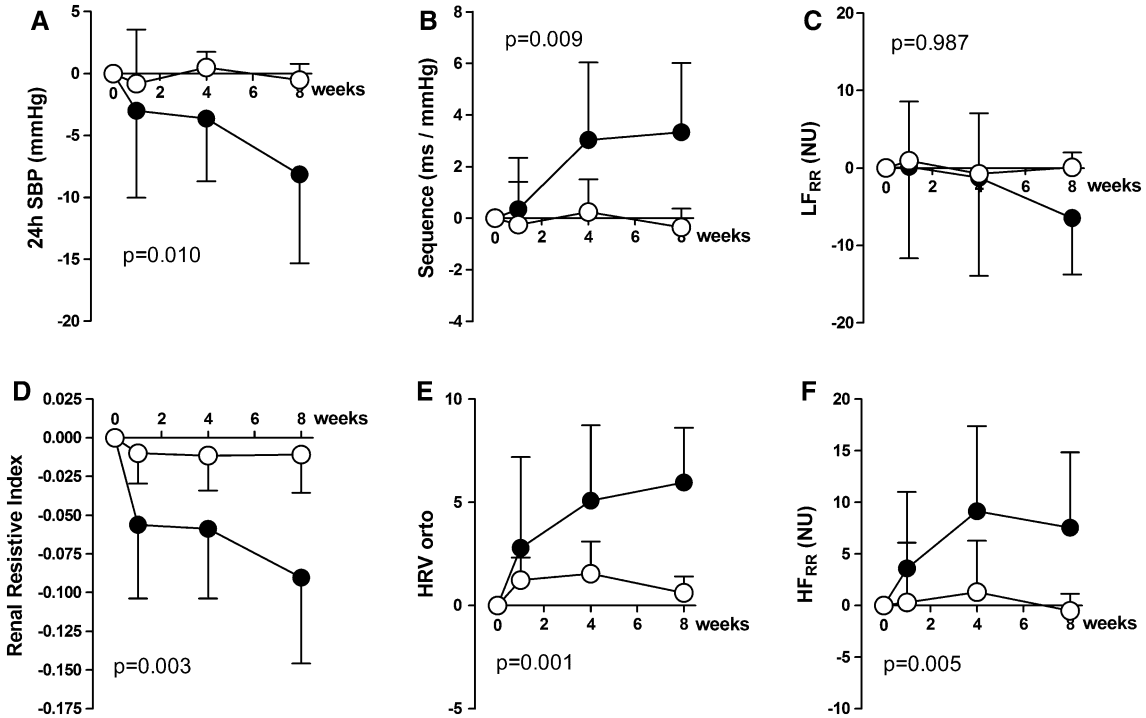

Fig. 1 Changes in 24h-ambulatory systolic blood pressure (24h SBP) **a** baroreflex sensitivity (sequence method) **b** low frequency component of heart rate variability (LFRR) **c** renal resistive index (RRI) **d** 30:15 ratio of HR response to standing (ms) (HRVorto) **e** and high frequency component of heart rate variability (HFRR) **f** from baseline to the end of the study in subjects assigned to intervention (*black circle n*=24) and controls (*empty circles n*=13).

autonomic changes induced by repeated slow-breathing sessions, the possibility to move SB into the clinical environment was investigated using a device that is able to guide patients in reaching a SB rate. Based on the favorable results seen in office BP measurements during pre- and post-observational studies, in prospective matched case-control studies, and in randomized control trials, the device received FDA approval as an "adjunctive treatment to reduce BP" (http://www.resperate.com) [14, 23]. However, a recent meta-analysis of eight studies [24], although confirming a decrease in office SBP of 3.1 mm Hg (95 % CI 1.4–4.7) after 8 weeks [24], showed no effects when excluding sponsored studies [24]. When considering the effects on 24 h BP, conflicting results were reported because there was either a significant reduction after 8 weeks [7, 25] or no effects after 4 [26] or 8 weeks [27] of observation. However, the duration of daily sessions adopted in all studies is less than 15 min [14, 24]. The possibility to restore the BRS [28], to increase the participation of the vagal autonomic component in cardiac control [29], and to reduce BP [28], was demonstrated for physical training, but in all these studies the duration of the training sessions was consistently longer (45–60 min) [28, 29]. The duration of a session of physical activity should last more than 30 min according to current guidelines [14], a time duration which is also utilized in regular yoga regular practice [30]. Daily sessions of 30 min voluntary SB were adopted only in a single independent, controlled, randomized study [13] where a significant reduction of 24 h SBP at the 6 months' follow-up was detected [13]. The duration of the SB session could thus be important for inducing favorable changes in cardiovascular autonomic control in the long term. term. In contrast to previous studies performed using a device [24], we integrated listening to music with additional instructions on how to perform SB. Therefore, we cannot exclude the possibility that the additional instructions given to patient may have potentiated the simple biofeedback provided by the device adopted in other studies [24]. The possibility that music-guided SB performed without the use of the device might have an effect is of potential practical use.

According to our study, BP changes parallel the enhancement in BRS in the time domain (sequence technique) and in the frequency domain (spectral analysis). In contrast to observations with physical exercise [28, 31], the restoration of BRS was not associated with baseline reductions of HR. The increase in BRS was, however, preceded by an enhancement of HF_{RR} power reflecting an enhancement of parasympathetic tone [20] as also observed in healthy individuals assigned to physical training [32].

Table 3 Autonomic changes in control ($n = 24$) and intervention ($n = 13$) groups

Variable	Time (w)	Control ($n = 24$)		Intervention ($n = 13$)		Mixed model analysis		
		Mean ± SD	Variation (95 % CI)	Mean ± SD	Variation (95 % CI)	Time	Gr	$T \times$ Gr
Baroreflex sensitivity								
Sequence method (m sec/mm Hg) (up and down)								
	0	8.64 ± 2.90	0	8.84 ± 3.34	0			
	1	8.38 ± 2.88	−0.256 (−1.317 to 0.804)	9.18 ± 3.81	0.346 (−0.434 to 1.127)	–	–	–
	4	8.87 ± 3.32	0.240 (−1.191 to 1.67)	11.87 ± 4.07	3.035 (1.982 to 4.088)	–	–	–
	8	8.28 ± 3.16	−0.357 (−1.605 to 0.891)	12.17 ± 3.26	3.334 (2.415 to 4.252)	–	–	–
						0.01	0.33	0.01
Sequence method (m sec/mm Hg) (Up)								
	0	10.18 ± 4.56	0	9.35 ± 3.37	0			
	1	10.37 ± 4.51	0.191 (−1.015 to 1.397)	10.30 ± 3.75	0.956 (0.069 to 1.843)	–	–	–
	4	10.88 ± 4.88	−0.452 (−2.118 to 1.215)	11.82 ± 4.69	2.726 (1.499 to 3.953)	–	–	–
	8	11.82 ± 4.58	0.410 (−1.072 to 1.891)	12.56 ± 3.67	3.514 (2.424 to 4.605)	–	–	–
						0.06	0.01	0.05
Sequence method (m sec/mm Hg) (Down)								
	0	7.54 ± 4.75	0	8.08 ± 3.29	0			
	1	8.05 ± 3.86	0.513 (−1.076 to 2.102)	8.59 ± 3.86	0.519 (−0.651 to 1.689)	–	–	–
	4	8.26 ± 4.40	0.723 (−0.984 to 2.431)	10.17 ± 4.31	2.092 (0.835 to 3.349)	–	–	–
	8	7.44 ± 3.78	−0.093 (−1.688 to 1.502)	11.24 ± 3.40	3.164 (1.990 to 4.338)	–	–	–
						0.28	0.01	0.05
Spectral analysis								
α-LF (at rest during spontaneous respiration)								
	0	5.59 ± 1.85	0	5.91 ± 3.51	0			
	1	5.98 ± 2.11	0.394 (−0.715 to 1.502)	7.22 ± 4.35	1.313 (0.497 to 2.129)	–	–	–
	4	6.19 ± 1.38	0.604 (−0.583 to 1.792)	8.02 ± 3.66	2.116 (1.242 to 2.99)	–	–	–
	8	5.70 ± 1.92	0.117 (−0.942 to 1.177)	8.46 ± 3.44	2.549 (1.769 to 3.329)	–	–	–
						0.01	0.52	0.05
α-HF (at rest during spontaneous respiration)								
	0	8.13 ± 2.39	0	9.39 ± 3.61	0			
	1	8.46 ± 1.88	0.322 (−0.759 to 1.403)	10.81 ± 3.86	1.425 (0.629 to 2.221)	–	–	–
	4	8.89 ± 3.17	0.751 (−1.263 to 2.766)	12.44 ± 4.51	3.051 (1.568 to 4.534)	–	–	–
	8	8.56 ± 2.76	0.426 (−1.191 to 2.043)	13.94 ± 4.25	4.554 (3.363 to 5.744)	–	–	–
						0.01	0.27	0.05
Heart rate variability								
Time domain tests								
HRVdeep (beats/min)								
	0	18.38 ± 5.04	0	14.96 ± 3.97	0			
	1	17.54 ± 4.72	−0.846 (−3.148 to 1.456)	16.29 ± 4.37	1.333 (−0.361 to 3.028)	–	–	–
	4	18.69 ± 5.20	0.308 (−1.605 to 2.22)	20.60 ± 4.09	5.643 (4.236 to 7.05)	–	–	–
	8	17.58 ± 4.19	−0.805 (−3.433 to 1.823)	23.04 ± 3.50	8.083 (6.149 to 10.018)	–	–	–
						0.01	0.01	0.01
The 30:15 ratio of HR response to standing (ms)								
	0	12.85 ± 1.63	0	14.96 ± 2.60	0			
	1	14.08 ± 1.04	1.231 (−0.807 to 3.268)	17.75 ± 4.75	2.792 (1.292 to 4.291)	–	–	–
	4	14.38 ± 2.66	1.538 (−0.2 to 3.277)	20.04 ± 4.69	5.082 (3.802 to 6.362)	–	–	–
	8	13.46 ± 1.44	0.610 (-0.625 to 1.846)	20.38 ± 3.70	5.958 (5.049 to 6.868)	–	–	–
						0.01	0.01	0.01

Table 3 continued

Variable	Time (*w*)	Control ($n = 24$)		Intervention ($n = 13$)		Mixed model analysis		
		Mean ± SD	Variation (95 % CI)	Mean ± SD	Variation (95 % CI)	Time	Gr	$T \times$ Gr
Frequency domain tests								
LF_{RR} (normalized units)								
	0	31.01 ± 7.80	0	33.67 ± 9.94	0			
	1	31.96 ± 8.31	0.952 (−5 to 6.905)	33.81 ± 9.87	0.145 (−4.237 to 4.526)	–	–	–
	4	30.25 ± 6.95	−0.756 (−7.082 to 5.569)	32.40 ± 12.65	−1.272 (−5.927 to 3.383)	–	–	–
	8	31.09 ± 8.21	0.084 (−3.316 to 3.485)	27.21 ± 8.75	−6.460 (−8.962 to −3.957)	–	–	–
						0.94	0.79	0.99
HF_{RR} (normalized units)								
	0	19.92 ± 7.03	0	15.04 ± 4.99	0			
	1	20.22 ± 7.23	0.295 (−3.589 to 4.18)	18.64 ± 8.23	3.602 (0.744 to 6.461)	–	–	–
	4	21.22 ± 8.41	1.293 (−2.81 to 5.396)	24.16 ± 11.23	9.125 (6.106 to 12.145)	–	–	–
	8	19.40 ± 6.66	−0.526 (−3.908 to 2.857)	22.56 ± 8.24	7.521 (5.032 to 10.01)	–	–	–
						0.01	0.01	0.01
LF_{RR}/HF_{RR} ratio								
	0	1.82 ± 1.04	0	2.43 ± 0.86	0			
	1	1.87 ± 0.95	0.055 (−0.434 to 0.543)	2.07 ± 0.77	−0.364 (−0.723 to −0.004)	–	–	–
	4	1.74 ± 1.13	−0.074 (−0.434 to 0.286)	1.55 ± 0.67	−0.883 (−1.148 to −0.618)	–	–	–
	8	1.86 ± 1.09	0.042 (−0.262 to 0.347)	1.30 ± 0.58	−1.135 (−1.359 to −0.91)	–	–	–
						0.01	0.86	0.01

HRVdeep heart rate variability during deep breathing, *α-HF* square root of the ratio between RR and SBP variability in the band of high frequency, *α-LF* square root of the ratio between RR and SBP variability in the band of low frequency

The early parasympathetic response was associated with a reduction in RRI, probably mediated by the parasympathetic modulation of renal sympathetic activity [33]. The renal response is indeed unlikely due to a direct parasympathetic effect on renal blood flow autoregulation, as there are no efferent renal vasomotor fibers present in the vagus nerve [34, 35]. It is unlikely that the significant RRI reduction observed at the first follow-up visit could be a result of structural changes in the blood vessel wall during the 2-month study period. RRI changes observed in the present study may thus reflect changes in vasomotor stimuli.

The present study is the first to provide evidence of stable modifications of cardiovascular control induced by the long-term practice of SB (pranayama yoga) in hypertensive subjects. Four months of respiratory training in pranayama yoga reduced the sympathovagal balance and the LF component of HRV in healthy elderly subjects [36], indicating a positive shift towards parasympathetic predominance. The reduced sympathovagal balance may be due to a central modulator regulatory effect, with an impact on BRS in our hypertensive patients. The activation of the Hering-Breuer reflex due to an increase in tidal volume during SB [5] might have also reduced chemoreflex sensitivity, thus ameliorating baroreflex function [5, 6]. Additionally, reducing the respiratory rate to six breaths per min induces R-R interval fluctuations, which when merged with the respiratory cycle, show a considerable increase in amplitude relative to BP changes. This may also lead to enhanced baroreflex efficiency [4].

Table 4 Variables independently associated with changes of 24 h SBP (A) and RRI (B)

Variables	*B* (95 % CI)	*β*	*p*
(A) 24th SBP			
(Constant)	−1.932 (−5.737 to 1.873)	–	0.317
Office SBP	0.492 (0.388 to 0.596)	0.582	0.001
Seq	−0.410 (−0.726 to −0.094)	−0.180	0.010
HRVdeep	0.059 (−0.114 to 0.233)	−0.052	0.500
HRVorto	0.132 (−0.110 to 0.373)	0.082	0.282
LF_{RR}/HF_{RR} ratio	2.265 (1.236 to 3.294)	0.309	0.001
(B) RRI			
(Constant)	−0.007 (−0.043 to 0.028)	–	0.682
BRS Seq	−0.001 (−0.004 to 0.002)	0.037	0.613
HRVdeep	−0.002 (−0.004 to −0.001)	−0.251	0.003
HRVorto	−0.004 (−0.006 to −0.002)	−0.279	0.001
LF_{RR}/HF_{RR} ratio	0.020 (−0.011 to 0.029)	0.322	0.001

Adjusted for age, heart rate, and breathing rate at rest

RRI renal resistive index, *Office SBP* office systolic blood pressure, *HRVdeep* heart rate variability during deep breathing, *HRVorto* heart rate variability during standing, *BRS Seq* baroreflex sensitivity at sequence method

Hypertensive patients included in our study had normal absolute values of RRI, normal renal function, and no microalbuminuria. Therefore, even minor absolute RRI changes could be appreciated. It is reasonable to expect that the improvement in BRS and the reduction of renal resistance may play a protective role in hypertensive patients.

The methods of assessing sympathetic and parasympathetic modulation in the present study are indirect, using HRV and the assessment of BRS involves only noninvasive measurements. Other more direct techniques such as microneurography or pharmacological challenges were not used. Although these invasive techniques are not feasible for routine clinical measurements, they may provide greater insight into the mechanisms underlying the autonomic changes investigated in this study [15]. A further limitation of the present study is the lack of serum catecholamine measurements. The use of more direct measures should be considered in future studies.

In conclusion, the antihypertensive effects of repeated 30 min daily sessions of music guided SB are associated with favorable changes in the autonomic nervous system and a reduction of RRI. Although this treatment method is effective, its time consuming nature may limit its use in the clinical setting. However, this limitation is shared by most non-pharmacological treatment and prevention strategies, such as physical exercise.

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Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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