Cardiology Journal
2024, Vol. 31, No. 3, 418–426
DOI: 10.5603/cj.96299
Copyright © 2024 Via Medica
ISSN 1897–5593
eISSN 1898–018X

VIA MEDICA

ORIGINAL ARTICLE

Acute effects of the 4-4-8 breathing technique on arterial stiffness in healthy young men

Ryota Kobayashi[1], Hideyuki Negoro[2]

[1]Department of Natural and Environmental Science, Teikyo University of Science, Tokyo, Japan
[2]Department of Medicine, Nara Medical University, Nara, Japan

Abstract

Background: *Increased arterial stiffness is a risk factor for cardiovascular disease. Slow, deep breathing decreases blood pressure related to arterial stiffness. The objective of the present study was to determine the acute effects of a single session of slow breathing on arterial stiffness, blood pressure, and cardiac autonomic function.*

Methods: *Fifteen healthy men (20 ± 0 years) were administered (a) a slow breathing condition (12 consecutive breaths of 4 s of inhalation, 4 s of pause, and 8 s of exhalation through the nose, approximately 5 min per breath) and (b) a control, two-condition crossover design. Carotid-femoral artery pulse wave velocity (cfPWV), brachial-ankle PWV (baPWV), brachial blood pressure, high frequency (HF) and low frequency (LF) were measured at baseline, 30 min, 60 min and 24 h after respiratory control.*

Results: *Brachial-ankle PWV and brachial systolic pressure on the 4-4-8 breathing trial decreased after 30 min of respiratory control compared to baseline ($p < 0.05$), but did not change on the CON trial. Carotid-femoral PWV on both trials was unchanged; HF on the 4-4-8 breathing trial increased ($p < 0.05$) and LF decreased ($p < 0.05$) after 30 min of respiratory control compared to baseline, but was unchanged on the CON trial.*

Conclusions: *These results suggest that slow breathing techniques may be effective in modulating autonomic function and improving arterial stiffness in healthy young adults.* (Cardiol J 2024; 31, 3: 418–426)

Keywords: arterial stiffness, 4-4-8 breathing technique, heart rate variability, blood pressure, healthy young men

Introduction

Increased central (aortic) and systemic (brachial–ankle) arterial stiffness is an independent risk factor for the development of cardiovascular disease [1, 2]. Arterial stiffness increases with age and with various pathological conditions such as obesity, diabetes, smoking, and dyslipidemia, and arterial stiffness has important implications for cardiovascular health [3].

Breathing can directly affect the activity of the autonomic nervous system, including heart rate [4]. Voluntary control of breathing, especially slowing its rate, comes from Eastern traditions and has been used for thousands of years as an important part of meditation and relaxation [4–6]. Heart rate variability (HRV), which is regulated by the activity of the cardiac autonomic nervous system, varies with respiration [7]. For example, controlled breathing at 6 breaths/min increases baroreflex sensitivity [8]. In healthy individuals, slow breathing induces a shift to parasympathetic activity, which is dependent on vagal activity and is primarily associated with energy conservation, rest, and relaxation [9]. Furthermore, even in younger participants, 5 min of slow, deep breathing

Address for correspondence: Dr. Ryota Kobayashi, Department of Natural & Environmental Science, Teikyo University of Science, 2-2-1 Senju, Sakuragi, Adachi-ku, Tokyo 120-0045, Japan, tel: +81 3 6910 1010, fax: +81 3 6910 3800, e-mail: rkobayashi@ntu.ac.jp

Received: 01.07.2023 Accepted: 23.12.2023 Early publication date: 13.02.2024

significantly increased parasympathetic activity and reduced the state of anxiety [10]. Chang et al. [11] reported changes in parasympathetic balance during slow, deep breathing compared to that during breathing at typical rates.

Slow, deep breathing is a useful nonpharmacological intervention to control hypertension [12]. It causes periodic fluctuations in intrathoracic pressure, which affects venous return to the heart, cardiac output, and ultimately blood pressure (BP) [13, 14]. In patients with hypertension, 2 min of slow, deep breathing has been demonstrated to lower systolic blood pressure (SBP) and diastolic blood pressure (DBP) by 8.6 mmHg and 4.9 mmHg, respectively [15]. Changes in BP are strongly associated with arterial stiffness [16]. In addition, slow, deep breathing stimulates stretch receptors in the aortic arch and carotid sinus, thereby activating the baroreflexes and exerting a favorable effect on the cardiovascular system [17]. Thus, a single session of slow, deep breathing may reduce arterial stiffness.

Several breathing control methods are available for this purpose. One of them is the 4-4-8 breathing technique, a breathing pattern developed by a Harvard Medical School physician named Professor Hideyuki Negoro. The 4-4-8 breathing technique involves inhaling for 4 s, holding the breath for 4 s, and exhaling for 8 s. This technique is thought to increase parasympathetic activity and decrease sympathetic activity, thus reducing anxiety and fear [18, 19]. In other words, slow, deep breathing may decrease arterial stiffness via changes in autonomic function and BP. However, the acute effects of the slow, deep, 4-4-8 breathing technique on arterial stiffness is unclear.

Therefore, this study aimed to identify the immediate effects of the 4-4-8 breathing technique on HRV, BP, and arterial stiffness in healthy young men. The hypothesis herein, was that the 4-4-8 breathing technique would decrease arterial stiffness.

Methods

Participants

Because HRV changes differ between men and women owing to hormonal differences [20], only men were recruited in this study to minimize variability. Fifteen healthy men aged 20 years were recruited from the Teikyo University of Science student programs. Prior to the start of the study, all participants provided written informed consent after receiving a complete verbal and written explanation of the purpose and methods of the study. Young men with no medical history who were in good health were included. Individuals with a history of abnormal blood/urine tests, chest radiographs, or electrocardiograms; those with a history of hypertension; and those taking medications that may alter BP were excluded. All participants had no experience with meditation. This study was conducted in accordance with the Declaration of Helsinki and was reviewed and approved by the Ethics Committee of Teikyo University of Science (approval number: 22A009).

This study is a cross-over study. Prior to the testing session, the order in which the two conditions were to be performed was randomly assigned to each participant by an individual not affiliated with the study. None of the participants performed abdominal breathing in the (a) 4-4-8 breathing technique and (b) control (CON) conditions.

To determine the appropriate sample size, a power analysis was performed using IBM SPSS Statistics (ver. 25; IBM Corp., Armonk, NY, USA). The magnitude of the effect of 4-4-8 breathing technique on arterial stiffness was assumed to be 0.5. Using an analysis of variance, it was determined that to detect a difference with 80% power and a two-sided alpha of 5%, nine participants should be included. To account for dropouts, 15 participants were included in the study. This study was conducted in compliance with the Declaration of Helsinki in terms of ethics, human rights, and protection of the participants' personal information.

Study design

Participants were asked to come to the laboratory early in the morning after an 8-h of overnight fasting. Next, the participants were asked to assume the supine position in a quiet room and underwent resting HRV, BP, and arterial stiffness testing. The researcher then explained and demonstrated the 4-4-8 breathing technique using a simplified instruction manual so that the participants could fully understand it. The participants performed the 4-4-8 breathing technique in a quiet room, lying in the relaxed supine position with their eyes closed. HRV, BP, and arterial stiffness were tested in the supine position at 30 min, 60 min, and 24 h after the 4-4-8 breathing technique was completed.

Body composition

Body height was measured in 0.1-cm increments using a height meter (Sanwa Corporation, Tokyo, Japan), and body weight and fat percentage were measured noninvasively (impedance method) using a body composition analyzer (InnerScan Dual Black RD-E04BK, Tanita Corporation, Tokyo, Japan). Body mass index (BMI) was calculated by dividing weight (kg) by height squared (m^2) (kg/m^2).

Arterial stiffness

Pulse wave velocity (PWV) is an established measure of arterial stiffness. To evaluate arterial stiffness, PWV was calculated from the pulse wave propagation time between two arterial points as follows: PWV = arterial length/pulse wave propagation time. As an index of systemic arterial stiffness, brachial–ankle PWV (baPWV) was evaluated using a blood pressure pulse wave testing device (BP-203RPEII; Fukuda Colin Co., Tokyo, Japan) by attaching oscillometric sensors to the left and right upper arms and ankles [21]. Carotid–femoral PWV (cfPWV) was measured by recording arterial pressure waveforms (SphygmoCor XCEL TM-2805V; A&D Corp., Tokyo, Japan) using tonometric and oscillometric sensors affixed to the left common carotid and left femoral arteries, respectively. The linear distance between the sensors was measured using a tape measure and divided by the automatically calculated time difference in the increase in arterial pressure between the sensors, which was used as an index of aortic stiffness [22]. The intra- and inter-rater coefficients of variation for the PWV measurements were 3% and 4%, respectively.

Cardiovascular indices

Arterial BP waveforms were recorded using oscillometric sensors attached to both upper arms with a BP pulse wave testing device (PWV/ABI; Fukuda Colin, Inc., Tokyo, Japan) [23] to evaluate the BP in the brachial arteries. The same device with electrocardiograph sensors attached to both wrists [23] was used to evaluate the heart rate. A tonometric sensor attached to the left carotid artery was used to record arterial BP waveforms using a BP pulse wave testing device (SphygmoCor XCEL TM-2805V; A&D Corp., Tokyo, Japan) to evaluate the SBP of the aortic artery, augmentation index (AIx), ejection pulse pressure and reflex pulse pressure. The intra- and inter-rater coefficients of variation for BP readings were 2% and 3%, respectively.

HRV analysis

The high-frequency (HF) and low-frequency (LF) HRV components reflect parasympathetic and sympathetic activation is generally assessed as the overall sympathetic vagal balance and degree of autonomic excitation [24]. The RR intervals recorded from the wearable heart rate sensor WHS-1/RRD-1 (Union Tool Corporation, Tokyo, Japan) were downloaded and analyzed using the HRV analysis software RRI Analyzer 2 (Union Tool Corporation, Tokyo, Japan). The software converted the RR intervals into the frequency domain indices LF (ms^2) and HF (ms^2). Each index was calculated at 2-min intervals based on the recommendations of the standard. It is generally accepted that 1 min is needed to evaluate the HF component of HRV, whereas approximately 2 min are needed to address the LF component [25]. Automatic artifact correction was performed on all recordings before analysis. Data were subjected to HRV spectral analysis; the LF and HF bands were defined as 0.04–0.15 Hz and 0.15–0.4 Hz, respectively [26].

4-4-8 breathing technique

The participants were guided by a video of a droplet on a computer screen moving up and down. As the droplet rose, the participants inhaled, and as the droplet fell, they exhaled. Initially, the participants had to (1) exhale completely through the mouth, (2) breathe in through the nose while placing their hands on the belly while it expanded (4 s), (3) hold their breath (4 s), and (4) breathe out through the nose to depress the belly (8 s). The exercise lasted for 5 min (19 cycles).

Statistical analysis

A total of 15 participants were included in the full analysis set. Clinical response rates and 95% confidence intervals were calculated, and statistical analyses were performed in a blinded manner. Data for the outcome variables were tested for normality and log normality using the Shapiro–Wilk test. Repeated-measures two-way analysis of variance was used to evaluate the between-trial change in each measure in each intervention using a post hoc test (Bonferroni method). All statistical analyses were performed using IBM SPSS Statistics (ver. 25; IBM Corp., Armonk, NY, USA), with a statistical significance level of 5%. All data are presented as mean ± standard deviation.

Results

Physical characteristics

Twenty subjects were recruited, screened, 5 excluded, and 15 included. All the enrolled participants (n = 15) completed the study sessions without any adverse events. The participants' height, weight, body fat percentage, BMI, and resting BP are summarized in Table 1.

Table 1. Characteristics of study participants.

	Value
Age [years]	20 ± 0
Height [cm]	170 ± 5
Weight [kg]	56 ± 3
Body fat [%]	16 ± 2
BMI [kg/m²]	19 ± 2
Brachial SBP [mmHg]	106 ± 6

Values are expressed as mean ± standard deviation; BMI — body mass index; SBP — systolic blood pressure

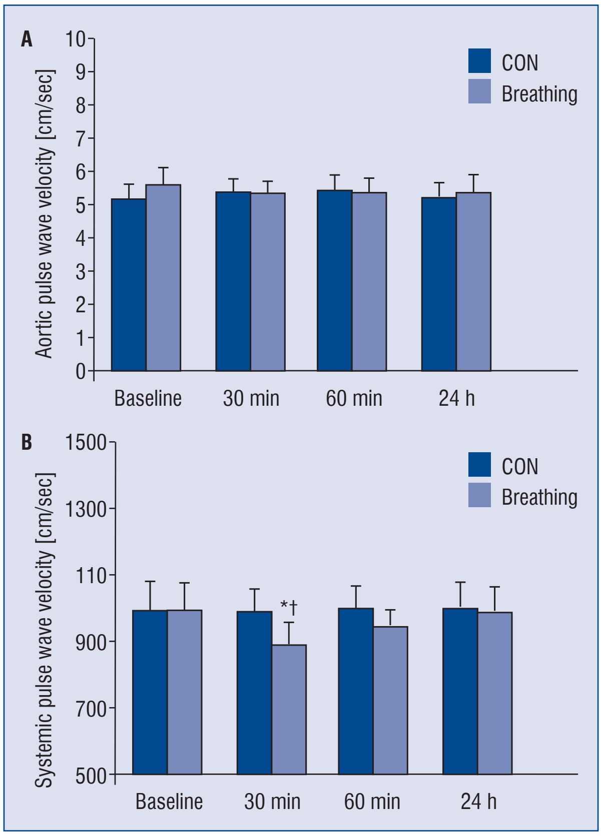

Figure 1. Changes in arterial stiffness before and after 4-4-8 breathing. Values are expressed as mean ± standard deviation; PWV — pulse wave velocity; CON — control conditions; *p < 0.05 vs. baseline; †p < 0.05 vs. CON.

Arterial stiffness

The cfPWV values are shown in Figure 1A. The cfPWV values for both trials did not significantly change at 30 min, 60 min, or 24 h compared to those at baseline, and there were no between-trial differences. baPWV values are shown in Figure 1B. In the 4-4-8 breathing technique trial, the baPWV values significantly decreased at 30 min ($p < 0.05$), but no significant change was observed at 60 min, and 24 h. In the CON trial, baPWV values at 30 min, 60 min, and 24 h were not significantly different from those at baseline. The baPWV at 30 min was significantly lower in the 4-4-8 breathing technique trial than in the CON trial ($p < 0.05$).

Cardiovascular indices

Blood pressure values are shown in Table 2. In the 4-4-8 breathing technique trial, brachial SBP, PP and AIx decreased significantly at 30 min ($p < 0.05$) compared to baseline, but there was no significant change in the value at 60 min and 24 h. In the CON trial, brachial SBP, PP and AIx did not change significantly at 30 min, 60 min, and 24 h compared to that at baseline. Brachial SBP, PP and AIx at 30 min was significantly lower in the 4-4-8 breathing technique trial than in the CON trial ($p < 0.05$). In both trials, brachial DBP, ankle SBP, DBP, PP, aortic SBP, aortic ejection pulse pressure, and aortic reflex pulse pressure did not change significantly from baseline at 30 min, 60 min, or 24 h, and were not significantly different in both trials.

Sympathetic response (LF)

The LF values are shown in Figure 2A. In the 4-4-8 breathing technique trial, the LF value was significantly lower at 30 min ($p < 0.05$) than at baseline, but there was no significant change in the value at 60 min and 24 h. In the CON trial, In the CON trial, LF values at 30 min, 60 min, and 24 h were not significantly different from those at baseline. The LF value at 30 min was significantly lower in the 4-4-8 breathing control trials than in the CON trial ($p < 0.05$).

Parasympathetic response (HF)

The HF values are shown in Figure 2B. In the 4-4-8 breathing technique trial, the HF value was significantly higher at 30 min ($p < 0.05$) than at baseline, but there was no significant change in the value at 60 min and 24 h. In the CON trial, HF values at 30 min, 60 min, and 24 h were not significantly different from those at baseline. The HF value at 30 min was significantly higher in the 4-4-8 breathing control trials than in the CON trial ($p < 0.05$).

Discussion

According to available research, this is the first study to investigate the acute effects of the 4-4-8 breathing technique on PWV in young participants.

Table 2. Changes in cardiovascular indices before and after 4-4-8 breathing.

	Baseline	After 30 min	After 60 min	After 24 h
Brachial SBP [mmHg]				
4-4-8 breathing	105 ± 10	99 ± 4*†	106 ± 12	105 ± 7
CON	106 ± 6	104 ± 6	105 ± 6	106 ± 10
Brachial DBP [mmHg]				
4-4-8 breathing	67 ± 6	67 ± 5	67 ± 5	67 ± 4
CON	68 ± 5	68 ± 5	68 ± 6	66 ± 6
Brachial PP [mmHg]				
4-4-8 breathing	38 ± 5	32 ± 4*†	39 ± 4	37 ± 6
CON	37 ± 6	36 ± 8	37 ± 4	39 ± 8
Ankle SBP [mmHg]				
4-4-8 breathing	117 ± 10	117 ± 6	117 ± 8	117 ± 6
CON	118 ± 6	118 ± 6	118 ± 6	117 ± 6
Ankle DBP [mmHg]				
4-4-8 breathing	61 ± 6	60 ± 8	63 ± 8	62 ± 6
CON	61 ± 6	63 ± 4	62 ± 6	59 ± 6
Ankle PP [mmHg]				
4-4-8 breathing	55 ± 8	56 ± 8	54 ± 8	54 ± 4
CON	56 ± 10	55 ± 8	55 ± 6	57 ± 8
Aortic SBP [mmHg]				
4-4-8 breathing	95 ± 10	96 ± 10	95 ± 7	95 ± 10
CON	96 ± 10	95 ± 8	96 ± 6	96 ± 8
Ejection pulse pressure [mmHg]				
4-4-8 breathing	22 ± 2	23 ± 4	23 ± 4	23 ± 4
CON	23 ± 6	23 ± 6	24 ± 4	23 ± 4
Reflex pulse pressure [mmHg]				
4-4-8 breathing	11 ± 4	10 ± 4	11 ± 5	12 ± 4
CON	11 ± 4	11 ± 5	11 ± 4	11 ± 5
AIx [%]				
4-4-8 breathing	14 ± 5	10 ± 6*†	12 ± 5	14 ± 5
CON	14 ± 5	14 ± 6	14 ± 5	14 ± 7

Values are expressed as mean ± standard deviation; SBP — systolic blood pressure; DBP — diastolic blood pressure; PP — pulse pressure; AIx — Augmentation Index; CON — control conditions; *p < 0.05 vs. baseline; †p < 0.05 vs. CON

This study included young men to avoid the influence of sex on HRV [20]. In this study, the HF value increased and the LF, baPWV, and SBP values decreased after performing the 4-4-8 respiratory technique. However, values after 24 h were not significantly different between the two trials.

The typical number of breaths per minute is 12–20 [27], but the 4-4-8 breathing technique is similar to slow, deep breathing as it takes 16 s per breath, which is approximately 4 less breaths per minute. According to Vierra et al. [28], slow breathing helps improve HRV. Magnon et al. [10] reported that slow, deep breathing in healthy young participants significantly increased the HF value and restored vagal tone, similar to the findings of the present study. Thus, long, slow, and deep breathing with prolonged exhalation may increase parasympathetic activity and decrease sympathetic activity.

This study also found that the 4-4-8 breathing technique reduced BP. The results of this study are consistent with those of previous studies. Many studies have shown that deep breathing reduces BP by 4–54 mmHg in individuals of various ages and with various BP levels [12]. In this study, the 4-4-8 breathing technique reduced values by an average of 5.8 mmHg after 30 min. In previous studies, slow, deep breathing decreased SBP in patients with hypertension and increased the HF

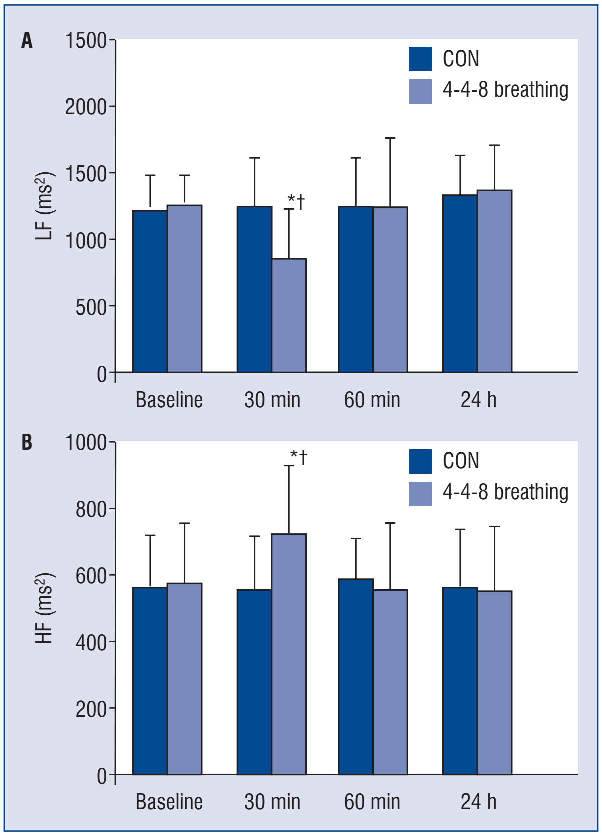

Figure 2. Changes in heart rate variability before and after 4-4-8 breathing. Values are expressed as mean ± standard deviation; HF — high frequency; LF — low frequency; CON — control conditions; *$p < 0.05$ vs. baseline; †$p < 0.05$ vs. CON.

value [29, 30]. Lin et al. [31] instructed healthy college students to breathe at 6.0 breaths/min and 5.5 breaths/min and found that the HRV was higher at 5.5 breaths/min. However, no positive effect on HRV has been identified when the ratio of inhalation to exhalation is 1 to 1 [32]. In this study, the 4-4-8 breathing technique resulted in longer exhalation times. Thus, practicing slow, deep breathing with a relatively long exhalation time may decrease BP owing to oxygenation and activation of the parasympathetic nervous system. This issue needs to be investigated in detail in future studies.

Most vascular functions are regulated by the contractile state of smooth muscle cells in the arterial wall [33]. Heart rate has an important influence on PWV and is closely related to HRV [34]. Changes in HRV are associated with changes in PWV [35]. In young adults, baPWV correlates with sympathetic nervous system activation [36, 37]. In the present study, the 4-4-8 breathing technique increased the HF value and decreased the LF and baPWV values. In other words, long, slow, and deep breaths with longer exhalations may decrease the PWV by regulating autonomic function. Another study examining the effects of the autonomic nervous system activity on arterial stiffness reported that the autonomic nervous system did not play a direct role in the regulation of cfPWV in healthy participants [38]. Consistent with these results, cfPWV did not change after the 4-4-8 breathing technique trial in this study. This may involve the organic effect of central arteries having more elastic tissue and less smooth muscle than peripheral arteries to adapt to the high pressures caused by ventricular ejection [39].

In humans, altering the contractile state of the brachial artery smooth muscle with the sympathetic neurotransmitters norepinephrine and nitroglycerin increases and decreases brachial artery stiffness, respectively [40]. Exposure to acute intermittent hypoxia and increased PWV increase norepinephrine levels [41, 42]. In other words, slow, deep breathing may suppress the PWV by increasing norepinephrine inhibition. However, norepinephrine was not measured in this study and is a subject for future research; important predictors of PWV are age and SBP [43]. As noted above, a recent study demonstrated that SBP decreased with slow, deep breathing compared to spontaneous breathing [30]. In this study, SBP and PWV decreased after the 4-4-8 breathing technique trial. Therefore, SBP may be involved in the decrease in baPWV with the 4-4-8 breathing technique. Further details need to be verified in future studies. Decreased respiratory sinus arrhythmias during deep breathing are associated with increased coronary atherosclerosis [44]. In this study, 4-4-8 breathing technique increased HF value and decreased baPWV in healthy young men, suggesting that the phenomenon of respiratory sinus arrhythmias occurred normally. Further detailed studies are needed in the future.

Arterial stiffness depends on arterial structural characteristics (calcification, intimal tunica media thickening, increased collagen, and decreased elastin) [45]. Increased sympathetic activity damages elastin fibers and decreases arterial compliance [46]. Improvements in HRV were observed after 4 weeks of respiratory training, and respiratory training lowered BP [47]. In other words, although this study had an acute effect, long-term breathing exercises might have resulted in organic changes and a lower baseline baPWV. Therefore, future studies should investigate the relationships among long-term 4-4-8 respiratory control, autonomic function, and PWV. Future long-term studies should also investigate whether breathing exercises contribute to better sleep quality.

Limitations of the study

This study has several limitations. First, because the participants were healthy young adults, caution should be exercised when generalizing the results to older adults or those with impaired sleep quality, such as those with metabolic syndrome. Second, norepinephrine or electroencephalogram was not assessed, which might have had important effects on arterial stiffness. The small sample size warrants caution when interpreting the results and limits the generalizability of the present findings.

Conclusions

In this study, the 4-4-8 breathing technique transiently reduced the BP and baPWV. According to available research, this is the first study to evaluate the effects of the 4-4-8 breathing technique on arterial stiffness. The results of this study are significant because they highlight the positive effects of slow, deep breathing on vascular function.

Data availability statement: Data described in the manuscript, code book, and analytic code will be made available upon request pending [e.g., application and approval, payment, other]. Before the study began, all participants received a complete explanation of the study's purpose and methods before providing their written informed consent.

Ethics statement: This study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Teikyo University of Science (approval No.: 22A009).

Author contributions: R.K. and H.N. designed the research; R.K. and H.N. conducted the research; R.K. and H.N. analyzed data; and R.K. wrote the paper. R.K. had the primary responsibility for the final content. All authors read and approved the final manuscript.

Funding: None.

Acknowledgments: None.

Conflict of interest: None declared.

Supplementary material: None.

References

1. Ohkuma T, Ninomiya T, Tomiyama H, et al. Collaborative Group for J-BAVEL (Japan Brachial-Ankle Pulse Wave Velocity Individual Participant Data Meta-Analysis of Prospective Studies). Brachial-Ankle Pulse Wave Velocity and the Risk Prediction of Cardiovascular Disease: An Individual Participant Data Meta-Analysis. Hypertension. 2017; 69(6): 1045–1052, doi: 10.1161/HYPERTENSIONAHA.117.09097, indexed in Pubmed: 28438905.
2. van Sloten TT, Protogerou AD, Henry RMA, et al. Association between arterial stiffness, cerebral small vessel disease and cognitive impairment: A systematic review and meta-analysis. Neurosci Biobehav Rev. 2015; 53: 121–130, doi: 10.1016/j.neubiorev.2015.03.011, indexed in Pubmed: 25827412.
3. Miyoshi T, Ito H. Arterial stiffness in health and disease: The role of cardio-ankle vascular index. J Cardiol. 2021; 78(6): 493–501, doi: 10.1016/j.jjcc.2021.07.011, indexed in Pubmed: 34393004.
4. Russo MA, Santarelli DM, O'Rourke D. The physiological effects of slow breathing in the healthy human. Breathe (Sheff). 2017; 13(4): 298–309, doi: 10.1183/20734735.009817, indexed in Pubmed: 29209423.
5. Gerritsen RJS, Band GPH. Breath of life: the respiratory vagal stimulation model of contemplative activity. Front Hum Neurosci. 2018; 12: 397, doi: 10.3389/fnhum.2018.00397, indexed in Pubmed: 30356789.
6. Zaccaro A, Piarulli A, Laurino M, et al. How breath-control can change your life: a systematic review on psycho-physiological correlates of slow breathing. Front Hum Neurosci. 2018; 12: 353, doi: 10.3389/fnhum.2018.00353, indexed in Pubmed: 30245619.
7. Aysin B, Aysin E. Effect of respiration in heart rate variability (HRV) analysis. Conf Proc IEEE Eng Med Biol Soc. 2006; 2006: 1776–1779, doi: 10.1109/IEMBS.2006.260773, indexed in Pubmed: 17946068.
8. Bernardi L, Gabutti A, Porta C, et al. Slow breathing reduces chemoreflex response to hypoxia and hypercapnia, and increases baroreflex sensitivity. J Hypertens. 2001; 19(12): 2221–2229, doi: 10.1097/00004872-200112000-00016, indexed in Pubmed: 11725167.
9. Zhang PZ, Tapp WN, Reisman SS, et al. Respiration response curve analysis of heart rate variability. IEEE Trans Biomed Eng. 1997; 44(4): 321–325, doi: 10.1109/10.563302, indexed in Pubmed: 9125815.
10. Magnon V, Dutheil F, Vallet GT. Benefits from one session of deep and slow breathing on vagal tone and anxiety in young and older adults. Sci Rep. 2021; 11(1): 19267, doi: 10.1038/s41598-021-98736-9, indexed in Pubmed: 34588511.
11. Chang Q, Liu R, Shen Z. Effects of slow breathing rate on blood pressure and heart rate variabilities. Int J Cardiol. 2013; 169(1): e6–e8, doi: 10.1016/j.ijcard.2013.08.121, indexed in Pubmed: 24063918.
12. Herawati I, Mat Ludin AF, Mutalazimah M, et al. Breathing exercise for hypertensive patients: A scoping review. Front Physiol. 2023; 14: 1048338, doi: 10.3389/fphys.2023.1048338, indexed in Pubmed: 36760529.
13. Malpas SC. Neural influences on cardiovascular variability: possibilities and pitfalls. Am J Physiol Heart Circ Physiol. 2002; 282(1): H6–H20, doi: 10.1152/ajpheart.2002.282.1.H6, indexed in Pubmed: 11748042.

14. Chen YF, Huang XY, Chien CH, et al. The effectiveness of diaphragmatic breathing relaxation training for reducing anxiety. Perspect Psychiatr Care. 2017; 53(4): 329–336, doi: 10.1111/ppc.12184, indexed in Pubmed: 27553981.
15. Joseph CN, Porta C, Casucci G, et al. Slow breathing improves arterial baroreflex sensitivity and decreases blood pressure in essential hypertension. Hypertension. 2005; 46(4): 714–718, doi: 10.1161/01.HYP.0000179581.68566.7d, indexed in Pubmed: 16129818.
16. Mitchell G. Arterial stiffness and hypertension. Hypertension. 2014; 64(2): 210–214, doi: 10.1161/hypertensionaha.114.03449.
17. Heusser K, Tank J, Engeli S, et al. Carotid baroreceptor stimulation, sympathetic activity, baroreflex function, and blood pressure in hypertensive patients. Hypertension. 2010; 55(3): 619–626, doi: 10.1161/HYPERTENSIONAHA.109.140665, indexed in Pubmed: 20101001.
18. Negoro H. Harvard & Sorbonne University Dr. Negoro's Special Class Cellular Breathing Lesson to Avoid Disease. SHUEISHA Inc. 2019.
19. Negoro H. Negoro H Harvard & Sorbonne Professor Negoro's Super Breathing Method. KADOKAWA 2018.
20. Zhang J. Effect of age and sex on heart rate variability in healthy subjects. J Manipulative Physiol Ther. 2007; 30(5): 374–379, doi: 10.1016/j.jmpt.2007.04.001, indexed in Pubmed: 17574955.
21. Sugawara J, Hayashi K, Yokoi T, et al. Brachial-ankle pulse wave velocity: an index of central arterial stiffness? J Hum Hypertens. 2005; 19(5): 401–406, doi: 10.1038/sj.jhh.1001838, indexed in Pubmed: 15729378.
22. Fryer S, Stone K, Paterson C, et al. Central and peripheral arterial stiffness responses to uninterrupted prolonged sitting combined with a high-fat meal: a randomized controlled crossover trial. Hypertens Res. 2021; 44(10): 1332–1340, doi: 10.1038/s41440-021-00708-z, indexed in Pubmed: 34334790.
23. Kobayashi R, Sakazaki M, Nagai Y, et al. Effects of different types of carbohydrates on arterial stiffness: a comparison of isomaltulose and sucrose. Nutrients. 2021; 13(12), doi: 10.3390/nu13124493, indexed in Pubmed: 34960045.
24. Shaffer F, Ginsberg JP. An overview of heart rate variability metrics and norms. Front Public Health. 2017; 5: 258, doi: 10.3389/fpubh.2017.00258, indexed in Pubmed: 29034226.
25. Laborde S, Mosley E, Thayer JF. Heart rate variability and cardiac vagal tone in psychophysiological research - recommendations for experiment planning, data analysis, and data reporting. Front Psychol. 2017; 8: 213, doi: 10.3389/fpsyg.2017.00213, indexed in Pubmed: 28265249.
26. Lombardi F, Malliani A, Pagani M, et al. Heart rate variability and its sympatho-vagal modulation. Cardiovasc Res. 1996; 32(2): 208–216, doi: 10.1016/0008-6363(96)00116-2, indexed in Pubmed: 8796106.
27. Moore T. Respiratory assessment in adults. Nurs Stand. 2007; 21(49): 48–56; quiz 58, doi: 10.7748/ns2007.08.21.49.48.c4605, indexed in Pubmed: 17844906.
28. Vierra J, Boonla O, Prasertsri P. Effects of sleep deprivation and 4-7-8 breathing control on heart rate variability, blood pressure, blood glucose, and endothelial function in healthy young adults. Physiol Rep. 2022; 10(13): e15389, doi: 10.14814/phy2.15389, indexed in Pubmed: 35822447.
29. Elliot WJ, Izzo JL, White WB, et al. Graded blood pressure reduction in hypertensive outpatients associated with use of a device to assist with slow breathing. J Clin Hypertens (Greenwich). 2004; 6(10): 553–560, doi: 10.1111/j.1524-6175.2004.03553.x, indexed in Pubmed: 15470284.
30. Li C, Chang Q, Zhang J, et al. Effects of slow breathing rate on heart rate variability and arterial baroreflex sensitivity in essential hypertension. Medicine (Baltimore). 2018; 97(18): e0639, doi: 10.1097/MD.0000000000010639, indexed in Pubmed: 29718876.
31. Lin IM, Tai LY, Fan SY. Breathing at a rate of 5.5 breaths per minute with equal inhalation-to-exhalation ratio increases heart rate variability. Int J Psychophysiol. 2014; 91(3): 206––211, doi: 10.1016/j.ijpsycho.2013.12.006, indexed in Pubmed: 24380741.
32. Bae D, Matthews JJL, Chen JJ, et al. Increased exhalation to inhalation ratio during breathing enhances high-frequency heart rate variability in healthy adults. Psychophysiology. 2021; 58(11): e13905, doi: 10.1111/psyp.13905, indexed in Pubmed: 34289128.
33. Brozovich FV, Nicholson CJ, Degen CV, et al. Mechanisms of vascular smooth muscle contraction and the basis for pharmacologic treatment of smooth muscle disorders. Pharmacol Rev. 2016; 68(2): 476–532, doi: 10.1124/pr.115.010652, indexed in Pubmed: 27037223.
34. Lantelme P, Mestre C, Lievre M, et al. Heart rate: an important confounder of pulse wave velocity assessment. Hypertension. 2002; 39(6): 1083–1087, doi: 10.1161/01.hyp.0000019132.41066.95, indexed in Pubmed: 12052846.
35. Soltani M, Baluchi MJ, Boullosa D, et al. Effect of intensity on changes in cardiac autonomic control of heart rate and arterial stiffness after equated continuous running training programs. Front Physiol. 2021; 12: 758299, doi: 10.3389/fphys.2021.758299, indexed in Pubmed: 34955880.
36. Nakao M, Nomura K, Karita K, et al. Relationship between brachial-ankle pulse wave velocity and heart rate variability in young Japanese men. Hypertens Res. 2004; 27(12): 925–931, doi: 10.1291/hypres.27.925, indexed in Pubmed: 15894832.
37. Ahn JH, Kong M. The relationship among pulse wave velocity, ankle-brachial pressure index and heart rate variability in adult males. Korean J Fam Med. 2011; 32(7): 406–411, doi: 10.4082/kjfm.2011.32.7.406, indexed in Pubmed: 22745879.
38. Mäki-Petäjä KM, Barrett SML, Evans SV, et al. The role of the autonomic nervous system in the regulation of aortic stiffness. Hypertension. 2016; 68(5): 1290–1297, doi: 10.1161/HYPERTENSIONAHA.116.08035, indexed in Pubmed: 27672029.
39. Chirinos JA, Segers P, Hughes T, et al. Large-Artery stiffness in health and Disease: JACC state-of-the-art review. J Am Coll Cardiol. 2019; 74(9): 1237–1263, doi: 10.1016/j.jacc.2019.07.012, indexed in Pubmed: 31466622.
40. Bank AJ, Wilson RF, Kubo SH, et al. Direct effects of smooth muscle relaxation and contraction on in vivo human brachial artery elastic properties. Circ Res. 1995; 77(5): 1008–1016, doi: 10.1161/01.res.77.5.1008, indexed in Pubmed: 7554135.
41. Zanella S, Doi A, Garcia AJ, et al. When norepinephrine becomes a driver of breathing irregularities: how intermittent hypoxia fundamentally alters the modulatory response of the respiratory network. J Neurosci. 2014; 34(1): 36–50, doi: 10.1523/JNEUROSCI.3644-12.2014, indexed in Pubmed: 24381266.
42. Hwang CL, Piano MR, Thur LA, et al. The effects of repeated binge drinking on arterial stiffness and urinary norepinephrine levels in young adults. J Hypertens. 2020; 38(1): 111––117, doi: 10.1097/HJH.0000000000002223, indexed in Pubmed: 31503138.

43. Safar ME, Asmar R, Benetos A, et al. French Study Group on Arterial Stiffness. Interaction between hypertension and arterial stiffness. Hypertension. 2018; 72(4): 796–805, doi: 10.1161/HYPERTENSIONAHA.118.11212, indexed in Pubmed: 30354723.
44. Engström G, Hamrefors V, Fedorowski A, et al. Cardiovagal function measured by the deep breathing test: relationships with coronary atherosclerosis. J Am Heart Assoc. 2022; 11(7): e024053, doi: 10.1161/JAHA.121.024053, indexed in Pubmed: 35352566.
45. Chen Y, Zhao X, Wu H. Arterial stiffness: a focus on vascular calcification and its link to bone mineralization. Arterioscler Thromb Vasc Biol. 2020; 40(5): 1078–1093, doi: 10.1161/ATVBAHA.120.313131, indexed in Pubmed: 32237904.
46. Omar NM, Marshall JM. Age-related changes in the sympathetic innervation of cerebral vessels and in carotid vascular responses to norepinephrine in the rat: in vitro and in vivo studies. J Appl Physiol (1985). 2010; 109(2): 314–322, doi: 10.1152/japplphysiol.01251.2009, indexed in Pubmed: 20466800.
47. Nagarajan S. Effect of slow breathing training for a month on blood pressure and heart rate variability in healthy subjects. Natl J Physiol Pharm Pharmacol. 2014; 4(3): 245, doi: 10.5455/njppp.2014.4.050520141.