An Acute Bout of a Controlled Breathing Frequency Lowers Sympathetic Neural Outflow but not Blood Pressure in Healthy Normotensive Subjects

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ABSTRACT

Controlled or paced breathing is often used as a stress reduction technique but the impact on blood pressure (BP) and sympathetic outflow have not been consistently reported. The purpose of this study was to determine whether a controlled breathing (12 breaths/min, CB) rate would be similar to an individual’s spontaneous breathing (SB) rate. Secondly, would a CB rate of 12 breaths/min alter heart rate (HR), BP, and indices of muscle sympathetic nerve activity (MSNA). Twenty-one subjects (10 women, 11 men) performed two trials: SB, where the subject chose a comfortable breathing rate; and CB, where the subject breathed at a pace of 12 breaths/min. Each trial was 6 min during which respiratory waveforms, HR, BP (systolic, SBP; diastolic, DBP), and MSNA were recorded. During CB, the 6 min average breathing frequency (14±4 vs 12±1 breaths/min, P<0.05 for SB and CB, respectively), MSNA burst frequency (18±12 vs 14±10 bursts/min, P<0.01) and MSNA burst incidence (28±19 vs 21±6 bursts/100 heart beats, P<0.01) were significantly lower than during SB. HR (66±9 vs 67±9 beats/min, P<0.05) was higher during CB. SBP (120±13 vs 121±15 mmHg, P=0.741), DBP (56±8 vs 57±9 mmHg, P=0.768), and MSNA total activity (166±94 vs 145±102 a.u./min, P=0.145) were not different between the breathing conditions. In conclusion, an acute reduction in breathing frequency such as that observed during CB elicited a decrease in indices of MSNA (burst frequency and incidence) with no change in BP.

KEY WORDS: Muscle sympathetic nerve activity, heart rate, breathing frequency

INTRODUCTION

Respiration can be controlled by voluntary and involuntary functions of the nervous system (11). The medulla contains inspiratory and expiratory centers in the ventral respiratory group that coordinate the breathing cycle (11). During quiet, or spontaneous breathing (SB), inspiratory and expiratory neurons send alternating signals to the integrating centers in the spinal cord to contract or relax the muscles involved in respiration, yielding a respiration rate of approximately 12-15 breaths/min (10, 11). The same pathways are not utilized during
voluntary control of respiration (such as paced or controlled breathing, CB). Conscious control of respiration originates in the motor cortex of the frontal lobe and projects along corticospinal tracts to the integrating centers in the spinal cord (11).

Associated with normal respiration is a fluctuation in heart rate (HR). HR increases upon inspiration and decreases upon expiration. This occurrence likely stems from vagal suppression, which will decrease HR, during inspiration (8) and/or a baroreflex mediated response (3). A baroreflex response refers to how baroreceptors on the aorta and carotid arteries detect mechanical deformation from the blood on the arterial walls and the resultant moderation of cardiovascular components like HR to adjust to the either increased or decreased pressure (17). While CB is not “natural” breathing, it is commonly used by singers and people who participate in yoga or meditative breathing practices (11). Additionally, biathletes, archers, and hunters use a CB-like technique just before shooting, in order to improve accuracy (1). For example, biathletes strive to quickly (<1 min) lower their HR to no more than 80% of maximal HR. In this range, the athlete can control his/her breathing in order to time the shot with bodily movements associated with the higher breathing frequency due to the intense exercise (19).

The natural respiration cycle has also been shown to affect blood pressure (BP) and neural outflow of muscle sympathetic nerve activity (MSNA), with MSNA decreasing during inspiration and increasing during expiration in a cyclical fashion (4, 13). Low frequency CB has been shown to lower MSNA. For example, Hering et al. (2013) implemented a protocol of <10 breaths/min in hypertensive males. They found that an acute (one 15 min session) exposure elicited a reduction in MSNA but not BP. However, long-term use (15 min per day for 8-weeks) showed no reduction in MSNA however a lowering of some, but not all, BP indices (7). It is unclear as to why there is a difference between acute and long-term interventions. This may be related to a reduction in other vasoactive agents and hormones during the long-term treatment. CB or guided breathing is often used as a relaxation technique and has been shown to be quite effective in reducing BP in a variety of populations, especially the hypertensive and diabetic populations (6, 9, 12). However, it is unclear if very acute changes (reduction) in respiration rate could also demonstrate positive improvements in BP and sympathetic outflow.

Therefore, the purpose of this study was to determine whether a very acute (6 min) exposure to CB could have a demonstrable impact on HR, BP, and MSNA. However, we first needed to determine whether an individual’s SB rate (12-15 breaths/min) was similar to a CB rate of 12 breaths/min. Next, we sought to determine if the acute 6 min bout of CB would elicit a reduction in HR, BP, and MSNA when compared to SB. It was hypothesized that there would be a decrease in both cardiovascular variables and MSNA because CB would produce a slower respiration rate than that observed during SB.

**METHODS**

**Participants**
Twenty-one healthy subjects (10 women, 11 men) at least 18 years old gave written informed consent to participate in the study, which was approved by the Institutional Review Board at Northern Arizona University.

Table 1 illustrates the subject characteristics. Exclusionary criteria were: evidence of sleep apnea, smoking or living with a smoker, obesity (body mass index of $\geq 30 \text{ kg/m}^2$), hypertension, currently taking or have taken in the last 6 months hormonal birth control or hormonal replacement therapy, donated plasma or blood within the prior two months, displayed any signs of cardiovascular, respiratory, renal diseases, or neurological disorders as assessed through the medical history questionnaire.

Table 1. Subject characteristics.

| Characteristic          | Value       |
|-------------------------|-------------|
| Age (years)             | 29±17       |
| Height (cm)             | 172±11      |
| Weight (kg)             | 69±13       |
| BMI (kg/m$^2$)          | 23±3        |
| Resting HR (beats/min)  | 65±9        |
| Resting SBP (mmHg)      | 121±14      |
| Resting DBP (mmHg)      | 66±8        |

Values are means±SD.

Protocol

Subjects reported to the lab for one visit. Prior to the study visit, subjects were asked to refrain from exercise for $\geq$12 hours, to avoid consuming alcohol or caffeine for $\geq$48 hours, and to arrive to the lab fasted for $\geq$8 hours. All studies were performed in the Cardiovascular Regulation Lab at Northern Arizona University, which was maintained at a temperature of 21.6 ± 1.3 °C. During instrumentation, subjects were at rest in the supine position on the patient table. A 3-lead electrocardiogram (ECG module, Finapres Medical Systems, Amsterdam, The Netherlands) was used for continuous monitoring of heart rate. Beat-by-beat BP (Finometer MIDI, Finapres Medical Systems, Amsterdam, The Netherlands) was obtained from the subject’s left middle or index finger while brachial artery pressure was obtained from the subject’s right arm (SunTech Tango Plus, Morrisville, NC). Respiration was measured with a piezoelectric respiratory belt transducer (ADInstruments, Colorado Springs, CO). Microneurography was used to record MSNA from a recording electrode that was placed into the peroneal nerve at the popliteal fossa. A subcutaneous reference electrode was placed 2-3 cm away from the recording electrode. Criteria for adequate MSNA recording included: 1) pulse synchrony; 2) facilitation during the hypotensive phase of the Valsalva maneuver and suppression during the hypertensive overshoot after release; 3) increases in response to breath holding; and 4) insensitivity to a gentle skin touch or a loud shout (18).

Once instrumentation was complete and a clear MSNA signal was found, baseline data were recorded. During SB, the subject was instructed to breathe at his/her normal pace. During CB, the subject followed a computer program that prompted the subject when to inhale and exhale (12 breaths/min). This respiration rate was chosen to avoid changes in heart rate variability, as is seen during spontaneous breathing patterns, while the 6 minute trial time allowed for equal
intervals in the breathing cycle (14). Subjects were given a 1 minute “pre-breathing” period to familiarize themselves to each breathing protocol. Both trials lasted six minutes while HR, BP, and MSNA were recorded continuously. There was a 5 min rest between SB and CB.

Statistical Analysis
Data were sampled and analyzed using LabChart (ADInstruments, Colorado Springs, CO). All data were averaged for the 6 min data collection period. MSNA bursts were identified from the integrated neurogram (2) and then were confirmed by trained personnel. Burst areas of the integrated neurogram and BP were measured simultaneously on a beat-to-beat basis. Burst frequency was defined as the number of bursts per min and burst incidence was used to normalize burst frequency per 100 heart beats. Total activity was defined as the burst area of the rectified and integrated neurogram. We assigned the largest burst amplitude during baseline a value of 100 which represented our calibration burst (6, 16). Therefore, all other bursts within a testing session were normalized against this value.

Data are reported as means ± SD. The 6 min averages of breathing frequency, HR, BP, and MSNA variables (burst frequency, burst incidence, and total activity) were compared between SB and CB using paired t-tests. All statistical analyses were performed in Sigma Plot using the average of all 6 minutes of data collection. Significance was declared as $P < 0.05$.

RESULTS

The subject characteristics are outlined in Table 1. Resting HR and BP values were obtained during the screening process.

Figure 1 illustrates the breathing frequencies for SB and CB. CB frequency was lower than SB (12 ± 1 vs 14 ± 4 breaths/min, $P < 0.05$).

HR, SBP, and DBP are illustrated in Figure 2. CB induced a slight but significant increase in HR (66±9 vs 67±9 beats/min for SB and CB, respectively, $P<0.05$). There was no difference in SBP or DBP between the two trials (SBP: 120±13 vs 121±15 mmHg, $P=0.741$; DBP: 56±8 vs 57±9 mmHg, $P=0.768$).

MSNA burst frequency and burst incidence (Figure 3) were lower during CB compared to SB (burst frequency: 18±2 vs 14±10 bursts/min, $P<0.01$; burst incidence: 28±19 vs 21±16 bursts/100 heart beats, $P<0.01$). There was no significant difference in MSNA total activity between the two trials (166±94 vs 145±102 a.u./min, $P=0.145$).

Figure 1. Individual breathing frequency for each subject during SB and CB. Breathing frequency was lower during CB compared to SB. *Difference between SB and CB, $P < 0.05$. 
Figure 2. Cardiovascular responses during SB and CB. HR was slightly but significantly elevated during CB compared to SB. SBP and DBP were not different between the two breathing trials. *Difference between SB and CB, P<0.05.

Figure 3. MSNA indices during SB and CB trials. MSNA burst frequency and burst incidence were lower during CB compared to SB. MSNA total activity was not different between the two trials. *Difference between SB and CB, P < 0.01.
**Table 2.** Individual changes from SB to CB at minute 6 in HR, MSNA burst frequency, and MSNA burst incidence.

| Subject | ΔHR (beats/min) | ΔMSNA BF (bursts/min) | ΔMSNA BI (bursts/100 heart beats) |
|---------|----------------|------------------------|----------------------------------|
| 1       | -13            | 9                      | 14                               |
| 2       | 4              | 5                      | 5                                |
| 3       | 2              | -7                     | -10                              |
| 4       | 3              | 8                      | 12                               |
| 5       | 1              | 3                      | 7                                |
| 6       | -1             | 4                      | 6                                |
| 7       | -4             | 2                      | 7                                |
| 8       | 1              | 24                     | 33                               |
| 9       | 3              | 18                     | 28                               |
| 10      | -3             | 7                      | -10                              |
| 11      | -8             | 4                      | 14                               |
| 12      | 2              | 18                     | 25                               |
| 13      | 2              | 4                      | 8                                |
| 14      | -5             | 0                      | 0                                |
| 15      | -7             | 7                      | 15                               |
| 16      | 2              | 3                      | 6                                |
| 17      | 1              | 2                      | 10                               |
| 18      | -4             | 4                      | 5                                |
| 19      | 3              | 0                      | 0                                |
| 20      | 0              | 0                      | 0                                |
| 21      | -3             | 4                      | 7                                |

HR, heart rate. MSNA BF, muscle sympathetic nerve activity burst frequency. MSNA BI, muscle sympathetic burst incidence. SB, spontaneous breathing. CB, controlled breathing.

**DISCUSSION**

The primary findings of this study were: 1) CB produced a lower breathing frequency than SB; 2) SBP and DBP were not different between the two trials; and 3) MSNA burst frequency and incidence were lower during CB. These findings partially support our hypothesis in that CB reduced MSNA indices but not BP.

Initially, we estimated that subjects would voluntarily choose a SB frequency around 12-15 breaths/min, consistent with the normal rates of respiration that are often reported in physiology textbooks (10, 11). However, we observed a large range of SB rates, as low as 5 breaths/min to as high as 23 breaths/min. Only 38% of the subjects demonstrated a SB frequency between 12 and 15 breaths/min.

When breathing rate was controlled (12 breaths/min) we observed no change in BP and a reduction in MSNA burst frequency and incidence when compared with a SB frequency condition. This is consistent with several studies that found BP responses have varied depending on the length of the intervention. For example, Hering et al (2013) reported no change in BP during a 15 min CB intervention, but a decrease in MSNA. Our study showed similar findings (7).
What is interesting about the current study, however, is that our subjects demonstrated a large range in SB frequencies (5-23 breaths/min) but consistent MSNA reductions in response to CB. Despite that some subjects increased their breathing frequency (24%), some decreased their breathing frequency (71%), and others had no change (5%), we noted that 76% of subjects showed a reduction in MSNA burst frequency and 81% in MSNA burst incidence when comparing SB to CB. These data suggest that other factors, in addition to breathing frequency, influence BP and MSNA. For example, MSNA was lower during CB in the four subjects who had the lowest SB frequencies (i.e., 5-9 breaths/min.). Theoretically, as breathing frequency increased in the CB condition, MSNA indices should have also increased.

It is likely that lung volume also plays a critical role in the BP and MSNA responses during respiration. Others (4, 15) have reported that a low lung volume induces an increase in MSNA. For example, Dempsey et al. (2002) reported that during low lung volume conditions, respiratory motor output is at its lowest. Conversely, at higher lung volumes central respiratory control is at its peak and is a strong inhibitor of MSNA (4). While we did not directly measure tidal volume, we speculate that during the CB trial, subjects increased lung (tidal) volume, which consequently inhibited sympathetic outflow. In subsequent studies, the lung volume should be measured to confirm whether this is true.

Taken together, these findings might suggest that to gain the most benefit from meditative breathing, or CB to reduce stress, there should be focus on both breathing frequency and depth. We found that even an acute trial (6 min) of CB could elicit a reduction in sympathetic outflow. We did not observe a concomitant decrease in BP but this may be due to the very acute CB intervention. It is likely that in the long-term this may lower BP and could be beneficial for those with hypertension (12). For example, some have reported decreases in BP by as much as 9 mmHg after adopting a CB-type protocol (5, 7, 12). This provides evidence for the benefits of non-pharmacologic interventions in the treatment of hypertension; however, the full dose-response curve needs to be further characterized. In a normotensive population, the effects of reduced MSNA indices may seem insignificant in the short term, but in the long term the reduction of these parameters may reduce the risk for hypertension later in life. In populations where the health of the cardiovascular system is in question, such as the hypertensive population, a reduction in sympathetic outflow could influence their plan of care.

One limitation of this study was that lung volume (tidal volume) was not measured. Therefore, we cannot verify that during the CB trial the subjects increased their tidal volume. We do not believe this changes the interpretation of our results, but further research should be conducted considering this variable. Based on the work of St Croix et al (1999) and Dempsey et al (2002) it is clear that a larger tidal volume inhibits MSNA.

In conclusion, a very acute CB intervention successfully lowers sympathetic outflow. The data from this study suggest that this could be related to both altering the breathing frequency and, likely, increasing the depth of breathing.
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