special communication

Controlled breathing protocols probe human autonomic cardiovascular rhythms

WILLIAM H. COOKE,[1] JAMES F. COX,[1] ANDRÉ M. DIEDRICH,[2] J. ANDREW TAYLOR,[3]
LARRY A. BEIGHTOL,[1] JAMES E. AMES IV,[1] JEFFREY B. HOAG,[1]
HENRIK SEIDEL,[4] AND DWAIN L. ECKBERG[1]
[1]Departments of Medicine, Physiology, and Mathematical Sciences, Medical College of Virginia of
Virginia Commonwealth University, and Hunter Holmes McGuire Department of Veterans Affairs
Medical Center, Richmond, Virginia 23249; [3]Hebrew Rehabilitation Center for the Aged, Brookline,
Massachusetts 02167; [2]Deutsche Forschungsanstalt für Luft- und Raumfahrt, Institute of Aerospace
Medicine, Cologne 51147; and [4]Institute for Theoretical Physics, University of Berlin,
Berlin D-10623, Germany

Cooke, William H., James F. Cox, André M. Diedrich, J. Andrew Taylor, Larry A. Beightol, James E. Ames IV, Jeffrey B. Hoag, Henrik Seidel, and Dwain L. Eckberg. Controlled breathing protocols probe human autonomic cardiovascular rhythms. *Am. J. Physiol.* 274 (*Heart Circ. Physiol.* 43): H709–H718, 1998.—The purpose of this study was to determine how breathing protocols requiring varying degrees of control affect cardiovascular dynamics. We measured inspiratory volume, end-tidal CO_2, R-R interval, and arterial pressure spectral power in 10 volunteers who followed the following 5 breathing protocols: *1*) uncontrolled breathing for 5 min; *2*) stepwise frequency breathing (at 0.3, 0.25, 0.2, 0.15, 0.1, and 0.05 Hz for 2 min each); *3*) stepwise frequency breathing as above, but with prescribed tidal volumes; *4*) random-frequency breathing (~0.5–0.05 Hz) for 6 min; and *5*) fixed-frequency breathing (0.25 Hz) for 5 min. During stepwise breathing, R-R interval and arterial pressure spectral power increased as breathing frequency decreased. Control of inspired volume reduced R-R interval spectral power during 0.1 Hz breathing ($P < 0.05$). Stepwise and random-breathing protocols yielded comparable coherence and transfer functions between respiration and R-R intervals and systolic pressure and R-R intervals. Random- and fixed-frequency breathing reduced end-tidal CO_2 modestly ($P < 0.05$). Our data suggest that stringent tidal volume control attenuates low-frequency R-R interval oscillations and that fixed- and random-rate breathing may decrease CO_2 chemoreceptor stimulation. We conclude that autonomic rhythms measured during different breathing protocols have much in common but that a stepwise protocol without stringent control of inspired volume may allow for the most efficient assessment of short-term respiratory-mediated autonomic oscillations.

respiratory sinus arrhythmia; power spectra; R-R interval

CARDIOVASCULAR RHYTHMS are modulated by central mechanisms and afferent input from arterial baroreceptors, chemoreceptors, cardiac receptors, and pulmonary and thoracic stretch receptors. In short-term recordings, cardiovascular rhythms may be dominated by respiration. Because of this, humans hold a unique advantage over other species as subjects for autonomic research: they can control their breathing. The ability to control breathing, however, may be at once an advantage and a disadvantage. Control of breathing (or at least measurement of, and factoring in of, breathing) may be essential if sense is to be made of R-R interval power spectra; Brown and co-workers (4) showed that respiratory frequency R-R interval spectral power varies as much as 10-fold at different breathing frequencies. Moreover, the possibility exists that carefully conceived breathing algorithms might inform actual mechanisms underlying human autonomic rhythms. Conversely, the conscious mental effort necessary to control breathing may itself alter the physiology being studied.

If advantages accruing from use of human subjects are to be realized, it is necessary to know how the actual control of breathing affects the variables being measured. For this reason, we evaluated how breathing protocols requiring varying degrees of tidal volume and respiratory frequency control influence autonomic cardiovascular dynamics. Our purpose was twofold: *1*) to determine whether control of inspired tidal volume adjusted for changes in respiratory frequency is necessary to properly assess frequency domain analyses of cardiovascular dynamics and *2*) to determine the impact of various controlled breathing algorithms on measured cardiovascular rhythms.

Our results suggest that, although autonomic rhythms measured during different breathing protocols have much in common, there are some statistically significant differences. Stringent control of inspired tidal volume reduces R-R interval oscillations, and random- and fixed-frequency breathing may decrease CO_2 chemoreceptor stimulation. Similar transfer functions between respiration and R-R intervals and systolic pressure and R-R intervals are achievable with both stepwise and random protocols, but only stepwise and fixed-frequency breathing allow for clear separation between low- and high-frequency oscillations. We suggest that, for short-term recordings, breathing protocols incorporating stepwise changes in frequency without stringent control of inspired volume may allow

for the most efficient assessment of respiratory-mediated autonomic oscillations.

METHODS

Subjects. We studied 10 healthy supine volunteers, 5 men and 5 women (mean age $\pm$ SE: 25.5 $\pm$ 1.7 yr; weight: 68.1 $\pm$ 4.7 kg). This research was approved by the human research committees of the Hunter Holmes McGuire Department of Veterans Affairs Medical Center and the Medical College of Virginia of Virginia Commonwealth University. All subjects gave written informed consent.

Measurements. We recorded the electrocardiogram, integrated tidal volume (Fleisch pneumotachograph), beat-by-beat finger photoplethysmographic arterial pressure (Ohmeda Finapres), and end-tidal CO_2 concentration (infrared analyzer; Gambro Engineering). Data were sampled at 250 Hz, recorded on digital tape, and transferred to computer for off-line analysis.

Tidal volume control. At the beginning of each experiment, subjects rested quietly in the supine position and breathed at a comfortable, uncontrolled rate and tidal volume for 5 min, with a face mask connected to a pneumotachograph and a two-way respiratory valve (Hans Rudolph). End-tidal CO_2 was measured from samples withdrawn from the face mask during the first 5 min. Mixed CO_2 was measured from samples withdrawn from a 5-liter mixing chamber during the last 2 min. We calculated average expiratory tidal volume and breathing interval and entered these, along with measurements of end-tidal and mixed CO_2, into a personal computer. We calculated physiological dead space with the Bohr equation (6) and alveolar volume by subtracting physiological dead space from tidal volume. We calculated inspired tidal volume from measurements made during quiet uncontrolled breathing, as follows

$$V_I \cdot breath^{-1} = V_D \cdot breath^{-1} + (V_A \cdot min^{-1}/F)$$

where V_I is inspired volume, V_D is physiological dead space, V_A is alveolar volume, and F is target frequency (breaths/min). Subsequently, we used this equation to calculate inspiratory tidal volume at different breathing rates to maintain normal alveolar ventilation regardless of breathing frequency.

Controlled breathing. The target breathing sequence was displayed graphically in real time on a laptop computer (Thinkpad; IBM). During testing, the computer was positioned above and in front of the subject on an adjustable stand so that she or he could view the screen comfortably, at a distance of ~0.6 m. Subjects attempted to match their breathing to target waveforms, as these waveforms scrolled off the left side of the computer display. Each breath was represented by a single triangle, the height and width of which were directly proportional to the targeted inspired volume and breathing interval. The leading side of each triangle represented inspiration, and the trailing edge represented expiration. A flat horizontal cursor positioned to the left of a stationary line moved up and down over time, exactly matching its vertical position to that of the waveform display; this cursor provided additional visual feedback to assist the subject in maintaining the required breathing rate.

During stepwise breathing with tidal volume control, a second horizontal cursor, the vertical position of which was dependent on the integrated pneumotachograph signal, was positioned adjacent to the first cursor. This signal was fed into a data acquisition module (model DI-205; DATAQ Instruments), which performed an analog-to-digital conversion. The pneumotachograph integrator was configured to reset at the

end of each inspiration and did not begin reporting measurements again until the beginning of the next inspiratory phase. Thus the computer displayed tidal volume only during inspiration.

Experimental protocol. Subjects performed breathing protocols in random order: *1*) 12 min stepwise frequency breathing with uncontrolled tidal volume (at 0.3, 0.25, 0.2, 0.15, 0.1, and 0.05 Hz sequentially, for 2 min each); *2*) 12 min stepwise frequency breathing as above, but with tidal volume control (inspiratory volume was calculated for each breathing rate to maintain normal alveolar ventilation); *3*) 6 min random, or "white noise" frequency breathing with uncontrolled tidal volume [the computer generated a random breathing interval series with a uniform distribution, within the range of ~0.5–0.05 Hz; the "whiteness" of the signal was verified with commercial software (DADiSP; DSP Development)]; and *4*) 5 min fixed-frequency (0.25 Hz) breathing with uncontrolled tidal volume.

Data analysis. We calculated power spectra as follows. The nonequidistant R-R interval time series and arterial pressure waveforms (7) were spline interpolated (cubic), resampled at 4 Hz, and passed through a finite low-pass impulse response filter with a cut-off frequency of 0.5 Hz. Data sets comprising 64 s (256 samples), sliding every 10 s, were trend eliminated (linear regression), windowed (Hanning method), and fast-Fourier transformed. We used the periodogram method to estimate power distribution (18). Power was expressed as the area under the spectrum over the frequency range of interest. For analysis of coherence and for calculation of transfer functions, spline interpolations (cubic) of tidal volumes and R-R intervals and systolic pressures and R-R intervals were made at 4 Hz. Power spectral densities were calculated with the Welch algorithm for 7 overlapping sections of 256 points (or 64 s) staggered by 128 points. We calculated the coherence between each of the pairs of measurements by dividing the cross-spectral densities by the product of the individual power spectral densities. We calculated the transfer function by dividing the cross-spectra of the two signals by the power spectra of the input signals (3). We analyzed some responses with a damped oscillator model of the form

$$X(f) = \frac{a}{2\pi \sqrt{(2\pi)^2(f^2 - b^2)^2 + 4c^2 \ f^2}}$$

where $X(f)$ is system output, a is amplitude of driving force, b is resonance frequency, and c is damping parameter.

We integrated spectral power over the following three frequency ranges: total (0.02–0.5 Hz), low (0.02–0.12 Hz), and high (0.12–0.5 Hz). To determine how stringent tidal volume control affects spectral power, we compared integrated low, high, and total spectral powers at each breathing frequency during stepwise breathing with and without controlled tidal volume. We also compared spectral power during fixed-frequency breathing (over 5 min) with that derived during 0.25 Hz stepwise breathing with and without tidal volume control (2-min segment).

Statistical analysis. We determined that our data were distributed normally with the Kolmogorov-Smirnov test (15). Statistical comparisons among each variable at different respiratory rates and volumes were performed with repeated-measures analysis of variance (ANOVA). Significant global F ratios were examined further with Student-Newman-Keuls post hoc analysis to identify significantly different means. To determine the effects of breathing at different respiratory rates on low, high, and total spectral power, we evaluated each dependent variable using a two (group)-by-six (respiratory frequency) ANOVA with repeated measures on the respira-

tory frequency factor. Significant interactions were probed with the analysis of simple main effects. We considered differences significant at $P < 0.05$.

RESULTS

Stepwise breathing protocol: Tidal volume control. Figure 1, *A–C*, shows data obtained from one subject during stepwise frequency breathing without tidal volume control. Figure 1*A* shows that mean R-R intervals, arterial pressures, and end-tidal CO_2 levels are stable during stepwise frequency breathing and that, as expected (22), inspired tidal volume increases as breathing rate decreases. Figure 1, *B* and *C*, shows the R-R interval and systolic pressure time series depicted in Fig. 1*A* and their three-dimensional power spectra and contour plots. Both analyses show peaks of spectral power at the breathing frequencies; and, to a lesser degree, both analyses reveal underlying low (less than ~0.05 Hz)-frequency spectral power throughout much of the stepwise frequency protocol [this is more apparent with systolic pressures (Fig. 1*C*) than R-R intervals (Fig. 1*B*)].

Figure 2 shows mean inspiratory volumes (corrected for body temperature, ambient pressure, saturated) and SD of inspiratory volumes for all subjects for all frequencies of stepwise breathing. During stepwise frequency breathing, inspired tidal volumes with or without tidal volume control were comparable ($P \geq 0.05$) to targeted tidal volumes (Fig. 2*A*). However, as Fig. 2*B* shows, tidal volumes were more constant when subjects breathed at slow breathing rates with than without tidal volume control ($P < 0.05$).

Figure 3 shows average end-tidal CO_2 levels during stepwise breathing with and without tidal volume control. During the initial 5-min period of uncontrolled breathing (not shown), end-tidal CO_2 concentrations averaged $5.25 \pm 0.14\%$. Average end-tidal CO_2 levels during stepwise frequency breathing, with or without tidal volume control, were comparable ($P \geq 0.05$) to the baseline level during uncontrolled breathing.

Figure 4 shows average R-R intervals during stepwise breathing, with and without tidal volume control. During the initial 5-min period of uncontrolled breathing (not shown), R-R intervals averaged 0.95 ± 0.04 s. Average R-R intervals during stepwise frequency breathing, with or without tidal volume control, were comparable ($P \geq 0.05$) to the baseline level during uncontrolled breathing.

Figure 5 depicts mean total R-R interval spectral power (0.02–0.5 Hz) during stepwise breathing with and without tidal volume control. Total spectral power was significantly ($P < 0.05$) greater at 0.1 Hz than at more rapid breathing rates, during both uncontrolled and controlled tidal volume protocols (see Table 1). Stringent control of inspired tidal volume yielded lower total R-R interval spectral power at 0.1 Hz during stepwise breathing ($P < 0.05$, uncontrolled vs. controlled tidal volume). Figure 6 shows that total systolic pressure spectral power was significantly higher ($P < 0.05$) at 0.1 Hz than at more rapid breathing rates, during both uncontrolled and controlled tidal volume

protocols (see Table 2). Control of inspired volume, however, had no effect ($P \geq 0.05$) on total systolic pressure power at any breathing frequency.

Stepwise vs. other breathing protocols. Figure 7 shows mean end-tidal CO_2 concentrations measured during each of the five breathing protocols. Uncontrolled breathing and stepwise breathing with or without tidal volume control yielded similar ($P \geq 0.05$) end-tidal CO_2 concentrations. Random (or white noise)- and fixed-frequency (0.25 Hz) breathing yielded significantly ($P < 0.05$) lower end-tidal CO_2 concentrations than uncontrolled breathing.

Total R-R interval spectral power was comparable when the 2-min segments of stepwise breathing (0.25 Hz), with or without tidal volume control, were compared with the 5-min segment of fixed-frequency breathing (see Table 1). Mean total R-R interval spectral power for all subjects was 0.0018 ± 0.0004 during stepwise breathing without tidal volume control, 0.0020 ± 0.0006 during stepwise breathing with tidal volume control, and 0.0028 ± 0.0004 s² during fixed-frequency breathing ($P \geq 0.05$).

Figure 8 shows mean total R-R interval spectral power derived at each breathing frequency during the stepwise protocol with and without tidal volume control and during fixed frequency, random frequency, and normal breathing. Average R-R interval spectral power and systolic pressure spectral power are also given in Tables 1 and 2. During normal, stepwise, and fixed-frequency breathing, spectral power peaks were present in both respiratory and low-frequency bands, except during 0.15, 0.1, and 0.05 Hz breathing, when the respiratory and low-frequency oscillations coincided. Breathing at higher frequencies (>0.15 Hz) did not affect low-frequency (0.02–0.12 Hz) oscillations during stepwise breathing with or without tidal volume control ($P \leq 0.05$), as may be seen more clearly from the data presented in Table 1. Similar responses were noted for systolic arterial pressure power, as shown in Table 2.

Figure 9, *A* and *B*, shows mean (solid line $\pm$ SE) cross-spectral analyses of the relation between respiration and R-R intervals and systolic pressure and R-R intervals during random-frequency breathing. Figure 9, *A* and *B*, shows total (0.02–0.5 Hz) integrated measurements obtained at each breathing frequency during the stepwise protocol with and without tidal volume control. All three breathing protocols yielded a significant relationship (≥ 0.5 coherence) between respiration and R-R intervals over most of the frequency ranges. Coherence was higher during both stepwise protocols compared with random breathing at every breathing frequency. Phase relations and transfer magnitudes were similar for the stepwise frequency and white noise breathing protocols.

Figure 9*B* shows data for systolic pressure and R-R intervals. Coherence was again higher during stepwise than random breathing. Phase and transfer function analyses for the three protocols were similar. Because of the short data segments we evaluated, we questioned whether our coherence estimates might be biased,
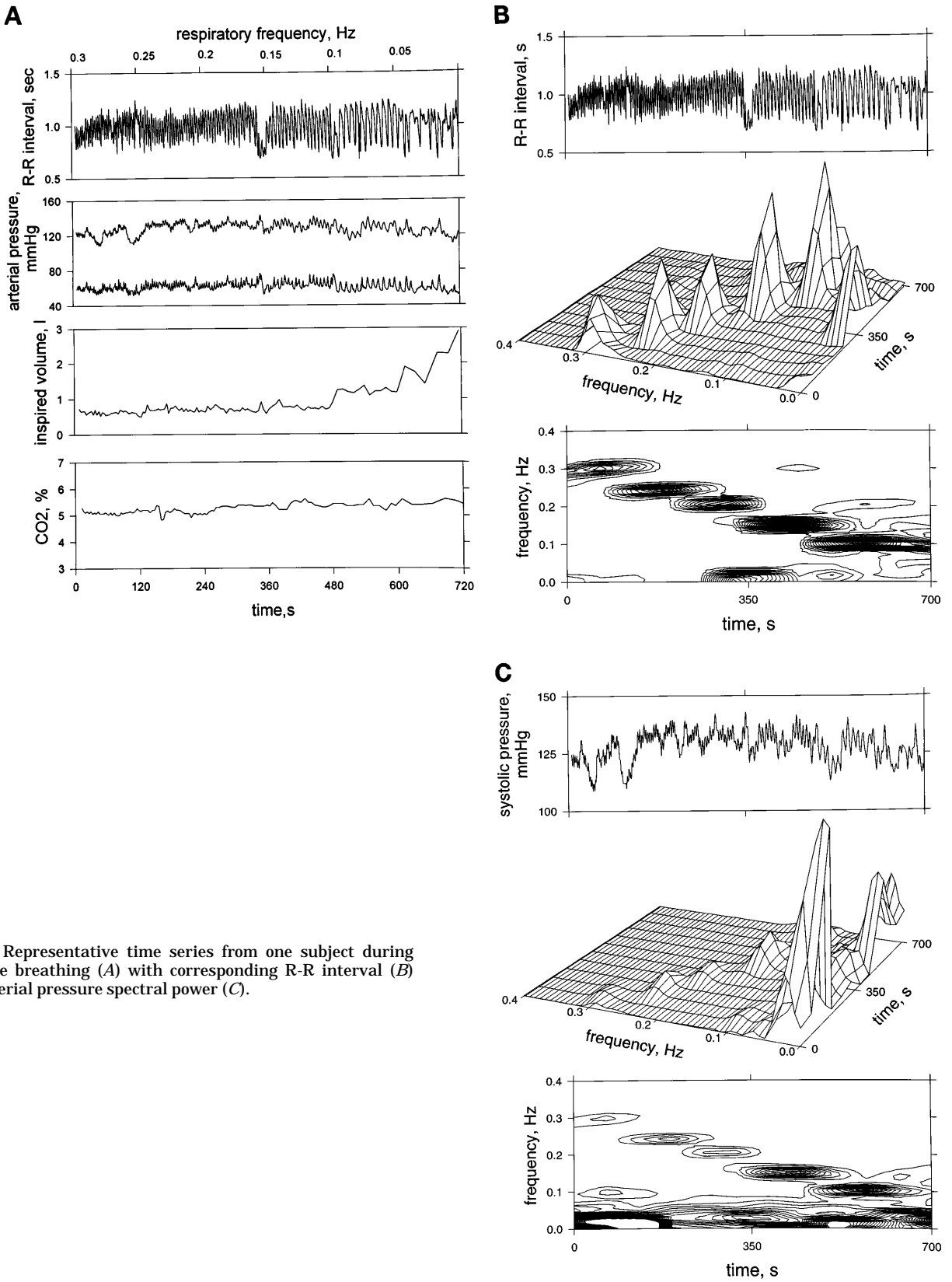
Fig. 1. Representative time series from one subject during stepwise breathing (*A*) with corresponding R-R interval (*B*) and arterial pressure spectral power (*C*).
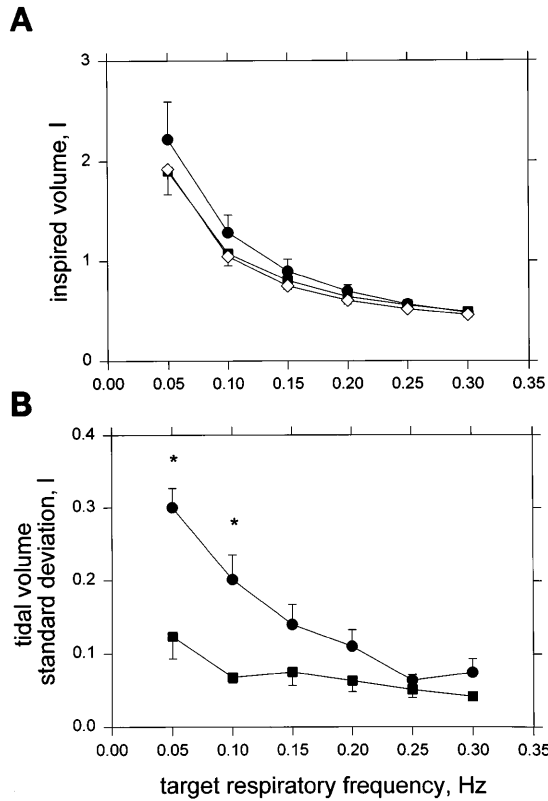
A

B

Fig. 2. Inspired volumes shown for all subjects during stepwise breathing with (■) and without (●) tidal volume control. Calculated inspired volumes are plotted for comparison (◇). Values are means ± SE; $n = 10$. *Significantly different between protocols, $P < 0.05$.

especially at 0.05 Hz breathing. We present average data for all subjects, aligned on the first inspiration with corresponding changes in R-R intervals in Fig. 10. Figure 10 shows that subjects were able to follow the breathing cues extremely well at 0.05 Hz and that R-R interval responses were remarkably consistent between subjects.
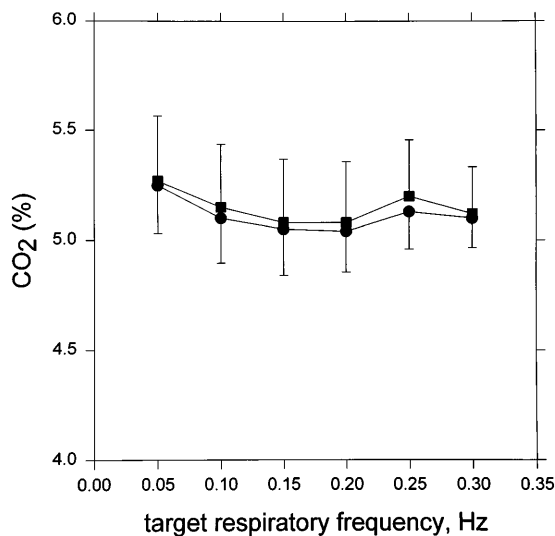

Fig. 3. Mean end-tidal CO_2 concentrations for all subjects during stepwise breathing with (■) and without (●) tidal volume control.
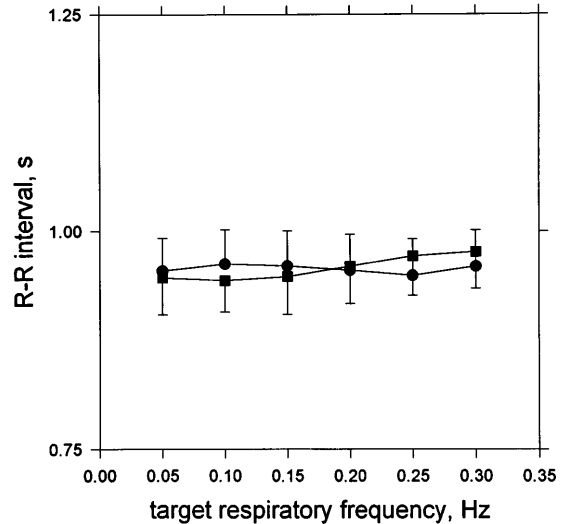

Fig. 4. Mean R-R intervals for all subjects during stepwise breathing with (■) and without (●) tidal volume control.

Figure 11 shows total R-R interval spectral power (0.02–0.5 Hz), integrated at each breathing frequency, during stepwise frequency breathing without tidal volume control (Fig. 11A) and white noise breathing (Fig. 11B). The solid line in each panel indicates the results of modeling of the data as a damped oscillator (see METHODS). The high correlation coefficients obtained indicate that the damped oscillator model fits data obtained with both methods extremely well.

DISCUSSION

We measured cardiovascular rhythms during the following five types of breathing: uncontrolled breathing; "stepwise" frequency breathing, with and without rigorous control of inspired tidal volume; random-frequency breathing; and fixed-frequency breathing. Our protocol had two complementary objectives. First, we evaluated stepwise frequency breathing and ad-
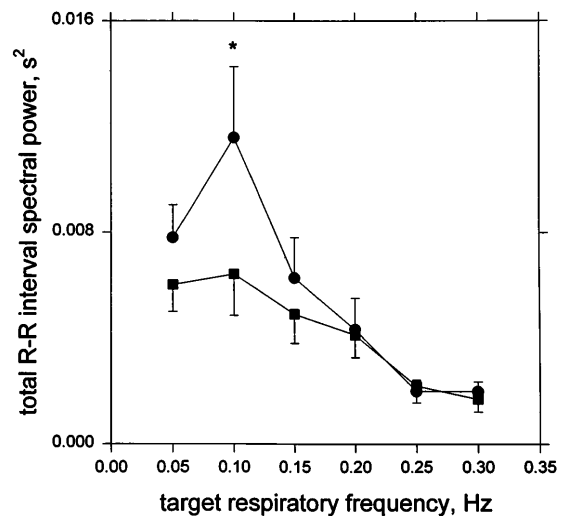

Fig. 5. Total R-R interval spectral power for all subjects during stepwise frequency breathing with (■) and without (●) tidal volume control. *Significantly different between protocols, $P < 0.05$.
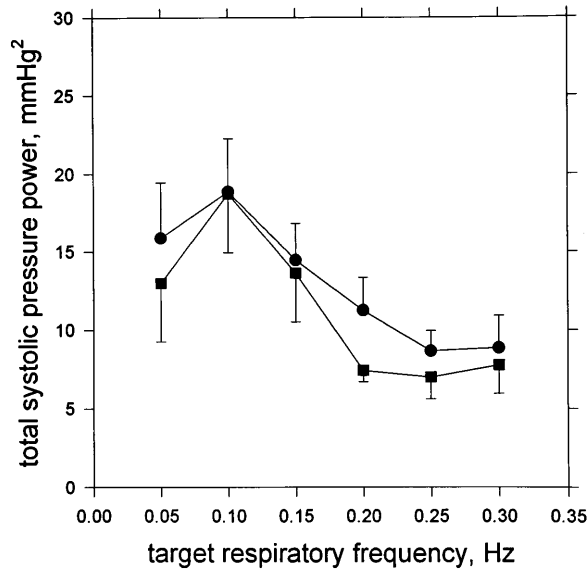
Fig. 6. Total systolic pressure spectral power for all subjects during stepwise breathing with (■) and without (●) tidal volume control.

Fig. 8. Total R-R interval spectral power for all protocols and subjects. Spectral power at each breathing frequency during stepwise frequency breathing without (solid line) and with (dashed line) tidal volume control.

dressed one question explicitly. Is control of inspired tidal volume, adjusted for changes in respiratory frequency, necessary to properly assess frequency domain analyses of cardiovascular dynamics? Second, we studied the impact of voluntary breathing control on measured cardiovascular rhythms. Our study provides new quantitative information on stepwise frequency breathing and indicates that, with exceptions, cardiovascular rhythms are comparable with and without tidal volume control. Furthermore, although autonomic cardiovascular rhythms measured during different breathing protocols have much in common, there are some important differences, as follows: strict tidal volume control decreases R-R interval oscillations during 0.1 Hz breath-
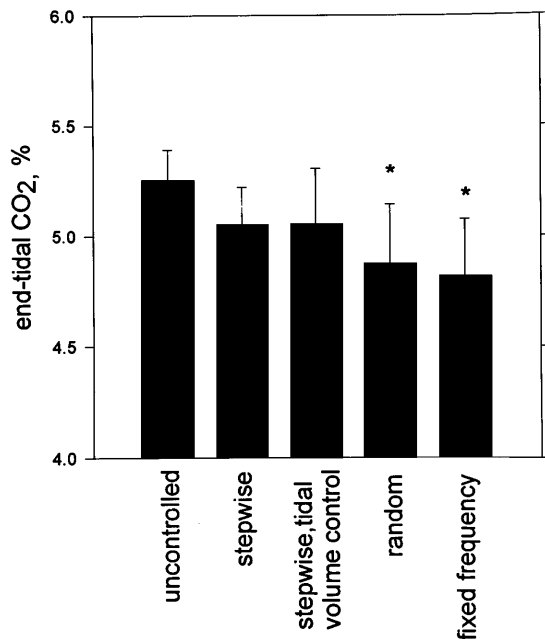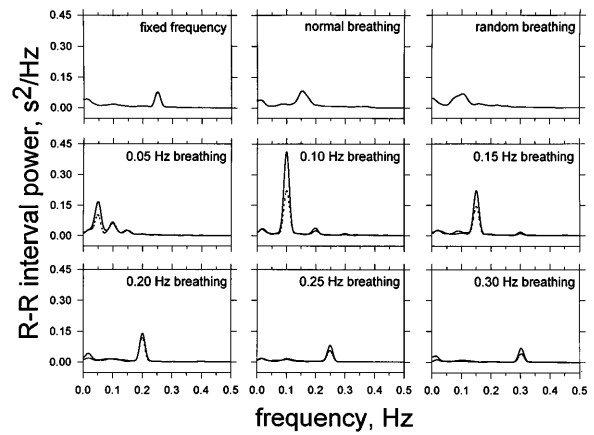
ing, and random- and fixed-frequency breathing modestly reduce end-tidal CO_2 concentrations. Similar transfer functions between respiration and R-R interval and arterial pressure and R-R interval are achievable with both stepwise and random protocols, but only stepwise and fixed-frequency breathing allow for the clear separation between low- and high-frequency oscillations when subjects breathe at frequencies greater than ~0.15 Hz.

Stepwise frequency breathing: technical considerations. Ours is by no means the first study of the influence of breathing frequency on cardiovascular rhythms. We (8) and others (2, 5, 12) documented the dependence of respiratory peak minus valley R-R interval fluctuations on breathing rate when subjects breathe at discrete breathing frequencies. We (4) and others (2, 23) also studied the effects of breathing at discrete

Fig. 7. End-tidal CO_2 for all protocols and subjects. *Significantly different from uncontrolled, normal breathing ($P < 0.05$).

Table 1. *R-R interval spectral power*

Protocol	Low Frequency (0.02–0.12 Hz)	High Frequency (0.12–0.5 Hz)	Total (0.02–0.05 Hz)
NB	0.0016 ± 0.0003	0.0031 ± 0.0009	0.0048 ± 0.0015
FF	0.0012 ± 0.0002	0.0016 ± 0.0004	0.0028 ± 0.0004
RB	0.0031 ± 0.0005	0.0014 ± 0.0003	0.0045 ± 0.0008
SW-U frequency, Hz			
0.30	0.0009 ± 0.0002^a	0.0011 ± 0.0001	0.0021 ± 0.0003^a
0.25	0.0006 ± 0.0001^a	0.0013 ± 0.0004	0.0018 ± 0.0004^a
0.20	$0.0016 \pm 0.0009^{a,c}$	0.0027 ± 0.0006	$0.0043 \pm 0.0011^{a,b}$
0.15	0.0021 ± 0.0005^c	0.0042 ± 0.0011	0.0062 ± 0.0015^b
0.10	0.0103 ± 0.0035^b	0.0013 ± 0.0003	$0.0116 \pm 0.0035^{c*}$
0.05	0.0064 ± 0.0011^c	0.0013 ± 0.0003	0.0078 ± 0.0111^b
SW-C frequency, Hz			
0.30	0.0008 ± 0.0002^a	0.0009 ± 0.0002	0.0017 ± 0.0004^a
0.25	0.0001 ± 0.0003^a	0.0012 ± 0.0003	0.0020 ± 0.0006^a
0.20	0.0017 ± 0.0005^a	0.0023 ± 0.0006	$0.0041 \pm 0.0008^{a,b}$
0.15	0.0019 ± 0.0008^b	0.0029 ± 0.0008	0.0049 ± 0.0011^b
0.10	0.0051 ± 0.0011^b	0.0012 ± 0.0003	$0.0064 \pm 0.0015^{b*}$
0.05	0.0047 ± 0.0009^b	0.0012 ± 0.0003	0.0061 ± 0.0011^b

Values are means $\pm$ SE. Units are s^2. NB, normal breathing; FF, fixed-frequency breathing; RB, random breathing; SW-U, stepwise with uncontrolled tidal volume; SW-C, stepwise with controlled tidal volume. For each protocol, values within a column without a letter or that share the same letter are not significantly different. *Significantly different between stepwise protocols.

Table 2. *Systolic arterial pressure spectral power*

Protocol	Low Frequency (0.02–0.12 Hz)	High Frequency (0.12–0.5 Hz)	Total (0.02–0.05 Hz)
NB	10.86 ± 1.5	5.23 ± 1.78	16.11 ± 2.10
FF	8.86 ± 2.36	2.44 ± 1.02	11.31 ± 2.42
RB	12.85 ± 2.21	2.01 ± 1.01	14.86 ± 2.12
SW-U frequency, Hz			
0.30	7.53 ± 1.96^a	1.31 ± 0.21^a	8.85 ± 2.04^a
0.25	6.08 ± 1.37^a	2.56 ± 0.39^a	8.65 ± 2.67^a
0.20	7.52 ± 2.06^a	3.37 ± 0.51^a	11.22 ± 2.12^{ab}
0.15	8.62 ± 1.85^a	6.12 ± 1.08^b	14.45 ± 2.35^b
0.10	17.14 ± 3.71^b	1.30 ± 0.48^a	18.87 ± 3.78^b
0.05	14.02 ± 3.59^b	1.29 ± 0.33^a	15.32 ± 3.59^b
SW-C frequency, Hz			
0.30	6.23 ± 1.6^a	1.51 ± 0.41^a	7.75 ± 1.82^a
0.25	5.51 ± 1.38^a	1.45 ± 0.27^a	6.97 ± 1.38^a
0.20	4.83 ± 0.58^a	2.57 ± 0.41^a	7.42 ± 0.72^{ab}
0.15	8.44 ± 3.23^a	5.16 ± 0.81^b	13.62 ± 3.13^b
0.10	17.99 ± 3.92^b	1.02 ± 0.13^a	18.72 ± 3.78^b
0.05	16.57 ± 8.64^b	1.41 ± 0.35^a	12.98 ± 3.72^b

Values are means $\pm$ SE. Units are $mmHg^2$. For each protocol, values within a column without a letter or that share the same letter are not significantly different.

frequencies on R-R interval spectral power. Novak et al. (17) and Akselrod (1) studied the effects of ramped breathing protocols on R-R interval fluctuations (registered as Wigner distributions and spectral power). All of these studies indicate that the magnitude of respiration-related R-R interval fluctuations, however measured, is critically dependent on breathing rate. A corollary of this evidence is that respiration must be taken into account (or at least measured and factored in) if sense is to be made of short-term human cardiovascular rhythms.

We made several observations on the effects of stepwise frequency breathing on cardiovascular rhythms. It is not necessary for subjects to control their tidal volumes voluntarily during stepwise frequency breathing; they automatically (presumably with the aid of chemoreceptors) adjust their tidal volumes (Fig. 2A) and maintain normal end-tidal CO_2 levels (Figs. 3 and 7). This conclusion merely documents in a new way the well-known inverse relation between breathing frequency and tidal volume (22). However, variability (SD) of tidal volumes is greater at 0.05 and 0.1 Hz during stepwise frequency breathing when subjects are permitted to vary their tidal volumes according to chemoreceptor inputs than when target tidal volumes are provided to maintain constant alveolar ventilation (Fig. 2B). Of interest, greater variability of tidal volumes and R-R intervals without than with tidal volume control does not translate into greater variability of arterial pressure (Fig. 6). We did find, however, [as did Elghozi et al. (10)], that total systolic pressure power is greater at slower than faster breathing frequencies (Fig. 6).

The stepwise frequency breathing algorithm that we used does not significantly affect mean R-R intervals (Fig. 4). The lack of changes of R-R intervals supports several inferences regarding modulation of human cardiovascular rhythms. Because, during short-term recordings, R-R intervals are linear functions of vagal-cardiac nerve traffic (13), the constancy of R-R intervals
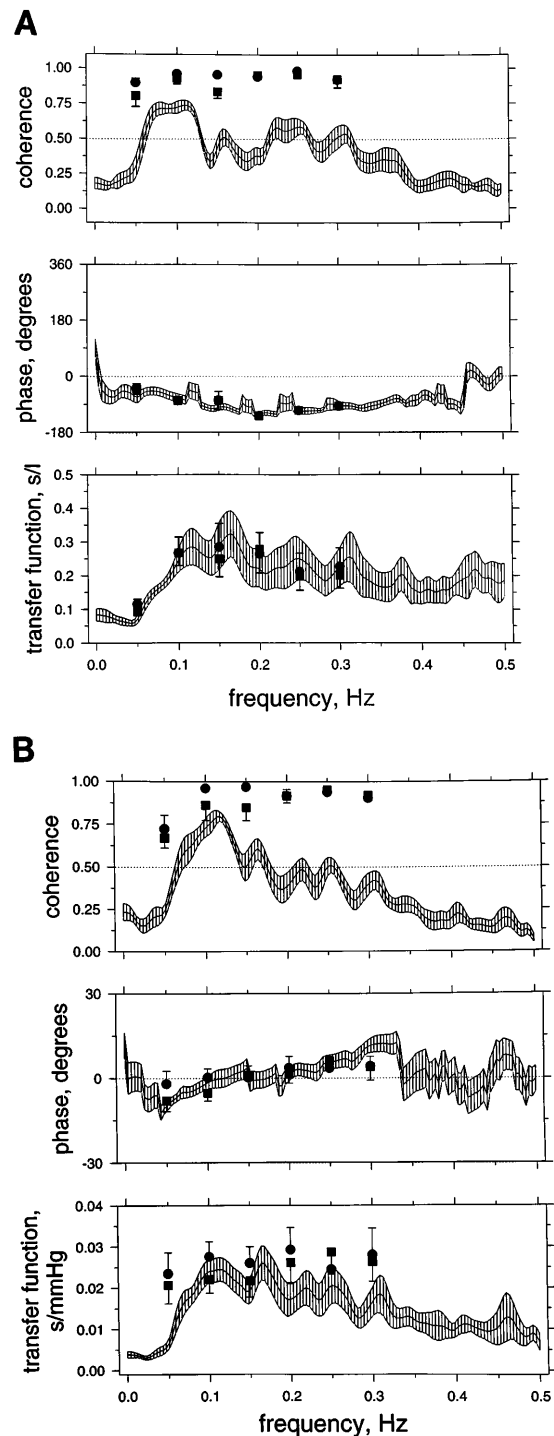

Fig. 9. Associations between respiration and R-R interval (A) and arterial pressure and R-R interval (B) with mean coherence, transfer magnitude, and phase. In A and B, total integrated spectral power for each 2-min section of stepwise breathing is plotted at its frequency on the transfer function derived during 6 min random-frequency breathing (solid line $\pm$ SE, indicated by the shaded area).

suggests that changes of breathing frequency do not influence the absolute level of vagal-cardiac nerve traffic (assuming sympathetic activity does not change reciprocally, an assumption that has not been tested during stepwise breathing). Thus these data challenge the notion (16, 19) that voluntary control of breathing is
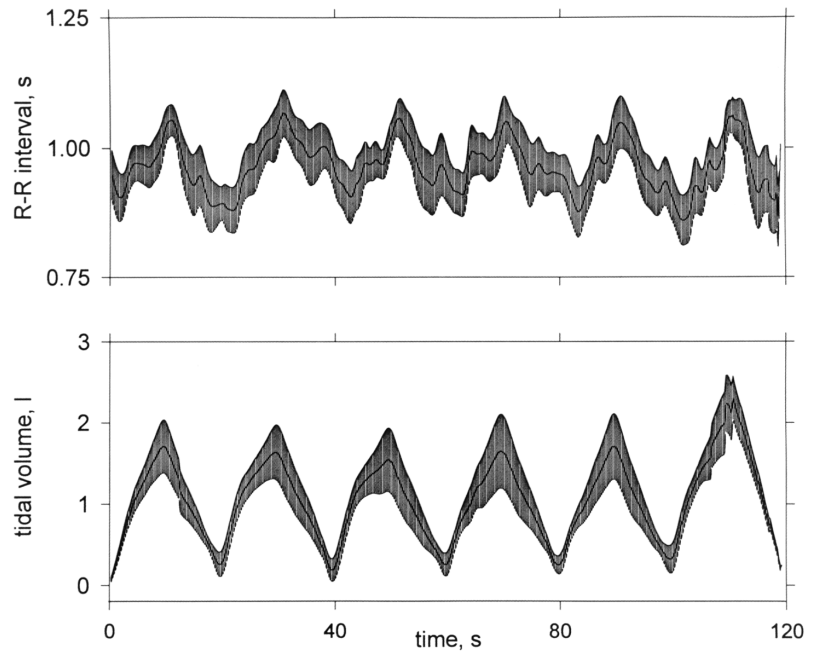
Fig. 10. Transfer of respiration to R-R interval. Average responses (solid line) with SE (shaded area) above (solid line) and below (broken line) the mean for all subjects during 0.05-Hz breathing.

a "vagal maneuver," which increases the level of vagal-cardiac nerve traffic.

The constancy of R-R intervals at each breathing frequency with and without tidal volume control (Fig.

Fig. 11. Total R-R interval spectral power plotted with a damped oscillator model prediction. *A*: mean total spectral power for each respiratory frequency during stepwise frequency breathing without tidal volume control (●), plotted with the model prediction (solid line). *B*: mean total spectral power during random-frequency breathing (dashed line), plotted with the model prediction (solid line).

4) suggests further that the mental alertness required for subjects to match their actual to targeted tidal volumes does not reduce relative vagal-cardiac nerve fluctuations. Published literature is divided sharply on the question of whether voluntary control of breathing affects the cardiovascular rhythms being measured. Hirsch and Bishop (12) measured peak minus valley R-R interval oscillations during spontaneous and controlled breathing and found that R-R interval fluctuations during spontaneous breathing are comparable to fluctuations occurring during controlled-frequency breathing. In a less rigorous analysis, Eckberg and co-workers (9) showed that changes of heart period and muscle sympathetic nerve activity are superimposable during uncontrolled breathing, frequency-controlled breathing, and frequency- and tidal volume-controlled breathing.

Two recent studies by Patwardhan et al. (20, 21) support opposite conclusions. One (20) shows that voluntary control of breathing does not affect, and the other [based on a different experimental algorithm (21)] shows that voluntary control of breathing reduces, R-R interval spectral power. If, as likely, control of breathing requires more mental effort than spontaneous breathing, control of breathing should affect autonomic activity. This assertion is supported by the study of Wallin and colleagues (24), which showed that mental stress increases arterial pressure, heart rate, muscle sympathetic nerve activity, and cardiac norepinephrine spillover. We conclude, however, based on our results and the highly contradictory literature cited above, that the influence of voluntary control of breathing on human autonomic activity is probably small.

We cannot explain the peculiar reduction of R-R interval spectral power that occurred at 0.1 Hz during stepwise frequency breathing with tidal volume control (Fig. 5). We considered and excluded several possibili-
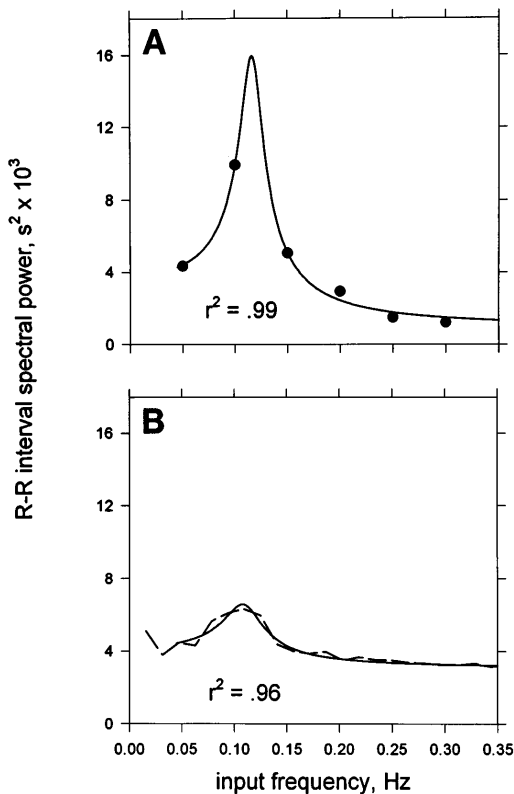
ties. Reduced R-R interval spectral power at 0.1 Hz was not due to reduced arterial pressure variability, which was similar with and without tidal volume control (Fig. 6). It probably was not due to reduced tidal volume variability, because tidal volume variability was also less for stepwise breathing with tidal volume control when the two ramped protocols were compared at 0.05 Hz (Fig. 2B), but R-R interval spectral power at 0.05 Hz was not different (Fig. 5). Similarly, it probably was not due to the mental stress associated with slow breathing. Such stress might have been expected to result in an increase in sympathetic activity (24), which likely would have been greater or at least similar during 0.1 Hz breathing with (as subjects are concentrating on matching inspired volumes to visual targets) compared to without tidal volume control, and yet R-R interval spectral power was less during controlled tidal volume breathing. Erratic changes (or lack thereof) in R-R interval consequent to extremely large breaths necessary at 0.05-Hz breathing might provide clues into potential contributions from modifications of central command. However, subjects were able to track the breathing cues easily at 0.05 Hz, and R-R interval responses to such breathing cues were consistent (see Fig. 10).

Total R-R interval (Fig. 5) and systolic pressure spectral power (Fig. 6) were less at the lowest breathing frequency used (0.05 Hz) than at 0.1 Hz. Transfer function analysis (Fig. 9A) and damped oscillator modeling (Fig. 11) also documented reductions in the low breathing frequency range and showed that they are present during white noise, as well as stepwise frequency breathing. Although it is impossible to directly address this observation with our current data, we speculate that efferent sympathetic traffic is measurably higher during 0.1 Hz compared with 0.05-Hz breathing.

Comparisons among different breathing protocols. A major conclusion from our study is that the several breathing protocols that we evaluated yield similar information regarding cardiovascular rhythms. We identified an exception to this conclusion, however. Random- and fixed-frequency breathing provoked statistically significant reductions of end-tidal CO_2 levels compared with uncontrolled breathing (Fig. 7). Unpublished data (R. A. Henry, I.-L. Lu, L. A. Beightol, and D. L. Eckberg) suggest that the reductions we observed (from end-tidal CO_2 levels slightly above to slightly below 5%) have negligible effects on R-R interval spectral power. Nevertheless, it is interesting that decreases in end-tidal CO_2 were recorded during two but not during all breathing protocols. Because the visual display was identical for all protocols, it is possible that some aspect of the protocol itself, such as dramatic breath-by-breath changes in tidal volume and frequency during random breathing or the lack of an equilibration period before the beginning of fixed-frequency breathing (causing subjects to overestimate the required inspired volume), contributed to the mild hyperventilation observed during these two protocols.

Berger and co-workers (3) developed an elegant method to characterize cardiovascular rhythms; they asked volunteers to breathe at a wide range of physiologically relevant frequencies, according to computer-generated random frequency, or "whitened" cues. Random-frequency breathing was conceived as an improvement over the stepwise frequency protocols used previously (which employed different, discrete respiratory rates) primarily because of the long duration of studies necessary to evaluate all physiologically relevant discrete frequencies and the possibility that slow deep breaths might produce hypercapnia (3). Our stepwise frequency breathing algorithm may dispel both concerns, since the entire protocol requires only 12 min and does not change end-tidal CO_2 levels significantly. Our comparison of stepwise frequency and random breathing methods suggests [as did the study by Berger et al. (3)] that the two protocols yield nearly identical results, with two important exceptions: stepwise frequency breathing does not significantly reduce end-tidal CO_2 and allows for a clear separation between respiratory and low-frequency cardiovascular oscillations when subjects breathe at higher frequencies. It should be noted that, although we confirm the findings of Berger et al. (3) that similar transfer functions are achievable with fixed- and random-frequency protocols (Fig. 9), we concede that using 64-s data sets during stepwise frequency breathing (resulting in <2 independent averages/stage) may have limited the reliability of our coherence estimates.

Interactions between respiratory and low-frequency cardiovascular oscillations. Both stepwise and random-breathing protocols provide information on how changes of inputs translate (or "transfer") into changes of outputs. The primary input with both methods is respiration (presumably, arterial pressure changes, which also can be treated as inputs, are secondary to respiratory changes). Thus both methods characterize system responses. Stepwise and fixed-frequency breathing also allow for the analysis of naturally occurring rhythms that exist at rates different from respiration. For example, during relatively rapid breathing, separation between respiratory and lower-frequency rhythms is complete (Fig. 1, B and C, bottom). This separation of rhythms is apparent during stepwise breathing at 0.2, 0.25, and 0.3 Hz breathing, allowing us to suggest that, contrary to research published earlier (17), breathing at frequencies substantially >0.1 Hz does not influence low-frequency (0.02–0.12 Hz) R-R interval or arterial pressure rhythms.

As mentioned, Pagani et al. (19) and Malliani et al. (16) suggested that breathing control might be used to alter neural outflow (16, 19), and they proposed that, when subjects attempt to regulate their breathing, they increase their vagal-cardiac nerve traffic. Although the constant R-R intervals over all of the breathing frequencies that we studied with our stepwise frequency breathing protocol (Fig. 4) make it highly unlikely that vagal-cardiac nerve activity changed, we do not rule out the possibility that the use of different controlled breathing algorithms might help to answer fundamen-

tal questions regarding the organization of human autonomic rhythms. It is beyond the scope of the present study to probe this possibility. However, we suggest that use of a stepwise frequency breathing protocol (similar, but probably not identical, to the one we report here) might inform mechanisms underlying questions such as: Does slow breathing increase the strength of muscle sympathetic bursts? When breathing frequency approaches the frequency of slower R-R interval, arterial pressure, and muscle sympathetic nerve rhythms, does breathing entrain those rhythms or pull their frequencies toward that of respiration? If it does, does this mean that naturally occurring, lower-frequency rhythms arise from rhythm generators located in the medulla? [The answer to this question might be obtained by contrasting responses of healthy subjects with those of tetraplegic patients, who, although they have low-frequency R-R interval and arterial pressure rhythms (11, 14), lack connections between the medulla and spinal cord, where preganglionic sympathetic motoneurons are located.]

In conclusion, we studied the effects of controlled breathing protocols on cardiovascular rhythms. We found that, during stepwise frequency breathing, total R-R interval and arterial pressure spectral power at the breathing frequency tend to increase as breathing frequency decreases. Additionally, control of tidal volume reduces R-R interval spectral power during 0.1 Hz breathing through unknown mechanisms. Comparisons among different breathing protocols indicate that random- and fixed-frequency breathing reduce end-tidal CO_2 modestly but significantly compared with uncontrolled breathing. We conclude that, although autonomic rhythms measured during different breathing protocols have much in common, there are some statistically significant differences. We suggest that, for short-term recordings, breathing protocols incorporating stepwise changes in frequency without stringent control of inspired volume may allow for the most efficient assessment of autonomic cardiovascular rhythms.

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