A Standardization Method for Respiratory Sinus Arrhythmia at Supine Rest as an Index of Cardiac Parasympathetic Activity Using Breathing Frequency

Takayuki KAGEYAMA, Hideki IMAI and Michinori KABUTO

Urban Environment and Health Project, National Institute for Environmental Studies

Abstract: A Standardization Method for Respiratory Sinus Arrhythmia at Supine Rest as an Index of Cardiac Parasympathetic Activity Using Breathing Frequency: Takayuki KAGEYAMA, *et al.* Urban Environment and Health Project, National Institute for Environmental Studies—Two spectral components of heart rate variability, respiratory sinus arrhythmia (RSA) and Mayer wave-related sinus arrhythmia (MWSA) provide noninvasive indices of cardiac vagal activity and systemic sympathetic activity with vagal modulation, respectively. But the basic problem that breathing frequency (BF) appears to modify RSA without any change in the tonic level of vagal activity remains, indicating the need for respiratory standardization. In the present study, RSA amplitude at supine rest was examined as a function of BF intentionally maintained constant (0.18–0.33 Hz) for 11 nonsmokers (5 males and 6 females aged 19–28) with consideration of time after meals, which may affect RSA. The amplitude calculated by autoregressive spectral analysis linearly decreased with the increase in BF on a log-log scale. This held in the case of RSA for nonpaced regular breathing with the mean BF corresponding to the center frequency of RSA. The amplitude during nonpaced regular breathing could therefore be statistically standardized to that at BF of, e.g., 0.25 Hz, which agreed well with that at the paced BF of 0.25 Hz ($r=0.958$). This suggests a simple, efficient method for the reliable assessment of vagal activity based on RSA amplitude and of sympathetic activity indicated by the ratio of MWSA amplitude to RSA amplitude. (*J Occup Health* 1996; 38: 20–24)

Key words: Heart rate variability, Respiratory sinus arrhythmia, Mayer wave-related sinus arrhythmia, Parasympathetic activity, Sympathetic activity, Eating, Breathing frequency, Paced breathing; Standardization

Received May 15, 1995; Accepted July 18, 1995

Correspondence to: T. Kageyama, Urban Environment and Health Project, Onogawa 16-2, Tsukuba, Ibaraki 305, Japan

Heart rate variability includes two major spectral components, respiratory sinus arrhythmia (RSA) and Mayer wave-related sinus arrhythmia (MWSA). Their amplitudes are respectively proposed as effective noninvasive indices of cardiac vagal activity and systemic sympathetic activity with vagal modulation^{1–5}. The amplitudes obtained at supine rest are expected to be useful in neurological assessment of possible effects caused by exposure to neurotoxins or stressors in the fields of occupational and environmental health^{6–11} as well as other clinical tests^{12–15}. But there remains the basic problem that RSA amplitude appears to be modified by breathing frequency (BF) without any change in the heart rate or tonic level of parasympathetic activity, indicating a need for respiratory standardization for reliable assessment^{4, 16–20}. In the present study, RSA amplitude at supine rest was examined as a function of BF intentionally maintained at various frequencies (0.18–0.33 Hz) with a pacemaker, in order to develop a simple standardization method, taking time after meals, which may influence RSA and MWSA, into consideration.

According to a previous study²¹, supine RSA amplitude at 30 min after meals as well as supine MWSA amplitude at 90 min after meals are affected by the meal intake, while those at 150 min after meals are not. Experiment I was therefore performed to determine more precisely the time points after a meal at which the amplitudes of RSA and MWSA are no longer affected by the meal intake. This is important in an experimental situation such as the following one, as well as in, e.g., routine physical examination at a work site.

Many studies have shown that slow and/or deep breathing increases RSA amplitude^{4, 16–20} without any change in the tonic level of cardiac vagal activity^{18–20}. The effect can be avoided by main-

taining BF constant with a pacemaker^{4, 5, 12, 14, 18}. This method simultaneously controls the tidal volume, because, in general, the tidal volume for resting subjects who were instructed to maintain BF constant depends on BF²⁰, but this is not easy for inexperienced subjects in a routine physical examination. Another way to exclude the effect is statistically standardizing the amplitude of RSA as that at a certain standard BF based on the actual BF^{16, 18, 19} which is usually measured with a pneumogram (PMG). But the mean BF during nonpaced respiration is known to correspond to the center frequency of RSA at rest^{1 4, 16 20}, which can be obtained by autoregressive spectral analysis followed by component wave analysis of heart rate variability^{3-10, 20-23}. We therefore intended to simplify the latter method in Experiment II, using the BF calculated in the analysis, with consideration of time after meals.

Subjects and Methods

The subjects were 11 healthy nonsmokers, 5 males and 6 females, who gave written informed consent to participate in the following experiments. The mean $\pm$ S.D. of their ages was 22.0 ± 3.3 (range 19–28), and that of their body mass indices (BMI, kg/m^2) was 20.5 ± 1.7 . The experiments were conducted in a sound-insulated and electrically shielded room (temperature 24°C , relative humidity 50%, and background sound level 30 dBA).

Experiment I: The subjects started eating the standard lunch of 673 kcal at 12:00, and finished it within 20–25 min. Their electrocardiograms (ECG, standard lead I) were recorded in the supine position immediately before the meal as well as 0, 30, 60, 90, and 120 min after finishing the meal, for 3 min each, during quiet breathing at a frequency set by 0.25 Hz metronome signals. They spent their time sitting and reading or relaxing in a study suite between the data collections. A similar experiment was conducted once on another day.

Successive R-R intervals (about 150–240 beats) including no extrasystole or abnormal arrhythmia were measured on the ECG with a tachograph (CERX Co., Ltd., TM55). Autoregressive spectral analysis and component wave analysis^{3 12, 14} were applied to the data to calculate power density and center frequencies of MWSA and RSA, by means of the “Autoregressive Component Wave Analysis Program” (Bio-Information Lab. Co., Ltd.). Amplitudes of MWSA and RSA were expressed as a coefficient of component variation for MWSA ($\text{C-CV}_{\text{MWSA}}$, %) and that for RSA (C-CV_{RSA} , %), according to the following formula^{4-12, 14}: $\text{C-CV}_k = 100 \cdot P_k^{1/2} / \text{RR}$, where $k = \text{MWSA or RSA}$, $P_k =$

power density of k component (msec^2), and $\text{RR} =$ mean of R-R intervals (msec). Difference between RR , C-CV_{RSA} , $\text{C-CV}_{\text{MWSA}}$, and $\text{C-CV}_{\text{MWSA}}/\text{C-CV}_{\text{RSA}}$ before and after meals were examined by 3-way ANOVA (subjects $\times$ days $\times$ time), and, in the case that the effect of time was significant, Bonferroni multiple comparison was applied to examine the difference from premeal values.

Experiment II: Following a rest period of at least 120 min after a standard breakfast or lunch, the subjects lay quietly for 10 min, after which their ECG were recorded 4 times, for 3 min each, with quiet breathing at a frequency set by 0.18, 0.20, 0.25, or 0.33 Hz metronome signals (paced breathing, PB), but the order of BFs was randomized among the subjects. This experiment was repeated once on another day, but following the 4th session, ECGs were recorded for 3 more min with the subjects in the same position and instructed to breathe regularly without taking deep breaths, and without using metronome signals (nonpaced breathing, NPB).

The data were analyzed by the procedure described for Experiment I. The effects of BF on RR , C-CV_{RSA} , $\text{C-CV}_{\text{MWSA}}$, and f_{MWSA} for PB were examined by 3-way ANOVA (subjects $\times$ days $\times$ BF). Values other than C-CV_{RSA} were pooled for comparison with those for NPB by 2-way ANOVA (subjects $\times$ PB/NPB). Finally C-CV_{RSA} were expressed as percentages of those at the BF of 0.25 Hz for the same subject on the same day and plotted vs f_{RSA} on a log-log scale, according to the method used in previous studies^{16, 17}. Regression analysis was applied to f_{RSA} and the relative C-CV_{RSA} values for PB, and then, whether the relationships fit the data for NPB was also examined.

Results

Experiment I: Premeal parameters are summarized in Table 1. Fig. 1 shows their % of the premeal values. Significant decreases in RR and C-CV_{RSA} were observed immediately after finishing the meal (0 min), while $\text{C-CV}_{\text{MWSA}}$ was increased significantly at 30–90 min after the meal, and consequently, $\text{C-CV}_{\text{MWSA}}/\text{C-CV}_{\text{RSA}}$ was increased significantly at 0–90 min after the meal. All of these

Table 1. Premeal parameters

Parameters	Mean (S.E.)
RR (msec)	1025.6 (30.6)
C-CV_{RSA} (%)	3.4 (0.3)
$\text{C-CV}_{\text{MWSA}}$ (%)	1.6 (0.1)
$\text{C-CV}_{\text{MWSA}}/\text{C-CV}_{\text{RSA}}$	0.47 (0.07)

$n = 22$ (11 subjects $\times$ 2 days).

Fig. 1. Change in heart rate variability after meals. Dots and bars respectively show mean and S.E. of changes with respect to premeal values. Significance of difference from premeal values; $\star p < 0.05$, $\star\star p < 0.01$.

values recovered to the premeal levels by 120 min after the meal. There was no significant difference according to sex or the day of the experiment in the premeal values or the above changes, and they were not correlated to age or BMI.

Experiment II: f_{RSA} values during PB always agreed with the frequency of the metronome signals. As shown in Table 2, $C-CV_{RSA}$ for PB signifi-

Fig. 2. Relationship between breathing frequency and $C-CV_{RSA}$ at supine rest. $C-CV_{RSA}(f)$, $C-CV_{RSA}(\%)$ at breathing frequency of f (Hz); $\blacksquare$, Mean (S.E.) of % values of $C-CV_{RSA}(f)$ for paced breathing to $C-CV_{RSA}(0.25)$ in the same series for the same subjects; $\bullet$, data obtained during nonpaced breathing. Regression lines are calculated from the data at paced breathing (dashed lines show $\pm 10\%$ ranges).

cantly decreased with the increase in the frequency, and other parameters were not significantly affected by the frequency or PB/NPB. No significant difference by day of experiment was found for any of the parameters. Regression analysis which compared BF with changes in $C-CV_{RSA}$ for PB demonstrated a good linear relationship on a log-log scale (Fig. 2). The empirical equation shown in Fig. 2 can be rewritten as follows:

$$C-CV_{RSA}(0.25) = \exp(\ln(C-CV_{RSA}(f)) + 0.916 \ln(f) + 1.270),$$

where $f = f_{RSA}$ or BF (Hz), and $C-CV_{RSA}(f) = C-CV_{RSA}$ at f_{RSA} of f .

There was no significant correlation of age, sex, or BMI to the above trend.

The mean (S.D.) of f_{RSA} (=BF) for NPB was 0.25 (0.03) Hz. For NPB, f_{RSA} and the relative $C-CV_{RSA}$ values were distributed within the $\pm 10\%$ range of the regression line obtained for PB (Fig. 2). The converted $C-CV_{RSA}$ values obtained by

Table 2. Changes in parameters according to breathing frequency (BF)

Parameter	Breathing		Paced BF (Hz)						$p^{1)}$	Nonpaced breathing		$p^{2)}$
	0.18		0.20		0.25		0.33					
RR (msec)	990.1	(6.5)	980.4	(6.0)	980.6	(6.0)	996.7	(6.5)	NS	996.0	(6.8)	NS
C-CV _{RSA} (%)	4.4	(0.3)	4.0	(0.3)	3.2	(0.2)	2.6	(0.2)	***	3.2	(0.3)	
C-CV _{MWSA} (%)	1.6	(0.3)	1.8	(0.3)	1.5	(0.3)	1.3	(0.3)	NS	1.7	(0.2)	NS
f _{MWSA} (Hz)	0.090	(0.008)	0.095	(0.006)	0.104	(0.006)	0.104	(0.006)	NS	0.096	(0.009)	NS

Mean (S.E.). ¹Significance of difference among BFs by 3-way ANOVA (subjects $\times$ days $\times$ BF; but no day effect was significant); *** $p < 0.001$; NS, not significant. ²Significance of difference between paced and nonpaced breathing by 2-way ANOVA (subjects $\times$ paced/nonpaced); NS, not significant.

applying the regression equation to the observed $C\text{-CV}_{\text{RSA}}$ and f_{RSA} for NPB on the assumption that BF was 0.25 Hz agreed well with the $C\text{-CV}_{\text{RSA}}$ (0.25) for PB: the coefficient of correlation between the converted $C\text{-CV}_{\text{RSA}}$ and the $C\text{-CV}_{\text{RSA}}$ (0.25) for PB was 0.958 ($p < 0.001$), and, the relative values for the former compared with the latter ranged from 96.4 to 105.5%. Since the raw $C\text{-CV}_{\text{RSA}}$ values for NPB were moderately correlated with the $C\text{-CV}_{\text{RSA}}$ (0.25) for PB ($r = 0.639$; $p < 0.05$), the correlation was highly improved by the above standardization.

Discussion

It has been shown that RSA and MWSA amplitudes at supine rest can be affected by age^{4,22}, obesity^{23,24}, smoking^{22,25}, eating²¹, and the time of day²¹, but the present study was designed to exclude the effects of these factors as much as possible: only young nonsmokers were included, and all the experiments were performed in the late morning or early afternoon because RSA amplitude may decrease significantly in the late afternoon²¹. Time after meals was also considered in Experiment II, in view of the results of Experiment I, and neither the amplitudes of RSA and MWSA nor their changes showed any variation with age or BMI based on an examination of the present results. Furthermore, they did not show any difference according to sex, in agreement with a previous study²².

Decreases in $C\text{-CV}_{\text{RSA}}$ and RR observed immediately after meals in Experiment I have been related to vasodilative responses to eating and digestion which cause a decrease in venous return and total systemic resistance, thus suppressing vagal cardiac control activity^{21,26,27}. An increase in $C\text{-CV}_{\text{MWSA}}/C\text{-CV}_{\text{RSA}}$, which has been proposed as an index of sympathovagal interaction², after meals can be explained by a specific dynamic action or postprandial sympathetic activation^{21,28,29}, but the increase in $C\text{-CV}_{\text{MWSA}}$ was observed only at 30–90 min after meals, suggesting that the increase in $C\text{-CV}_{\text{MWSA}}$ at 0 min was canceled by the above decrease in vagal activity which modifies $C\text{-CV}_{\text{MWSA}}$. In view of the results which confirmed those reported previously²¹, Experiment II was conducted following a rest period of at least 120 min after finishing the meal, in order to avoid the effects of eating and digestion on RSA and MWSA.

In Experiment II, supine $C\text{-CV}_{\text{RSA}}$ for PB significantly decreased with the increase in BF, while $C\text{-CV}_{\text{MWSA}}$ was confirmed to be independent of BF. These results confirm those reported previously^{3,4,16–20}. As can be seen in Fig. 2, for example, $C\text{-CV}_{\text{RSA}}$ (0.20) is 1.4 times greater than

$C\text{-CV}_{\text{RSA}}$ (0.30), although the change in $C\text{-CV}_{\text{RSA}}$ not accompanied by a change in the mean heart rate does not reflect the change in the tonic level of vagal cardiac control^{18–20}. Such modification by BF is not negligible for reliable assessment of vagal activity with $C\text{-CV}_{\text{RSA}}$, showing the need for respiratory standardization.

The fact that the regression equation of BF and $C\text{-CV}_{\text{RSA}}$ for PB was applicable in the case of NPB when BF was between 0.18 and 0.33 Hz shows that pacing of BF did not affect $C\text{-CV}_{\text{RSA}}$, in agreement with results obtained in previous studies^{16,19,20}. This suggests a simple method to standardize RSA amplitude obtained during spontaneous regular breathing; once $C\text{-CV}_{\text{RSA}}$ and f_{RSA} are obtained for a subject who was instructed to breathe regularly without taking deep breaths, the $C\text{-CV}_{\text{RSA}}$ at a certain standard BF (e.g., 0.25 Hz) can be predicted by means of the above formula. The feasibility of the standardization method was supported by the agreement of the converted $C\text{-CV}_{\text{RSA}}$ with $C\text{-CV}_{\text{RSA}}$ (0.25) for PB. Although the $C\text{-CV}_{\text{RSA}}$ for NPB without the standardization was moderately correlated with the $C\text{-CV}_{\text{RSA}}$ (0.25) for PB, the respiratory standardization highly improved the correlation.

The method is efficient for reliable assessment of vagal activity based on RSA amplitude, and consequently can be applied to the assessment of sympathetic activity or sympathovagal balance based on $C\text{-CV}_{\text{MWSA}}/C\text{-CV}_{\text{RSA}}$ ². It should also be noted, however, that the method has a limitation in that it was based on the data for young, healthy and non-obese nonsmokers. It remains to be determined whether the method can be applied to the aged, the obese and/or heavy smokers.

Conclusion

It was shown that RSA and MWSA amplitudes at supine rest should be assessed at least 120 min after finishing a meal in order to exclude the effects of eating and digestion. The relationship between BF and RSA amplitude at supine rest was experimentally determined when the BF was between 0.18 and 0.33 Hz. The relationship was confirmed to hold in the case of RSA for nonpaced regular breathing with the mean BF corresponding to the center frequency of RSA. This suggests a simple statistical method of respiratory standardization for the RSA amplitude during nonpaced regular breathing at supine rest, which is efficient for reliable assessment of cardiac parasympathetic activity.

References

- 1) Akselrod S, Gordon D, Madwed JB, Snidman NC,

- Shannon DC, Cohen RJ. Hemodynamic regulation: investigation by spectral analysis. *Am J Physiol* 1985; 249: H867-875.
- 2) Pomerantz B, Macaulay RJB, Caudill MA, et al. Assessment of autonomic function in humans by heart rate spectral analysis. *Am J Physiol* 1985; 248: H151-153.
 - 3) Pagani M, Lombardi F, Guzzetti S, et al. Power spectral analysis of heart rate and arterial pressure variabilities as a marker of sympatho-vagal interaction in man and conscious dog. *Circ Res* 1986; 59: 178-193.
 - 4) Hayano J. Quantitative assessment of autonomic function by autoregressive spectral analysis of heart rate variability: Effect of posture, respiratory frequency, and age. *Jiritsu Shinkei* 1988; 25: 334-344.
 - 5) Hayano J, Sakakibara Y, Yamada A, et al. Accuracy of assessment of cardiac vagal tone by heart rate variability in normal subjects. *Am J Cardiol* 1991; 67: 199-204.
 - 6) Murata K, Araki S. Autonomic nervous system dysfunction in workers exposed to lead, zinc, and copper in relation to peripheral nerve conduction: A study of R-R interval variability. *Am J Ind Med* 1991; 20: 663-671.
 - 7) Murata K, Araki S, Maeda K. Autonomic and peripheral nervous system dysfunction in workers exposed to hand-arm vibration: A study of R-R interval variability and distribution of nerve conduction velocities. *Int Arch Occup Environ Health* 1991; 63: 205-211.
 - 8) Murata K, Araki S, Yokoyama K. Assessment of the peripheral, central and autonomic nervous system function in styrene workers. *Am J Ind Med* 1991; 20: 775-784.
 - 9) Murata K, Araki S, Yokoyama K, Maeda K. Autonomic and peripheral nervous system dysfunction in workers exposed to mixed organic solvents. *Int Arch Occup Environ Health* 1991; 63: 335-340.
 - 10) Murata K, Araki S, Yokoyama K, Yamashita K, Okajima F, Nakaaki K. Changes in autonomic function as determined by ECG R-R interval variability in sandal, shoe and leather workers exposed to n-hexane, xylene and toluene. *Neuro Toxic* 1994; 15: 867-876.
 - 11) Kageyama T, Kabuto M. Combined effects of mental workload and following exposure to road traffic noise on sympathetic activities assessed by a spectral component of heart rate variability. *Arch Complex Environ Studies* 1995; 7: 13-20.
 - 12) Hayano J, Sakakibara Y, Yamada M, et al. Decreased magnitude of heart rate spectral components in coronary artery disease. *Circulation* 1990; 81: 1217-1224.
 - 13) Comi G, Sora MGN, Bianchi A, et al. Spectral analysis of short-term heart rate variability in diabetic patients. *J Auton Nerv Syst* 1990; 30: S45-S50.
 - 14) Hayano J, Yamada A, Mukai S, et al. Severity of coronary atherosclerosis correlates with the respiratory component of heart rate variability. *Am Heart J* 1991; 121: 1070-1079.
 - 15) Ziegler D, Spüler M, Karsten Dannehl, DV, Gries FA, Mühlen H. Prevalence of cardiovascular autonomic dysfunction assessed by spectral analysis and standard tests of heart-rate variation in newly diagnosed IDDM patients. *Diabetes Care* 1992; 15: 908-911.
 - 16) Hirsch JA, Bishop B. Respiratory sinus arrhythmia in humans: how breathing pattern modulates heart rate. *Am J Physiol* 1981; 241: H620-629.
 - 17) Bernaldi L, Rossi M, Ricordi L. Clinical assessment of respiratory sinus arrhythmia by computerized analysis of RR interval and respiration. *G Ital Cardiol* 1992; 22: 517-529.
 - 18) Grossman P, Stemmler G, Meinhardt E. Paced respiratory sinus arrhythmia as an index of cardiac parasympathetic tone during varying behavioral tasks. *Psychophysiol* 1990; 27: 404-416.
 - 19) Grossman P, Karemaker J, Wieling W. Prediction of tonic parasympathetic cardiac control using respiratory sinus arrhythmia: the need for respiratory control. *Psychophysiol* 1991; 28: 201-216.
 - 20) Hayano J, Mukai S, Sakakibara M, Okada A, Takata K, Fujinami T. Effects of respiratory interval on vagal modulation of heart rate. *Am J Physiol* 1994; 267: H33-40.
 - 21) Hayano J, Sakakibara Y, Yamada M, et al. Diurnal variations in vagal and sympathetic cardiac control. *Am J Physiol* 1990; 258: H642-646.
 - 22) Murata K, Landrigan PJ, Araki S. Effects of age, heart rate, gender, tobacco and alcohol ingestion on R-R interval variability in human ECG. *J Auton Nerv Syst* 1992; 37: 199-206.
 - 23) Hirsch J, Leibel RL, MacKintosh R, Aguirre A. Heart rate variability as a measure of autonomic function during weight change in humans. *Am J Physiol* 1991; 261: R1418-1423.
 - 24) Kabuto M, Nishikido N, Kageyama T, Imai H, Honda Y, Kaneko T. A cardiac autonomic function test using heart rate variability (the 2nd report): Association with obesity, aging and smoking. *Jpn J Ind Health* 1994; 36: S316.
 - 25) Hayano J, Yamada M, Sakakibara Y, et al. Short- and long-term effects of cigarette smoking on heart rate variability. *Am J Cardiol* 1990; 65: 84-88.
 - 26) Brandt JL, Castleman L, Ruskin HD, Greenwald J, Kelly JJ, Jones A. The effect of oral protein and glucose feeding on splanchnic blood flow and oxygen utilization in normal and cirrhotic subjects. *J Clin Invest* 1955; 34: 1017-1025.
 - 27) Vatner SF, Franklin D, Citters RLV. Mesenteric vasoactivity associated with eating and digestion in the conscious dog. *Am J Physiol* 1970; 219: 170-174.
 - 28) Lipsitz LA, Pluchino FC, Wei JY, Minaker KL, Rowe JW. Cardiovascular and norepinephrine responses after meal consumption in elderly (older than 75 years) persons with postprandial hypotension and syncope. *Am J Cardiol* 1986; 58: 810-815.
 - 29) Jansen RWMM, Penterman BJM, Lier HJJV, Hoefnagels WHL. Blood pressure reduction after oral glucose loading and its relation to age, blood pressure and insulin. *Am J Cardiol* 1987; 60: 1087-1091.