A Standardization Method for Respiratory Sinus Arrhythmia Using Breathing Frequency (the 2nd Report): Efficiency of Assessing Changes in Cardiac Parasympathetic Activity with Posture

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 Using Breathing Frequency (the 2nd report): Efficiency of Assessing Changes in Cardiac Parasympathetic Activity with Posture: Takayuki KAGEYAMA, *et al.* Urban Environment and Health Project, National Institute for Environmental Studies Respiratory sinus arrhythmia (RSA) provides a noninvasive index of cardiac parasympathetic activity, although it appears to be modified by breathing frequency (BF) without any change in the tonic level of vagal activity. In the previous study, we proposed a statistical method for respiratory standardization of RSA amplitude at supine rest. Although RSA amplitude in other postures provide useful information on cardiac autonomic function or activity, they also appear to be modified by BF. We therefore examined RSA amplitude at sitting rest and that at standing rest as functions of BF intentionally maintained constant (0.18–0.33 Hz) for 11 nonsmokers (5 males and 6 females aged 19–28) in order to develop similar respiratory standardization methods in those postures. In those postures, RSA amplitudes linearly decreased with the increase in BF on a log-log scale, regardless of whether it was paced or nonpaced regular breathing. The respiratory modification of RSA amplitude at standing rest was greater than that at sitting rest, and both of them were greater than that at supine rest. This shows that RSA amplitude in the postures during nonpaced regular breathing can be statistically standardized based on the BF according to posture. Applying these methods to the data obtained from another experiment concerned with changes in RSA amplitude with posture, the efficiency of the standardization was confirmed.

(*J Occup Health* 1996; 38: 107–112)

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

Respiratory sinus arrhythmia (RSA), a component of heart rate variability, has been associated with cardiac parasympathetic activity. Mayer wave-related sinus arrhythmia (MWSA), another component of the variability, has been associated with systemic sympathetic activity with vagal modification^{1–5}. Their amplitudes at supine rest are expected to be useful indices to assess the possible autonomic effects caused by neurotoxicants or stressors in the fields of occupational and environmental health^{6–14}.

RSA amplitude appears to be modified by breathing frequency (BF) without any change in the heart rate or tonic level of parasympathetic activity, showing a need for respiratory standardization for the reliable assessment of vagal activity based on RSA amplitude^{15–19}. In our previous study²⁰, therefore, the RSA amplitude at supine rest calculated by autoregressive spectral analysis was examined as a function of BF. Based on their simple relationship regardless of paced or nonpaced regular breathing, we proposed a method to standardize RSA amplitude based on the BF corresponding to the center frequency of RSA (f_{RSA}).

Although our previous study dealt with the RSA at supine rest, the heart rate variability in other postures also provides useful information on cardiac autonomic function and activity. For example, the variability during task presentation, such as mental arithmetic, watching, and counting, in the sitting position has often been measured to assess the effects of the task on autonomic activities²³, whereas the decrease in the RSA amplitude accompanied by transition from supine rest to a tilting or stand-

Received Oct 26, 1995; Accepted Feb 8, 1996

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

ing position has been proposed as an index of cardiac parasympathetic function²⁴⁻²⁷⁾. The decrease can be diminished by aging^{4, 28, 29)}, obesity^{28, 30)}, eating a meal^{20, 31)}, smoking³²⁾, or diabetic autonomic neuropathy³³⁾ or other diseases. The decrease has also been used to assess the possible effects of exercise³⁰⁾ and shift work³⁴⁾ on autonomic functions.

The data from previous studies^{16, 19)} indicate that the degree of respiratory modification of the RSA amplitude may depend on posture. If this is true, a method for standardizing the amplitudes based on BF should be developed according to posture. The present study therefore aimed at the development of methods for standardizing RSA amplitude at sitting rest and that at standing rest, and the results were compared with those obtained at supine rest in our previous study²⁰⁾.

Subjects and Methods

Experiment I: The subjects were 11 healthy non-smokers, 5 males and 6 females, who gave written informed consent to participate in the following experiments. The mean $\pm$ S.D. of their age was 22.0 ± 3.3 (range 19–28), and that of their body mass indices (BMI, kg/m²) 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).

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 was recorded 4 times for 3 min each time, 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. ECGs were consecutively recorded at sitting rest (90° tilt) and standing rest in a similar way. Their postures were actively changed, and the ECG recording started after the heart rate became stable. This experiment was repeated once on another day, but following the 4th session at every posture, ECGs were recorded for 3 more min with the subjects in the same position. During this period, the subjects were instructed to breathe regularly without taking deep breaths and without using metronome signals (nonpaced breathing, NPB).

Successive R-R intervals (about 150–240 beats) including no extrasystole or abnormal arrhythmia were measured on the ECG with a tachograph (Model TM55, CERX Co., Ltd., Tokyo). Autoregressive spectral analysis and component wave analysis^{3, 10, 12)} were applied to the data to calculate the power density and center frequencies of MWSA and RSA, following the method previously

reported²⁰⁾. The amplitudes of MWSA and RSA were expressed as a component coefficient of variation for MWSA ($C-CV_{MWSA}$, %) and that for RSA ($C-CV_{RSA}$, %), according to the following formula^{4-10, 12, 14)}: $C-CV_k = 100 * P_k^{1/2} / RR$, where k = MWSA or RSA, P_k = power density of k component (msec²), and RR = mean of R-R intervals (msec).

All of the data were analyzed according to posture. The effects of BF on RR , $C-CV_{RSA}$, $C-CV_{MWSA}$, and $C-CV_{MWSA}/C-CV_{RSA}$ 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^{15, 16, 20)}. Regression analysis was applied to the f_{RSA} and the relative $C-CV_{RSA}$ values for PB, and then whether their relationships fit the data for NPB was also examined.

Experiment II: Fifty-nine healthy nonsmokers (18 males and 41 females) aged 20.2 ± 1.7 (Mean $\pm$ S. D.), who gave written informed consent, participated in the following experiments conducted in the above-mentioned room. Following a rest period of at least 120 min after eating a meal, they lay quietly for 5 min, after which their ECGs (standard lead I) and pneumatogram (PMG; Model TR762-T, Nihon-Kohden Co., Ltd., Tokyo) were recorded for 5 min at supine rest, sitting rest (90° tilt), and standing rest in that order. Their postures were actively changed, and the recording started after the heart rate became stable. They were instructed to breathe regularly without the use of any metronome signals during the recording.

The ECG data were analyzed by the same procedure as in Experiment I. Based on the PMG recorded during the same period as that for ECG recording, the mean BF (Hz) was calculated. $C-CV_{RSA}$ and BF for the three postures were compared with or without applying the respiratory standardization methods for BF obtained in Experiment I and our previous study²⁰⁾ on the assumption that BF was 0.25 Hz.

Results

Experiment I: f_{RSA} values during PB always agreed with the frequency of the metronome signals. As shown in Table 1, $C-CV_{RSA}$ for PB both at sitting rest and standing rest significantly decreased with the increase in f_{RSA} , while the other parameters were not significantly affected by f_{RSA} or PB/NPB. No significant difference by day of ex-

Table 1. Changes in parameters according to breathing frequency (BF) at sitting rest and at standing rest

Parameter	Breathing Posture	Paced BF (Hz)				$p^{(1)}$	Nonpaced breathing	
		0.18	0.20	0.25	0.33		$p^{(2)}$	
RR (msec)	Sitting	896.1(7.1)	908.5(7.5)	921.1(5.6)	864.2(7.5)	NS	905.1(7.1)	NS
	Standing	726.4(6.9)	699.3(5.8)	704.2(6.8)	713.4(6.8)	NS	709.5(6.1)	NS
C-CV _{RSA} (%)	Sitting	3.0(0.4)	2.5(0.4)	2.2(0.6)	1.9(0.2)	***	2.4(0.2)	
	Standing	2.8(0.1)	2.3(0.1)	1.9(0.1)	1.4(0.2)	***	2.0(0.2)	
C-CV _{MWSA} (%)	Sitting	2.1(0.4)	2.1(0.3)	2.2(0.3)	1.9(0.2)	NS	2.5(0.5)	NS
	Standing	2.7(0.1)	2.6(0.3)	2.7(0.2)	3.0(0.2)	NS	3.0(0.2)	NS
f _{MWSA} (Hz)	Sitting	0.086(0.005)	0.079(0.003)	0.096(0.007)	0.097(0.005)	NS	0.085(0.006)	NS
	Standing	0.073(0.006)	0.075(0.005)	0.078(0.003)	0.085(0.004)	NS	0.080(0.005)	NS

Mean (S.E.). ¹⁾ Significance of difference among BFs by 3-way ANOVA (subjects $\times$ days $\times$ BFs; but no day effect was significant);

*** $p < 0.001$; NS, not significant. ²⁾ Significance of difference between paced and nonpaced breathing by 2-way ANOVA (subjects $\times$ paced/nonpaced); NS, not significant.

periment was found for any of these parameters. f_{RSA} (=BF) and relative changes in C-CV_{RSA} for PB demonstrated good linear relationships on a log-log scale both for sitting rest and standing rest (Fig. 1). According to the General Linear Model analysis of $\ln$ (relative change in C-CV_{RSA}) in which

Fig. 1. Relationships between breathing frequency and C-CV_{RSA} at sitting rest and standing rest. C-CV_{RSA}(f), C-CV_{RSA}(%) at breathing frequency of f (Hz); ■, Mean (S.E.) of % percentages of C-CV_{RSA}(f) for paced breathing to C-CV_{RSA}(0.25) in the same series for the same subjects; ●, data obtained during non-paced breathing. Regression lines are calculated from the individual data obtained during paced breathing (dashed lines show $\pm 10\%$ ranges).

$\ln$ (BF), subjects, and their interaction were assumed as independent variables, neither the effects of subjects nor the interaction was significant for these two postures. The empirical equation obtained from the regression analyses for the individual data are shown in Fig. 1. They can be rewritten as follows:

$\text{C-CV}_{\text{RSA}}(0.25) = \exp(\ln(\text{C-CV}_{\text{RSA}}(f)) + 1.093 * \ln(f) + 1.515)$ at sitting rest, and

$\text{C-CV}_{\text{RSA}}(0.25) = \exp(\ln(\text{C-CV}_{\text{RSA}}(f)) + 1.144 * \ln(f) + 1.586)$ at standing rest,

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

There was no significant correlation of age, sex, or BMI with the above trends.

The mean (S.D.) of f_{RSA} (=BF) for NPB was 0.26 (0.05) Hz at sitting rest and 0.25 (0.04) Hz at standing rest. For NPB, f_{RSA} and the relative C-CV_{RSA} values were distributed within the $\pm 10\%$ range of the regression lines obtained for PB (Fig. 1). The standardized C-CV_{RSA} values calculated by applying the regression equations to the observed C-CV_{RSA} and f_{RSA} for NPB on the assumption that BF was 0.25 Hz agreed well with the C-CV_{RSA}(0.25) for PB: the coefficients of correlation between the standardized C-CV_{RSA} and the C-CV_{RSA}(0.25) were 0.950 ($p < 0.001$) at sitting rest and 0.906 ($p < 0.001$) at standing rest, and the relative values for the former compared with the latter ranged from 92.0 to 108.5% at sitting rest and from 95.8 to 108.5% at standing rest, respectively.

Experiment II: The mean BF obtained from PMG always agreed with the f_{RSA} obtained from ECG.

The mean (S.D.) of f_{RSA} (= mean BF) according to posture is shown in Table 2. The correlations between the f_{RSA} at the three postures were not strong (Table 3), although they were significant. The changes in f_{RSA} with the changes in

Table 2. Changes in f_{RSA} with posture

Parameter	Mean (S.D.)
a) f_{RSA} at supine rest (Hz)	0.26 (0.05)
b) f_{RSA} at sitting rest (Hz)	0.26 (0.04)
c) f_{RSA} at standing rest (Hz)	0.26 (0.05)
b-a (Hz)	0.00 (0.06)
c-b (Hz)	0.00 (0.06)

N=59, no postural change was statistically significant.

Table 3. Coefficients of correlation between f_{RSA} at three postures, supine rest, sitting rest, and standing rest

	Sitting	Standing
Supine	0.502***	0.458***
Sitting		0.415***

Pearson's correlation coefficient; N=59, *** $p < 0.001$.

posture were not correlated with any of the f_{RSA} before or after the changes in posture.

Table 4 shows the changes in C-CV_{RSA} due to the changes in posture with or without respiratory standardization. Although the C-CV_{RSA} as raw values decreased with the transition from supine rest to the sitting position as well as that from the sitting position to the standing position on average, the changes were not statistically significant. Once the respiratory standardization was applied, however, the variances in the relative changes in C-CV_{RSA} significantly diminished compared with those without the standardization ($p < 0.001$ both for sitting and standing rest). As a result, multiple comparison by 2-way ANOVA (subjects $\times$ postures) demonstrated the significant difference between C-CV_{RSA} at standing rest and that at supine rest. The relative decrease in C-CV_{RSA} at standing rest expressed as percentages of those at supine rest was $70.2 \pm 21.4\%$ (Mean $\pm$ S.D.).

The changes in f_{RSA} with posture were not correlated with any of the C-CV_{RSA} before or after the

changes in posture with or without the respiratory standardization. There was no correlation of age or sex with the changes in C-CV_{RSA} with posture regardless of the respiratory standardization.

Discussion

The present study was designed to control factors such as age^{4, 28, 29}, smoking³², time of day³¹ and time after meals^{31, 20} which may affect the RSA amplitude. There was no sex difference in the results. As for the results of Experiment I, there was no effect of obesity on the RSA amplitudes or their changes.

As expected, C-CV_{RSA} both at sitting rest and at standing rest decreased with the increase in BF without any change in the mean heart rate. The observed changes in C-CV_{RSA} do not reflect the change in the tonic level of vagal cardiac control. C-CV_{RSA} was independent of BF regardless of posture. These trends agreed with the results of previous studies^{3, 4, 15-20}.

The empirical equations of f_{RSA} and C-CV_{RSA} for PB both at sitting rest and at standing rest held in the case of NPB when BF was between 0.18 and 0.33 Hz, showing that pacing BF did not affect the C-CV_{RSA} at the postures in agreement with the case of supine rest²⁰. Once C-CV_{RSA} and f_{RSA} at sitting or standing rest are obtained for the subject who was instructed to breathe regularly without taking deep breaths, the C-CV_{RSA} at a certain standard BF (e.g., 0.25 Hz) can be predicted by means of the above equations. This statistical standardization without a pacemaker for BF enables avoidance of a dual-task situation under task presentation such as mental arithmetic at these postures, providing merit to examine the effects of the task on heart rate variability, as pointed out by Grossman *et al.*¹⁸.

The decline in C-CV_{RSA} with the increase in BF at standing rest was greater than that at sitting rest (Fig. 1), and both of them were greater than that

Table 4. Changes in C-CV_{RSA} with posture with or without respiratory standardization

Respiratory standardization	Posture	C-CV_{RSA} (%) ^{c)}	Percentage of the values at supine ^{d)}
Without standardization ^{a)}	Supine	4.0 (2.2)	100.0 (0.0)
	Sitting	3.2 (1.6)	95.3 (65.7)
	Standing	2.2 (1.4)	70.3 (59.8)
With standardization ^{b)}	Supine	4.1 (2.6)	100.0 (0.0)
	Sitting	3.2 (1.5) *	93.9 (26.4*)
	Standing	2.2 (1.3)	70.2 (21.4*)

^{a)} C-CV_{RSA} is shown as raw values. ^{b)} C-CV_{RSA} is standardized for BF ($-f_{\text{RSA}}$) by means of the empirical equations in Experiment I or Reference 20) with the assumption that BF is 0.25 Hz. ^{c)} Mean (S.D.), N=59; significance of difference between postures by 2-way ANOVA (subjects $\times$ postures), * $p < 0.05$ (multiple comparison by the least square means method). ^{d)} Mean (S.D.) of relative changes in C-CV_{RSA} expressed as the percentages of those at supine rest; significance of difference in variances with respiratory standardization compared with those without respiratory standardization, * $p < 0.001$.

at supine rest, because the empirical equation at supine rest previously obtained by us²⁰⁾ is as follows (Fig. 2):

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

These differences according to posture agree with the implications of early data^{16, 19)}.

These results mean that the degree of the relative change in $C-CV_{RSA}$ due to the change in posture appears to be affected by the BF before and after the change. Suppose that BF for a subject was 0.25 Hz at supine rest and it became 0.30 Hz at standing rest with the change in posture (Fig.2). According to the above empirical equation, the $C-CV_{RSA}$ at standing rest is underestimated by 0.81 times, compared with the case in which BF was kept at 0.25 Hz after the change in posture, resulting in underestimation of the relative change in $C-CV_{RSA}$ accompanied with the change in posture by 0.81 times.

This is the reason why the changes in $C-CV_{RSA}$ due to the change in posture did not appear to be

Fig. 2. Comparison in relationships between breathing frequency and $C-CV_{RSA}$ at supine rest, sitting rest, and standing rest. $C-CV_{RSA}(f)$, $C-CV_{RSA}(\%)$ at breathing frequency (BF) of f (Hz). Equations for supine rest (see ref. 20), sitting rest, and standing rest are as follows: $\log_{10}(100 * C-CV_{RSA}(f) / C-CV_{RSA}(0.25)) = -0.916 * \log_{10}(f) + 1.449$, $\log_{10}(100 * C-CV_{RSA}(f) / C-CV_{RSA}(0.25)) = -1.093 * \log_{10}(f) + 1.342$, and $\log_{10}(100 * C-CV_{RSA}(f) / C-CV_{RSA}(0.25)) = -1.144 * \log_{10}(f) - 1.311$. If BF at supine rest was 0.25 Hz and it became 0.30 Hz at standing rest with the change in posture, $C-CV_{RSA}$ at standing rest is underestimated by 0.81 times compared with the case in which BF was kept at 0.25 Hz.

significant in the results of Experiment II, when the changes in BF were neglected. In an actual situation, as in Experiment II, such a change in BF with a change in posture cannot be predicted by any of the BF or the $C-CV_{RSA}$ values before or after the change in posture. Once the respiratory standardization based on f_{RSA} ($=BF$) was applied according to posture, however, the variations in the relative changes in $C-CV_{RSA}$ significantly diminished, demonstrating a significant difference between the $C-CV_{RSA}$ at standing rest and that at supine rest. This agreed with the results of the studies where BF was controlled^{4, 19)}, showing the efficiency of the above respiratory standardization.

In conclusion, the results of the present study suggest simple statistical methods of respiratory standardization for the RSA amplitude during non-paced regular breathing at sitting rest and that at standing rest. The standardization methods according to posture will be efficient for reliable assessment of cardiac parasympathetic activity based on the RSA amplitude and of sympathetic activity indicated by the ratio of MWSA amplitude to RSA amplitude^{3, 11, 25)}. It should also be noted, however, that the methods have a limitation that they are based on the data for young, healthy and nonobese nonsmokers. It remains to be determined whether the methods can be applied to the aged, obese and/or heavy smokers.

References

- 1) Akselrod S, Gordon D, Madwed JB, Snidman NC, Shannon DC, Cohen RJ. Hemodynamic regulation: investigation by spectral analysis. *Am J Physiol* 1985; 248: 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; 249: 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 (in Japanese).
- 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) Murata K, Araki S, Yokoyama K, Yamashita K, Sata F, Ono Y. Autonomic neurotoxicity of alcohol assessed by heart rate variability. *J Auton Nerv Syst* 1994; 39: 1-7.
- 12) Kamada T, Miyake S, Kumashiro M, Monou H, Inoue K. Power spectral analysis of heart rate variability in Type As and Type Bs during mental workload. *Psychosom Med* 1992; 54: 462-470.
- 13) Kamada T, Sato N, Miyake S, Kumashiro M, Monou H. Power spectral analysis of heart rate variability in Type As during solo and competitive mental arithmetic task. *J Psychosom Res* 1992; 36: 543-551.
- 14) 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.
- 15) Hirsch JA, Bishop B. Respiratory sinus arrhythmia in humans: how breathing pattern modulates heart rate. *Am J Physiol* 1981; 241: H620-629.
- 16) 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.
- 17) 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.
- 18) 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.
- 19) 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.
- 20) Kageyama T, Imai H, Kabuto M. A standardization method for respiratory sinus arrhythmia as an index of cardiac parasympathetic activity using breathing frequency. *J Occup Health* 1996; 38: 20-24.
- 21) Aasman J, Mulder G, Mulder LJM. Operator effort and the measurement of heart-rate variability. *Human Factors* 1987; 29: 161-170.
- 22) Lee DH, Park KS. Multivariate analysis of mental and physical load components in sinus arrhythmia scores. *Ergonomics* 1990; 33: 35-47.
- 23) Murata A. Measurement of mental workload by heart rate variability indexes. *Ningen-Kohgaku* 1992; 28: 91-98 (in Japanese).
- 24) Lipsitz LA, Mietus J, Moody GB, Goldberger AL. Spectral characteristics of heart rate variability before and during postural tilt. *Circulation* 1990; 81: 1803-1810.
- 25) Montano N, Ruscone TG, Porta A, Lombardi F, Pagani M, Malliani A. Power spectrum analysis of heart rate variability to assess the changes in sympathovagal balance during graded orthostatic tilt. *Circulation* 1994; 90: 1826-1831.
- 26) Hopf HB, Skyschally A, Heusch G, Peters J. Low-frequency spectral power of heart rate variability is not a specific marker of cardiac sympathetic modulation. *Anesthesiology* 1995; 82: 609-619.
- 27) Lippman N, Stein KM, Lerman BB. Failure to decrease parasympathetic tone during upright predicts a positive tilt-table test. *Am J Cardiol* 1995; 591-595.
- 28) Kabuto M, Nishikido N, Kageyama T, Kurokawa Y, Kobayashi T, Kaneko T. A cardiac autonomic function test using heart rate variability (the 4th report): Association with age, obesity, smoking. *J Occup Health* 1995; 36: S342 (in Japanese).
- 29) 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.
- 30) Watanabe S, Shinkai S, Kurokawa Y, Asai H, Torii J, Shirasaki M. Effects of 15 weeks' exercise training on autonomic function in moderately obese middle aged women. *Jpn J Hyg* 1993; 48: 487 (in Japanese).
- 31) Hayano J, Sakakibara Y, Yamada M, et al. Diurnal variations in vagal and sympathetic cardiac control. *Am J Physiol* 1990; 258: H642-646.
- 32) 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.
- 33) Comi S, 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.
- 34) Watanabe S. Effects of shift work on circadian rhythm in autonomic function estimated by power spectrum analysis of heart-rate fluctuations and postural test. *Ehime-Igaku* 1990; 9: 46-61 (in Japanese).