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Assessment of the primary effect of aging on heart rate variability in humans

Chiho Fukusaki, M.Sc.,[1] Kiyoshi Kawakubo, M.D., Ph.D.,[2] and Yoshiharu Yamamoto, Ph.D.[1]

[1]Educational Physiology Laboratory, Graduate School of Education, and [2]Department of Health Sciences, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan

Address correspondence and reprint requests to Yoshiharu Yamamoto, Ph.D., Associate Professor, Educational Physiology Laboratory, Graduate School of Education, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan.
Tel: 81-3-5841-3971; Fax: 81-3-5689-8069
E-Mail: yamamoto@p.u-tokyo.ac.jp

Beat-to-beat heart rate variability (HRV), reflecting cardiac autonomic control mechanisms, is known to change with age. However, the degree to which this change is mediated by aging per se or by physiologic changes characteristic of normative aging is still unclear. This study was designed to examine the association of aerobic fitness, body habitus or obesity, and blood pressure with age-related changes in HRV. Resting HRV data was recorded from 373 healthy subjects (124 men, 249 women; age range, 16–69 y) and analyzed by coarse-graining spectral analysis to decompose the total spectral power into its harmonic and fractal components. The low- and high-frequency (LF, 0.0–0.15 Hz; HF, >0.15 Hz) harmonic components were calculated from the former, whereas the latter was used to calculate the integrated power (FR) and the spectral exponent β, which were, in turn, used to evaluate the overall complexity of HRV. Factor analysis was performed to test whether potentially age-related changes in the components of HRV might be observed secondarily through other variables affecting HRV. Significant ($p < 0.05$) age-related changes in the harmonic (HF and LF) and fractal (FR and β) components of HRV were generally consistent with those described in the literature. In addition, factor analysis showed that there was a unique common factor that primarily explained correlations among age, HF, and β ($p < 0.05$) without the contributions from LF, FR, aerobic fitness, body habitus or obesity, and blood pressure. It was concluded that, in this population-based sample, age-related changes in HF and β, both of which reflect vagal modulation of heart rate, were primarily mediated by aging per se and not by physiologic changes characteristic of normative aging.

Key words: aging, autonomic nervous system, aerobic fitness, heart rate variability, spectral analysis, fractal.

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In humans, beat-to-beat heart rate variability (HRV), or the variation in R-R intervals, is thought to reflect gross outflow from the autonomic centers in the brain through sympathetic and parasympathetic nervous innervation of pacemaker cells in the sinoatrial node [1]. This view is supported by the fact that variability in R-R intervals and, therefore, HRV are dramatically decreased in denervated human hearts (eg, orthotopic heart transplants) [2,3]. Short-term HRV shows seemingly irregular or complex dynamics consisting of periodic oscillations within high-frequency (HF, >0.15 Hz) and low-frequency (LF, 0.0–0.15 Hz) bands [4], with the variability being characterized by a broad band spectrum with fractal scaling [5,6]. In addition, it appears that aging is associated with decreased periodic oscillations of HRV [7–9], as well as with a loss of complexity within the fractal component [6,10,11]. Despite general agreement that the components of HRV change with age, the degree to which these changes are mediated by aging per se or by physiologic changes characteristic of normative aging remains unclear [12].

Aging is accompanied by a variety of usually irreversible changes known to affect HRV, including changes in physical fitness, body habitus, blood pressure and, of course, chronologic age. Therefore, it is possible that changes in aerobic fitness [13,14], body habitus or obesity [15,16], and blood pressure [17] might indirectly lead to the age-related changes in HRV, especially when the variables are correlated with components of HRV. Statistical factor analysis [18] is a technique used to detect common factors, unobservable or hypothetical variables, that may contribute to the variance of at least two observed variables, therefore accounting for the correlations among all the observed variables. With this technique, the aforementioned question is reduced to whether there is a unique common factor that would solely explain correlations between age and the components of HRV (see appendix for details). In this study, we have examined this hypothesis by analyzing HRV, aerobic fitness, body habitus or obesity, and blood pressure in a population-based sample of healthy subjects.

Materials and methods

Subjects

The study participants included 373 healthy subjects, 124 men (age range, 16–69 y; mean age ± SD, 45.4 ± 13.2 y) and 249 women (age range, 16–69 y; mean age ± SD, 44.8 ± 12.1 y), who generally had no apparent medical problems, although 18 had borderline hypertension. None of the subjects had electrocardiographic abnormalities, nor were any taking medications that might affect blood pressure. In ad-

dition, none had been engaged in regular athletic activities in the past few years.

Procedures

Subjects reported to a community health and medical center by 9:00 AM after an overnight fast. After a medical history was taken, resting systolic blood pressure (SBP) and diastolic blood pressure were measured with a sphygmomanometer, and a venous blood sample was taken for analysis. A resting ECG was recorded at approximately 9:30 AM, and then an exercise stress test was performed, which entailed using either a motor-driven treadmill or a cycle ergometer to volitional fatigue. Height, weight, and skinfold thickness were measured after lunch.

HRV

ECGs were recorded with use of a standard lead II (CardiMax FX-601, Fukuda Denshi, Tokyo, Japan). To measure HRV, the subjects rested quietly for approximately 10 minutes in the supine position, breathing normally. The first 5 minutes acclimatized the subject before data was collected (data from the first 5 minutes were discarded). The analog ECG signal was processed in real time with use of a personal computer (PC9801, NEC, Tokyo, Japan) interfaced to an analog-to-digital converter (PCN-2198, PC Technology, Tokyo, Japan), sampling at a frequency of 1,000 Hz. The digitized data were differentiated, and the length of one cardiac cycle was determined as the R-R interval. Series of R-R intervals (approximately 300 beats) from which HRV was determined were recorded on diskettes. Before analysis, the HRV data were manually searched for artifactual R-R intervals, which, when found, were corrected with use of summation and integer division. From these corrected HRV recordings, the mean heart rate (mean R-R) and the standard deviation of the R-R intervals (SD_{R-R}) were calculated. Linear trends in the recordings were eliminated by linear regression. Coarse-graining spectral analysis [19] was used to break down the total spectral power of HRV into regular oscillatory or harmonic components and a nonharmonic fractal component. The integrated powers in the LF and HF ranges were calculated from the harmonic components, and the overall complexity of the HRV was evaluated from the fractal component [5]. The latter, showing $1/f^{\beta}$ scaling (f = frequency), was used to calculate its integrated power (FR), after which the spectral exponent β was estimated as the slope of the linear regression of a log-fractal power, versus log-frequency plot.

Aerobic fitness

An exhausting incremental exercise test was performed with use of either a motor-driven treadmill (SPR761, Sakai Medical, Tokyo, Japan) or a cycle ergometer (Aerobike 710, Combi, Tokyo, Japan), randomly assigned to each of the subjects. The work load was increased during the treadmill test according to a modified Bruce protocol [20], whereas during cycling, the workload was increased in ramp fashion (20 W/min for men and 15 W/min for women). Peak oxygen uptake ($\dot{V}O_2$ peak) was estimated from the maximal velocity and the slope (in the treadmill test) or the power output attained (in the cycle ergometer test) with use of the equations recommended by the American College of Sports Medicine [21].

Body habitus and blood chemistry

Each subject's height, weight, and tricep skinfold thickness, and subscapular skinfold thickness were measured. The percentage of body fat (%Fat) was estimated from the skinfold thickness using equations reported for the Japanese population [22]. Blood samples were collected from the antecubital vein for measurement of triglyceride (TG), total cholesterol (Tcho), high density lipoprotein (HDL), and glucose (Glu). Approximately 10 ml of blood were collected in a sampling tube, centrifuged at 2,000 rpm for 10 minutes (Kubota-2010, Kubota, Tokyo, Japan), and stored at 4°C until analyzed. TG and Tcho were measured enzymatically (RX-30, Nihon Koden, Tokyo, Japan), HDL was also measured enzymatically after precipitating the low and very low density fractions with phosphotungstic acid magnesium, and Glu was measured with use of the hexokinase method (RX-20, Nihon Koden).

Statistical analyses

The analyses were conducted with use of the Statistical Analysis System (SAS) Ver. 6.07 [23]. Because the values of HF, LF, and integrated power (FR) were not normally distributed as judged by the Shapiro-Wilk test, the values were log-transformed before analysis. To evaluate the effects of age and gender on the observed variables, the subjects were categorized according to age (16–29 y, 30–39 y, . . . , 60–69 y), and a two-way analysis of variance (age × gender) was carried out with use of the general linear model procedure. P values <0.05 were considered statistically significant. To assess whether potentially age-related changes in HRV might be observed secondarily through variables affecting the components of HRV, the FACTOR procedure was used to conduct a factor analysis [18] among HRV-related variables (LF, HF, FR, and β), variables for body habitus or obesity (height, weight, and %Fat), aerobic fitness ($\dot{V}O_2$ peak), blood chemistry (TG, Tcho, HDL, and Glu), SBP, and age (see appendix for detailed procedures). Before the analysis, these variables, which were not normally distributed (Shapiro-Wilk test), were also log-transformed. Because the primary purpose of this study was to investigate whether age might have direct effects on the components of HRV, we further evaluated the validity of a factor loading for age on the factors related to the components of HRV. To that end, we first generated 50 datasets for both men and women, of which only age was randomly sorted. Factor analysis was then performed on these datasets 50 times. Because this procedure would eliminate coupling between age and the other variables, the factor loadings for age were expected to be low if they existed in the actual data. The factor loadings for age from the actual data were compared

with those from dummy datasets with the same factor structure, which produced the 95% confidence intervals (ie, null effect ranges).

Results

Population characteristics

As shown in Table 1, as expected, men were significantly taller and heavier than women and had lower %Fat levels. $\dot{V}O_2$ peak in men was significantly higher than in women, and men had higher levels of TG, lower levels of Tcho and HDL, and higher SBP than women. Overall, decreases in height and $\dot{V}O_2$ peak and increases in TG, Tcho, and SBP were significant with age.

HRV

Figure 1 shows representative examples of HRV and power spectra from a young woman and an old woman. Typically, younger subjects had more HRV and larger harmonic spectral components than older subjects, whereas β was larger in older subjects than in younger subjects. In sum, as shown in Table 2, although mean R-R was unrelated to age, there were significant age-related increases in β and decreases in SD_{R-R}, HF, LF, and FR. HF was significantly lower in men than in women, whereas β was significantly lower in women than in men, indicating that HRV in women was more complex than in men.

Factor analysis

As shown in Table 3, among men, the ratio of the sum of the eigenvalues to the total variance of the data (ie, the cumulative contribution) was 0.865 for up to five factors. This indicates that more than 80% of the variance was explained by those five factors. Weight, %Fat, and TG had highly positive loadings, and $\dot{V}O_2$ peak and HDL had highly negative loadings on the first factor in men, indicating that these variables were directly correlated. Glu and β had highly positive loadings and HF had highly negative loadings on the second factor. The factor loading for age on the second factor was also highly positive, indicating that older age was primarily associated with higher Glu, higher β, and lower HF. Both LF and FR, which reflect the magnitude of the low-frequency HRV component, had highly positive loadings on the third factor; loading for age on the third factor was small. Tcho and TG had highly positive loadings on the fourth factor, whereas SBP and height had highly positive loadings on the fifth factor. From 47 dummy datasets from men, in which only age was randomly

Table 1. Population characteristics

Age group	16–29 y	30–39 y	40–49 y	50–59 y	60–69 y
n (m, f)	16, 30	25, 48	32, 83	23, 58	28, 30
Height (cm)*					
Male†	168.9 ± 7.0	171.2 ± 6.1	170.3 ± 5.2	166.8 ± 5.4	163.4 ± 5.2
Female	158.4 ± 5.7	158.3 ± 5.2	155.2 ± 5.2	154.0 ± 4.7	152.5 ± 5.7
Weight (kg)					
Male†	66.3 ± 16.9	68.6 ± 9.2	69.7 ± 10.2	62.8 ± 8.1	63.9 ± 8.0
Female	54.2 ± 7.6	53.7 ± 7.0	55.6 ± 7.7	53.8 ± 5.4	54.3 ± 9.0
% Fat					
Male†	15.9 ± 3.2	17.2 ± 5.9	17.4 ± 4.2	15.0 ± 4.5	16.4 ± 3.9
Female	25.6 ± 8.5	24.6 ± 7.2	28.0 ± 6.6	27.3 ± 6.0	26.8 ± 7.1
$\dot{V}O_2$ peak (ml/kg/min)*					
Male†	38.7 ± 7.6	36.5 ± 5.8	36.9 ± 6.4	37.2 ± 5.9	31.8 ± 5.9
Female	32.5 ± 2.9	32.7 ± 4.7	30.6 ± 4.0	29.7 ± 3.3	28.2 ± 4.3
TG (mg/dl)*‡					
Male†	90.1 ± 42.8	145.1 ± 107.6	148.3 ± 98.1	106.7 ± 80.6	125.8 ± 69.7
Female	58.9 ± 22.7	81.5 ± 58.0	83.6 ± 35.6	112.6 ± 67.1	108.8 ± 65.1
Tcho (mg/dl)*‡					
Male†	183.1 ± 42.6	196.1 ± 36.2	200.9 ± 29.3	204.4 ± 23.2	198.6 ± 37.4
Female	180.3 ± 32.9	197.0 ± 40.3	205.8 ± 32.1	224.2 ± 36.9	232.1 ± 31.9
HDL (mg/dl)‡					
Male†	50.8 ± 9.5	52.2 ± 10.8	47.5 ± 11.2	61.6 ± 16.4	50.6 ± 14.6
Female	67.8 ± 16.7	66.8 ± 15.3	62.9 ± 12.1	60.0 ± 15.3	61.6 ± 14.8
Glu (mg/dl)					
Male	85.3 ± 7.5	92.2 ± 24.8	93.5 ± 9.9	99.2 ± 12.7	95.2 ± 11.6
Female	91.1 ± 38.5	87.0 ± 8.3	90.9 ± 12.1	91.6 ± 11.7	94.5 ± 10.3
SBP (mm Hg)*					
Male†	118.4 ± 11.8	113.4 ± 11.4	121.0 ± 14.4	119.3 ± 13.6	124.4 ± 16.9
Female	101.7 ± 9.2	102.3 ± 11.7	109.3 ± 14.3	114.2 ± 17.1	117.8 ± 17.9

Values are mean ± SD.

$\dot{V}O_2$ = oxygen uptake; TG = triglyceride; Tcho = total cholesterol; HDL = high-density lipoprotein; Glu = glucose; SBP = systolic blood pressure.

*P <0.05 for the age effect.

†P <0.05 for the gender effect.

‡P <0.05 for the age × gender interaction.

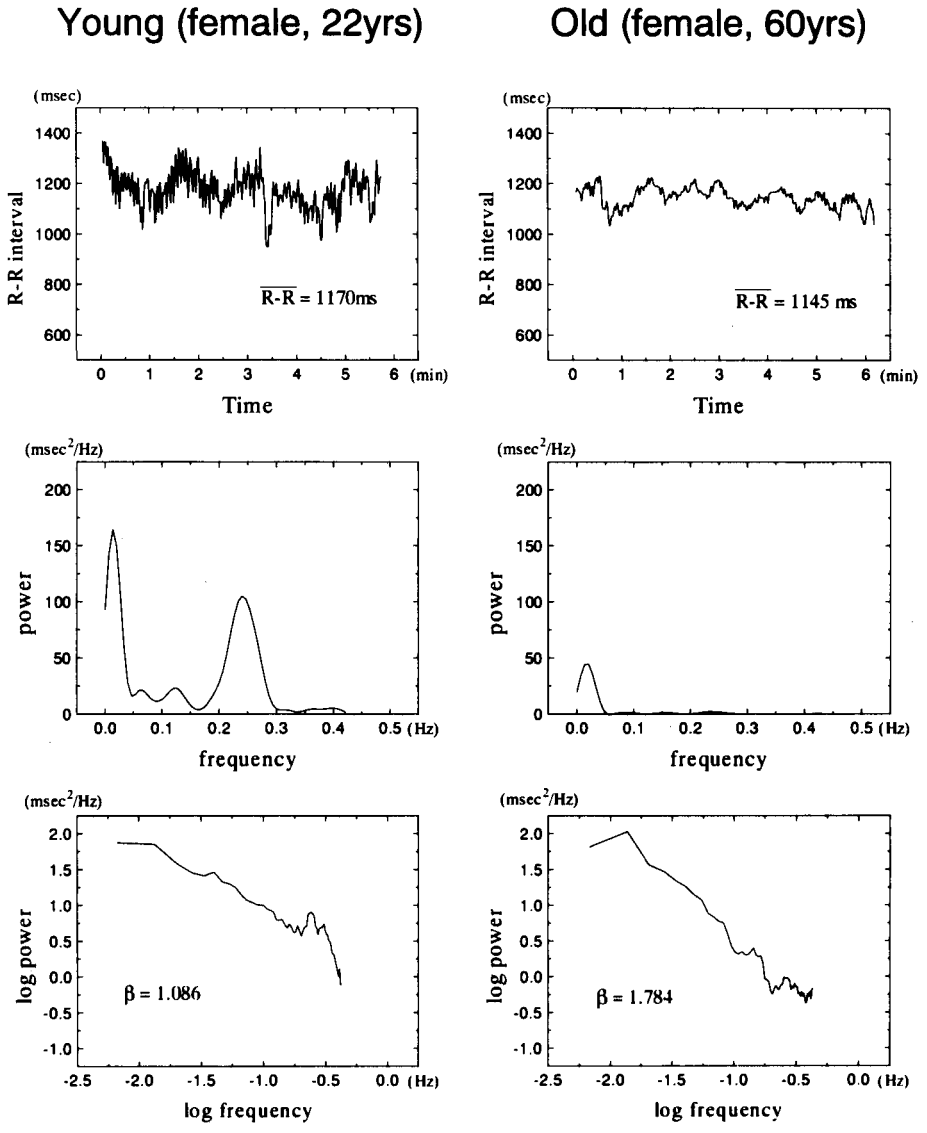

Figure 1. Representative examples of heart rate variability and the power spectra from a young woman (22 y; left) and an old (60 y; right) woman. The top panels show raw heart rate variability data derived from approximately 300 heartbeats. The middle and bottom panels show the spectra of the harmonic and fractal heart rate variability components, respectively.

shuffled, we were able to extract the same five factors obtained for the actual dataset. The actual factor loadings for age were significantly larger than the null effect ranges only for the second factor.

As shown in Table 4, among women, the cumulative contribution by the first five factors was 0.669. Weight, %Fat, Glu and SBP had highly positive loadings on the first factor, whereas $\dot{V}O_2$ peak had highly negative loadings. LF and FR had highly positive loadings and HF had moderately positive loadings on the second factor. The factor loading for age on the second factor was moderately negative. HF had highly negative loadings and β had highly positive loadings on the third factor. Moreover, the factor loading for age on the third factor was the highest among the five factors. Again, older age was associated with higher β and lower HF. Height and Tcho had higher absolute factor loadings on the fourth factor, but there were also substantial levels of factor loadings for weight and age. TG and HDL had higher loadings on the fifth factor. From 22 dummy datasets from women, we extracted the same five factors as obtained from the actual dataset. The actual factor loadings for age on the second, third, and fourth factors were significantly higher than the null effect ranges.

Discussion

This study examined the association of aerobic fitness, body habitus or obesity, and blood pressure with age-related changes in the harmonic and fractal components of short-term HRV in a healthy population-based sample. The observed age-related changes in the components of HRV in these volunteers were generally consistent with those described in the literature [6–12]. In addition, factor analysis revealed that there was a unique common factor in both men and women that largely accounted for the correlations among age, HF, and β without including potential contributions from aerobic fitness, body habitus or obesity, and blood pressure. Therefore, in this sample, the age-related changes in HF and β, both of which are known to reflect vagal modulation of heart rate [1,4,24], are primarily mediated by aging per se, not by physiologic changes characteristic of normative aging.

Table 2. Age-related changes in heart rate variability and the harmonic and fractal components

Age group	16–29 y	30–39 y	40–49 y	50–59 y	60–69 y
n (m, f)	16, 30	25, 48	32, 83	23, 58	28, 30
Mean R-R (msec)*					
Male	895 ± 117	938 ± 132	979 ± 127	990 ± 114	932 ± 142
Female	1028 ± 145	978 ± 139	935 ± 110	969 ± 96	979 ± 120
SD_{R-R} (msec)†					
Male	48.7 ± 9.9	47.4 ± 20.4	40.4 ± 14.3	38.6 ± 13.2	34.1 ± 11.5
Female	62.1 ± 17.4	51.1 ± 16.1	40.4 ± 15.0	40.9 ± 15.7	33.2 ± 10.5
Ln (HF) ($msec^2$)†					
Male‡	5.16 ± 1.01	4.50 ± 1.21	4.32 ± 0.72	3.91 ± 1.05	3.40 ± 1.35
Female	6.35 ± 0.86	5.09 ± 1.25	4.53 ± 1.03	4.14 ± 1.28	3.98 ± 0.89
Ln (LF) ($msec^2$)†					
Male	5.52 ± 0.75	5.40 ± 1.28	5.47 ± 1.03	5.24 ± 1.09	4.62 ± 1.13
Female	5.82 ± 0.71	5.41 ± 0.96	4.85 ± 1.32	5.07 ± 0.94	4.61 ± 1.10
Ln (FR) ($msec^2$)†					
Male	5.93 ± 0.68	5.96 ± 0.84	5.59 ± 1.00	5.62 ± 1.09	5.45 ± 0.87
Female	6.41 ± 0.65	6.14 ± 0.74	5.76 ± 0.94	5.65 ± 0.96	5.21 ± 0.88
β†					
Male‡	1.04 ± 0.33	1.19 ± 0.32	1.29 ± 0.32	1.43 ± 0.36	1.42 ± 0.41
Female	0.84 ± 0.32	1.06 ± 0.35	1.26 ± 0.39	1.36 ± 0.33	1.24 ± 0.37

Values are mean ± SD.
HF = high frequency; LF = low frequency; FR = integrated power.
*P <0.05 for the age × gender interaction.
†P <0.05 for the age effect.
‡P <0.05 for the gender effect.

HRV

Understanding the effects of age and other factors on HRV and its spectral components is important for two main reasons. First, decreased HRV in patients after infarction is associated with an increased risk for sudden cardiac death. This effect is independent of other prognostic measures [25,26], although the prognostic value of HRV is currently thought to be influenced by confounding factors [9]. Second, HF components of HRV are associated solely with cardiac vagal activity, whereas LF components are associated with both vagal and sympathetic activity [4]. Therefore, appropriate analysis of HRV components could provide a noninvasive method of evaluating the autonomic neural balance of the heart in a variety of physiologic contexts, including normative aging.

The significant age-related changes in HRV and the harmonic components observed in this study are in general agreement with those described in previous studies [7–9,11,12]. In those studies, the magnitudes of HRV (SD_{R-R} and/or the total spectral power) and its harmonic (LF and HF) components were found to decrease with advancing age. Such age-related decreases in SD_{R-R}, LF, and HF were also observed in this study. In addition, we observed that the power of the aperiodic fractal component (FR) of short-term HRV, which accounted for the majority of very low-frequency HRV [27], also decreased with age. Much attention has recently been paid to the irregular or complex dynamics of HRV [5,6,28]. Although physiologic interpretation of the complexity of the fractal component of short-term HRV has not been fully established, it has been proposed that it might reflect a homeostatic regulation of heart rate [24] through extensively coupled control systems and feedback loops, acting on different time scales [28]. Lipsitz and Goldberger [28] hypothesized that aging is associated with the loss of HRV complexity because of degeneration of these multiple control systems. Our finding that β, which is inversely related to the complexity of the fractal component of HRV, increased with age supports this hypothesis. This finding is also in agreement with results showing that the approximate entropy of HRV, another measure of the complexity of time series, was less in older subjects than in younger ones [10,11], as well as with a more recent study by Iyengar *et al.* [6], who obtained similar results with use of yet another measure. By using a larger, more uniform population sample, we have been able to extend these earlier studies and to establish a rationale for the further study of the functional significance of the fractal HRV on cardiovascular homeostasis in the elderly.

Factor analysis

Because aging is accompanied by a variety of usually irreversible changes in physical fitness, body habitus, blood pressure, and chronologic age, the age-related changes in HRV observed in the population studied may have been brought about indirectly (ie, by changes in variables for aerobic fitness, which are known to decrease with aging, especially when they are correlated with HRV-related variables). This is a well-known problem of multicolinearity; therefore, we adopted factor analysis to examine whether such correlations were present in our datasets. It was found that, although the factor loadings for age on the factors related to the components of HRV (the second and third factors in both men and women) were significant, those for obesity (%Fat), aerobic fitness ($\dot{V}O_2$ peak), and blood pressure (SBP) were substantially lower. Lower loadings on factors related to the components of HRV by variables other

Table 3. Factor loadings on the first five factors for men

Variable	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
Weight	**0.758**	−0.136	0.069	−0.009	0.471
% Fat	**0.758**	0.113	−0.064	0.105	0.167
$\dot{V}O_2$ peak	**−0.585**	−0.258	0.347	−0.004	−0.022
TG	**0.640**	−0.055	−0.080	0.618	−0.100
HDL	**−0.789**	0.024	0.094	0.120	0.239
Glu	−0.008	**0.616**	−0.175	0.132	−0.043
HF	−0.313	**−0.692**	0.349	0.111	−0.264
β	0.211	**0.692**	0.468	−0.240	0.239
Age	−0.029	***0.767**	−0.123	0.182	−0.024
LF	−0.128	−0.326	**0.783**	0.196	−0.160
FR	−0.156	−0.081	**0.871**	−0.078	−0.040
Tcho	−0.041	0.193	0.074	**0.884**	0.167
SBP	−0.017	0.212	−0.239	0.153	**0.632**
Height	0.133	−0.548	0.113	−0.027	**0.594**
EV	2.727	2.522	1.979	1.377	1.253
CC	0.239	0.460	0.634	0.755	0.865

The factor loadings after Varimax rotation [18] are shown. The maximal loading for each variable is emphasized with boldface. $\dot{V}O_2$ = oxygen uptake; TG = triglyceride; HDL = high-density lipoprotein; Glu = glucose; HF = high frequency; LF = low frequency; FR = integrated fractal power; Tcho = total cholesterol; SBP = systolic blood pressure; EV = eigenvalue; CC = cumulative contribution.

*P <0.05 from the null effect range calculated with use of age-shuffled, surrogate data.

than age, combined with the absence of contributions of HRV-related variables to those other factors, suggests that age is directly and primarily influencing the harmonic and fractal components of HRV. The results of the factor analysis further revealed that, in both men and women, LF and FR were extracted as constituents of different factors than were HF and β. The former reflect the magnitude of low- and very low-frequency HRV [27] and are reported to be mediated by both sympathetic and vagal activity [1,4], whereas the latter are reported to selectively reflect vagal modulation of heart rate [1,4,24]. The fact that the factor loading for age on HF and β was higher than on LF and FR may suggest that, in healthy subjects, such as those participating in this study, age-related changes in the vagal modulation of heart rate predominate over changes caused by sympathetic influences.

The factor loading patterns for men and women were generally similar, with some differences. One clear similarity was that, in both sexes, increased obesity was accompanied by lower aerobic fitness in the first factor, which had the largest eigenvalue, and age-related changes in the HRV components were extracted as the second or third factor. Still, the detailed structures of factor loading patterns were not exactly the same in men and women, perhaps because of the smaller size of the male sample.

Our finding that age primarily influenced components of HRV in sedentary subjects is in partial agreement with the findings of a recent study by Byrne *et al.* [12]. They used a multiple regression model for the harmonic components of HRV depending on age, $\dot{V}O_2$ peak, and body mass index and found that the partial correlation coefficient for age was significantly greater than that for $\dot{V}O_2$ peak or body mass index. Strictly speaking, however, results with a multiple regression model should be interpreted cautiously in the presence of multicolinearity. For example, whereas Byrne *et al.* reported a significant Pearson correlation coefficient for $\dot{V}O_2$ peak and HF, the partial correlation coefficient for $\dot{V}O_2$ peak in the regression equation containing HF as a dependent variable was insignificant and almost 0 [12]. In contrast, by using a factor analysis model, the details of the correlation structure among observed variables could be investigated (see appendix). For example, an estimated Pearson correlation coefficient (r) between $\dot{V}O_2$ peak and HF in women, calculated by Eq. (4) (see appendix and Table 4), was 0.250 (the actual r was 0.207, p <0.001). Table 4 also shows that, for the most part, this correlation resulted from contributions of factor loadings on the first (−0.477 × −0.118 = 0.056) and the second (0.266 × 0.589 = 0.156) factors. The corresponding value for the third factor, which was closely related to age and to vagal modulation of heart rate, was very small, indicating that, whereas $\dot{V}O_2$ peak was in fact correlated with HF, age had less colinearity with this relationship. Therefore, our approach has the capacity to provide deeper insight into factors affecting the components of resting HRV in humans than does multiple regression.

Limitations

Because they were conducted within community-based health and medical examinations, the time allotted to make ECG recordings was limited to approximately 10 minutes per person, which may not, at first, seem long enough to obtain stable measurements of HRV. Under these conditions, we were forced to limit each HRV recording to approximately 300 cardiac cycles (4–5 minutes) obtained after

Table 4. Factor loadings on the first five factors for women

Variable	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
Weight	**0.756**	−0.005	0.171	0.523	−0.062
% Fat	**0.756**	−0.019	0.128	0.071	−0.170
$\dot{V}O_2$ peak	**−0.477**	0.266	−0.021	0.209	0.296
Glu	**0.530**	−0.158	−0.151	−0.112	0.022
SBP	**0.500**	−0.123	0.285	−0.277	0.099
LF	−0.109	**0.833**	−0.102	−0.049	0.096
FR	−0.138	**0.867**	0.000	0.158	−0.024
HF	−0.118	0.589	**−0.693**	0.077	0.050
β	0.026	0.058	**0.893**	0.034	−0.119
Age	0.112	*−0.325	***0.551**	*−0.502	−0.109
Height	−0.002	0.044	−0.003	**0.804**	0.190
Tcho	0.398	−0.023	0.393	**−0.480**	0.289
TG	0.404	−0.085	0.261	−0.273	**−0.544**
HDL	−0.026	0.031	−0.045	0.035	**0.923**
EV	2.282	2.023	1.967	1.652	1.436
CC	0.163	0.307	0.448	0.566	0.669

The factor loadings after Varimax rotation [18] are shown. The maximal loading for each variable is emphasized with boldface. $\dot{V}O_2$ = oxygen uptake; Glu = glucose; SBP = systolic blood pressure; LF = low frequency; FR = integrated fractal power; HF = high frequency; Tcho = total cholesterol; TG = triglyceride; HDL = high-density lipoprotein; EV = eigenvalue; CC = cumulative contribution.

*P <0.05 from the null effect range calculated with use of age-shuffled, surrogate data.

a 5-minute acclimatization period, during which it was hoped that the physiologic responses of the subjects would stabilize. In that regard, we previously evaluated the effects of the length of the period of data collection on HRV by comparing LF, HF, and β derived from coarse-graining spectral analysis [5,19] with HRV data collected during long periods (up to 8,500 beats). We found that, for HRV derived from 256 beats and 512 beats, values of β were not significantly different from those derived from long-term HRV. In contrast, values for LF and HF obtained from short-term data were significantly smaller than those from the long-term data [5], although, even in that case, the results for short- and long-term HRV were still correlated. Therefore, the limited period during which HRV data were collected did not likely affect the primary findings of this study.

The clinical setting in which the examinations took place did not allow us to measure or control the subjects' breathing, which has been previously reported to affect HRV [29,30]. In particular, the HF component of HRV, which primarily represents the respiratory modulation of heart rate [1,4], should be evaluated with caution when breathing frequency is smaller than the boundary frequency (0.15 Hz). In this study, we asked the subjects to breathe as normally as possible to minimize this effect. Moreover, changes in respiration patterns would not be expected to affect calculation of β [31,32], which reflects vagal modulation of heart rate [24].

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Appendix

A brief background and details of the procedures used in our factor analysis are introduced in this appendix. Statistical factor analysis [18] is a technique to detect common factors—unobservable and hypothetical variables—that contribute to the variance of at least two of the observed variables, therefore accounting for the correlations among the observed variables. It models the j-th variable (j = 1, . . . , p) for the i-th subject (i = 1, . . . , n), or x_{ij}, through linear combination of factor scores common to all the variables, f_{ik} (k = 1, . . . , m), plus a unique factor score unique to each variable, u_{ij}. Therefore, for each subject, the j-th variable is modeled by:

$$x_{ij} = a_{j1}f_{i1} + a_{j2}f_{i2} + \cdots + a_{jm}f_{im} + d_j u_{ij}. \qquad (1)$$

As in this study, the coefficient a_{jk}, called a factor loading, is usually determined so that any of two factors, including the unique factor, are uncorrelated with each other:

$$\begin{cases} \sum_{i=1}^{n} f_{ik}f_{ik'} = 0 & (k \neq k') \\ \sum_{i=1}^{n} f_{ik}u_{ij} = 0 \\ \sum_{i=1}^{n} u_{ij}u_{ij'} = 0 & (j \neq j'). \end{cases} \qquad (2)$$

The direct consequence of this is that the covariance between the two variables x_{ij} and $x_{ij'}$, or $s_{jj'}$ (the same as the Pearson correlation coefficient $r_{jj'}$ when the variance of each variable is set to unity), is:

$$s_{jj'} = r_{jj'} = \sum_{i=1}^{n} x_{ij}x_{ij'} = a_{j1}a_{j'1}\sum_{i=1}^{n} f_{i1}^2 + a_{j2}a_{j'2}\sum_{i=1}^{n} f_{i2}^2 + \cdots + a_{jm}a_{j'm}\sum_{i=1}^{n} f_{im}^2 + \sum_{k\neq k'}\left(a_{jk}a_{jk'}\sum_{i=1}^{n} f_{ik}f_{ik'}\right) + \sum_{j,k}(a_{jk}d_j\sum_{i=1}^{n} f_{ik}u_{ij}) + d_j d_{j'}\sum_{i=1}^{n} u_{ij}u_{ij'}. \qquad (3)$$

Using the second equation and assuming $\Sigma f_{ik}^2 = 1$ by previous standardization, we obtain:

$$r_{jj'} = a_{j1}a_{j'1} + a_{j2}a_{j'2} + \cdots + a_{jm}a_{j'm}. \qquad (4)$$

This equation shows that the standard Pearson correlation coefficient is greatly influenced by two variables (j and j') of which the absolute factor loadings on selected factors are higher. In addition, if the absolute factor loadings on a selected factor are high in more than two variables, this indicates that mutually linear relationships (or multicolinearity) exist among these variables. In this study, for example, the greatest factor loadings (among extracted factors) were found for weight, percentage of body fat, peak oxygen uptake (negative), glucose, and systolic blood pressure on the first factor for women (Table 4). These variables have been frequently reported to correlate with each other, suggesting that higher body weight, percentage of body fat, glucose, and systolic blood pressure are associated with a lower peak oxygen uptake [33]. The factor loading patterns further suggested that, for women (Table 4), the factor loading for age on the first factor was much smaller than on the third factor, which explains a great portion of correlations among HF, β, and age. In other words, the effect of age on the decrease in HF and the increase in β was stronger than on, for example, the decrease in peak oxygen uptake. Therefore, the factor analysis was capable of examining the correlation structure behind multiple observed variables.

To approximate the $p \times m$ factor loading matrix (Tables 3 and 4), the $p \times p$ correlation matrix was first subjected to principal component analysis to calculate the p eigenvalues (λ_k) corresponding to the variance of each principal component. The number of factors (m) was set so that all the $\lambda_k$values were greater than unity [23]. At this stage, five factors were extracted for both men and women (Tables 3 and 4). Finally, the a_{jk} was calculated as a weight for the standardized principal component scores [18]. To help in interpreting the results of the factor loading matrix, the m factor score vectors (ie, the standardized principal component vectors, which are uncorrelated with each other) were rotated toward simple structure. We applied Varimax rotation [18] to the five factors to demonstrate the factor loading patterns more clearly (Tables 3 and 4).