Research Article

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Standardized tests of heart rate variability: normal ranges obtained from 309 healthy humans, and effects of age, gender, and heart rate

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The authors undertook this study to determine the effects of age, gender, and heart rate (HR) on the results of cardiac autonomic function tests for measuring heart rate variability (HRV) in a large sample of healthy subjects (n = 309). Conventional tests (deep breathing, maximum/minimum 30:15 ratio), and a standardized 5-minute resting study, including spectral analysis of HR, were used. The main findings included (1) the indices of all tests, except for the ratio of the low- (LF) to high-frequency (HF) spectral power (LF/HF ratio) and HR itself, are inversely related to age in both sexes; (2) the 5-minute spectral bands (except for the LF/HF ratio), the variation coefficient, expiratory–inspiratory ratio during deep breathing, and the maximum/minimum 30:15 ratio are independent of HR; (3) women up to the age of 55 years have a higher resting HR compared with men; (4) young and middle-aged women show a significantly lower LF power and LF/HF ratio compared with age-matched men, whereas no significant gender differences are observed in the absolute HF power. The authors computed age- and gender-dependent normal values for each of the HRV indices studied here and discuss the clinical consequences arising from gender differences in HRV.

Key words: autonomic nervous system, human, cardiovascular regulation, heart rate variability, 30:15 ratio, deep breathing test.

Autonomic nervous system dysfunction has been described not only in cardiovascular disorders [1], but also in various endocrinologic [2,3] neurologic [4,5], and, recently, psychiatric disorders [6,8]. Several noninvasive tests of heart rate variability (HRV) are commonly used to evaluate cardiovascular autonomic nervous system (ANS) function, including well-established conventional tests (eg, maximum/minimum 30:15 ratio, deep breathing test, Valsalva maneuver) and standardized, computerized measurements of HRV involving spectral analysis. Standardized, computerized measurements of HRV involving spectral analysis provides quantitative information on ANS function differentiated into vagal and sympathetic components [1]. There has recently been a growing interest in this particular technique because several studies in patients who had a previous myocardial infarction have consistently shown an association between a reduced HRV and an increased risk of cardiovascular morbidity and mortality [9–15]. Moreover, an association between reduced HRV and poor prognosis has also been shown for many other diseases, including diabetes mellitus, alcoholism, and dilatative cardiomyopathy [16–19]. Even though expert committees still have not made any final judgments on the future clinical value of HRV studies [1,20], it is beyond dispute that a wider clinical application of HRV requires appropriately defined normal values for the various HRV indices. Therefore, studies of adequately large samples of healthy subjects are needed and should investigate the relation between HRV and physiologic variables, such as age, gender, and heart rate (HR), in detail.

Most of the studies that address this point have focused on the influence of age on the various HRV indices. Convincing evidence has emerged from the literature that the results of these tests decrease with increasing age [21]. In contrast, the influence of HR itself on various HRV indices has only rarely been investigated [22–27]. Regarding possible gender differences in ANS function, some data are available in the literature [23–26,28–39], but the results are inconsistent in that a lack of gender differences, and a higher or even lower HRV in women, have been reported. Much of the data relating to this topic was found only by chance in studies designed to investigate other matters.

Moreover, these studies varied considerably in their methodology: Differences existed not only in the types of ANS function tests used (conventional tests or computerized HRV analyzes) or the patient sample (number, race, age distribution), but also in the duration of the HRV analyses (short-term assessment between 2 and 15 minutes, 24-hour long-term recordings), the subjects' body position (recumbent, sitting), and the control of breathing (free breathing versus metronomic breathing) during the recording session, the selection of HRV indices (time-domain or frequency-domain variables), and the statistical procedures used (calculation of power spectra by fast-Fourier transformation or autoregressive models, consideration or disregard of HR itself as a covariate).

A task force report published recently by the European Society of Cardiology and the North American Society of Pacing and Electrophysiology [1] focused on the problem of reduced comparability of HRV study results arising from these methodological variations. After a critical assessment of this problem, quality guidelines for performing and evaluating HRV studies were developed, and adequately large studies in healthy subjects for defining normal HRV values were demanded.

In the current study, and considering the guidelines of the task force report, a standardized 5-minute HRV resting study [1], the deep-breathing test (DBT), and an orthostatic test (maximum/minimum 30:15 ratio) were performed in 309 healthy subjects. The DBT and orthostatic tests are components of the autonomic test battery proposed by Ewing and Clarke in the mid 1970s, which was recommended for the standardized assessment of autonomic dysfunction in diabetic neuropathy [40]. The aim of this study was to answer the following questions: (1) which of the HRV indices are dependent on age; (2) does HR itself influence the time- or the frequency-domain (or both) HRV indices; and (3) are there any significant effects of gender on the HRV indices?

Methods

Study population

Three-hundred fifty-six subjects (173 male, 183 female), with an average age of 36.7 ± 13.8 years (18–77 years), were recruited for the study. They included medical and nonmedical employees of the hospital clinics in Gelsenkirchen, Düsseldorf, and Weimar; medical and nonmedical students of the Ruhr-University of Bochum; and the family members of both groups. After receiving an explanation of the planned study, prospective subjects decided whether to participate. Evaluation of the medical condition of the patients was based on raising full medical histories and undertaking a general physical and neurologic examination. Exclusion criteria included the presence of diseases known to affect HRV (eg, cardiovascular, endocrinologic, and test-interfering neurologic and psychiatric disorders [including alcoholism or polytoxemia]). No subject took medications regularly, the only exceptions being oral contraceptives and the occasional intake of nonsteroidal anti-inflammatory drugs (eg, acetylsalicylate or paracetamol).

The HRV tests were performed in the order described below. Heart rate variability examinations were performed between 9:00 and 11:30 AM to avoid diurnal variations. All participants were instructed to have a light meal only and to abstain from smoking and drinking caffeine-containing beverages or alcohol. They were also asked to avoid demanding physical activities for at least 2 hours before the recording session.

Five-minute resting study of heart rate variability

The evaluation and execution of HRV measurements was based on previously published experimental procedures [1,8,22]. For analyzing HRV, we used the Neurodiag software program (R. Lambeck, Munich, Germany). With this system, a fast machine-code algorithm recognizes the typical form of the QRS complexes from a set of parameters characterizing the time course of the signal: steepness of the ascending and descending slopes, rise time, and amplitude. The optimal setting of these parameters is evaluated from individual electrocardiograms (ECGs) during a learning phase that is automatically activated before the actual measurement phase. During measurement, the optimal settings of the QRS recognition parameters are continuously adjusted to the changes in electrocardiographic waveforms observed (eg, with deep breathing). For controlling the reliability of QRS detection, the electrocardiographic signal is continuously displayed on a computer screen, and the correctly recognized QRS complexes are automatically marked on the monitor. The R-R intervals were measured to an accuracy of ± 1 ms. The artifact-free, digitized signal was stored on a personal computer for later analysis. The examination was started after the subjects had rested on the examination bench for 10 minutes in a relaxing and comfortably temperate room.

Recording HRV while the patients are lying down best enables the patients to relax. The subjects were asked to breathe regularly and calmly, and to speak and to move as little as possible; they were also asked to stay awake. The resting heart rate (HRr) was defined as the average heart rate during the 5-minute resting examination. Time-domain variables included the coefficient of variation at rest (CVr), defined as the ratio of the standard deviation of the R-R intervals divided by the average duration of the R-R intervals, and the root mean square of successive differences at rest (RMSSDr); both parameters represent parasympathetic activity [1,22]. Data used for calculating the spectral analyses were the artifact-free R-R intervals registered over a period of 5 minutes at a temporal resolution of 1 ms. The interval series was converted using a mathematical algorithm [41] into a discrete signal of 1024 levels (computed at equidistant sampling points 290 msec apart). The resulting power spectrum was calculated using a fast-Fourier transformation, whereby three frequency bands were automatically separated: very-low-frequency (VLF) band (0.003–0.04 Hz), low-frequency (LF) band (0.04–0.15 Hz), and high-frequency (HF) band (0.15–0.4 Hz).

The LF band is probably associated with parasympathetic [42] and sympathetic activity both [43–45], and represents a reflection of the baroreflex response. The HF band coincides with the respiratory frequency and reflects mainly respiration-linked variations of HR (respiratory sinus arrhythmia) resulting from centrally mediated cardiac vagal control [1].

Deep-breathing test

The subjects were instructed to take six deep breaths per minute (6 seconds inspiration, 4 seconds expiration). The respiratory cycles were depicted on a computer monitor by rising and falling columns. The resulting changes in HR, the breath-dependent amplitudes of the R-R deflections, and properly recognized QRS complexes were also registered. The coefficient of variation during deep breathing (CVd) and the root mean square of successive differences during deep breathing (RMSSDd) were calculated from at least 100 artifact-free R-R intervals [8,46]. During the breathing cycle with the maximum HRV, the longest R-R interval during expiration (E) and the shortest R-R interval during inspiration (I) were determined to obtain the expiratory–inspiratory (E/I) ratio and the E-I difference. Moreover, a geometrically constructed variable, the mean circular resultant, was calculated by vector analysis [22,47].

Orthostatic test (maximum/minimum 30:15 ratio)

The electrocardiographic analysis was started after the patients were instructed to stand up rapidly. The 30:15 ratio (the ratio of the longest R-R interval between the 21st and 45th heart beat divided by the shortest interval between the 5th and 25th heart beat after standing up) was calculated [7,22,46]. The predominant component of this reflex is also subject to parasympathetic influences.

Statistics

Because the HRV indices were skewed, all HRV indices (except the E/I ratio and 30:15 ratio) were log transformed (log10 transformation); after logarithmic transformation, all HRV indices showed a normal distribution. Because by definition the E/I and 30:15 ratios cannot yield values between 0 and 1, it was not the ratio, but rather the ratio minus 1 that revealed a normal log distribution [22]. The age-dependent lower limits of normal were defined as the 2.5-percentile points of the distributions. Correlations between different HRV indices and between the HRV indices and age or HR were computed using Pearson correlation coefficients. Gender differences of the HRV indices were calculated with the independent factor gender (male versus female) and the covariates age and HR by multivariate analysis of variance (MANCOVA). In an alternative statistical procedure, five different age subgroups were created (17–25, 26–35, 36–45, 46–55, greater than 55 years), which were considered as additional independent factors in the MANCOVA in place of the covariate age. For comparing the means of the HRV indices of the age subgroups, a one-way analysis of variance was applied with a *post hoc* comparison using the Scheffé test. The level of significance was set uniformly at $\alpha = 0.05$.

Results

Forty-seven subjects were excluded for numerous reasons (previous diseases, abnormal findings during examination or resting electrocardiography, increased appearance of artifacts or extrasystoles during electrocardiographic recording); data from 309 healthy subjects were available for further analysis. Table 1 shows age and gender distribution. The percentage of correctly recognized QRS complexes during the 5-minute resting study was 99% or more for each of the subjects included in the study.

Influence of age and heart rate on heart rate variability

All HRV variables studied, except HRr and LF/HF ratio, were significantly age dependent (Table 2). A significant inverse correlation between age and the HRV indices was observed for both sexes (data not shown).

Unlike the spectral bands (VLF, LF and HF bands), the LF/HF ratio showed a weak correlation with HRr (Table 2). Regarding the time-domain indices at rest, significant inverse correlations were noted between HRr and both CVr and RMSSDr. The influence of HRr on RMSSDr was stronger than that of age. In contrast, the CVd, E/I ratio and the maximum/minimum 30:15 ratio were all independent of HR.

Most of the HRV indices correlated with one another. High correlation coefficients ($r > 0.5$) were found between HF power and DBT. Both the lower frequency components (VLF, LF power) and the LF/HF ratio were not correlated with the 30:15 ratio, and the LF/HF ratio showed no correlation with the DBT results.

Influence of gender on heart rate variability

Because age and HR both markedly influence HRV, both these variables have to be taken into account when statistically determining any potential gender differences concerning HRV. Multivariate analysis for the total group of healthy subjects ($n = 309$), with the covariates age and HR (MANCOVA) and the HRV indices of the 5-minute resting study as dependent variables, showed a significant influence of gender (male versus female) on the absolute LF

Table 1. Age and sex distribution in the study population

Age subgroup (y)	Male (*n*)	Female (*n*)	Total no. (%)
17–25	33	25	*n* = 58 (18.8)
26–35	66	57	*n* = 123 (39.8)
36–45	22	25	*n* = 47 (15.2)
46–55	16	26	*n* = 42 (13.6)
56–65	8	16	*n* = 24 (7.8)
>65	6	9	*n* = 15 (4.9)
Total	151	158	N = 309 (100)

Table 2. Correlation coefficients between the HRV indices and age or heart rate

	Age		Heart rate	
	Coefficient *r*	p value	Coefficient *r*	p value
5-min resting study				
HRr (bpm)	−0.02	NS	Not done	Not done
CVr (%)	−0.46	<0.001	−0.26	<0.001
RMSSDr (ms)	−0.41	<0.001	−0.57	<0.001
VLF power ($msec^2$)	−0.31	<0.001	0.09	NS
LF power ($msec^2$)	−0.44	<0.001	0.11	NS
HF power ($msec^2$)	−0.52	<0.001	−0.05	NS
Total power ($msec^2$)	0.50	<0.001	0.07	NS
LF/HF ratio	0.10	NS	0.22	<0.01
Deep breathing test				
CVd (%)	−0.55	<0.001	−0.10	NS
RMSSDd (msec)	−0.45	<0.001	−0.50	<0.001
MCR	−0.52	<0.001	−0.15	<0.05
E-I difference (msec)	−0.46	<0.001	−0.36	<0.001
E-I ratio	−0.53	<0.001	−0.10	NS
Orthostatic test				
30:15 ratio	−0.30	<0.001	−0.13	NS

HRV = heart rate variability; HRr = heart rate at rest; bpm = beats per minute; NS = not significant; CVr = coefficient of variation at rest; RMSSDr = root mean square of successive differences at rest; VLF = very low frequency; LF = low frequency; HF = high frequency; CVd = coefficient of variation at deep breathing; RMSSDd = root mean square of successive differences at deep breathing; MCR = mean circular resultant during deep breathing; E-I difference = longest R-R interval during expiration (E) minus the shortest R-R interval during inspiration (I); E-I ratio = E divided by I; 30:15 ratio = the longest R-R interval between the 21st and 45th heart beat divided by the shortest interval between the 5th and 25th heart beat after standing.

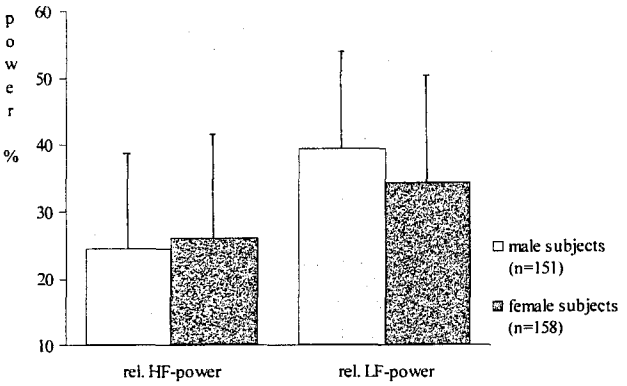

Figure 1. Mean (± SD) of the percentage low-frequency (LF) and high-frequency (HF) powers as a proportion of the total power (defined as the sum of very low frequency [VLF]+LF+HF) separated according to gender. The multivariate analysis (covariates age and heart rate) confirmed the presence of significant gender differences: women showed a higher relative HF power (F = 5.61, p = 0.019) and a lower relative LF power (F = 8.91, p = 0.003) than men.

power (F = 8.06, p = 0.005) and the LF/HF ratio (F = 5.49, p = 0.02): women showed a lower average LF power (2.85 ± 0.48 versus 3.03 ± 0.44 $msec^2$) and a lower average LF/HF ratio (2.13 ± 2.12 versus 2.50 ± 2.15) compared with men. There were no significant gender differences for the absolute HF power and the total power (defined as the sum of VLF + LF + HF). When calculating the percentage LF and HF powers as a proportion of the total power (Fig. 1), women showed a higher relative HF power (F = 5.61, p = 0.019) and a lower relative LF power (F = 8.91, p = 0.003) compared with men. The multivariate analysis for all HRV indices of each of the autonomic reflex tests (DBT; 30:15 ratio) showed no significant gender differences.

Tables 3 and 4 illustrate the averages for each of the HRV indices computed separately for each gender and each of the 5 different age subgroups. If the covariate age is substituted in the above MANCOVA analysis with the additional factor "age subgroup" (the HR itself remains a covariate), this statistical procedure confirms the expected significant effect of the factor age subgroup on all the time- and frequency-domain HRV indices listed in Table 3 (all p values ≤ 0.001). Interaction effects between age and gender were not found (p values 0.5–1.0). Regarding the factor gender, the significant differences remained, that is, for the absolute (F = 4.57, p = 0.033) and relative LF power (F = 5.10, p = 0.025) and for the relative HF powers (F = 4.02, p = 0.046). There were no significant gender differences for the absolute HF power. As illustrated in Table 3, the most prominent gender differences for the absolute LF power were noted at ages between 26 and 55 years, whereas no gender differences were observed at ages older than 55.

Interestingly, healthy women up to the age of 55 years compared with age-matched healthy men showed a higher mean HRr (Table 3). A two-factor analysis (factors "age subgroup" and "gender") showed a significant effect only for gender (F = 4.66, p = 0.032), but not for the factor age subgroup (F = 2.27, p >0.05), whereas there was a lack of a significant interactive effect between these two factors (p = 0.31).

Definition of normal values taking age and gender into account

To obtain the age-dependent 2.5 percentiles of the HRV indices, all data were transformed logarithmically, except for the E/I and 30:15 ratios, which were calculated using a log (y-1) transformation [22]. The 2.5 percentiles of all the age-dependent HRV variables derived from these transformations are given in Tables 5 and 6. Because significant gender differences were found for the LF power, and all HR–dependent HRV indices differed depending on gender (women have a significantly higher HR than men), we provide separate values for the 2.5 percentiles of the HRV indices for men and women. By applying the definition suggested here for the normal ranges of the HRV indices (values >2.5 percentile), 2.5% of the healthy men and 2.5% of the healthy women showed an abnormal test result; that is, the specificity for each of the HRV indices is 97.5%.

Table 3. Results of the 5-minute HRV resting study in 309 healthy individuals with respect to age and gender

	Age subgroups (in years)				
	17–25 (n = 58)	26–35 (n = 123)	36–45 (n = 47)	46–55 (n = 42)	>55 (n = 39)
HRr (bpm)					
Male	68.9 ± 10.5	68.9 ± 9.1	72.8 ± 13.0	69.1 ± 7.8	69.8 ± 9.6
Female	76.7 ± 13.5	71.3 ± 12.7	76.5 ± 11.1	71.8 ± 8.8	68.0 ± 9.2
CVr (%)					
Male	0.78 ± 0.20[4,5]	0.76 ± 0.16[4,5]	0.69 ± 0.12[5]	0.59 ± 0.15	0.48 ± 0.18
Female	0.77 ± 0.16[3,4,5]	0.72 ± 0.19[4,5]	0.59 ± 0.16	0.53 ± 0.16	0.53 ± 0.21
RMSSDr (msec)					
Male	1.65 ± 0.32[4,5]	1.58 ± 0.22[4,5]	1.45 ± 0.30	1.31 ± 0.21	1.25 ± 0.21
Female	1.58 ± 0.32[4]	1.54 ± 0.30[4]	1.35 ± 0.23	1.28 ± 0.22	1.33 ± 0.27
Total power (msec^2)					
Male	3.62 ± 0.35[4,5]	3.56 ± 0.36[4,5]	3.39 ± 0.24	3.20 ± 0.30	3.02 ± 0.26
Female	3.63 ± 0.22[3,4,5]	3.48 ± 0.35[4,5]	3.29 ± 0.36	3.14 ± 0.37	3.07 ± 0.35
VLF (msec^2)					
Male	3.04 ± 0.38	3.03 ± 0.40	2.95 ± 0.33	2.83 ± 0.36	2.68 ± 0.32
Female	3.05 ± 0.27[5]	3.00 ± 0.35[5]	2.86 ± 0.44	2.76 ± 0.31	2.66 ± 0.34
LF (msec^2)					
Male	3.14 ± 0.39[5]	3.15 ± 0.42[4,5]	2.99 ± 0.35[5]	2.77 ± 0.35	2.49 ± 0.42
Female	3.11 ± 0.33[4,5]	3.01 ± 0.40[4,5]	2.79 ± 0.43	2.60 ± 0.54	2.54 ± 0.46
HF (msec^2)					
Male	3.01 ± 0.50[4,5]	2.88 ± 0.40[4,5]	2.70 ± 0.29[5]	2.30 ± 0.30	2.25 ± 0.31
Female	3.04 ± 0.39[3,4,5]	2.87 ± 0.47[4,5]	2.59 ± 0.35	2.35 ± 0.44	2.34 ± 0.42

All data except HRr were log10 transformed; the values given are mean ± SD. $p < 0.05$, one-way analysis of variance with a *post hoc* comparison using the Scheffé test.
HRV = heart rate variability; HRr = heart rate at rest; bpm = beats per minute; CVr = coefficient of variation at rest; RMSSDr = root mean square of successive differences at rest; VLF = very low frequency; LF = low frequency; HF = high frequency.
Superscript numbers indicate significant differences in HRV indices between younger patient subgroups compared with [3]subgroup 3 (36–45 y), [4]subgroup 4 (46–55 y), and [5]subgroup 5 (>55 y).

Discussion

This study was performed to determine the effects of age, gender, and HR on the results of HRV during standardized tests in a large sample of healthy subjects. Conventional tests (deep breathing, max/min 30:15 ratio), and a standardized 5-minute resting study, were used. The latter method particularly is a simple, well-tolerated, and economical procedure that can be used without problems in clinical routine procedures. It allows adequate quantitative estimation of cardiac autonomic nervous system regulation [1] in a manner largely independent of patient cooperation.

The most important results included the following: (1) the HRV indices of all tests (except for the LF/HF ratio and HR) are inversely age dependent in both sexes; (2) the spectral bands of the 5-minute resting study (except for the LF/HF ratio), the coefficient of variation (CVd), and the E/I ratio during deep breathing, and the max/min 30:15 ratio, are independent of HR (unlike the remaining HRV indices studied); (3) women up to the age of 55 years have a higher HRr compared with men in the same age group; and (4) young and middle-aged women have a significantly lower LF power and LF/HF ratio compared with age-matched men, whereas no significant gender differences are seen for the absolute HF power.

Age dependence of the heart rate variability indices

The age dependence of all the HRV indices measured confirms our earlier results [22] and is consistent with the results of others, who measured HRV in healthy subjects during a short-term resting study [29,30,32,48–50], during 24-hour Holter recording [23,25,33,35,51], or by conventional tests such as the DBT or the max/min 30:15 ratio [26,28,52–56]. Only a few studies found no association between age and the low- or high-frequency components of spectral power [57–59] or the DBT (summary in Low *et al.*) [52], but these studies used very small samples (n = 10–63). Low *et al.* [52] reported that vagal modulation of HRV decreases with increasing age, whereas postganglionic sympathetic function is barely affected by age [52]. Others reported that sympathetic nerve activity increases with age [60–62]. Sega et al. [63] reported a significant decrease in total, LF, and HF power with increasing age, whereas the LF/HF ratio while the patient is in the supine posture increased. These results were interpreted as evidence for a shifting of autonomic neurocardiac balance with age [63]. Our findings (ie, the decreasing LF power with age and the lack of an association with age for the LF/HF ratio) contradict such a theory and are consistent with most other studies, which found no associations between the LF/HF or mid-frequency/HF ratios and age [24,29,35,44,49]. Only isolated reports showed correlations between age and the LF/HF or mid-frequency/HF ratios [30,48]. However, compared with our study their samples were significantly smaller (n = 34–67), and differing age groups were investigated: Yeragani *et al.* [48] evaluated children aged 4–12 years, whereas in Ryan's study [30] almost a third of the healthy subjects were older than 70 years.

Table 4. Results of the deep-breathing and the orthostatic test (30:15 ratio) in 309 healthy individuals with respect to age and gender

	Age subgroups (in years)				
	17–25 (n = 58)	26–35 (n = 123)	36–45 (n = 47)	46–55 (n = 42)	>55 (n = 39)
CVd (%)					
Male	1.00 ± 0.17[4,5]	1.02 ± 0.14[4,5]	0.90 ± 0.19[5]	0.79 ± 0.22[5]	0.57 ± 0.18
Female	0.99 ± 0.14[4,5]	0.98 ± 0.19[4,5]	0.86 ± 0.15[5]	0.79 ± 0.18	0.66 ± 0.18
RMSSDd (msec)					
Male	1.76 ± 0.24[4,5]	1.77 ± 0.22[3,4,5]	1.55 ± 0.24[5]	1.52 ± 0.20	1.27 ± 0.23
Female	1.65 ± 0.25[5]	1.70 ± 0.29[4,5]	1.55 ± 0.20	1.50 ± 0.19	1.36 ± 0.25
E-I difference (msec)					
Male	2.52 ± 0.15[4,5]	2.51 ± 0.14[4,5]	2.42 ± 0.17[5]	2.33 ± 0.22[5]	2.09 ± 0.21
Female	2.46 ± 0.18[5]	2.45 ± 0.23[5]	2.34 ± 0.17	2.31 ± 0.19	2.19 ± 0.23
E/I ratio					
Male	−0.83 ± 0.04[5]	−0.83 ± 0.04[4,5]	−0.86 ± 0.05[5]	−0.89 ± 0.05	−0.93 ± 0.03
Female	−0.82 ± 0.07[4,5]	−0.83 ± 0.06[4,5]	−0.88 ± 0.03	−0.88 ± 0.05	−0.91 ± 0.04
MCR					
Male	−1.23 ± 0.25[5]	−1.24 ± 0.21[5]	−1.41 ± 0.23	−1.41 ± 0.19	−1.64 ± 0.16
Female	−1.26 ± 0.20[4,5]	−1.24 ± 0.18[4,5]	−1.39 ± 0.27[5]	−1.51 ± 0.22	−1.66 ± 0.21
30:15 ratio					
Male	−0.92 ± 0.06	−0.88 ± 0.06	−0.90 ± 0.05	−0.92 ± 0.03	−0.94 ± 0.05
Female	−0.87 ± 0.06	−0.89 ± 0.06	−0.90 ± 0.04	−0.93 ± 0.04	−0.93 ± 0.06

All data were log10 transformed; values are mean ± SD. $p < 0.05$, one-way analysis of variance with a *post hoc* comparison using the Scheffé test.

CVd = coefficient of variation at deep breathing; RMSSDd = root mean square of successive differences at deep breathing; E-I difference = longest R-R interval during expiration (E) minus the shortest interval during inspiration (I); E/I ratio = E divided by I; MCR = mean circular resultant during deep breathing; 30:15 ratio = the longest R-R interval between the 21st and 45th heart beat divided by the shortest interval between the 5th and 25th heart beat after standing.

Superscript numbers indicate significant differences in HRV indices between younger patient subgroups compared with [3]subgroup 3 (36–45 y), [4]subgroup 4 (46–55 y), and [5]subgroup 5 (>55 y).

In our study, the strongest correlations were seen between age and all DBT indices and the HF and LF power. The former two HRV indices are known to reflect parasympathetic influences, while the LF power is at least partly under sympathetic influence. These findings indicate a decrease with age in parasympathetic and sympathetic activity. This is consistent with the finding that HR, influenced by both parts of the autonomic nervous system, is not correlated with age, a finding that confirms our previous results [22] and those from others [23,26,28,35].

Influence of heart rate on heart rate variability

Consistent with our previous results [22], the time-domain, but not the frequency-domain, HRV indices of the 5-minute resting study are dependent on HRr. The influence of HRr on RMSSDr might be stronger than the

Table 5. Normal ranges for age-dependent tests relating to control of heart rate in healthy women

	Supine resting study					Deep-breathing test					
Age (y)	CVr (%)	RMSSDr (msec)	VLF band (msec²)	LF band (msec²)	HF band (msec²)	CVd (%)	RMSSDd (msec)	MCR	E-I difference (msec)	E/I ratio	Standing up 30:15 ratio
15	2.78	12.24	296	230	194	5.92	21.47	0.029	145	1.117	1.104
20	2.57	11.07	260	193	154	5.35	19.28	0.026	133	1.113	1.102
25	2.38	10.01	228	161	122	4.83	17.30	0.023	123	1.110	1.101
30	2.20	9.05	200	135	97	4.36	15.54	0.021	113	1.107	1.099
35	2.04	8.18	176	113	77	3.94	13.95	0.018	103	1.104	1.098
40	1.88	7.39	154	94	62	3.56	12.52	0.016	95	1.102	1.096
45	1.74	6.68	135	79	49	3.21	11.24	0.014	87	1.099	1.095
50	1.61	6.04	119	66	39	2.90	10.09	0.013	80	1.096	1.093
55	1.49	5.46	104	55	31	2.62	9.06	0.011	73	1.094	1.092
60	1.38	4.94	92	46	25	2.37	8.13	0.010	67	1.091	1.090
65	1.28	4.47	81	39	20	2.14	7.30	0.009	62	1.089	1.089

Values given are the 2.5 centiles.

CVr = coefficient of variation at rest ; RMSSDr = root mean square of successive differences at rest; VLF = very low frequency; LF = low frequency; HF = high frequency; CVd = coefficient of variation at deep breathing; RMSSDd = root mean square of successive differences at deep breathing; MCR = mean circular resultant during deep breathing; E-I difference = longest R-R interval during expiration (E) minus the shortest R-R interval during inspiration (I); E/I ratio = E divided by I; 30:15 ratio = the longest R-R interval between the 21st and 45th heart beat divided by the shortest interval between the 5th and 25th heart beat after standing.

Table 6. Normal ranges for age-dependent tests relating to control of heart rate in healthy men

	Supine resting study					Deep-breathing test					
Age (y)	CVr (%)	RMSSDr (msec)	VLF band ($msec^2$)	LF band ($msec^2$)	HF band ($msec^2$)	CVd (%)	RMSSDd (msec)	MCR	E-I difference (msec)	E/I ratio	Standing up 30:15 ratio
15	3.32	16.00	244	362	236	6.34	27.25	0.028	200	1.129	1.107
20	3.05	14.22	219	300	185	5.61	23.76	0.025	178	1.125	1.105
25	2.80	12.63	197	249	145	4.97	20.72	0.022	159	1.121	1.103
30	2.58	11.22	176	207	113	4.40	18.06	0.020	141	1.118	1.101
35	2.37	9.96	158	172	89	3.89	15.75	0.018	126	1.114	1.099
40	2.18	8.85	142	142	69	3.44	13.73	0.016	112	1.111	1.097
45	2.00	7.86	128	118	54	3.05	11.98	0.014	100	1.107	1.096
50	1.88	6.98	114	98	42	2.70	10.44	0.013	89	1.104	1.094
55	1.69	6.20	103	81	33	2.39	9.11	0.011	79	1.101	1.092
60	1.55	5.51	92	68	26	2.11	7.94	0.010	71	1.098	1.091
65	1.43	4.89	83	56	20	1.87	6.92	0.009	63	1.095	1.089

Values given are the 2.5 centiles.
CVr = coefficient of variation at rest; RMSSDr = root mean square of successive differences at rest; VLF = very low frequency; LF = low frequency; HF = high frequency; CVd = coefficient of variation at deep breathing; RMSSDd = root mean square of successive differences at deep breathing; MCR = mean circular resultant during deep breathing; E-I difference = longest R-R interval during expiration (E) minus the shortest R-R interval during inspiration (I); E/I ratio = E divided by I; 30:15 ratio = the longest R-R interval between the 21st and 45th heart beat divided by the shortest interval between the 5th and 25th heart beat after standing.

influence of age. In contrast to our previous study, the E/I ratio and the maximum/minimum 30:15 ratio were independent of HR (for both variables our previous study found only low correlation coefficients of r = −0.20), and there was a weak correlation (r = −0.15) between the mean circular resultant and HR (in the previous study it was not significant). Because such low correlation coefficients reflect little or even no relation between the two variables [64], it is our opinion that the mentioned findings only appear to be contradictory and are in fact clinically irrelevant.

This study clearly shows that the time-domain variables of the 5-minute resting study cannot be adequately judged without taking the instantaneous HR into account. If HR itself is not accounted for in the statistical analysis, the evaluation should focus on HR–independent HRV indices. In the 5-minute resting study this applies to the spectral bands. Regarding the DBT, the CVd should be given preference over the RMSSDd, and the E/I ratio rather than the E-I difference should be used. However, the common practice of adjusting HRV indices for HR cannot guarantee an optimized description of ANS function because in healthy individuals, at least, the actual HR reflects the result of a permanently acting autonomic influence on the intrinsic HR.

Influence of gender on heart rate variability

Our analysis showed that women up to 55 years of age have a lower LF power and a smaller LF/HF ratio compared with age-matched men. Some have asserted that gender differences are exclusively a result of differences in HR [23,36], a notion that is not supported by our results. Prospective studies on gender effects, including short-term recordings (2–15 minutes), have reported inconsistent results regarding the frequency-domain HRV indices. For women compared with men, no differences [30] or a lower LF power have been reported [29,31,32]. For HF power, no difference [29] or an increase was observed in women [30–32], whereas for the LF/HF ratio, a reduction was observed among women [29–32]. In two of these studies, various time-domain HRV indices were also taken into account, but their results were also inconsistent [31,32].

Inconsistencies among these studies may result from differences in the study populations or in analytical methods. Our results are largely compatible with the largest study performed to date (n = 1,984) by Liao *et al.* [29], who also showed a reduction in LF power and LF/HF ratio in women compared with men and no difference in the HF power [29]. In both their study and ours, HRV was evaluated at rest during uncontrolled normal breathing (ie, no metronomic breathing). However, we consider it highly unlikely that potential gender differences in the absolute HF power were undetected as a result of this procedure. Sinnreich *et al.* [32], when performing 5-minute resting studies during uncontrolled and metronomic breathing both, concluded that metronomic breathing is not needed to obtain reliable measures of HF power using short-duration recordings [32]. Moreover, as our results show, no gender differences are found for the indices of the DBT, confirming the results of some [22,28], albeit not all [26], studies. Because HF power and the DBT indices both reflect parasympathetic activity, the results consistently indicate that there are no gender-related differences in vagal tone. The reduction of the LF/HF ratio in women can therefore be considered to be caused by a reduction in LF power alone. A significant reduction of LF power among women without gender differences in total power means that there is a higher relative parasympathetic activity in women than in men.

Women up to 55 years of age had a higher HRr than men. This finding has been observed in many larger studies [23–26,29,32,35]. However, it cannot be explained solely on the basis of gender differences in autonomic regulation. The observation of a reduced LF power and the resulting

increase in relative HF power in women would let us expect the opposite, if anything, ie, a lower HR in women compared with men. Do women have higher intrinsic HRs, or do hormonal influences affect the intrinsic HR? The latter hypothesis is supported by the fact that significant gender differences in HR and LF power were seen only for individuals up to the age of 55, that is, before the menopause has occurred for most women or when it was just starting. Other investigators have also shown that significant gender differences in spectral L-F power [24] and various other HRV indices [25,26] are found only up to ages 40 to 50 years.

What are the clinical consequences of the gender differences in HRV? Provided one accepts the concept that the L-F power at least partly reflects sympathetic activity and, therefore, the LF/HF ratio reflects the sympathovagal balance [44], our results indicate a reduced sympathetic activity in women compared with men. Although the physiologic origin of the spectral LF component is a matter of considerable dispute [65], the conclusions drawn from the concept of sympathovagal balance are attractive. Accepting that a raised sympathetic activity is related to a higher susceptibility toward life-threatening cardiac arrhythmias and the development of coronary heart disease [66], and that vagal activity exerts a cardioprotective effect [67,68], it is tempting to speculate that the lower sympathetic and higher parasympathetic activity found in young and middle-aged women is related to their known lower cardiac morbidity and mortality compared with men of comparable age. Such an association has been discussed by some research groups [24,35], but it is likely that this view represents an oversimplification of a complex and still poorly defined relation. A reduction in L-F power is not necessarily associated with a better prognosis. It can even be connected to a worse prognosis concerning cardiac morbidity and mortality [69–71]. Therefore, any practical consequence emerging from the observed gender differences in ANS function require further study and explanation.

Study limitations

This study was performed in a large group of healthy subjects. We are aware of only two larger studies [29,50], whereby only the larger of the two showed sex differences in HRV [29]. Compared with the mean age in most other studies that evaluated HRV in healthy subjects [24,25,29–32], the mean age of our subjects was significantly lower. All subjects were healthy according to history and clinical examination and the electrocardiogram was normal. Because of ethical reasons, we abstained from performing coronarangiography or myocardial scintigraphy in our healthy subjects. Because we did not perform screening blood tests, we cannot rule out definitively the possibility that subclinical cardiac disease or other diseases, such as an unrecognized type 2 diabetes or hyperthyroidism, were present in isolated cases. Recently, it was suggested that salicylates might mediate autonomic modulation [72]. On questioning, a number of the subjects in our study reported that they occasionally took acetylsalicylic acid (eg, because of a cold). We assume that occasional intake of acetylsalicylic acid before study enrollment produced no systematic perturbation of the HRV indices measured here, particularly because none of the patients had taken acetylsalicylic acid over a long period of time, and all subjects were healthy at the time of investigation. If there is a significant association between HRV and the concentration of certain serum lipid fractions or leukocyte count [73,74], this could not be taken into account in the statistical analysis. The statistical calculations of the somewhat skewed distribution of our subjects in the various age subgroups (older patients were underrepresented) might have been distorted, but we consider this unlikely because the HRV indices were linearly age dependent for both sexes. Moreover, to reduce the disproportions among men and women patients older than 55 years and to ensure that the age subgroups were all similar in size, all participants older than 55 years were allocated to one age subgroup. Our study does not allow definitive conclusions to be drawn on the cause of gender differences in HRV. The hypothesis relating to a potential correlation between hormonal regulation in women and HRV is supported by our study, but it cannot be proven definitively, because no information was available regarding the hormonal status of the women included in the study.

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